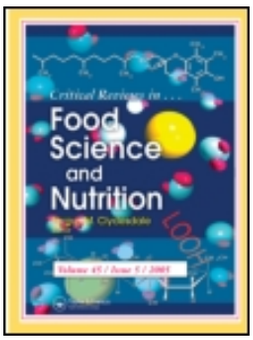

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Specificity of Infant Digestive Conditions: Some Clues for Developing Relevant In Vitro Models

CLAIRE BOURLIEU,[1,2] OLIVIA MÉNARD,[1,2] KARIMA BOUZERZOUR,[1,2] GIUSEPPINA MANDALARI,[3,4] ADAM MACIERZANKA,[3] ALAN R. MACKIE,[3] and DIDIER DUPONT[1,2]

[1]INRA, UMR 1253, Science & Technology of Milk and Egg, 35000 Rennes, France
[2]Agrocampus Ouest, Science & Technology of Milk and Egg, 35000 Rennes, France
[3]Institute of Food Research, Norwich Research Park, Norwich, Norfolk NR4 7UA, UK
[4]Pharmaco-Biological Department, University of Messina, Annunziata, 98168 Messina, Italy

Digestion of nutrients is an essential function of the newborn infant gut to allow growth and development and understanding infant digestive function is essential to optimize nutrition and oral drug delivery. Ethical considerations prohibit invasive in vivo trials and as a consequence in vitro assays are often conducted. However, the choice of in vitro model parameters are not supported by an exhaustive analysis of the literature and do not mimic precisely the digestive conditions of the infant. This review contains a compilation of the studies which characterized the gastroduodenal conditions in full-term or preterm infants of variable postnatal age from birth up to six months. Important data about healthy full-term infants are reported. The enzymatic (type of enzymes and level of activity) and nonenzymatic (milk-based diet, frequency of feeding, bile salt concentrations) conditions of digestion in infants are shown to differ significantly from those in adults. In addition, the interindividual and developmental variability of the digestive conditions in infants is also highlighted.

Keywords Digestion, newborn infant, gastrointestinal, in vitro model, digestive fluids, infant nutrition

INTRODUCTION

At birth, the digestive compartments and intestinal lumen of the newborn infants are exposed for the first time to external constituents: human milk or infant formulas are absorbed and digested. Digestion of nutrients is an essential function for the newborn to allow normal growth and development (Kelly and Coutts, 2000; Neu, 2007). Evaluation of this multistage complex function has been classically conducted through long, onerous, ethically delicate in vivo clinical studies (on animals and/or human). Valuable data about the luminal events which occur during digestion can also be obtained by in vitro tests which require the precise knowledge of newborns' digestive conditions. The recent reviews dealing with in vitro model to mimic digestion have demonstrated the interest of this rapid approach for preliminary studies of certain hypotheses such as digestibility, allergenicity, structural evolution, etc. (Boisen and Eggum, 1991; Oomen et al., 2003; Yoo and Chen, 2006; Wickham et al., 2009; McClements and Li, 2010; Hur et al., 2011). The maturation of digestive function occurs very early during uterine life allowing enteral feeding by 29-week GA (McClean and Weaver, 1993; Commare and Tappenden, 2007; Neu, 2007). However, several enzymatic and secretory functions are immature during the first months of life and may affect digestion. Their precise ranges of variation in infants (—zero to six months) have not been defined. Thus, an exhaustive review of the gastric and duodenal conditions in the healthy full-term infant (aged zero to six months) is needed to illustrate the specificity and variability of these digestive conditions as compared to adult. This review will be a valuable decision tool for scientists to make well-considered choice of digestive parameters for their models.

Adult in vitro digestion models generally consider three main phases: (i) oral phase in the mouth during which solid food are chewed and transformed into a soft bolus which can be swallowed, (ii) gastric phase, and (iii) duodenal phase (Oomen

Address correspondence to Claire Bourlieu, INRA, UMR 1253 and Agrocampus Ouest, Science & Technology of Milk and Egg, 65 rue de St Brieuc, 35000 Rennes, France. E-mail: claire.bourlieu@rennes.inra.fr

et al., 2003). A specificity of newborn infants (aged zero to six months) is to be fed exclusively on liquid milk-based meals which reduces considerably the oral phase of the digestive process: contact of the liquid meal with the oral cavity is limited to the swallowing time, and exposure of the meal to active components of the saliva (glycoprotein and salivary amylase mainly) will occur in the subsequent phases. Thus our review will present the physiologic data available in literature about the gastric and duodenal phases and will highlight the key parameters which influence gastroduodenal digestion in infants with the aim of defining them for in vitro modeling purpose.

GASTRIC PHASE

The first step of digestion occurs in the stomach under the action of gastric juice secreted by the nonparietal and parietal cells of the gastric mucosa. Collection of pure gastric juice is extremely difficult since the use of gastric tube only allows the aspiration of gastric content which also contain residual meal, swallowed saliva, bile, and pancreatic reflux. However, it is generally accepted that gastric secretions consist of water, acid, minerals, and enzymes (Geigy, 1973). The two main types of gastric enzymes contained in these secretions are the human gastric lipase (HGL) and pepsins which catalyze the hydrolysis of lipids and proteins, respectively. There is a nonparallel development of these two types of enzymes in infants, *i.e.* (i) a rapid development of lipase from the 11th week of gestation (Lindquist and Hernell, 2010) which reaches adult activity levels at birth, and (ii) a slow development of pepsins, also secreted early during development (14th week) but which reaches only 18% of adult activity four weeks after birth (DiPalma et al., 1991; Ménard et al., 1995; Armand et al., 1996; Henderson et al., 1998). The ontogeny of these two enzymes as compared to other digestive enzymes is proposed in Figure 1. It appears that the extent of fat digestion in the stomach is comparable to the one reported for adults, whereas a minimal protein digestion occurs (Mason, 1962) due to a combination of low pepsin output (Agunod et al., 1969; Yahav et al., 1987; Weisselberg et al., 1992) and high postprandial gastric pH (Mason, 1962; Harries and Fraser, 1968; Hamosh et al., 1978; Cavell, 1983; Smith et al., 1986). Indeed, bioactive human milk proteins such as lactoferrin and immunoglobulin A are present in the stools of infants fed human milk (Schanler et al., 1986; Davidson et al., 1989) suggesting impaired milk protein hydrolysis in the stomach as well as in the intestine.

The gastric phase in terms of enzymes and fluid composition is influenced both by hormones (*e.g.* gastrin, cholecystokinin, and secretin) and by other factors (gastric acidity, mixing, and gastric emptying rate), at least in part controlled hormonally, which differ in newborns as compared to children and adults.

Gastric Lipase

As first underlined by Hamosh (1983), lipase activity is essential for the newborn that has to face a sudden switch from its former high carbohydrate diet in utero to its high fat diet at birth. Indeed, fat mainly present in the form of triacylglycerols (TAG) supply 40 to 50% of the total calories in human milk or infant formulas.

Hydrolysis of TAG is initiated by HGL (398 AA, 45238 Da) which has a low pH optimum 3.0 to 5.0 (maximum at 5.4), does not require bile salts or cofactors to be active, and is stable to pepsin allowing its action in the gastric environment (Hamosh et al., 1978; Armand et al., 1996; Ville et al., 2002). HGL plays a qualitative rather than quantitative part in the digestion of fat: in healthy adults, gastric digestion leads to the hydrolysis of 10 to 30% of the total esterified fatty acids (Hamosh, 1983; Rudloff and Lonnerdal, 1992; Carrière et al., 1993; Lengsfeld et al., 2003; Mu and Hoy, 2004). Inhibition of the HGL by the long chain FA generated during the lipolytic reaction was hypothesized to explain such reduced degree of hydrolysis (Pafumi et al., 2002): released FFA form particles on the emulsion interface that trap gastric lipase and restrict its activity. Gastric lipolysis though limited has been presented very early as a key phenomenon in efficient fat digestion for newborn infants to compensate for the immaturity of exocrine pancreatic function, to allow the subsequent action of pancreatic lipases or milk bile salt-dependent lipase on human milk fat globules. Based on in vitro studies, it has been suggested that amphiphilic properties of the lipolysis products resulting from the action of gastric lipase participate in subsequent lipid emulsification (Bernback et al., 1989, 1990; Berton et al., 2009). HGL has a strong *sn*-3 regioselectivity which results in the preferential release of acyl moieties located in this position: long chain polyunsaturated FA in human milk and short to medium chain FA in bovine milk. Despite this specificity, the profile of FFA obtained from an infant formula enriched in medium chain triglycerides, remains dominated by palmitic and oleic acids (Roman et al., 2007).

Some values of HGL activity determined in gastric aspirates of healthy term or preterm infants in fasting state are summarized in Table 1. As can be seen, the reported activity levels of HGL is very variable (0–38.9 U/mL), which can be explained by (i) dilution of gastric aspirates with amniotic liquid in the case of gastric aspirates collected at birth, (ii) variation of lipase activity assay (variation in the chain length of substrate, in the solid fat content, and in emulsion ultrastructure), (iii) individual variability, and (iv) variation in the length of time between feeding (effect of dilution by meal and gastric emptying). Indeed, HGL activity in gastric content increases during meal digestion (Figure 2) as a result of meal-induced secretion and emptying (Fredrikzon and Hernell, 1977; Armand et al., 1996; Roman et al., 2007).

Conversely, HGL activity is little influenced by

(i) The nature of the meal (human milk or different types of formula). At similar fat content, this parameter does not affect gastric lipase activity (Armand et al., 1996; Roman et al., 2007);
(ii) The age of the infants. Though Hamosh et al. (1978, 1981) reported higher lipolytic activity in gastric aspirates col-

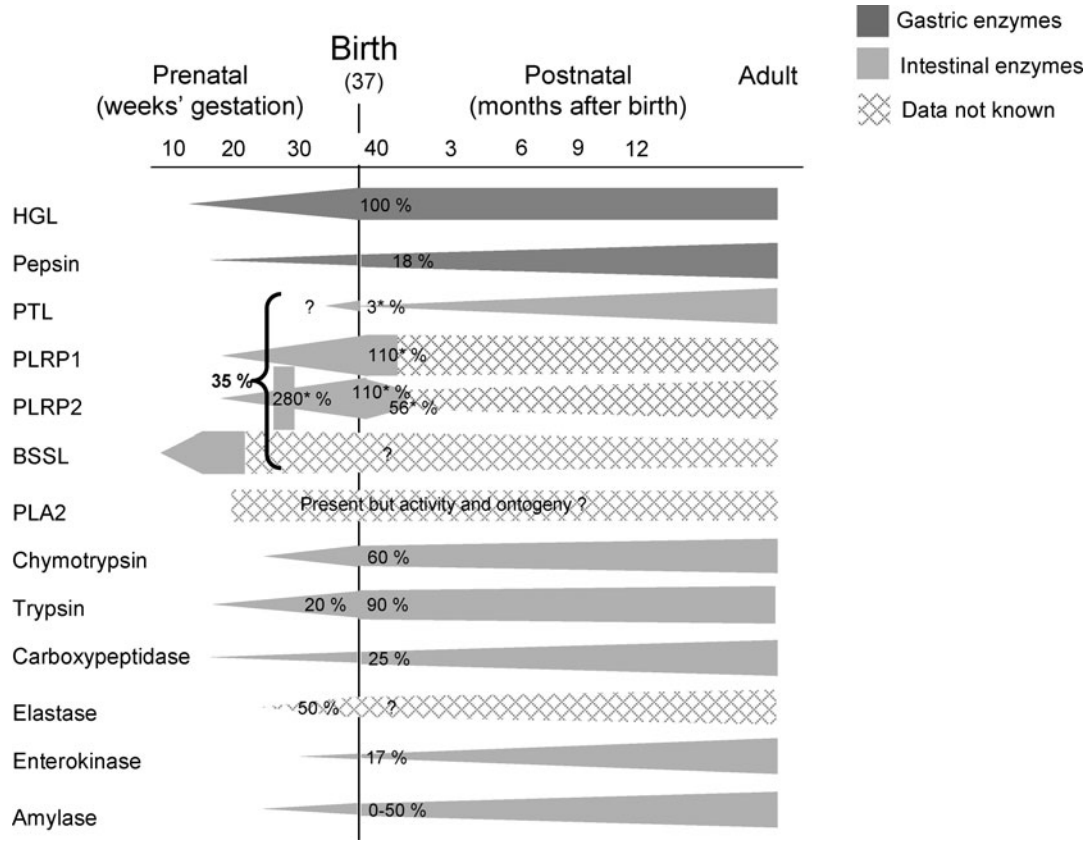

Figure 1 Ontogeny, levels of activity or of mRNA expression (*) of the main digestive enzymes reported in literature. Figure refers to the values at birth or at a given postconception age for preterm infants. Adapted from (McClean and Weaver, 1993).

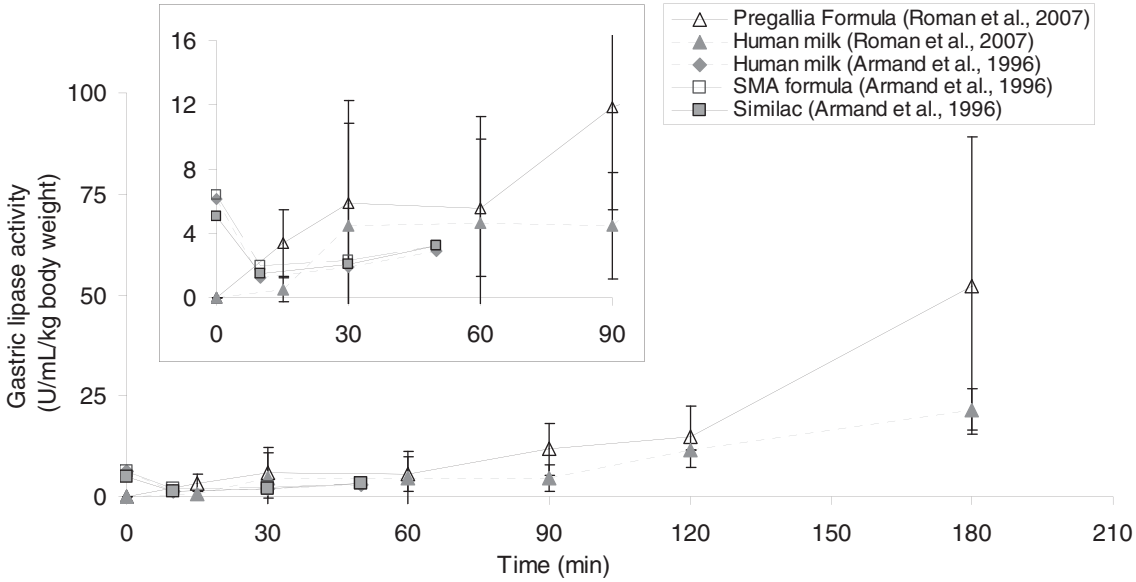

Figure 2 Gastric lipase activity and its postprandial evolution in newborn stomach after ingestion of breast milk or formulas. Composition of SMA formula (g/L): protein 19, fat 44, and carbohydrate 85; composition of Similac formula (g/L): protein 22, fat 84, and carbohydrate 86; composition of PreGallia formula (g/L): not published.

Table 1 Values of HGL activity determined in gastric aspirates of healthy full-term or preterm infants determined in fasting state

Group: *N*; GA; Age	Birth weight (kg)	pH of collected sample	Gastric activity (μmol/min/mL)	References	Additional information (collection time, type of assay)
$N = 7$; 38–41 wk; 2.1 ± 1.9 d	3.43 ± 0.51	2.5 ± 0.5	38.9 ± 22.8	(Fredrikzon and Hernell, 1977)	Collection before the first meal ($N = 4$)—activity against p-nitrophenyl acetate determined spectrophotometrically at pH 5.5
$N = 3$; 33–36 wk; 27 ± 9.2 d	1.94 ± 0.22	3.0 ± 0.7	18 ± 13.6	(Fredrikzon and Hernell, 1977)	Collection in fasting subjects who had already been fed—similar assay as above
$N = 9$; 27 ± 0.5 wk; n.a.	0.83 ± 0.03	5.56 ± 0.5	0.321 ± 0.117	(Hamosh et al., 1981)	Collection at birth—[tri^{3}H]oleate as emulsified substrate test at pH 4.2
$N = 22$; 32 ± 0.4 wk; n.a.	1.30 ± 0.03	5.74 ± 0.3	0.328 ± 0.028		
$N = 24$; 34 ± 0.4 wk; n.a.	1.77 ± 0.02	5.70 ± 0.19	0.350 ± 0.053		
$N = 35$; 35 ± 0.2 wk; n.a.	2.25 ± 0.03	6.25 ± 0.12	0.608 ± 0.034		
$N = 22$; 37 ± 0.3 wk; n.a.	2.76 ± 0.02	5.36 ± 0.30	0.564 ± 0.062		
$N = 30$; 39 ± 0.2 wk; n.a.	3.32 ± 0.05	4.93 ± 0.17	0.501 ± 0.041		
$N = 13$; 30–34 wk; n.a.	1.05–1.78	4.0 ± 0.17	0.048 ± 0.051 (0.004 to 0.140)	(Hamosh et al., 1978)	Collection before feeding – doubly labeled ^{3}Hglyceryl-^{14}C tripalmitin—optimal pH of lipase 5.4
$N = 11$; 28.9 ± 1.4 wk; 5.5–7.5 wk	1.18 ± 0.07	3.2 ± 0.3	7.9 ± 0.8	(Armand et al., 1996)	Collection before feeding—infant studied 1 to 5 times—[tri^{3}H]oleate as emulsified substrate test at pH 5.4
$N = 9$; 29.1 ± 0.9 wk; 4.6–7.0 wk	1.09 ± 0.09	3.4 ± 0.5	8.9 ± 1.8		
$N = 8$; 28.9 ± 1.4 wk; 5.8–7.4 wk	1.00 ± 0.14	3.4 ± 0.3	6.8 ± 0.9		
$N = 9$; 29 ± 1 wk; 5.3 ± 1.8 wk	1.53 ± 0.55	7.0–3.35 ± 0.87	Close to zero	(Roman et al., 2007)	Collection twice a day for five days before feeding—tributyrate substrate test using pH-stat

n.a, non available; wk, week; d, day.

lected at birth as compared to gastric aspirates after birth, other authors did not show an effect of age on HGL activity. Armand et al. (1996) indicated that gastric lipase activity (4–15 U/mL) of the gastric aspirates of premature newborns was in the range previously reported in adults (Armand et al., 1995). Roman et al. (2007) also reported similar lipase activity levels for premature newborns, reaching 75% of the maximum levels recorded in healthy adults (100.6 ± 56 vs. 130.0–156 U/mL). DiPalma et al. (1991) determined the lipase activity in biopsy specimens from the gastric body and gastric antrum, in subjects aged from three months to 26 years. They estimated that there was no significant difference in the level of lipase activity among age groups. Irrespective of age, a regional distribution of activity was shown: antrum activity values were very low as compared to body values.

HGL lipase output, which corresponds to the quantity of lipase excreted through the gastric pylorus over a given period of time, follows the same trends as HGL activity: lower fasting values (4–10 U/kg) as compared to postprandial values (22–33 after 10-minute feeding) were reported for instance in premature infants (Armand et al., 1996), independent of the type of meals (23.1 ± 5.1, 28.3 ± 6.6, and 22.5 ± 6.4 U/kg for human milk, SMA SP, or Similac SC, respectively), limited influence of age: the output of premature newborn is comparable to healthy adults fed a high fat diet (22.6 ± 3.0 U/kg) (Armand et al., 1995). The authors postulated that the high gastric lipase level of premature infants could be the result of an adaptation to infant high fat diet as had been previously reported in human adults (Armand et al., 1995).

Lastly, a summary of the extents of gastric lipolysis reported in full-term or a premature infants is proposed in Table 2. Most studies characterized an instantaneous rate of lipolysis (quantity of FFA in the gastric phase at sampling) and presented very similar final lipolysis levels of around 17 to 18% (total FA; Fredrikzon and Hernell, 1977; Roman et al., 2007). Global extent of gastric lipolysis was assessed only by Roman et al. (2007) who calculated the sum of the pyloric lipolysis product outputs and the residual lipolysis products remaining in the stomach after three-hour digestion: a global extent of lipolysis of 6.1 ± 2.1% (total FA) was shown. Similar contribution of gastric stage to overall fat absorption could be inferred from Roy et al. (1977) who compared fat absorption in two groups of infants (GA = 30 weeks) either nasogastric fed or nasojejunal fed. The study indicated much lower fat excretion (14.9 ± 1.7% of ingested fat) in nasogastric fed infants than in the nasojejunal fed group (23.0 ± 2.9% of ingested fat) and a global difference of 8.1% of ingested fat.

In contrast to HGL activity or output, extent of HGL lipolysis is affected by the nature of the meal: human milk had a significantly higher ($p < 0.05$) instantaneous degree of lipolysis (Armand et al., 1994, 1996) and overall gastric lipolysis extent than formulas (Roman et al., 2007). The difference in emulsion supramolecular structure (nature of the interface) and the possible, though limited, action of bile salt-stimulated lipase

Table 2 Extent of gastric lipolysis reported in literature in full-term or premature newborn infants

Group: *N*; GA; Age	Test meal (mL/kg body weight)	Intragastric lipolysis level	References
$N = 6$; 32–37 weeks; 23.8 ± 7.3 days	20–30 mL of pasteurized human milk	1.8, 16.3, 41.3% of ingested TAG in 30, 60, 150 minutes—estimation** of released FFA in 150 minutes ∼ 16.7% (total FA)	(Fredrikzon and Hernell, 1977)
$N = 4$; 32–34 weeks; n.a.*	20–25 mL test meal of Formulas**	15.6% of ingested TAG in 7 minutes—released FFA ∼ 23.6% (total FA)	(Hamosh et al., 1978)
$N = 11$; 28.9 ± 1.4 weeks; 5.5–7.5 weeks	8.4 mL Human milk	16.8 ± 2.4, 25.3 ± 1.8% of ingested TAG in 30 and 50 minutes—released FFA in 50 minutes 25.3% (total lipids) or 11.2 (total FA)%***	(Armand et al., 1996)
$N = 9$; 29.1 ± 0.9 weeks; 4.6–7.0 weeks	17.4 mL SMA formula	8.4 ± 1.3, 13.3 ± 2.7% of ingested TAG in 30 and 50 minutes—released FFA in 50 minutes 16.4% (total lipids) or 6.4% (total FA)***	
$N = 8$; 28.9 ± 1.4 weeks; 5.8–7.4 weeks	17.0 mL Similac formula	10.4 ± 2.6, 14.4 ± 4.TAG in 30, 50 minutes—released FFA in 50 minutes 17.4% (total lipids) or 6.9% (total FA)	
$N = 9$; 29 ± 1 weeks; 5.3 ± 1.8 weeks	PreGallia formula	Released FFA% (total FA): 11 ± 6 in 15 minutes, 15 ± 7 in 60 minutes and 18 ± 4 in 180 minutes	(Roman et al., 2007)
$N = 2$; 32 weeks; 2 weeks	Human milk	Released FFA% (total FA): 23.0–18.2%	

*n.a, nonavailable.
**Isomil 20 and isomil 24.
***Estimation considering the global evolution of partial glycerides (MAG, DAG) in the gastric aspirate and equation: $L\ (\%) = \frac{100 \times FFA}{(3 \times TAG + 2 \times DAG + MAG + FFA)}$ (1)
With *L*: extent of lipolysis, FFA: free fatty acids, TAG: triacylglycerides, DAG: diacylglycerides, MAG: monoacylglycerides.

(BSSL) were proposed as explanations of the higher lipolysis of human milk fat.

Pepsins

The major gastric proteases, pepsins, are activated from zymogen precursors, pepsinogens by selective cleavage of a small basic peptide. In a narrow pH range (1–2.5), the activation is autocatalytic. The major peptic digestion products are polypeptides with N-terminal amino acids including phenylalanine and leucine as well as small amounts of oligopeptides and amino acids (Freeman and Kim, 1978). Pepsin activity has often been measured on hemoglobin substrate (Anson and Mirsky, 1932; Schlamowitz and Petersen, 1959). A pepsin unit in this test has been defined as the amount of enzyme required to produce 0.1 μmol of tyrosine containing peptides at 37°C in 10 minutes at pH 1.8 from a 2% hemoglobin solution. Using this test, DiPalma et al. (1991) localized pepsin secretion in the stomach of infants (3–19 months) and determined the effect of age on this secretion. They preferentially localized pepsin activity to the gastric body, although activity was also present in gastric antrum and duodenum. No effect of age was seen.

Using the same pepsin activity assay, Weisselberg et al. (1992) characterized fasting and meal-stimulated pepsinogen secretory function in preterm infants ($N = 44$, GA = 31.4 range 28–36 weeks, aged 1–90 days) to determine the influence of feeding on pepsinogen secretory maturation. The subjects were divided into 3 GA (28–30, 31–33, and 34–36 weeks). Fasting pepsin activity did not change with postconceptional nor with postnatal age and was low, averaging 4.1 U/stomach volume/min/kg bodyweight. Significant meal-stimulated pepsinogen secretion appeared during the third postnatal week in the most preterm group of infants (28–30 weeks of gestation), whereas it was detected as soon as the first week in the two other groups. This study suggested that the maturation of meal-induced pepsinogen secretion appears at 31-week gestation. The presence of food in the gastrointestinal tract would not significantly stimulate pepsinogen secretion prior to this date.

Still using the hemoglobin pepsin activity assay, Armand et al. (1996) determined average higher fasting but lower postprandial pepsin activity in premature infants ($N = 28$, GA = 29.2 weeks, aged five to six weeks). Fasting activity of 125 ± 15 U/mL/kg body weight and average postprandial level of 63 ± 11 U/mL/kg (between 10- and 50-minute postfeeding) were recorded. At 50-minute postfeeding, this author reported a gastric volume of 5 mL with a pepsin activity of 87 U/mL for infants formulas, which gives a pepsin activity of 435 U/stomach volume 17-fold higher than the one reported by Weisselberg et al. (1992) using the same test. Such difference may be explained by slightly lower postnatal age of subjects in Weisselberg et al. (1992) study but also by sample handling and biological variability. Armand et al. (1996) also compared her data with adult pepsin activity which presented fasting levels of 942 ± 120 and 1333 ± 115 U/mL, respectively in low fat or high fat diet (Armand et al., 1995). Infants who were also on high fat diet, had pepsin fasting activity constituting only 10% of the fasting activity detected in adults on high fat diet. In both studies, the effect of different meals (human milk vs. formulas for the premature infants and high fat vs. low fat for adults) on pepsin activity and output was assessed. In premature infants, the type of meals had no effect on these two parameters as presented in Figure 3a & b. Conversely in adults, the type of diet (high fat 50% of energy vs. low fat 25% of energy) induced higher fasting pepsin activity and output: pepsin activity and output increased, respectively, by

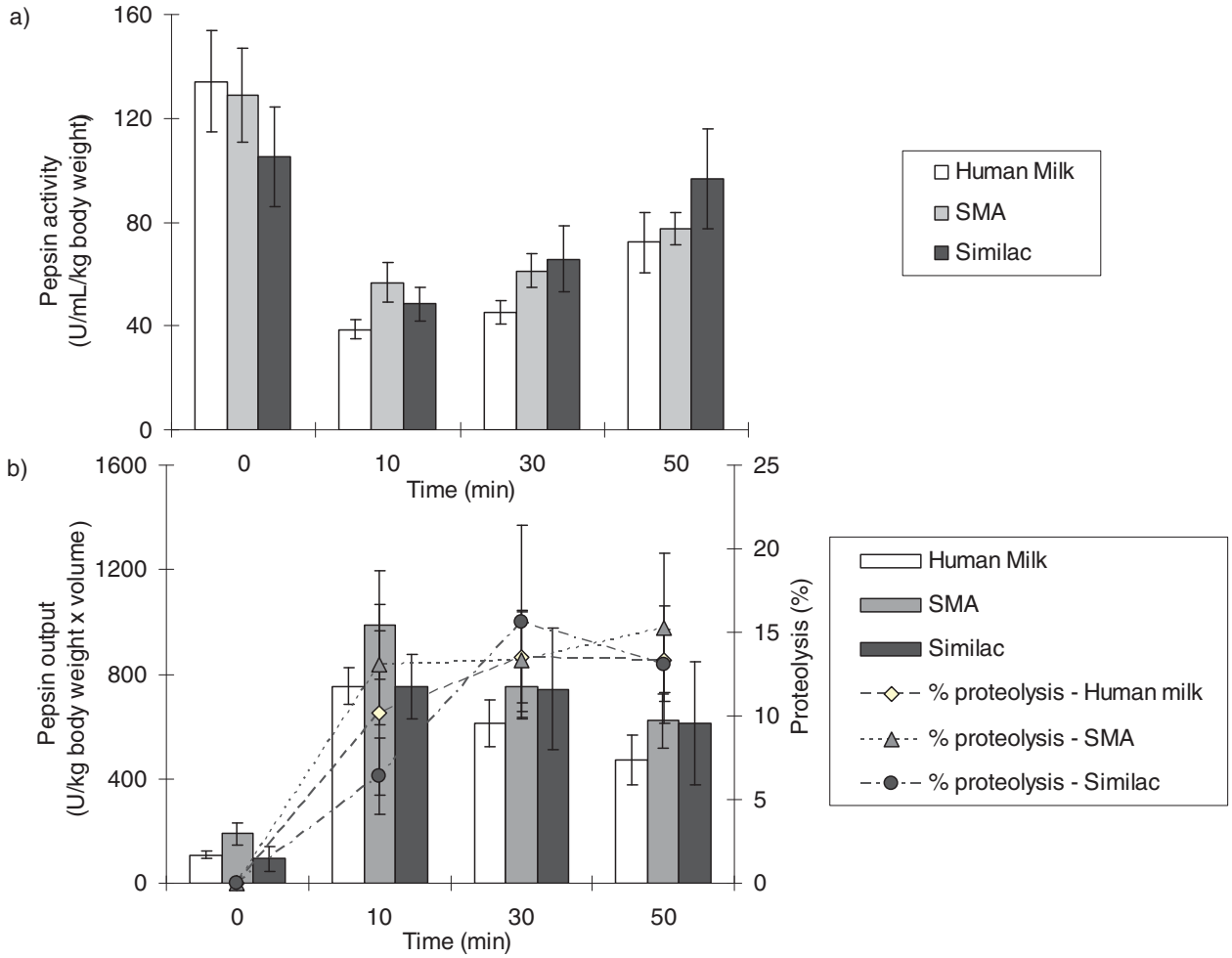

Figure 3 Effect of meals (human milk vs. 2 types of formula) on: (a) pepsin activity, (b) pepsin output, and gastric proteolysis extent. (Armand et al., 1996; Henderson et al., 1998). (Color figure available online.)

41 and 37% in the high fat diet (Armand et al., 1995). The mean postprandial pepsin output was significantly lower in preterm infants as compared to adults, 589 vs. 3352 U/kg (stimulation with pentagastrin), respectively. This result was in agreement with previous reports of low pepsinogen secretion in premature infants as compared to full-term infants and children (Adamson et al., 1988) and of low pepsinogen secretion in preterm and full-term infants as compared to children and adults (Agunod et al., 1969; Weisselberg et al., 1992; Henderson et al., 1998). Pepsinogen secretion reaches adult level by only two years of age. Agunod et al. (1969) published extremely precious data about the evolution of gastric secretion composition and pepsin output with age in full-term infants (aged 1–110 days) in comparison with children (four–nine years) and adults. A limit in this study is that the gastric contents were collected poststimulation with Histalog (1 mg/kg bodyweight), an histamine H_2 agonist used clinically to test gastric secretory functions, and thus cannot be representative of normal postprandial gastric function. The pepsin assay used in this experiment differed from the previously presented data. Pepsin activity at pH 2.0 was calculated from a standard curve of crystallized bovine pepsin and expressed as milligrams pepsin-equivalent per milliliter gastric juice (mg/mL). The values of pepsin output in collected gastric content after Histalog stimulation are presented in Table 3.

The extent of gastric proteolysis has been reported only for preterm infants and is rather limited (Figure 3b). A degree of proteolysis of 15% was reached on average, suggesting that most dietary proteins reached the intestine with only minimal change. This low degree of gastric proteolysis shown in premature infants is in accordance with Mason (1962). Data on 17 postprandial gastric aspirates from 9 full-term newborns aged between 5 and 13 days at 90 and 180 minutes after the start of the feeding found a small amount of hydrolyzed protein in only one sample. The limited protein hydrolysis was explained by the combination of low pepsin output (Agunod et al., 1969; Yahav et al., 1987; Weisselberg et al., 1992) and high postprandial gastric pH 5.0 to 6.0 (Mason, 1962; Harries and Fraser, 1968; Hamosh et al., 1978; Cavell, 1983; Smith et al., 1986). The disparity between low proteolysis and high lipolysis enhances the protective functions of milk by preserving the bioactivity

Table 3 Evolution of gastric secretion composition and pepsin output with age in full-term infants (aged one to 110 days) in comparison with children (four to nine years) and adults (Agunod et al., 1969)

Age of subjects	1 day	3–8 days	10–11 days	14–17 days	25–32 days	67–110 days	4–9 years	Adults
Number of subjects	10	7	5	4	3	4	2	(–)
Mean weight (kg)	3.4	3.3	3.0	3.4	3.9	4.9	(–)	70.0
Gastric juice								
Mean volume (mL)	3.3	3.7	4.0	6.4	3.1	13.4	42.5	143.2
Range	0.8–9.3	1.3–4.7	2.0–5.4	1.5–12.0	3.0–3.2	7.1–19.9	35.0–50.0	(–)
Pepsin (mg/mL)								
Mean	0.04	0.05	0.12	0.14	0.10	0.12	0.45	0.29
Range	0.07	0–0.09	0.09–0.14	0.12–0.16	0.07–0.12	0.06–0.18	0.41–0.50	0.23–0.36
Pepsin output (mg/mL/hr/kg)								
Mean	0.04	0.06	0.15	0.24	0.08	0.28	(–)	0.60
Range	0–0.19	0–0.12	0.11–0.22	0.06–0.36	0.06–0.10	0.23–0.32	(–)	0.47–0.73
Titratable acid mean (mM/L)	8.1	14.4	34.4	26.7	26.4	34.8	114.2	91.2
Range	4.2–16.7	9.2–26.0	20.6–55.0	15.4–35.0	10.0–45.3	19.6–50.0	111.0–117.5	50.1–132.3
Mean output (mM/h/kg)	0.01	0.02	0.04	0.05	0.02	0.10	(–)	0.19
Range	0–0.03	0–0.03	0.04–0.05	0.01–0.09	0–0.06	0.03–0.14	(–)	0.10–0.27

of specific milk proteins (immunoglobulin A, lactoferrin, and lysozyme) and by generating lipolysis products (FFA, monoglycerides) with antiviral, antibacterial, and antiprotozoan activity (Henderson et al., 1998).

Gastric Emptying

In the newborn, the stomach is the first important digestive compartment where meal bolus is transitorily stored. Within this role, the stomach has three key functions: (i) food storage in stomach body and fundus which is able to relax and adapt its volume so that intragastric pressure remains stable, and (ii) mixing of food and (iii) emptying mainly mediated by the antrum toward stomach pylorus and duodenum (Faure and Navarro, 2000). Considering this relaxation property, determining an average volume of gastric compartment for infants is difficult. Intragastric fasting volume reviewed by Geigy (1973) indicated that mean newborn content represented 30% of mean children content (∼nine years) and 11% of mean adult content (Table 4).

Clinical observations revealed that in preterm and very low birth weight infants, gastric emptying is slower than in full-term and normal weight infants (Faure and Navarro, 2000). Their immature gastrointestinal motility was presented as a key element of this impaired emptying (Berseth, 1996). Gastric emptying is thus supposed to evolve with age but it could not be shown in a study of 28 infants aged 1 to 10 weeks (including 3 prematures) (Signer and Fridrich, 1975). Similarly, Billeaud et al. (1990) indicated that gastric emptying does not vary with age nor gender during the first year of life. Several direct or indirect methods (marker dilution, scintigraphy, echography, etc.) have been applied to determine gastric emptying in neonates after feeding either human milk or formula. The results obtained on premature neonates mainly are summarized in Table 5.

Table 4 Mean intragastric fasting volume reported in literature (Geigy, 1973; Thompson, 1951)

Subjects (N; Age; birth weight)	Mean ± SD (mL)	Range (mL)	References
Newborn ($N = 15$; < 1 day; 2.07 - 4.11 kg)	2.00	1.0–7.0	(Gellis et al., 1949)
Newborn ($N = 154$; < 1 day; 3.37 ± 0.58 kg)	2.65 ± 2.05	0.4–12.3	(Thompson, 1951)
Infants ($N = 3$)	2.4 ± 0.7	1.0–3.5	(Wolman, 1946)
Children ($N = 59$; 9 years)	8.8 ± 8.2	0.4–80	
Adults ($N = 7$)	24 ± 5	(−)	(Dubois et al., 1977)

Large variations in half-emptying times among individuals in a group are generally reported and individual variation of 17 minutes for a given neonate (two studies over a 24-hour period) were indicated by Ewer et al. (1996). Despite these variations, the observations indicated generally that human milk empties faster than formula milk even when the formula has been optimized to reproduce precisely the chemical composition of human milk (fat content, type and concentration of carbohydrate, protein type, and content) osmolarity, and caloric density (Billeaud et al., 1990). Cavell (1979) evidenced a biphasic emptying pattern with an initial fast phase for human milk and more linear pattern for formulas. In most of the studies, however, the emptying pattern was exponential as seen in Figure 4a & b and can be accurately fitted using Elashoff model (Elashoff et al., 1982):

$$f = 2^{-(\frac{t}{T1/2})^{\beta}} \quad (1)$$

With f: fraction remaining in stomach at time t, $T1/2$: the time from the start of the meal until 50% of the meal has been emptied also called gastric half-emptying time, β: which determines the shape of the curve and intensity or lack of initial lag time.

Examples of fittings of experimental data reported for preterm infants (Ewer et al., 1994; Roman et al., 2007) using this model are presented in Figure 4. The gastric half-emptying times reported for infant formula ($T1/2 = 72$) and Human milk ($T1/2 = 36$) by Ewer et al. (1994) are in accordance with data reported by other authors and summarized in Table 5. Conversely, the short-gastric half-emptying time for formula

Table 5 Examples of gastric half-emptying times after ingestion of human milk or formula reported for infants (—zero to six months) in literature

Group: N; GA; Age	Birth weight (kg)	Test meal	Gastric half-emptying time (min)	Method	References
$N = 28$; GA n.a. 3 prematures; 25 days (1–10 wk)	n.a.*	Infant formula** (50 mL/feed)	87 ± 29	Radioisotope counting	(Signer and Fridrich, 1975)
$N = 11$; 33–38 weeks; 1–9 weeks	n.a.	Human milk Infant formula	25 51	Marker dilution technique with PEG 3000 as inert marker	(Cavell, 1979)
N = 17; full-term infants (> 37 weeks); 4 weeks to 6 months	4.94 (average bodyweight)	Human milk—$N = 8$ (32 mL/kg/feed) Infant formula***—$N = 9$ (32 mL/kg/feed)	48 ± 15 78 ± 14	Marker dilution technique with PEG 3000 as inert marker	(Cavell, 1981)
$N = 10$; healthy prematures	n.a.	Infant formulas	~ 30	Marker dilution technique	(Siegel et al., 1982)
N = n.a.; full-term infants (> 37 weeks); 0–12 months	n.a.	Human milk Infant formula	61 76	Scintigraphy	(Billeaud et al., 1990)
$N = 14$; 33 (30–35) weeks; 11 days (4–26)	1.65 (1.13–2.13)	Human milk	36	Ultrasound evolution of Sectional Area of the Gastric Antrum‡	(Ewer et al., 1994)
		Infant formula# (167 mL/kg/day)	72	Ultrasound evolution of Sectional Area of the Gastric Antrum‡	
$N = 19$; 32 (29–34) weeks; 21 days (7–37)	1.62 (0.98–2.37)	Infant formula# ($N = 9$) Pre-term formula§ ($N = 8$) Human milk ($N = 2$) (21 mL/kg/feed)	64 ± 7.1 34 ± 4.9 44 ± 4.7		(Ewer et al., 1996)
$N = 28$; 24–34 weeks; 5.5 (1–11) weeks;	0.50–1.70	Human milk or 2 infants formulas	~ 30	Gastric aspirate variation of volume‡‡	(Armand et al., 1996)
$N = 20$; 1–10 weeks (27–41 weeks); 24 days (range 7–74)	2.11 (0.96–4.10)	Human milk ($N = 9$) Infant formula ($N = 11$)	47 65	^{13}C-Octanoic acid breath test	(Van Den Driessche et al., 1999)
$N = 9$; 30 weeks (27–31 weeks); 4.3 ± 1.8 weeks	1.53 ± 0.55	Infant formula	17 ± 5	Total FA remaining in the stomach as markers‡‡‡	(Roman et al., 2007)
$N = 20$; 37 weeks (28–40); 31 days (6 days–13 weeks)	2.70 (0.72–3.69)	Intact (IF) Partially (PF) Extensively (EF) Hydrolyzed formula†—69 to 72 mL /feed	55 53 46	^{13}C-Octanoic acid breath test	(Staelens et al., 2008)

*n.a., nonavailable.

**Composition (g/100 mL): protein 1.7, fat 3.7, and lactose 7.2–113 mg gelatin introduced as microcolloids.

***Composition (g/100 mL): protein 1.6 with casein 0.65 and whey protein 0.95, fat 3.5, lactose 7.2, sodium 0.02, potassium 0.07, calcium 0.04, and phosphorus 0.03.

#Whey predominant formula: whey 60%, casein 40%, and Cow and gate premium.

§Cow and gate low birth weight formula.

†Composition (g/100 mL): IF 67 kcal, fat 3.57, protein 1.23, carbohydrate 7.48, minerals 0.2; PF 72 kcal, fat 3.67, protein 1.37, carbohydrate 8.4, minerals 0.2; EF 67 kcal, fat 3.43, protein 1.65, carbohydrate 7.50, minerals 0.2.

‡Measuring ultrasonically the changes in the cross sectional area which occurs after a feed showed a highly significant correlation between intragastric milk volume and an increase in ACSA after the administration of a feed (Newell et al., 1993).

‡‡Assuming that volume found in the stomach represents almost entirely the remaining volume of food.

‡‡‡Assuming that the emulsified milk lipids and the water phase to be simultaneously emptied.

($T1/2 = 17$) reported by Roman et al. (2007) seems less representative of usual emptying pattern but can be explained by more erratic and irregular gastric motility in very preterm infants and the influence of the method of quantification using lipids as markers. Experimental gastric emptying data reported in full-term infants are less numerous and less precise (Cavell, 1981, 1983; Billeaud et al., 1990). Elashoff fitting of Cavell (1983) data for full-term infants ($N = 8$) aged 3.5 months after infant formula feeding, is very close to the one obtained for Ewer et al. (1994) data on preterm infants: $T1/2 = 78$ and $\beta = 1.2$ to 1.5. Variations of gastric emptying patterns due to change in milk composition as will be detailed below can be approached for full-term infants using the data of Billeaud et al. (1990). Based on this data, adjustment of average half-emptying time of Elashoff fitting can be undertaken.

Gastric emptying regulation has been extensively described for adults: the release of amino/fatty acids in the duodenum stimulates cholecystokinin (CCK) secretion which slows

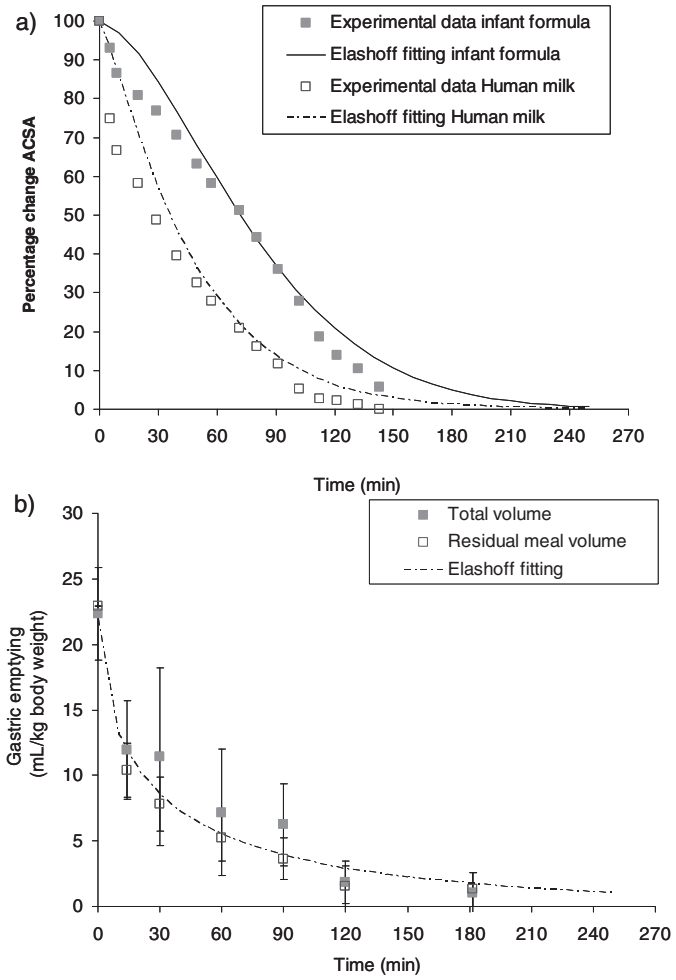

Figure 4 Kinetics of gastric emptying reported in preterm infants and their Elashoff fittings. (a) Antral cross-sectional area (ACSA) evolution after infant formula or Human milk feeding for preterm infants aged 11 days (33-week GA; Ewer et al., 1994). ACSA are well correlated with intragastric milk volume. Elashoff fitting parameters for infant formula: $T1/2 = 72$, $\beta = 1.6$, $r^2 = 0.99$, for Human milk: $T1/2 = 36$, $\beta = 1.15$, $r^2 = 0.97$. (b) Gastric emptying after infant formula feeding for preterm infants aged 4.3 weeks (30-week GA; Roman et al., 2007). Elashoff fitting parameters: $T1/2 = 17$, $\beta = 0.55$, $r^2 = 0.96$.

gastric contractions by limiting the contractions of the antrum and pylorus (Liddle et al., 1986; Moran and Kimberly, 2004). In infants, similar hormonal feedback is supposed to take place and the factors which were tested for their impact on gastric emptying are the following:

- Increasing caloric density slows down gastric emptying (Siegel et al., 1984)
- Changes in carbohydrate composition of feeds can influence emptying, however, this effect is less pronounced than change in quality of fat (Siegel et al., 1985): glucose polymers or maltodextrins resulted in faster emptying as compared to glucose or to lactose.
- Fortifying milk has no impact on gastric emptying at isocaloric values (McClure and Newell, 1996)
- Temperature differences (6–37°C) of the milk (formula milk) do not seem to have any effect on gastric emptying (Anderson and Berseth, 1996)
- Given an isocaloric content, the nature of proteins and their state of hydrolysis can affect gastric emptying. Casein dominant formulas are emptied more slowly than whey proteins which could be linked to their susceptibility to acid coagulation (Billeaud et al., 1990). Extensively hydrolyzed formulas are emptied faster than non or even partially hydrolyzed formulas (Billeaud et al., 1990; Garzi et al., 2002; Staelens et al., 2008). The level of heat induced denaturation of proteins and more specifically whey proteins which are specifically prone to heat denaturation might affect gastric emptying in human infants. However, this was shown in animals only (Scanff et al., 1992) where a slower gastric emptying of β-lactoglobulin was seen after ingestion of pasteurized milk as compared to raw milk. This behavior was explained by the formation of casein/β-lactoglobulin aggregates and subsequent entrapment of the β-lactoglobulin in the casein acid gel formed during the gastric digestion.
- The quality of fat: with regard to fat composition, short and medium chain FA (acetic up to decanoic acids) were less effective in slowing gastric emptying in comparison to longer chain FA (Hunt and Knox, 1968). Siegel et al. (1985) also reported that medium chain TAG were emptied faster than long chain TAG. The influence of fat ultrastructure was only tested in adults: acid-stable emulsions were associated with delayed gastric emptying in comparison with unstable emulsions (Foltz et al., 2009; Marciani et al., 2009). In the case of unstable emulsion, an initial release of a low fat watery phase into the duodenum induced a minimal hormonal feedback and hence a faster emptying rate was observed. With regard to the relative stability of human milk or formula emulsion structures in the gastric environment, Armand et al. (1996) using SEM investigation reported a good stability of the two types of emulsion over a 50-minute period. However, the technique of investigation which supposed dehydration/fixation of the samples was very denaturing.

The reason why infant formulas having chemical composition similar to human milk, with among other parameters a 40:60 casein/whey w/w proteins ratio, empty more slowly than human milk, remains to be elucidated. Billeaud et al. (1990) set the hypothesis that the fat composition of human milk (quickly assimilated medium chain FA in position 3 of TAG) and the presence of BSSL in human milk could contribute to accelerate gastric transit. More recent works focusing on the influence of food structure on digestion (Aguilera, 2006) have underlined the importance of considering protein structural state (level of process-induced denaturation; Dupont et al., 2010) and emulsion ultrastructure (Armand et al., 1999; McClements and Li, 2009; Singh et al., 2009; Golding and Wooster, 2010) as key parameters affecting kinetics of digestion.

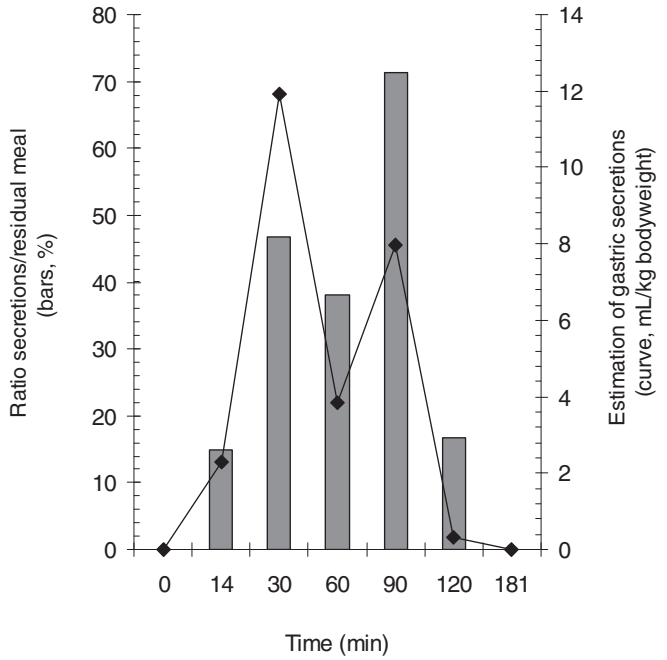

Figure 5 Estimation of postprandial gastric secretions in preterm infants fed infant formula ($N = 9$; 4.3 ± 1.8 weeks; 30 weeks range: 27–31 weeks). Calculated from (Roman et al., 2007).

Gastric Secretions

Fasting gastric secretions of around 1 mL/kg/hour in preterm ($N = 10$, GA = 32.6 ± 1.7 weeks) or full-term ($N = 14$, GA = 39.5 ± 1.0 weeks) newborns aged less than two days were reported by Marino et al. (1984). They increase to reach 2 to 3 mL/kg/hour in infants of one to two years (Agunod et al., 1969). Postprandially, in preterm infants aged 4.3 weeks (30-week GA), Roman et al. (2007) estimated that gastric secretions contributed significantly to gastric total volume (range of 28–48% v/v between 30 and 90 minutes). The volume of secretions estimated in their study, considering a similar rate of emptying of the secretions and of the meal, is represented in Figure 5. The ratio of residual secretion as compared to residual meal volume is also presented. This corresponds to postprandial gastric secretions of around 0.15 mL/kg/minute during the first 30 minutes and decreasing on the end of the kinetics to values ranging from 0.08 to 0 mL/kg/minute.

In addition to hydrolytic enzymes, gastric juice is typically composed of H^+, other electrolytes, mucus (mucins and surfactants), gastric intrinsic factor, and water. Structurally mature parietal cells with the proton pump have been shown to exist in the body and antrum of the fetal stomach as early as at 13 weeks of gestation (Kelly and Newell, 1994). Thus acid secretion is active at birth even in very premature neonates (GA = 24 weeks), for which gastric pH decreases below 4 during the first day of life (Avery et al., 1966). Fasting secretions of acid average 0.01 mM/kg/hour for preterm neonates (GA = 32 weeks) 48 hours after birth. They remain really close to this value, ranging from 0.01 to 0.03 mM/kg/hour, for full-term infants during their first year of life. Postprandial maximal secretion stimulated via secretagogues (histamine or pentagastrin) remains identical to fasting secretion until two to three months. At this stage, postprandial secretions of up to 0.1 mM/kg/hour are reported. These secretions rise up to 0.19 to 0.42 mM/kg/hour around the age of one year and remain at this level in adults.

Table 6 Monovalent electrolyte concentration of gastric aspirate (Hyde, 1968)

	No. of specimens	Na^+ (mm/L)	K^+ (mm/L)	Cl^- (mm/L)
Total	87	100 ± 22 (47–140)	11.7 ± 4.16 (4–22.4)	130 ± 17.6 (68–174)
Preterm infants	14	118 (93–131)	9.8 (6.1–13.6)	137 (120–148)
Full-term infants	30	94 (69–128)	13.2 (6.9–22.4)	122 (68–153)

With regard to other electrolyte concentrations of newborn gastric fluid, they were estimated during the investigation of postoperative gastric aspirates from 24 newborns aged of less than four weeks (Hyde, 1968). Concentrations are reported in Table 6.

A mucus bicarbonate barrier consisting mainly of mucins, surfactants, and bicarbonate ions is secreted by the gastric mucosa. This barrier has been described in adults as presenting a pH gradient from near-neutral pH at the epithelial surface to acidic at the gastric lumen, which protects the mucosa against corrosive effects of H^+ and pepsin secretions (Allen and Flemström, 2005). Mucins represent a mixture of glycoproteins. Fractions of mucins in adult gastric juice was estimated to 1.4 g/L of undissolved substances and 0.5 g/L dissolved mucoproteins (Geigy, 1973). Surfactants are mainly composed in adult of two glycerophospholipids (phosphatidylcholine 40–45% and phosphatidylethanolamine 28–33%), whereas other glycerophospholipids (phosphatidylglycerol, phosphatidylserine, and phosphatidylinositol) and sphingomyelin represent around 5 to 10% (Rubio et al., 1995). Gastric surfactant has been detected in low amount in preterm infant around 28 weeks. Evolution of the composition of these surfactants during the first postnatal weeks has been only studied in animal models (Shub et al., 1983). The precise levels of mucus and bicarbonate secretions in newborns have not been reported. However, newborn infants have higher vulnerability of their mucosa to gastric acidity but this disappears around—two to three weeks of postnatal age (Saliba et al., 2001). Prostaglandins, notably PGE2, play a central role in mucosal protection in the newborns, by stimulating mucus production and secretion of bicarbonate, by inhibiting acid secretion, and increasing mucosal surface hydrophobicity (Kelly and Newell, 1994).

Gastric pH

Several studies in preterm and full-term neonates revealed that immediately after birth the gastric pH is most of the time neutral to alkaline as a result of swallowed amniotic fluid in utero (Avery et al., 1966; Miclat et al., 1978). Singh et al. (1970), who monitored gastric juice pH during the first 72 hours of life in

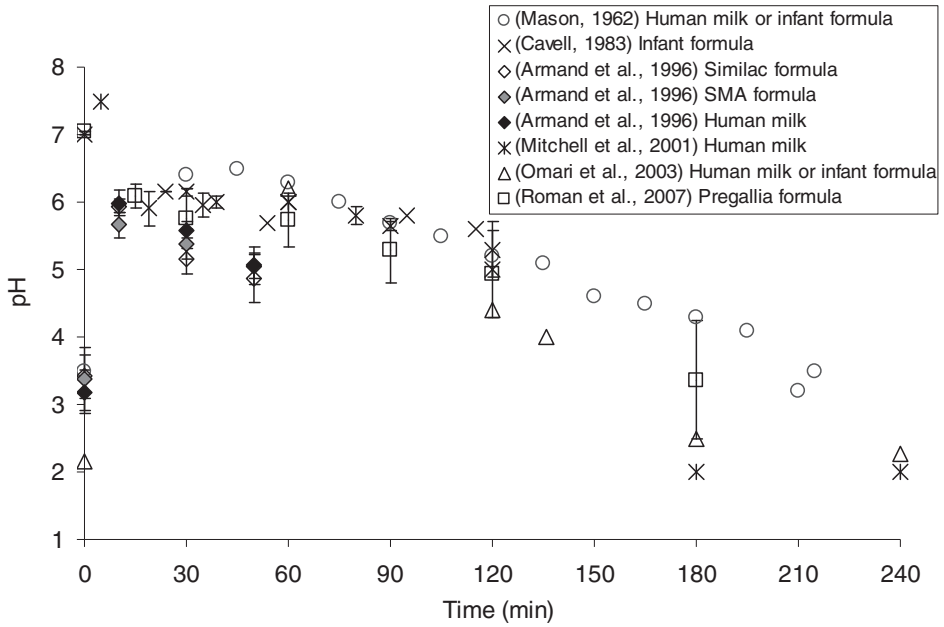

Figure 6 Gastric pH monitored in preterm infants before and throughout a meal (Cavell, 1983; Mason, 1962; Armand et al., 1996; Mitchell et al., 2001; Omari and Davidson, 2003; Roman et al., 2007). Composition of Cavell (1983) formula (g/L): protein 16 (casein 6.5, whey protein 9.5), fat 35, lactose 72, sodium 0.2, potassium 0.7, calcium 0.4, and phosphorus 0.3. Other formulas described earlier on in the review.

healthy newborns ($N = 30$), observed a sharp fall during the first three hours, followed by a 24-hour period of stability and a subsequent rise at 48 and 72 hours. These fluctuations have been explained by the impact of feed and, most specifically of milk or infant formulas, on gastric acidity because of their high buffering capacity. Since the frequency of feeding is extremely high during the first six months of life, the characterization of postprandial pH appeared really early as an important element of gastric digestion. Indeed, this gastric pH influences gastric digestive enzyme activity for reason elucidated above.

Gastric pH variations monitored in preterm infants before and throughout a meal are displayed in Figure 6 (Mason, 1962, Harries and Fraser, 1968; Hamosh et al., 1978; Cavell, 1983; Smith et al., 1986; Henderson et al., 2001). Gastric pH generally ranged from 3.2 to 3.5 before test meal and increased to 6.0 to 6.5 immediately after feeding. pH remained higher than pH 5.0 for at least 50 minutes after the meal. At this high pH, the level of conversion of pepsinogens to pepsins and activity of the proteases is very limited.

Armand et al. (1996) tested whether diet, human milk versus two infant formulas, could influence postprandial pH in preterm infants ($N = 28$, GA = 29.2 weeks, aged —five to six weeks). Gastric pH, both at baseline or after feeding, was comparable for all diets and in the range previously reported (Hamosh et al., 1978; Smith et al., 1986). It suggested similar buffering capacity of human milk and formulas as well as similar gastric secretions as suggested by comparable gastric lipase and pepsin output. These authors pointed out that the pH of gastric contents after ingestion of human milk or formula was in the range of optimal gastric lipase activity (4.0–6.0) but higher than the range of pepsin optimal activity (1.5–2.2) (Schlamowitz and Petersen, 1959). The observed increase in pH above 4.0 significantly reduces protein digestion by pepsin. Indeed, caseins have been shown to become partly resistant to pepsin digestion at pH 4.0, whereas they were fully digested in the range of 1.5 to 3.5 (Sakai et al., 2000).

Contribution of Swallowed Saliva to Gastric Digestion

Saliva is a complex viscoelastic fluid containing minerals and a complex mixture of proteins and peptides such as acidic and basic proline-rich proteins, α-amylases, salivary cystatins, histatins, and statherin (Humphrey and Williamson, 2001; Schipper et al., 2007). The normal flow of saliva is well known, though highly variable, for adults: the average unstimulated flow rate is 0.3 mL/minute and flow rises up to maximal values around 7 mL/minute under stimulation; this stimulated saliva is reported to contribute to as much as 80 to 90% of the average daily production (Humphrey and Williamson, 2001). Saliva flow rate is not as well characterized for infants. Seidel et al. (2001) studied the unstimulated salivary flow rate and composition of a large number of healthy newborns aged 6 to 24 hours ($N = 73$, GA = 37–41 weeks). They reported an average flow rate of 0.036 (0.03–0.186 mL/minute), *i.e.* 10% of the average unstimulated flow rate reported for adults. If the ratio between unstimulated and stimulated flow rates found for adults is extrapolated to infants, it would mean that maximal stimulated flow reaches around 0.8 mL/minute. In any case, swallowed saliva enhances the dilution of the meal in the gastric compartment in addition to the contribution of gastric secretions. The main components in saliva are (i) the glycosylated mucins

present at diluted concentration in newborn infants as compared to adults but which could in a minor way supplement in the mucins originating from gastric secretions, and (ii) α-amylase which is secreted at low levels in newborn infants as compared to adults and is inactivated by low pH but might remain slightly active in the poorly acidified neonatal stomach (Murray et al., 1986). More precisely, salivary amylase is detectable from 20-week gestation (Martin et al., 1999). Amylase activity levels in saliva are very low at birth (9.9 $\pm$ 9.2 U/mL) as compared with those of adults but increase rapidly to reach approximately two-thirds of adult values (90 U/mL) by three months (57.2 $\pm$ 40.7 U/mL). Large variations in α-amylase development of activity with age were reported by Sevenhuysen et al. (1990).

DUODENAL PHASE

After the gastric phase, the chyme (meal and gastric secretions) is progressively emptied into the duodenum where the digestive process is carried on by the concerted action of pancreatic enzymes and biliary secretions. Control of pancreatic and biliary secretions by secretagogues is immature in infants. For instance, children showed a response to pancreazymin only by the age of two years, thus indicating the secretory response of the human pancreas is absent or minimal at birth and is acquired during the postnatal period (Lebenthal and Lee, 1980).

Lipolytic Enzymes Active in Duodenal Phase

Several lipolytic enzymes are secreted by the exocrine pancreas into the duodenum which act together to achieve digestion and absorption of dietary fats: pancreatic colipase-dependent lipase also called pancreatic triglyceride lipase (PTL), pancreatic lipase-related proteins 1 and 2 (PLRP1 and 2), pancreatic phospholipase A_2 (PLA_2), and BSSL also called cholesterol esterase (CEase, EC3.1.1.13) or carboxyl ester hydrolase. The main enzyme responsible for efficient intestinal digestion of dietary TAG in adult is PTL which is estimated to account for 56% of the luminal hydrolysis of TAG (Carrière et al., 1993), whereas HGL is only responsible of 10 to 30% of this hydrolysis. Phospholipid digestion is catalyzed by PLA_2. In human infants, expression of PTL is low and studies in rodents also indicated low or nul levels of PTL and PLA_2 at birth (Fredrikzon and Olivecrona, 1978; Li et al., 2007; Andersson et al., 2011). Instead two lipases with broad substrate specificities, *i.e.* PLRP2 and BSSL have been presented as the major catalysts of fat digestion in early life as long as milk is the main food (Lindquist and Hernell, 2010).

These findings on the specificities of the lipolytic enzymes in the newborns are more recent than the studies which determined lipase activity in newborns' duodenal content. Indeed, the studies date back to the 70s to mid-90s (Mason, 1962; Norman et al., 1972; Zoppi et al., 1972; Fredrikzon and Olivecrona, 1978; Cavell, 1983; Boehm et al., 1995a, 1995b). These studies determined a global lipolytic activity in duodenal content which was most of the time roughly related to PTL action even though it represents the concerted action of all the different lipolytic enzymes. Contribution of HGL in this duodenal activity was considered to be negligible in these assays since the test of activity was conducting at neutral or alkaline pH, *i.e.* high above the optimal pH of the HGL.

Lipase Activity and Output Reported in Literature

The fasting or postprandial values of lipase activity were determined mainly for premature infants except for the study of Zoppi et al. (1972) which included full-term infants and are summarized in Table 7. Despite the variations in values and lipase assays, several trends can be deduced from this table.

(i) Fasting lipase activity varies greatly from one infant to another. For instance in full-term infants ($N = 8$, GA = 40 weeks, aged 8 $\pm$ 5 days), values ranging from 20 to 233 U were determined (Norman et al., 1972) (Figure 7).

(ii) Lipase secretion is influenced by gestational and postnatal age. Zoppi et al. (1972) compared duodenal lipase activity between full-term infants (birth weight = 3.6–4.0 kg) and pre-term infants (GA = 32–34 weeks, birth weight = 2.0–2.4 kg) after secretagogue stimulation. At birth, the preterm group had nonsignificant but generally lower values of lipase activity as compared to the full-term group (176 $\pm$ 232 vs. 267 $\pm$ 498 μmol/min/mL/kg). Considering the lack of significance between the two groups and high interindividual variations (Figure 8), this effect of GA is limited and the authors concluded that at birth the premature neonates from 32-week GA onwards had a fairly well-developed exocrine pancreatic lipolytic function. With regard to the influence of postnatal age, lipase activity of full-term infants at birth represented 70% of the activity detected in children (nine months to 13 years old) and 6% of the children lipase output over a 50-minute period poststimulation (Figure 8) but again with extremely high variability among the groups. One week after birth, output became higher in preterm than in full-term neonates, which was related to a functional demand for more rapid growth. Boehm et al. (1995a) established that the mean lipase activity in two groups of preterm infants (group I: $N = 33$, GA = 29–32 weeks vs. group II: $N = 22$, GA = 33–36 weeks) was not significantly different (13.7 $\pm$ 7.9/15.9 $\pm$ 9.8 U/mL), suggesting that the development of the exocrine pancreas reaches basal level before the 29th week of gestation. This means lipase activity reached approximately 35% of the children value at the end of the observation period.

(iii) Lipase activity decreases after a test meal which can be explained by the dilution of pancreatic juice as soon as the test meal reaches the intestine (Norman et al., 1972). Fredrikzon and Olivecrona (1978) also showed that lipase and esterase activities decreased in preterm newborns ($N =$ 7, 31- to 37-week GA, 16–38 days) after ingestion of a test

Table 7 Fasting or postprandial values of lipase activity determined mainly in the duodenum content of premature infants but for the study of Zoppi et al. (1972) which included full-term infants

Group: N; GA; Age	Birth weight (kg)	Duodenal lipase activity (μmol/min/mL/kg)	References	State	Additional information
$N = 8$; 40 (36–42); 8 ± 5 days (3–15)	3.71 (3.30–4.08)	128 ± 148 (20–233)	(Norman et al., 1972)	Fasting	Collection of duodenal juice 10 minutes before beginning of test meal—lipase assay continuous titration on olive oil emulsified by gum arabic 25°C, pH 8.6
$N = 36$; 30–34 weeks; from birth to 1 month	2.15 (2.00–2.40)	Birth: 176 ± 232 24 hours*: 120 ± 156 1 week: 400 ± 537 1 month: 317 ± 374	(Zoppi et al., 1972)	Postprandial	Collection of duodenal juice with distal marker near ligament of Treitz and proximal in first part of duodenum over a 50-minutes period—10–20 after feeding stimulation with pancreozymin and then with secretin—activity assay continuous titration
$N = 8$; full-term; from birth to 1 week	3.68 (3.60–4.00)	Birth: 267 ± 498 24 hours*: 96 ± 98 1week: 92 ± 97	(Zoppi et al., 1972)	Postprandial	
$N = 7$***; 36 ± 4; 12 ±10 days (1–25)	2.50 ± 0.41 (2.05–2.97)	124 ± 80	(Fredrikzon and Olivecrona, 1978)	Fasting	Activity assay continuous titration of butyric acid released from tributyrin pH 8.0
$N = 7$; 31–37 weeks; 16–38 days	(1.70–2.53)	254 ± 17(149–586)	(Fredrikzon and Olivecrona, 1978)	Postprandial	Test meal—before feeding
$N = 7$; 31–37 weeks; 16–38 days	(1.70–2.53)	59 ± 39 (31–104)	(Fredrikzon and Olivecrona, 1978)	Postprandial	Test meal—after feeding average over 180-minute continuous titration of butyric acid released from tributyrin pH 8.0
$N = 12$; 28.3 ± 1.2 weeks; 26.1 ± 3.1 days	1.41 ± 0.13	8.4 ± 3.5 (2.8–15.4) 15.1 ± 4.2 (9.6–25.3) 13.8 ± 4.8 (7.7–21.6)	(Boehm et al., 1995b)	Postprandial with 3 different diets	Infant fed standard preterm formula# - lipase assay nephelometrically estimated with test kit using triolein as substrate at pH 7.4
$N = 33$; 29–32 weeks; from birth to 6 weeks	n.a.**	13.7 ± 7.9	(Boehm et al., 1995a)	Fasting values	lipase assay nephelometrically estimated with test kit using triolein as substrate at pH 7.4
$N = 22$; 33–36 weeks; from birth to 6 weeks	n.a.**	15.9 ± 9.8			

*After 1st feeding.
**n.a., nonavailable.
***Infants normal or with mild respiratory distress other excluded from the study.
#Standard preterm formula (g/100 mL): protein 2, whey/casein ratio 60:40, lipids derived from vegetable oils and cow fat 3.5, and energy intake 531 ± 71 KJ/kg/d.

meal (Figure 9). These authors explained this decrease by incompletely developed pancreas and frequency of feeding.

(iv) The lipolytic activity of pancreatic juice is modulated by diet. Boehm et al. (1995b) compared the lipolytic activity of pancreatic juices of preterm infants fed three different meals: lipase activity was significantly higher than in the group fed with the experimental formula (with long chain polyunsaturated FA, 13.8 ± 4.8 U/mL) or with fortified human milk (15.1 ± 4.2 U/mL) as compared to preterm formula (without long chain polyunsaturated FA, 8.4 ± 3.5 U/mL). Fecal fat excretion, ranging from 12.4 to 14.8%, was similar in the three groups.

Pancreatic Triglyceride Lipase, Colipase, and Pancreatic Lipase-related Proteins 1 and 2

Human triglyceride pancreatic lipase (645 AA, 51157 Da) (Lowe et al., 1989; Winkler et al., 1990; Van Tilbeurgh et al., 1992; Lowe, 2002) is a carboxyl esterase with strong preference for acylglyceride as compared to other esters (Andersson et al., 1996). It has a *sn*-1,3 regiospecificity and typoselectivity for short chain FA and medium chain as compared to long FA (Brockerhoff, 1970; Savary, 1971; Yang et al., 1990). It is inhibited by bile salts and needs a small protein cofactor colipase (112 AA, 11954 Da), to restore its activity (Borgström et al., 1979). Colipase output greater than 1% of mean normal level in children (120 U/kg/hour) was defined as sufficient to prevent fat malabsorption (Gaskin et al., 1984). Colipase output of 5.1 U/kg/hour total volume of duodenal aspirate was reported in six-month-old infant with cystic fibrosis after stimulation with continuous infusion of secretin and CCK (Nouri-Sorkhabi et al., 2000).

Two PLRPs (PLRP1 and PLRP2), highly homologous to PTL (68 and 65%, respectively; Giller et al., 1992), are expressed at relatively high levels in fetuses as compared to adults (Figure 10). PLRP1 and PLRP2 are expressed from 16-week GA onwards, at 42 weeks their levels represent 110 and 60%, respectively, of the one reported in adults, whereas PTL level

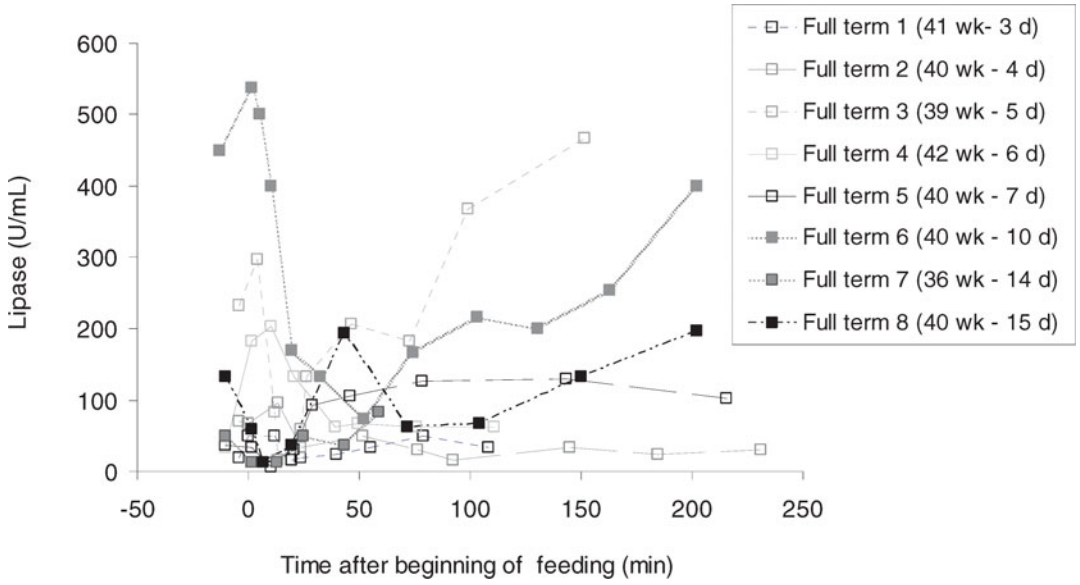

Figure 7 Evolution of lipase activity of pancreatic juice of full-term newborns after a test meal (pooled breast milk heated to 80°C and frozen). Adapted from (Norman et al., 1972). (Color figure available online.)

of expression is only 3% of the one of the in adults. PLRP1 has no known activity on TAG and its exact substrate and cofactor requirements have not yet been established (Giller et al., 1992; Berton et al., 2009). Berton et al. (2009) observed a decrease in lipolytic rate when PLRP1 was added simultaneously to PTL in milk fat emulsions and postulated a modulating role in pancreatic lipolysis. PLRP2 differs in substrate specificity and colipase dependence as compared to PTL (Andersson et al., 1996; Sias et al., 2004; Amara et al., 2010) and has a synergistic effect on PTL (Berton et al., 2009).

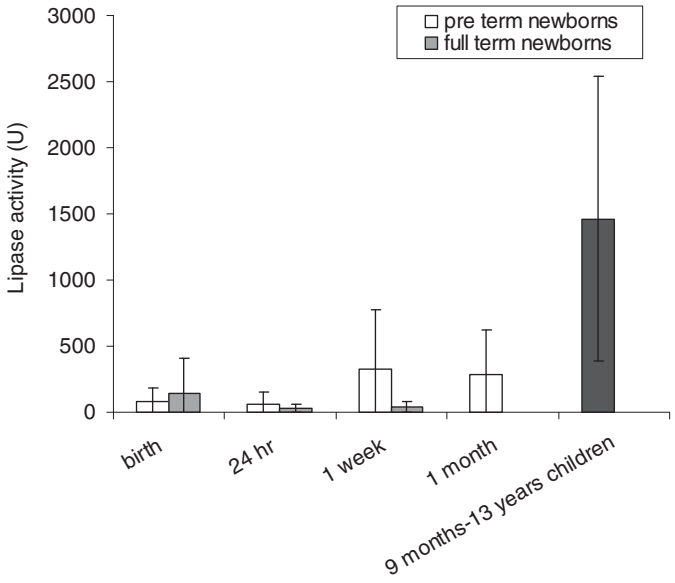

Figure 8 Mean postprandial lipase activity of pancreatic juice of preterm or full-term newborns as compared to children. Mean value determined over a 50-minute period after test meal/kg of bodyweight (composition meal g/L: lactose 70, protein 15, fat 36, minerals 2.5, and Cal 664) and injection of secretagogues (pancreozymin and secretin). Adapted from (Zoppi et al., 1972).

Bile Salt-Stimulated Lipase

BSSL (753 AA, 71834 Da) is a nonspecific lipolytic enzyme capable of hydrolyzing TAG, partial glycerides, cholesterol ester, and fat soluble vitamin esters such as retinyl esters, phospholipids, galactolipids, and ceramides (Hui and Howles, 2002). As its name suggests, it requires the presence of primary bile salts to be active (cholate and chenodeoxycholate). In contrast to gastric and pancreatic PTL, BSSL is able to hydrolyze the 3 *sn*-positions of acylglycerol esters without distinction and has been presented in infant digestion as being responsible for the shift from partial glycerides resulting from the action of other digestive lipases (gastric lipase, PLRP2 and PTL) towards end products of the hydrolysis reaction: FFA and glycerol (Bernback et al., 1990; Hernell and Blackberg, 1994). Levels of TAG hydrolysis higher than 90% were achieved in vitro with PTL, colipase and BSSL which was not the case without BSSL. The depletion of monoglycerides by hydrolysis to FA and glycerol could be favorable for fat absorption in a context of low intraluminal bile salt concentration which is found in newborns but this hypothesis remains to be tested (Signer et al., 1974; Carey et al., 1983; Lindquist and Hernell, 2010). BSSL also plays an important part in cholesterol ester, fat soluble vitamin ester, and long-chain polyunsaturated fatty acid hydrolysis in infant (Chen et al., 1989, 1994; Hernell et al., 1993; Manson and Weaver, 1997).

Expression of BSSL in fetal pancreatic tissues appears as early as the 6th week of gestation and is higher in 12- to 21-week-fetus pancreatic tissues than in adults (Roudani et al.,

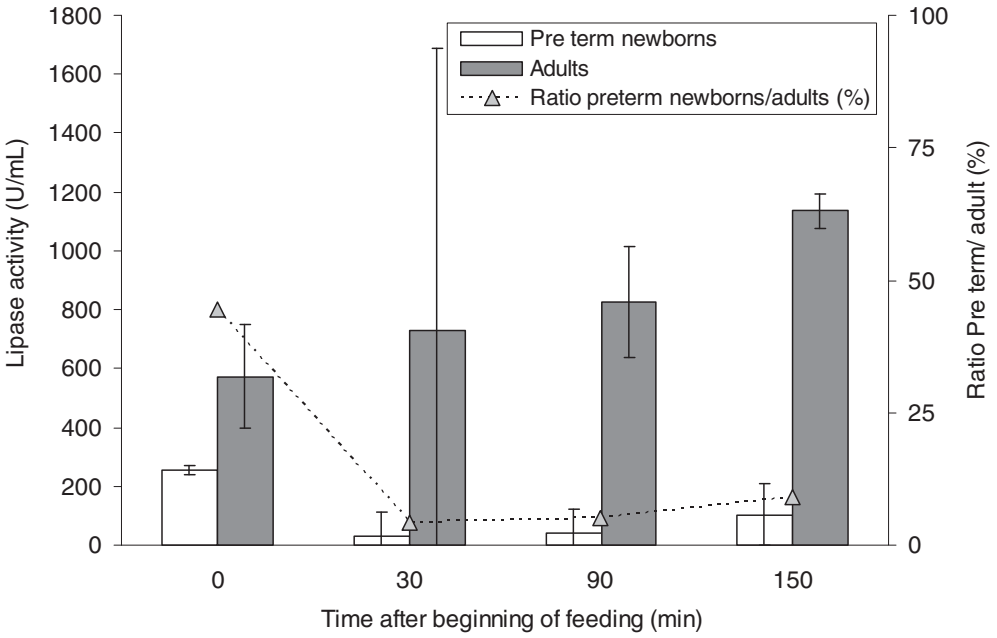

Figure 9 Evolution of lipase activity of pancreatic juice of preterm newborns as compared to adult after a test meal (heat treated pooled breast milk for newborns). Median values are presented and error bars stands for 3rd quartile values. Adapted from (Fredrikzon and Olivecrona, 1978).

1995). Little data are available about the level of secretion and activity of BSSL in the pancreatic juice of newborns. Low level of expression of this lipase in the newborn immature pancreas was reported by Lebenthal and Lee (1980).

The presence of BSSL in human milk, at levels of approximately 0.5 to 1% of milk protein, *i.e.* 4.25 to 8.5 g/L (Jenness, 1979; Baba et al., 1991) may supplement the possibly reduced activity of pancreatic lipolytic enzymes and allow better lipid absorption in low birth weight infants (Alemi et al., 1981; Sbarra et al., 1996). Indeed, BSSL is stable in the pH range of infant stomachs, although not active in the gastric environment where the concentration of BS is very low. BSSL activity investigated in the milk of 12 healthy mothers decreased significantly with length of lactation from 5.3 U/mL of milk at 2-week postpartum

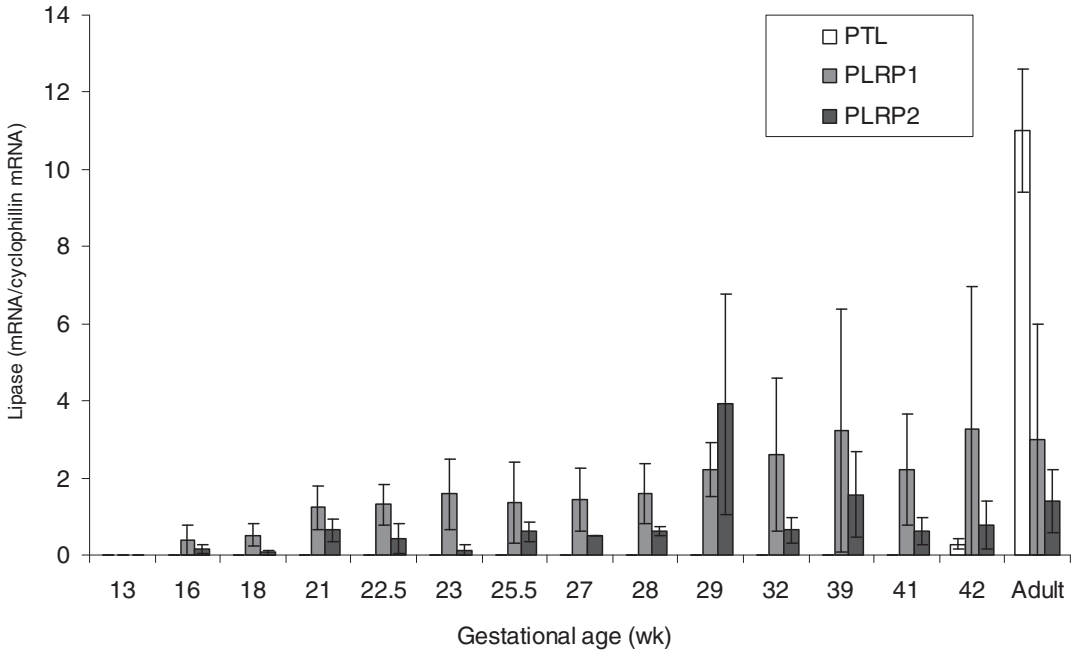

Figure 10 Evolution of level of expression of mRNA of pancreatic lipases during gestation ($N = 36$, GA = 13–42 weeks) as compared to adults. Values are normalized by the amount of mRNA-encoding cyclophilin used as endogenous standard. Adapted from (Yang et al., 2000).

to 3.6 U/mL at 16-week postpartum (Brown et al., 1988). A considerable number of studies support the hypothesis that milk-derived BSSL affects the digestion and absorption of milk fat and fat soluble vitamins in neonatal nutrition (Wang et al., 1989; Stromqvist et al., 1996; Howles et al., 1999).

A fetal glycoform of BSSL (fetoacinar pancreatic protein, FAP), which is expressed in fetal human pancreas and several other fetal tissues, has been shown to have a catalytic efficiency of only 10% of that of BSSL (Mas et al., 1993).

Phospholipase A_2

PLA_2 (14,000 Da, 125 AA) converts a significant nutrient pool of glycerophospholipids into absorbable products and is primarily responsible for hydrolyzing phosphatidylcholine (PC) to 2-lysophosphatidylcholine (2-lysoPC). PLA_2 is produced by the acinar cells of the exocrine pancreas for release into the duodenum via pancreatic juice. PLA_2 is released as inactive zymogens containing peptide extensions at the amino-termini and is activated by trypsin on entering the duodenum (Roy et al., 1988). The proteolytic event converting zymogens into active hydrolases is counterbalanced by inactivation of the hydrolases during transit to the small intestine due to additional proteolysis (Layer et al., 1986) which affects lipolityc enzymes more easily as compared to proteases (Borgström et al., 1993). In adults, PLA_2 makes up a small fraction of the pancreatic lipolytic enzymes, as the bulk of the lipase mass consists of BSSL and PTL (Sternby et al., 1991). Animal studies (mice and rat) suggest that its neonatal role is very limited until weaning (Li et al., 2007). The only pediatric study that determined PLA_2 secretion rates, (Nouri-Sorkhabi et al., 2000) showed that pancreatic phospholipase was cosecreted with lipase and colipase in nine male patients aged 0.5 to 16 years (seven pancreatic sufficient and two pancreatic insufficient) after stimulation with secretin and CCK. Similar secretion rates were found for colipase and PLA_2. While the normal PLA_2 secretion rate, expressed as the activity (mM/ml/hour), times the total volume of duodenal aspirate during 1 hour of stimulation per kg of body weight was 18.7 mM/h/kg/total period for a child aged 1.3 years, no PLA_2 activity was found in the two children with pancreatic insufficiency. Five children with pancreatic sufficiency were found with cystic fibrosis and their PLA_2 secretion rates ranged between 24.7 and 33.6 mM/h/kg/total period, with no significant age-related differences (0.5–16.8 years).

Proteolytic Enzymes Acting in Duodenal Phase

The pancreas is the major source of proteases of the digestive system. Pancreatic proteases are secreted as inactive zymogens that are activated by trypsin. Trypsin itself is activated from trypsinogen by intestinal enterokinase whose activity is limited in newborn infants ($\sim$ 20% of the activity detected in childhood —one to four years; Antonowicz and Lebenthal, 1977). This activation cascade and its dependence with secretagogues (CCK, secretin, etc) is represented in Figure 11. The properties of pancreatic proteases as compared to other digestive proteases and their specificity and level of activity in newborn infants is summarized in Table 8.

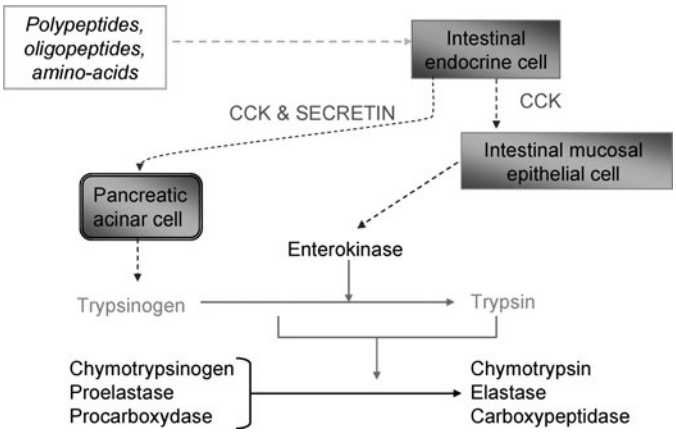

Figure 11 Activation of pancreatic proteases. Adapted from (Freeman and Kim, 1978).

Trypsin

Trypsinogen, because of its central role in the activation of other digestive enzymes is often considered the most important of the pancreatic enzymes. It makes up 19% w/v of the protein in pancreatic juice in adults and is also the most abundant of all the pancreatic enzymes. Four forms of trypsinogen are secreted in adults: a cationic form (at pH 8.6) also called protein serine 1 (PRSS1) which makes up two-third of the global tryptic activity, an anionic form also called protein serine 2 (PRSS2), a third form mesotrypsinogen or PRSS3 which represents less than 5% of total activity, and a fourth form called pancreasin was identified recently (Whitcomb and Lowe, 2007; Borulf et al., 2011). Their respective contribution in infant pancreatic secretions is not documented. However, several studies have quantified the global tryptic activity in infant duodenal fluid. Once again, the use of different enzyme activity assays makes comparison between studies difficult and the variability in methodologies and range of substrate is even greater than for pepsin activity. A summary of these studies, which will be discussed below is proposed in Table 9.

With regard to the ontogeny of tryptic activity, it has been detected in the pancreas as early as 16-week GA (Keene and Hewer, 1929) and secretion has been demonstrated by 20-week GA (Britton and Koldovsky, 1989).

With regard to the effect of GA and postnatal age on tryptic activity, Borgström et al. (1960) reported a decrease in trypsin concentrations for premature newborns as compared to full-term infants of the same age (7–10 days). However, trypsin levels in a two-week older premature group (22 days) had reached the values of the full-term group suggesting compensatory phenomena in newborn premature infants. Using the

Table 8 Biochemical properties and ontogeny of pancreatic proteases as compared to other digestive proteases in newborn infants. Adapted from (Hamosh et al., 1996)

Name of enzyme	Biochemical nature	Secretion site	Site of action	Ontogeny—Level of activity in newborn
Pepsins A (I), C (II or gastricin)	Aspartic acid residue as AS	Gastric mucosa	Stomach	14th-week GA—13% of activity of adult in full-term at birth increase to 30% at 4 weeks—10% of activity of adult in 4 weeks preterms
Chymosin (Rennin)	Aspartic acid residue as AS	Gastric mucosa	Stomach	(–)—adequate
Trypsins: Cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2), mesotrypsin (PRSS3)	Serine endopeptidase—cleaves bond at lysine or arginine residues	Pancreas	Intestinal lumen	20th-week GA—90% of childhood level at birth
Chymotrypsin	Serine endopeptidase—cleaves bonds at aliphatic AA residue	Pancreas		(–)
Elastase	Serine endopeptidase—cleaves bonds after small AA residue (Alanine, glycine, serine)	Pancreas		Low activity in newborn
Carboxypeptidases A, B	Exopeptidase cleaves aromatic, acid or neutral AA for form A or arginine/lysine for form B from carboxyl end of protein or peptide	Pancreas		At birth 8% and at 4 weeks 27% of activity in children (> 2 years)
Enterokinase	Endopeptidase	Intestinal mucosa		26th-week GA—at birth 17% of activity found in 1–4-year-old children

same methodology, these authors (Borgström et al., 1961) did not show any age-induced difference in trypsin levels between three full-term infants aged of three, four, and five months but the reduced number of infants ($N = 3$) biased the study. This limited effect of age on tryptic activity in full-term infants was also reported by Norman et al. (1972) who did not observe any correlation between age of the neonate ($N = 8$, 3–15 days) and level of activity (Figure 12). Lebenthal and Lee (1980) found similar tryptic activity in the duodenal fluid of infants ($N = 15$, nine full-term, six preterm with GA > 32 weeks) as compared to infants (>2 years). High prematurity (31 vs. 34 weeks) did not induce lower tryptic activity according to Boehm et al. (1995a) study which determined in both group average fasting values of 8 U/mL. In both groups of premature infants, the trypsin activity increased significantly with postnatal age and reached values found in duodenal aspirates of two- to six-year-old children within the first month of life (evolution for group II reported in Figure 13). In conclusion, tryptic activity in full-term

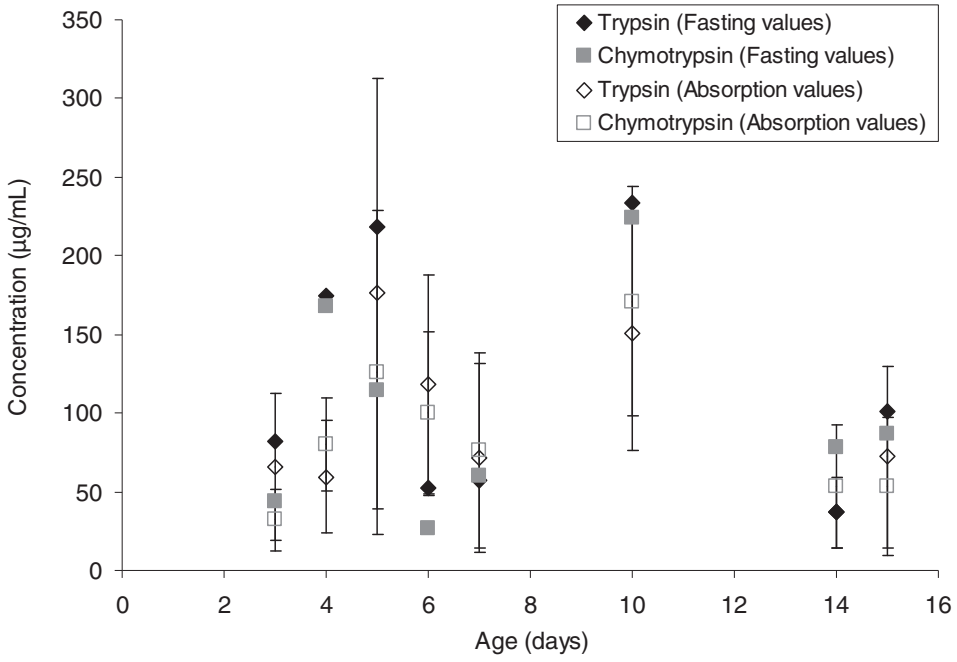

Figure 12 Influence of age on trypsin and chymotrypsin activity reported in duodenal juice of eight healthy full-term newborns (3–15 days). Adapted from (Norman et al., 1972).

Table 9 Trypsin and other pancreatic proteases activity in preterm or full-term infants reported in literature

Group: *N*; GA; Age	Birth weight (kg)	Protease activity (μg/mL or U/mL)	Substrate	References	State
Group I pre-term: $N = 5$; GA: n.a.*;7 days (6–9); Group II pre-term: $N = 5$; GA: n.a.; 22 d (14–32); Group III full-term: $N = 5$; GA: n.a.; 10 days (5–16)	Group I: 2.06 (1.86–2.18); Group II: 1.73 (1.70–1.82); Group III: 3.56 (3.00–4.40)	Trypsin (μg /mL) Group I : < 40; Group II ∼ Group III: 40 (0–175)	*N*-benzoyl-arginine ethyl esters	(Borgström et al., 1960)	Postprandial values**
$N = 3$; full-term; 3, 4, and 5 months	4.30, 3.90, 3.60;	Trypsin (μg/mL) Infant 1: 90 (50–175), infant 2: 113 (63–188), infant 3: 92 (62–154)	*N*-benzoyl-arginine ethyl esters	(Borgström et al., 1961)	Postprandial values***
$N = 8$; full-term 40 ± 1.8 weeks; 8 ± 4.5 (3–15) days	3.71 ± 0.24	Trypsin (μg/mL) fasting: 120 ± 78, postprandial: 94 ± 49; Chymotrypsin fasting: 100 ± 67, postprandial: 63 ± 35	Trypsin on p-toluene-sulfonyl-L-arginine methyl ester; Chymotrypsin on *N*-acetyl-L-tyrosine ethyl ester	(Norman et al., 1972)	Fasting or postprandial values after absorption of pooled breast milk
$N = 36$; preterm 32–34 weeks; 0 day 1 day 1 week 1 month	2.15 (2.0–2.4)	Trypsin (μg/kg) 59.7 ± 130 43.1 ± 58 233 ± 276 196 ± 258	Trypsin on *N*-benzoyl-DL-arginine-ethyl ester; μg of crystalline bovine enzyme with a specific activity of 13,500 BAEEU/mg	(Zoppi et al., 1972)	Stimulation with secretin and pancreozymin - Measurement over 50 min
$N = 8$; full-term; 0 day 1 day 1 week	3.68 (3.6–4.0)	66.1 ± 118 26.3 ± 36 96.6 ± 103		(Zoppi et al., 1972)	
$N = 15$ with 9 full-term with limited health problems and 6 preterm ≥ 32 week; 1 day to 1 month	Full-term (2.62–4.05)—Preterm (1.80–2.25)	Trypsin 1 d: 178 ± 18 nmol/min/mL, 1 month: 327 ± 3 nmol/min/mL; chymotrypsin 1 day: 5.3 ± 0.5, 1 month: 6.6 ± 0.3; carboxypeptidase B 1 day: 0.9 ± 0.0, 1 month: 2.9 ± 0.7	Trypsin on benzoyl-DL-arginine-p-nitroaniline; Chymotrypsin on *N*-benzoyl-DL-tyrosine athyl acetate; Carboxypeptidase B on hippuryl-L-arginine	(Lebenthal and Lee, 1980)	Fasting values
$N = 17$; n.a.; > 2 years with chronic diarrhea but normal pancreatic function	(−)	Trypsin: 166 ± 33 nmol/min/mL; chymotrypsin: 12 ± 2.3; carboxypeptidase B: 11 ± 2.2		(Lebenthal and Lee, 1980)	Fasting values
Group I: $N = 33$; 31.2 ± 0.7 (29–32) weeks; < 6 weeks; Group II: $N = 22$; 34.4 ± 0.8 (33–36) weeks; < 6 weeks	Group I: 1.58 ± 0.21; Group II : 2.00 ± 0.25	Trypsin Group I: 7.9 ± 4.7 U/mL ∼ Group II: 8.5 ± 5.1 U/mL	*N*-tosyl-L-arginine methyl esters	(Boehm et al., 1995a)	Fasting values
$N = 12$; 30.3 ± 1.2 weeks; 26 days $N = 12$; 29.8 ± 1.1 weeks; 23 days	1.41 ± 0.12 1.39 ± 0.14	Trypsin: 5.2 ± 3.6 U/mL (standard formula) 8.6 ± 3.0 U/mL (fortified human milk)	*N*-tosyl-L-arginine methyl esters	(Boehm et al., 1995b)	Postprandial values
$N = 18$; 26 (23–30) weeks; 8 (2–10) weeks	0.80 (0.47–1.41) at examination: 1.42 (0.82–2.40)	Trypsin fasting: 132 ± 117 μg/mL; postprandial: 52–121 μg/mL Elastase fasting 0.6 ± 0.7 μg/mL; postprandial: 0.19–0.6 μg/mL	Trypsin on *N*-benzoyl-DL-arginine-p-nitroanilide Elastase on succinyl-tri-alanine-p-nitroanilide	(Engberg et al., 1999)	Fasting or postprandial values

*n.a.: nonavailable.
**Formula composition (g/100 mL): Corn oil = 3.75, skim milk powder = 6.25, glucose = 7.
***Formula composition (g/100 mL): Corn oil = 5, skim milk powder = 9, glucose = 11.

babies is not significantly influenced by age except in the premature of less than one month as described by Boehm et al. (1995a).

All studies described high interindividual variability in tryptic levels which can be visualized for instance in Figure 14. Postprandial evolution of tryptic levels presents first a decrease due to the effect of meal dilution and a subsequent increase due to meal-induced pancreatic secretions. Parallel evolution of chymotrypsin was described by Norman et al. (1972) who determined trypsin/chymotrypsin ratio ranging from 0.5 to 2.0 and varying slightly during test meal but greatly from one infant to the other. The trypsin values they reported were in the range of those reported by Borgström et al. (1960, 1961) despite differences in the tryptic activity assay.

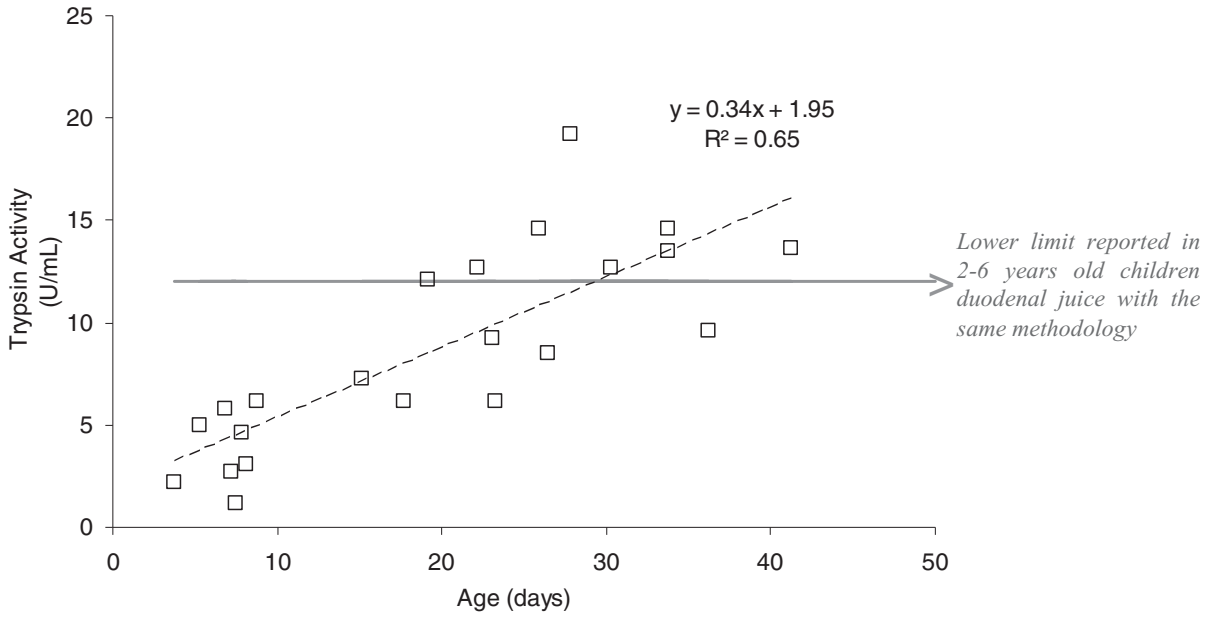

Figure 13 Evolution of tryptic activity with age in premature infants (group II, $N = 22$, GA = 33–36 weeks). Adapted from (Boehm et al., 1995a).

With regards to the influence of intestinal hormones, pancreozymin administration has no effect on infant tryptic activity in one-day-old and one-month-old infants, whereas it significantly enhances activity in children of more than two years old (Lebenthal and Lee, 1980). Secretin administration induced a decrease of tryptic activity in the three groups.

Boehm et al. (1995b) found no effect of diet on trypsin level of activity. They compared three different diets with similar nitrogen and energy contents (standard preterm formula, experimental formula enriched in polyunsaturated FA, and human milk fortified with protein) over a two-week period.

Chymotrypsin

Fewer studies report chymotryptic activities. These values are reported in Table 9 and Figure 15. Chymotrypsin activity in infants is about 50 to 60% of the level found in older children

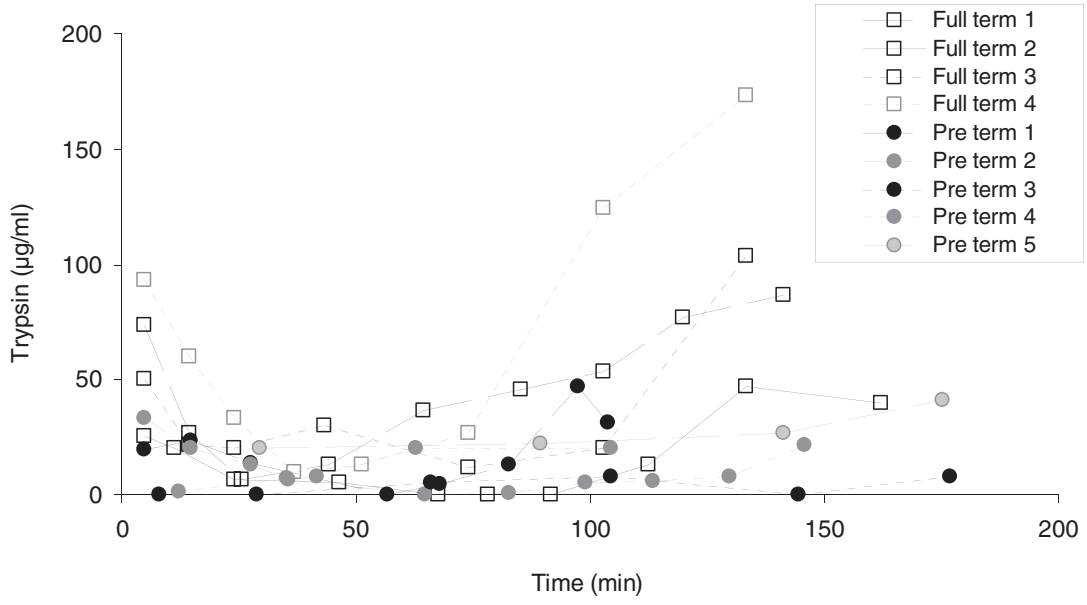

Figure 14 Fasting and postprandial tryptic activity reported in the duodenal juice of full-term normal infants (Full-term one to five) and preterm infants (Group I, N = five and seven days, range —six to nine days). Adapted from (Borgström et al., 1960).

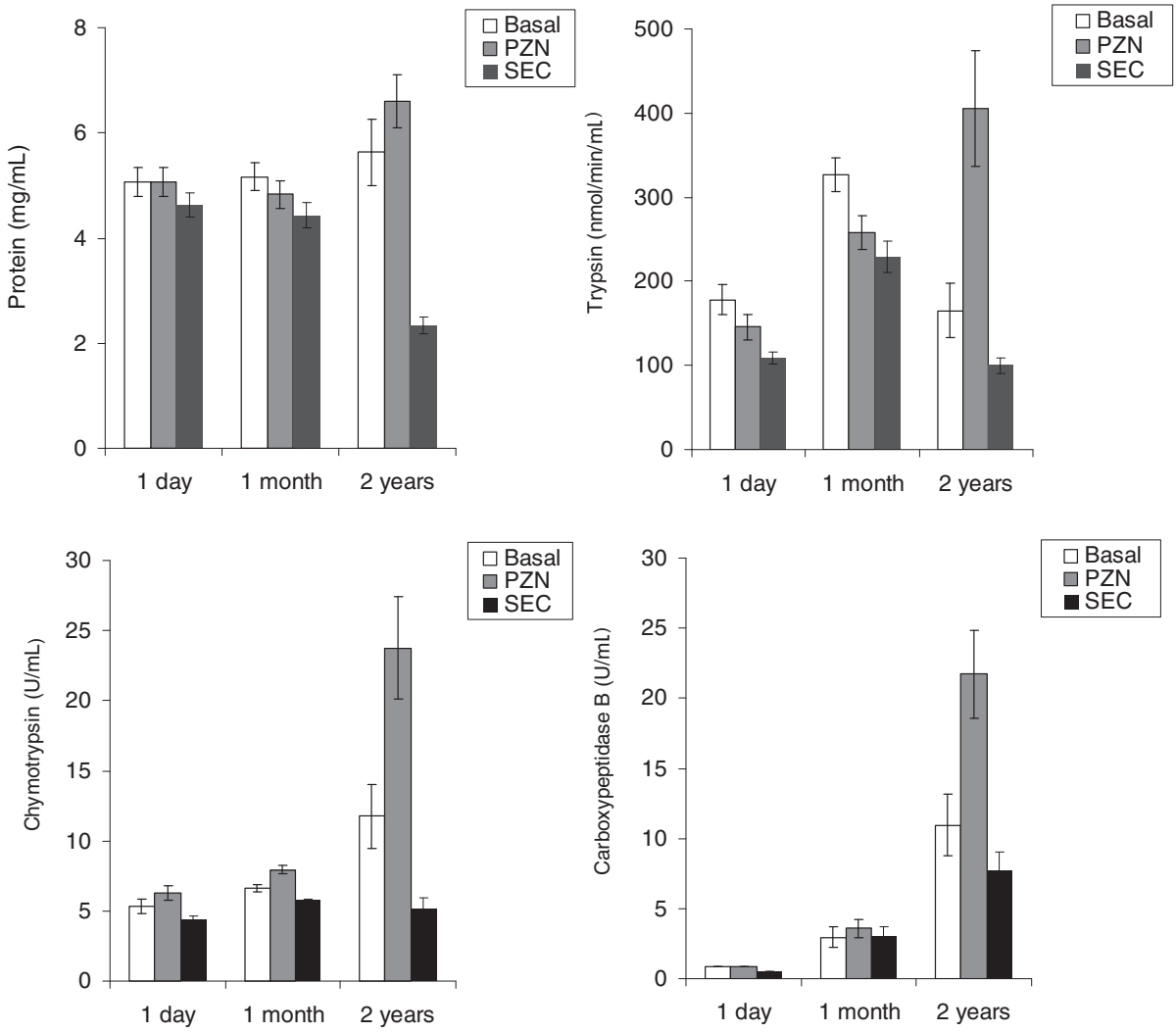

Figure 15 Basal and stimulated protein concentration and protease activity determined in duodenal juice in infants of one day or one month as compared to two-year-old children. PZN = pancreozymin administered intravenously at a dose of 2 U/kg bodyweight, SEC = secretin injected similarly to PZN after this latter. Adapted from (Lebenthal and Lee, 1980).

(> two years; Lebenthal and Lee, 1980). In a study of 30 children aged three weeks to 14 years (median 21 months) with pancreozymin stimulation, Brown et al. (1988) measured simultaneously duodenal and fecal chymotrypsin using acetyl tyrosine ethyl ester as synthetic substrate. Enzyme activities found in the duodenum ranged from approximately 100 to 1000 μg/kg bodyweight 50 minutes after pancreozymin administration. In the children who produced measurable enzyme activities in the duodenum after stimulation, mean fecal chymotrypsin concentration showed a highly significant positive correlation with apparent secretion rates of chymotrypsin ($p < 0.001$).

Other Proteases

The few elastase and carboxypeptidase B values determined in vivo in duodenal content of infants are reported in Table 9.

Amylases

Extremely low or absent pancreatic amylase activity has been reported in duodenal fluid of infants aged less than six months: Norman et al. (1972) determined that a small amount of amylase was present in the duodenal juice of 3 to 15 days healthy newborns without indicating the exact values; Zoppi et al. (1972) found values ranging from 1.2 to 5.9 U/mL in full-term infants from birth to one week after stimulation with secretagogues, whereas values in 9- to 13-year-old children similarly determined ranged around 170 U/mL; Borgström et al. (1960) presented no amylase activity in 7 of the 10 preterm infants they studied, whereas amylase activity ranged in average between 35 $\pm$ 23 U/mL in fullterm infants (Figure 16); similarly, Lebenthal and Lee (1980) found no amylase activity in the duodenal juice of preterm infants aged one day or one month, whereas basal values reached 221 $\pm$ 35 U/mL in children aged more than

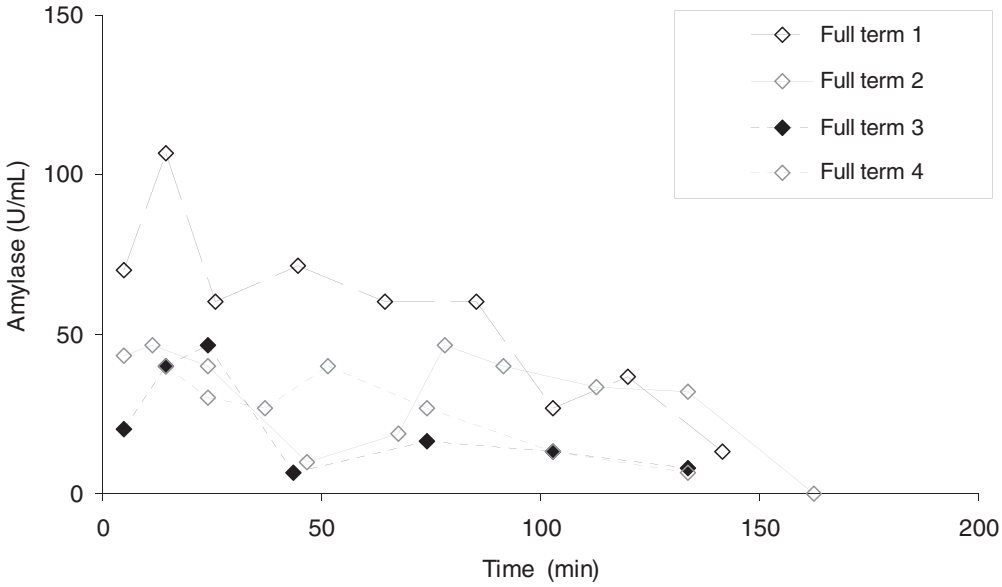

Figure 16 Example of amylase activity reported postprandially in full-term infants after ingestion of a test meal formula. Adapted from (Borgström et al., 1960).

two years. In adult, the amylase production in seven healthy patients ranged from 65.4 to 121.2 U/mL (Tribl et al., 2003). Values of 9170 ± 1915 U/hour were found by O'Keefe et al. (2005).

Salivary α-amylase introduced through swallowing saliva, remains active in infant duodenum thanks to moderately acidic gastric pH and proteolysis. This amylase also plays a role in starch or glucose polymers hydrolysis in the duodenum. A similar role has been suggested for mammary α-amylase.

Rate of Secretion and Nonenzymatic Composition of Pancreatic Fluid

The pancreatic secretion rates of fluid, concentration of proteins, and electrolytes in premature infants ($N = 25$, GA = 32–34 week, birth weight = 2.32 kg) as compared to full-term newborn infants ($N = 12$, birth weight = 3.84 kg) in fasting state or poststimulation are reported in Table 10a and b (Zoppi et al., 1973). The secretions rates are generally higher in premature than in full-term newborn infants, which was also observed for enzyme production in infants of the same age and indicated a more active pancreas in premature newborns (Zoppi et al., 1972). The values are always much lower for newborn infants as compared to children (nine months to three years) also reported for comparison purposes. For instance, basal pancreatic secretion rates at birth in full-term infants represents only 52% of the average value reported for children (nine months to 13 years). Hormonal stimulation induced an increase in pancreatic secretions for both groups of infants from birth onwards but a significant differentiated answer to the two hormonal stimulation is only observed after one month.

Bile Salts and Biliary Secretions

Within the gastrointestinal lumen, bile salts play an essential role in lipid lipolysis, absorption, and transport due to their high surface activity and self-assembly properties (Cowen and Campbell, 1977; Carey et al., 1983; Mu and Hoy, 2004; Maldonado-Valderrama et al., 2011). When bile salts are excreted into the intestinal lumen, they favor the emulsification of chyme and activate lipases (PTL/colipase and BSSL). They help to transport surface active lipolysis products away from the interface of the emulsion via the formation of mixed micelles which are then absorbed by the small intestine (Borgström and Patton, 2010; Lindquist and Hernell, 2010). In adults, well-balanced, enterohepatic circulation maintains hepatic bile salt secretion and provides a "feed-back" control of the bile salt and cholesterol metabolism. Several reports are available on adult gallbladder and hepatic bile composition. It is not the case for infants for which limited data on gallbladder bile (Setchell et al., 1988; Halpern et al., 1996) or bile-rich duodenal fluid are available.

In newborn infants, bile salts metabolism and turnover is active but immature (Watkins et al., 1973; De Belle et al., 1979; Balistreri, 1991). De Belle et al. (1979) studied intestinal (jejunum and ileum) absorption of sodium taurocholate in vivo in fetuses, neonates, infants, children, and adults. They demonstrated that the ideal mechanism for active transport of taurocholate is undeveloped in the fetus and newborn infant and suggested the hypothesis that the enterohepatic circulation of bile salt during the perinatal period is limited to that fraction of bile salt absorbed passively. In addition to this limitation in bile acid intestinal uptake, impaired bile acid conjugation, decreased synthesis, and decreased excretion due to lower ability of gallbladder to concentrate bile and lower gallbladder contractibility contribute to the immaturity of bile salt metabolism (Lehtonen

Table 10a Pancreatic fluid, protein and electrolytes secretion rates and concentration in preterm infants and in children before and after pancreozymin (PZN) and secretin (SEC) stimulation. Adapted from (Zoppi et al., 1973)

	Birth		2nd day		1 week		1 month		Children (9 month–13 years)	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Fluid (μL/min/kg)										
Basal	7.9	4.8	9.6	4.7	13.9	10.6	15.2	14.6	35.3	25.5
PZN	9.3	5.4	8.3	3.9	18.2	13.2	23.5	18.5	69.3	27.7
SEC	9.9	9	13.3	5.1	19.8	13.5	28.9	16.1	86.1	47.9
Protein (mg/mL)										
Basal	15.3	8.5	12.1	4.4	9.6	5.7	10.2	4.1	*n.a.*	n.a.
PZN	32.4	16.1	20.9	13.6	14.9	22.6	12.6	8.0	8.1	3.9
SEC	30.9	10.0	9.7	6.3	7.7	9.7	5.5	3.2	3.0	3.0
Na^+ (mM)										
Basal	105	67	72	66	119	102	93	77	n.a.	n.a.
PZN	214	194	176	82	188	144	137	112	1530	1552
SEC	204	158	130	94	190	129	140	109	1521	1816
K^+ (mM)										
Basal	3	27	3	3	4	8	3	2	n.a.	n.a.
PZN	8	7	6	8	7	8	3	2	61	69
SEC	9	4	5	6	6	5	4	3	45	33
Ca^{2+} (mM)										
Basal	41	58	23	43	78	115	56	34	n.a.	n.a.
PZN	72	59	65	85	99	110	83	65	26	25
SEC	68	34	37	53	60	67	41	36	7	8
HCO_3^- (mM)										
Basal	2	2	0	0	2	29	5	12	n.a.	n.a.
PZN	20	22	22	59	27	24	9	6	29	32
SEC	28	18	31	55	31	21	22	16	64	50

n.a., nonavailable.

Table 10b Pancreatic fluid, protein, and electrolytes secretion rates and concentration in full-term infants and in children before and after pancreozymin (PZN) and secretin (SEC) stimulation

	Birth		2nd day		1st week		Children (9 months to 13 years)	
	AV	SD	AV	SD	AV	SD	AV	SD
Fluid (μL/min/kg)								
Basal	6.9	5.5	8.2	4.3	8.3	3.7	35.3	25.5
PZN	8.3	5.3	8.6	4.8	8.4	5.9	69.3	27.7
SEC	8	7.2	8.5	6.8	11.4	6.6	86.1	47.9
Protein (mg/mL)								
Basal	76.2	317.9	62.2	156.4	60.3	71.7	n.a.	n.a.
PZN	50.9	97.4	32.5	46.4	25.1	31.2	1530	1552
SEC	48.3	123.7	36.2	104.1	80.9	106.5	1521	1816
Na^+ (mM)								
Basal	191	131	246	444	167	116	n.a.	n.a.
PZN	286	219	278	356	156	66	1530	1552
SEC	346	253	308	218	164	142	1521	1628
K^+ (mM)								
Basal	5	3	5	11	4	7	n.a.	n.a.
PZN	7	40	13	15	18	47	61	69
SEC	10	9	12	81	10	7	45	33
Ca^{2+} (mM)								
Basal	106	60	118	102	136	130	n.a.	n.a.
PZN	192	172	223	379	244	273	26	25
SEC	209	133	161	162	119	141	7	8

n.a., nonavailable.

Table 11 Gallbladder bile composition of infants and children. Adapted from (Halpern et al., 1996)

Subjects	Age (mo)	pH	CH (mM)	PL (mM)	BS (mM)	Total lipids (g/L)	CH (% mol/mol)	PL (% mol/mol)	BS (% mol/mol)
A	0.1	7.62	2.7	8	32.7	23	6.2	18.4	75.4
B	0.1	8.5	1.1	1.8	5.9	5	12.5	20.5	67.1
C	1.5	8.02	n.a	n.a	n.a	n.a	n.a	n.a	n.a
D	2	7.04	5.1	5.1	35.7	27	10.2	18.7	71.1
E	3	7.37	10.3	10.3	21.3	23.6	23.6	27.5	48.8
F	4	7.6	2.2	2.2	44.3	28	4.1	12.6	83.3
G	6	7.5	9.3	9.3	45.5	37	13.4	20.7	65.9
H	9	6.73	25.1	25.1	160.2	122	11	19	70
Mean (< 6 months)	1.8	7.7	4.3	5.5	28.0	21.3	11.3	19.5	69.1
SD (< 6 months)	1.6	0.5	3.7	3.7	14.8	9.4	7.6	5.3	12.9
Mean (< 12 months)	3.2	7.5	8.0	8.8	49.4	37.9	11.6	19.6	68.8
SD (< 12 months)	3.1	0.5	8.3	7.9	50.8	38.3	6.3	4.4	10.6
I	12	7.6	5.6	5.6	62.5	49	6.3	23.8	70
J	30	7.6	8.9	8.9	146.3	82	4.9	14.8	80.3
K	72	8.2	9	9	136.2	96	5	18.7	76.3
L	84	7.64	15.2	15.2	176.9	139	6	24	70
M	96	6.6	13.6	13.6	94.2	75	9.8	22.2	68
N	96	7.12	13.9	13.9	179	137	5.6	22.8	71.6
O	96	8.05	6	6	76.6	54	5.9	18.5	75.6
P	108	7.75	10.5	10.5	158.2	100	5.5	12.5	82
Q	120	7.85	6.2	6.2	103	76	4.4	21.7	73.9
R	144	8.5	7.4	7.4	134.1	100	4.1	22.2	73.7
Mean (> 12 months)	85.8	7.7	9.6	9.6	126.7	90.8	5.8	19.8	74.1
SD (> 12 months)	39.6	0.5	3.5	3.5	40.9	30.4	1.6	4.0	4.5

CH, cholesterol; PL, phospholipids; BS, bile salts; n.a., nonavailable.

et al., 1992, 1993). This results in a limited bile salt pool and low concentration of primary bile salt in the duodenum (Norman et al., 1972). The composition of gallbladder bile of infants ($N = 8$, aged 3.8 months) and children ($N = 10$, aged 7.1 years) determined by Halpern et al. (1996) is reported in Table 11.

The quality and conjugation of the pool of bile salt in fetuses and infants also differ from the one in adults: glycoconjugation is predominant in adult, whereas tauroconjugation is predominant in fetal and in breastfed newborns of less than three weeks (Brueton et al., 1978; Back and Walter, 1980). Brueton et al. (1978) demonstrated in low-weight birth babies ($N = 20$, aged 12–22 days) fed diets which differed in taurine content (human milk vs. two industrial formulas) that tauroconjugation was limited by substrate availability in the diet. A ratio of glycoconjugate/tauroconjugate lower than one in breast fed infants became higher than one after 12 days in infants fed formulas with a low content of taurine.

Lower biliary secretions result in low luminal concentration of biliary salts which have been presented specially for the preterm infants as a limiting factor for fat digestion: coefficient of fat absorption ranging between 74 to 91% in preterms after absorption of human milk, ranging between 68 to 85% in preterms after absorption of formula, ranging around 90% in full-terms after absorption of formula and generally > 95% in healthy adults (Lindquist and Hernell, 2010). Examples of duodenal bile salt composition determined in healthy newborns ($N = 8$, 3–15 days) have been reported by (Norman et al., 1972). The bile acids mainly consisted of the taurine and glycine conjugates of cholic and chenodeoxycholic acids. The total concentrations of cholic and chenodeoxycholic acids combined during the test meal ranged from 0.41 to 1.48 mM (range of variation 0.02–5.29 mM). This concentration was much lower than the one reported for adults and is probably too low to permit optimal fat digestion.

Again, human milk could benefit to digestive process by supplementing the low levels of bile salts in the newborn: bile salts have been detected in human colostrums and milk ($N =$ 28 mothers). Cholate and chenodeoxycholate were predominant (Forsyth et al., 1983).

Intestinal Mucus Secretions (Rubio et al., 1995)

The mechanisms of protection of the gastrointestinal epithelial cells rely on mucus secretions which contains glycoproteins and phospholipids. The hydrophobic character of gastric mucus barrier contributes to its protective effect. Immaturity of mucosal secretion disappears between the 2nd and 3rd weeks. Premature infants present gastric secretions of surfactants from 28-week GA onwards.

Small Intestine Motility and Transit Time

Small intestine motility in newborns and young infants is irregular with a pattern of peristaltic activity differing from that in adults though very limited data are available on this topic for full-term healthy infants (Dumont and Rudolph, 1994; Faure

and Navarro, 2000). Small intestine motility is more immature in preterm infants than in full-term infants (Berseth, 1989). More precisely, specific postprandial motility is absent at 31-week GA and appears by 35-week GA. Enteral feeding of preterm newborns also triggers postprandial motor activity (Berseth, 1990). This immaturity of motility results for preterm infants in longer transit times, generally considered four times longer than the ones reported for adults. Conversely for full-term infants and toddlers, though having lower amplitude of contractions, lower frequency and propagation speed than adults, a transit time quite similar to adults is generally considered. This can be explained by a higher relative gut surface area as compared to adults which speeds up absorption and counterbalances the slower motility (Faure and Navarro, 2000; Kauffman, 2011).

SYNTHESIS FOR INFANT IN VITRO DIGESTION MODELS AS COMPARED TO ADULTS

Both developmental factors and feeding specificities of infant (< six months) result in very singular conditions of digestion as compared to adults (Geigy, 1973; Kalantzi et al., 2006; McConnell et al., 2008; Golding and Wooster, 2010) and are summarized in Table 12a & b.

Nonenzymatic Specific Parameters of the Infant Digestion

These specificities cannot be restricted to enzymatic immaturity. Several nonenzymatic parameters modulate infant digestion. The frequency of feeding with feeding schedule of 6 to 12 times every 24 hours during the first month and approximately every —four to six hours afterwards has considerable impact on gastric pH, which in turn, influences digestive enzyme activities (Mitchell et al., 2001). The pH for optimal activity of gastric lipase and pepsins of 3 to 5.5 and 1.8, respectively, is not in line with the rise of pH following milk ingestion and so will impair hydrolysis reactions. In gavage-fed preterm newborns, pH remains higher than five for the hour following meal ingestion (Hamosh et al., 1981). The quantity of fluid and electrolytes also modulate enzyme activities and more specifically Ca^{2+}, though the gastric phase concentration of this cation has not been published for newborns. A similar remark can be made concerning bile salts, whose luminal concentration is

Table 12a Characteristics of gastric phase in full-term and preterm infants as compared to adults

	Full-term infants	Preterm infants	Adults
Total volume (mL)	F: 2–2.65 (1 d) PP: n.a.	PP: 30 mL/kg bodyweight + 15% meal volume secretion at 15 min (GA = 30 wk, aged 4.3 wk)	F: 24–45
Secretions (mL/h/kg)	F: 1 (1 wk)	F: 1 (1.7 wk), PP: 7.2 (first 30 min) then 1.2 (4.3 wk, GA = 30 wk)	Daily average: 1.46, F: 79.7 ± 37.9 mL/h, S: 210 ± 51.3 mL/h
Gas volume (mL)	n.a.	n.a.	36 ± 12
Ionic strength (mM)	115 (72–152)	132 (110–146)	F:100–150
Na^+ (mM)	94 (69–128)	118 (93–131)	F: 59.7 – S:25
K^+ (mM)	13.2 (6.9–22.4)	9.8 (6.1–13.6)	F: 14.2 – S:17.9
Ca^{2+} (mM)	n.a.	n.a.	F: 1.0 ± 0.4, S: 0.15 ± 0.25
Cl^- (mM)	122 (68–153)	137 (120–148)	F: 104 – S: 129
pH	Buffering effect of milk proteins—frequency of feeding—Fitting of literature data $pH = -0.015 \cdot \mathrm{T} + 6.53$ $r^2 = 0.73$ with T: time after feeding in min; 7.5 ($T0$) – 2.00 (240 min PP)		PP shift in pH depending on the buffering capacity of the meal. Fitting of Armand et al. (1995) data: pH = $-0.0001 \cdot T^2 - 0.0004 \cdot T + 2.59 r^2 =$ 0.88 with T: time after feeding in min; 2.5–1.0 (240 min PP)
HGL	Activity F: 38.9 ± 22.8 U/mL, PP: n.a.; Output: n.a.	No effect of diet—limited effect of age; Activity F: 0.00–31.6 U/mL, PP: 2–10.6 (10–180 min); Output F: 6.3 (1.4–13.3), S: 24.6 (16.1–34.9) U/h/kg	Activity F: 5.7–9.9 U/mL, S: 5.2–7.5 U/mL; Output F: 446–745 S: 845–1323 U/h, F: 7.3–12.15 S: 14.3–21.6 U/kg/h
Pepsins	Activity S: 0.12 (0.06–0.18) mg/mL (10–110 d); Output S: 0.23 (0.11–0.32) mg/mL/h/kg	Activity F: 4.1–125 U/mL/kg, PP: 63 ± 11 U/mL/kg; Output 569 U/h/kg	Activity: S: 0.29 (0.23–0.36) mg/ml; Output: S: 0.60 (0.47–0.73) mg/mL/h/kg; Activity F: 942–1333 U/mL, PP: 718–1042 U/mL; Output F: 79–108 kU/h, PP: 129–192 kU/h
Gastric emptying time (min)	$T1/2$ infant formula: 80 (76–87), Human milk: 54 (48–61)—rate of emptying to the duodenum ~ 0.63–0.43 Kcal/min Elashoff fitting (infant formula): $T1/2 =$ 78, $\beta = 1.2$–1.5	$T1/2$ infant formula: 50 (17–72), Human milk: 38 (25–47) Elashoff fitting (infant formula): $T1/2 =$ 72, $\beta = 1.6$, Elashoff fitting (Human milk): $T1/2 = 36$, $\beta = 1.15$	Emptying dependent on volume of meal, osmotic pressure and caloric content—fluid meal = > zero-order kinetics ($T1/2$: 10–60); solid meal = > first order kinetics ($T1/2$: 60–277)—rate of emptying to the duodenum ~2 Kcal/min

F, Fasting state; PP, Postprandial state; S: Stimulated; n.a., nonavailable; d, day; wk, week(s); mo, month(s), min, minutes.

Table 12b Characteristics of duodenal phase in full-term and pre-term infants as compared to adults

	Full-term infants	Pre-term infants	Adults
Bile salt concentration	Tauroconjugation dominant in breastfed infant; Cholic and chenodeoxycholic acids dominant; PP: 0.4–1.5 mM (3–15 days)	Tauroconjugation dominant in breastfed infant; Cholic and chenodeoxycholic acids dominant; Fetal gallbladder bile: 128.89 (98–186) μmol/L	Glycoconjugation dominant, F: 0.57–6.0 mM, PP:11.2 (8–16) mM
Gallbladder secretion and contractibility	Contraction index: 44–100%	Limited contraction index in pre-term infants—contraction index dependent on GA, significant contraction observed > 31 wk GA	(−)
Phospholipids; Cholesterol in gallbladder bile	19.5 ± 5.3% mol/mol total lipid; 11.3 ± 7.6% mol/mol total lipid	n.a.	22.2–27.8% w/w total lipid; 3.3–5.5% w/w total lipid
pH	6.2 (5.2–7.6)	n.a.	F: 5.8–6.5, PP: 5–5.5
Pancreatic juice secretion rate (μL/min)	By kg at 1 wk F: 8.3 ± 3.7, S: 11.4 ± 6.6; flow in newborn initially low matches flows in adults within the first months of life	By kg at 1 wk F: 13.9 ± 10.6, S: 19.8 ± 13.5	F< 100, By kg S: 48 ± 15, average daily secretion: 1700–2000 μL/d/kg
Ionic strength (mM)	F: 128 (115–140)	F: 137–163, S: 257	F: 140–150
Na^+ (mM)	F: 167 ± 116, S: 164 ± 142 (1 wk)	F: 119 ± 102, S: 190 ± 129 (1 wk)	F: 125 ± 14, S: 138
K^+ (mM)	F: 4 ± 7, S: 10 ± 7 (1 wk)	F: 4 ± 8, S: 6 ± 5 (1 wk)	F: 7.6 ± 1.7
Ca^{2+} (mM)	F: 136 ± 130, S: 119 ± 141 (1 wk)	F: 78 ± 115, S: 60 ± 67 (1 wk)	F: 0.6 ± 0.8
HCO_3^- (mM)	n.a.	F: 2 ± 29, S: 31 ± 21 (1 wk)	F: 10–45, S: 85 ± 11
Amylase (U/mL)	1.2–55 U/mL; Evolution with age $activity = 0.31 \cdot A + 19.4$ $r^2 = 0.8$, with A: age in days (validated 0–150 d)	Null in the first wks	65–121 U/mL
Lipase	Activity F: 128 (20–233) U/mL/kg 1100 (8 d)	Activity F: 14–124 U/mL/kg (0–6 wk, GA: 29–36 wk), PP: 8.4–400 U/mL/kg (1–38 d, GA: 28–37 wk)	Activity F: 100–400 U/ml, PP: 400–1500 U/mL
Trypsins	F: 104 (50–198) U/mL (1 wk–5 mo); PP: 94 ± 49 (3–15 d)	F: 104 (50–198) U/mL (1 wk-5 mo); PP: 94 ± 49 (3–15 d); Evolution with age $activity = 0.34 \cdot A + 1.95$ $r^2 = 0.65$, with A: age in days (validated 0–45 d)	Activity F: 20–50 U/ml
Other proteases	Ratio trypsin/chymotrypsin ∼1.2; Chymotrypsin F: 100 ± 67, PP: 63 ± 35 U/mL (3–15 d); Carboxypeptidase F: 0.9 to 2.9 U/mL (1 d to 1 mo), S: 0.9 to 3.6 U/mL (1 d to 1 mo); Elastase B: n.a.	Chymotrypsin F: 5.3 to 6.6 U/mL (1 d to 1 mo), S: 6.3 to 7.9 U/mL (1 d to 1 mo); Carboxypeptidase F: 0.9 to 2.9 U/mL (1 d to 1 mo), S: 0.9 to 3.6 U/mL (1 d to 1 mo); Elastase B F: 0.6 ± 0.7 μg/mL, PP: 0.2–0.6 μg/mL	Chymotrypsin S: 66 (28–154) U/min; Carboxypeptidase S: 61(23.9–157) U/min
Intestinal motility and transit time	Not completely mature. Transit time close to the one reported for adults	Immature postprandial motility up to 31 wk. Slower transit time than adults (∼ 4 times)	Elashoff fitting of small intestine emptying: $T1/2 = 160$ min; child T $1/2 = 200$ min and, $\beta = 2.2$ (Blanquet et al., 2004)

F, Fasting state; PP, Postprandial state; S, Stimulated; d, day; wk, week(s); mo, month(s).

10 times reduced in newborn infants as compared to adults and will specifically affect the duodenal solubilization of lipolysis products (Maldonado-Valderrama et al., 2011). It is one element in addition to immaturity of pancreatic lipolytic enzymes that helps to explain the limited fat absorption in preterm infants (68–91%) and full-term infants (90%) as compared to adults (≥ 95%; Lindquist and Hernell, 2010).

Specificity Induced by Milk-based Diet

The type of milk (infant formula vs. breast milk) has no impact on gastric and duodenal pH but significantly influences gastric emptying with half-times longer for formulas (80 minutes) as compared to human milk (5 minutes). This has been explained by some authors by a lower protein content of human milk. The type of proteins and presence of acid-coagulating proteins such as caseins in higher amount in formulas could also be an explanation for this delayed emptying (Billeaud et al., 1990). However, the maintained difference in gastric emptying times between infant formula and human milk having very similar chemical composition except for the profile of triacylglyceride acyl moieties is probably explained by structural differences. These structural differences have not been assessed in detail in infants' meal in comparison with adults. The type of milk (infant formula vs. breast milk) also influences the extent of gastric lipolysis which is higher for human milk than for formula (Armand et al., 1994; Roman et al., 2007) but always remains in the range reported for adults (10–30%). This higher extent of lipolysis of human milk has been explained both by the presence of prolipase factors in human milk and by structural differences between the two meals (native globules versus

homogenized fat emulsion). The type of milk was not reported to impact the limited proteolysis level which is reached in gastric phase in infant (15%) which is surprising considering the structural differences induced by process-denaturation in infant formulas (Rudloff and Lonnerdal, 1992; Dupont et al., 2010). Whether this difference of levels of milk protein denaturation affects the extent of duodenal proteolysis in infants has not been investigated in vivo.

Enzymatic Immaturity

Of course the immaturity of gastric protease (pepsins) on one side, and pancreatic lipolytic enzymes (PTL, PLA_2) and amylase on the other side, are also noticeable features of the infant digestive systems: (i)pepsins represent only 10% of the fasting levels detected in adult and 20% of the postprandial levels and (ii) PTL and PLA_2 are secreted in very limited levels, whereas PLRP2 and BSSL, two lipolytic enzymes with broad substrate specificity, are suspected to be the main actors of duodenal lipolysis in infants as long as milk is the main food (Lindquist and Hernell, 2010). Despite this suspected predominant physiological role, some basic data such as PLRP2 and BSSL concentrations in infant duodenal content have not yet been collected.

Influence of Gestational Age and Postnatal Age on Digestive Conditions

Gestational age and postnatal age influence some of the gastrointestinal parameters. More specifically preterm newborns (<—three to four weeks) as compared to full-terms of the same age have specifically high gastric pH resulting from frequent feeding, lower pepsin activity (10% of adult activity at four weeks vs. 30% in full-terms), faster gastric emptying, limited gallbladder contraction index, lower concentration of electrolytes in pancreatic fluid, and no amylase secretion and lower global pancreatic activity. However, compensatory mechanisms in preterm infants allow them to catch up with full-term infants within the first —three to four weeks of life. The parameters which have been specifically described as influenced by age for full-term infants are:

(i) The levels of pepsin activity which increases from 13% of activity of adult in full-term at birth increase to 30% at four weeks but subsequent evolution with age is not precisely given in literature,
(ii) Amylase activity level,
(iii) Pancreatic lipolytic enzymes and proteases other than trypsin, whose precise evolution with age is not available either.

As a consequence, the conditions of digestion of the infants cannot be restricted to a single model: preterm newborns should be differentiated from full-term newborns and from older infants (—two to six months). Of course, the gastrointestinal parameters were not determined precisely and exhaustively at each of these postnatal ages. However, the data collected in Table 12a & b allow differentiating these three models (preterm, newborn full-term, and older full-term infants using equations that describe the influence of postnatal age for some parameters).

Interindividual and Developmental Variability

Another important point which can be noted when considering the synthesis of parameters presented in this review is the high interindividual variations between parameters which is higher than the one reported for adults due to its inherent additional developmental variations. Ranges of variation for enzymatic activities and outputs specifically are large, which can only partially be explained by differences in the activity assays.

To integrate this variability, experimenters are advised to select average values of digestive parameters reported for infant and check that these values in their in vitro model trigger the levels of hydrolysis reported in literature for a similar milk-based type of meal.

CONCLUSIONS

Using a methodical comparison of the results of in vivo studies of infant upper gastrointestinal tract, this review has shed light on the high interindividual variability of parameters and variability in digestive enzymes developmental patterns. Despite these variations, we have elucidated the range of digestive conditions in premature infants as compared to full-term infants and to adults, using data extracted from studies sometimes dating back to the 1960s. A major issue in these sets of data is the variability in enzymatic assays, which makes comparison between studies very difficult. Systematic quantitative (for instance by ELISA using monoclonal antibodies) and functional (test of specific activity with harmonized methodology and substrate) characterization of digestive enzymes in vivo should be undertaken in the future to allow comparative approaches (Lengsfeld et al., 2003). The conditions of conservation of digestive fluids should also be harmonized to eliminate another source of variation, *i.e.* partial denaturation or hydrolysis of enzyme during the storage of the fluid (Kelly et al., 1991). Some physiological parameters remain to be elucidated such as the duodenal concentrations of PLRP1, of PLRP2, of PLA_2, and of some of the intestinal proteases (carboxypeptidase, elastase) in preterm and full-term infants.

Since several digestive parameters are influenced by postnatal age, it seems reasonable to state that there is not a single infant digestive model (—zero to six months) but several models depending on postnatal age. The parameters of these models should be used by scientists who focus on infant digestion instead of using general conditions of digestion which reflect the mature adult digestive system.

The application of this model could speed up the screening of structural parameters (ultrastructure of emulsion, state of interface, and level of denaturation of proteins) of infant formulas which have not been taken as much in consideration in infant nutrition as the chemical composition of the formula but could have important implications for digestion and absorption of nutrients in infants.

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ABBREVIATIONS

AA = Amino acids,
BSSL = Bile salt-stimulated lipase,
CCK = Cholecystokinin,
d = Day,
FA = Fatty acids,
FFA = Free fatty acids,
GA = Gestational age,
HGL = Human gastric lipase,
mo = Month,
N = Number of subjects,
n.a. = Non available,
PLRP1 = Pancreatic lipase related protein 1,
PLRP2 = Pancreatic lipase related protein 2,
PTL = Pancreatic triacylglyceride lipase,
PLA_2 = Phospholipase A_2,
TAG = Triacylglycerides,
$T1/2$ = Gastric half-emptying time,
wk = Week,
y = Year

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