Food Research International xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Holder pasteurization impacts the proteolysis, lipolysis and disintegration of human milk under *in vitro* dynamic term newborn digestion

Samira C. de Oliveira [a,b], Amélie Deglaire [a,b], Olivia Ménard [a,b], Amandine Bellanger [c], Florence Rousseau [a,b], Gwénaële Henry [a,b], Emelyne Dirson [c], Frédéric Carrière [d], Didier Dupont [a,b], Claire Bourlieu [a,b,*]

[a] INRA, UMR 1253, Science et Technologie du Lait et de l'Oeuf, 65 Rue de St Brieuc, 35042 Rennes, France
[b] Agrocampus Ouest, UMR 1253, Science et Technologie du Lait et de l'Oeuf, 65 Rue de St Brieuc, 35042 Rennes, France
[c] Centre Hospitalier Universitaire de Rennes, Département de Pédiatrie, 16 Boulevard de Bulgarie, 35203 Rennes, France
[d] CNRS, Aix Marseille Université, UMR 7282 Enzymologie Interfaciale & Physiologie de la Lipolyse, 31 Chemin Joseph Aiguier, 13402 Marseille, France

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 19 November 2015
Accepted 25 November 2015
Available online xxxx

Keywords:
Human milk
In vitro dynamic digestion
Lipolysis
Proteolysis
Holder pasteurization
Term newborn

ABSTRACT

When the mother's own human milk is unavailable or limited, pasteurized human milk from milk banks is preferentially administered instead of infant formula, especially for vulnerable hospitalized neonates. Holder pasteurization (62.5 °C, 30 min) may alter human milk composition and structure, which may modulate its digestive behavior. An *in vitro* dynamic system was set up to simulate the gastrointestinal digestion of term newborns in order to compare the kinetics of lipolysis, proteolysis and structural disintegration of raw *versus* pasteurized human milk. Human milk from 5 donors was pooled. Half of the pool was either administrated raw (RHM) or pasteurized (PHM). Digestions were conducted at least in duplicate for RHM and PHM. Heat-induced protein aggregation was observed in PHM. During gastric digestion, β-casein was proteolyzed significantly faster for PHM than for RHM ($p < 0.05$), whereas lactoferrin tended to be proteolyzed slower ($p = 0.07$) for PHM. Pasteurization selectively affected the intestinal release of some amino acids. At any time of the gastrointestinal digestion, the lipolysis of PHM was significantly lower than that of RHM, but no impact was observed on the profile of released fatty acids. RHM presented a structural destabilization after 60 min of gastric digestion, while there was no large variation for PHM. In the intestinal phase, the evolution of the particle sizes was rather similar. Overall, Holder pasteurization impacted the proteolysis, lipolysis and disintegration of human milk. However, this impact was limited and the physiologic and metabolic consequences remain to be investigated.

1. Introduction

Human milk is the ideal food for infant nutrition, allowing optimal growth and providing several short and long-term health benefits (Horta, Bahl, Martines, & Victora, 2007; Le Huërou-Luron, Blat, & Boudry, 2010). Numerous human milk components, or their digestive products, are known to play beneficial functions such as the improvement of digestive maturity, the regulation of microbiota development and of the immune system (Donovan, 2006; Goldman, 2000; Labbok, Clark, & Goldman, 2004). When the mother's own human milk is unavailable or limited, pasteurized human milk from milk banks is preferentially administered instead of infant formulas, especially for vulnerable hospitalized neonates (Wight, 2001). In order to meet the infant nutrient requirements and also to respect the digestive immaturity of the neonates, this practice is more and more encouraged by public health policies and is officially recommended (American Academy of Pediatrics, 2012; Arslanoglu et al., 2013). Several countries (e.g. France, Germany, Australia, Brazil) have developed their own guidelines to implement and regulate human milk banks (Arnold, 2006; Vieira, Soares, Pimenta, Abranches, & Moreira, 2011). These regulations concern all the steps of handling, processing and storing of human milk, in order to assure its microbiological safety and nutritional quality. For sanitary reasons, the Holder pasteurization (62.5 °C, 30 min) of human milk is imposed by the worldwide guidelines. In addition, two cycles of freezing and thawing are applied during milk storage: first from milk expression to pasteurization and last from pasteurization to administration (Borgo, Coelho Araujo, Conceicao, Sabioni, & Mendonca, 2014). These physical treatments (pasteurization and

Abbreviations: AA, amino acid; BSSL, bile salt stimulated lipase; CLSM, confocal laser scanning microscopy; DG, diglycerides; FA, fatty acid; FFA, free fatty acid; FID, flame ionization detector; GC, gas chromatography; MG, monoglycerides; Mw, molecular weight; PHM, pasteurized human milk; RGE, rabbit gastric extract; RHM, raw human milk; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; sn, stereospecific numbering; SDS, sodium dodecyl sulfate; TG, triglycerides; TLC, thin layer chromatography.

* Corresponding author at: INRA-AGROCAMPUS Ouest, UMR 1253, STLO, 65 Rue de St Brieuc, 35042 Rennes, France.
E-mail address: Claire.Bourlieu@rennes.inra.fr (C. Bourlieu).

http://dx.doi.org/10.1016/j.foodres.2015.11.022
0963-9969/

freeze–thaw) may partially modify the composition and the structure of the human milk, which could modulate its digestive behavior.

The consequences of pasteurization on the human milk composition have been explored previously, though some points remain unclear. Heating the human milk degrades some immunological and nutritional components such as immunoglobulins (IgA, IgG and IgM) (Koenig, de Albuquerque Diniz, Barbosa, & Vaz, 2005), vitamins (folacin, vitamins C and B6) (Molto-Puigmarti, Permanyer, Castellote, & Lopez-Sabater, 2011; Vanzoerengrobben, Schrijver, Vandenberg, & Berger, 1987) and enzymes (lysozyme, lactoperoxidase, lipoprotein lipase, bile salt stimulated lipase) (Akinbi et al., 2010; Henderson, Fay, & Hamosh, 1998). Besides these alterations, heat treatments together with freeze–thaw cycles of human milk can induce the disruption of the milk fat globules (Vieira et al., 2011; Wardell, Hill, & Dsouza, 1981), protein denaturation and some aggregation of proteins on the fat globule membrane (Raikos, 2010; Ye, Singh, Taylor, & Anema, 2004).

In terms of physiological and nutritional impacts, pasteurization of human milk may decrease lipid absorption (Andersson, Savman, Blackberg, & Hernell, 2007; Thomaz, Goncalves, & Martinez, 1999; Williamson, Finucane, Ellis, & Gamsu, 1978) and may reduce the mean weight gain in neonates (Williamson et al., 1978). This may be explained by two main differences. First of all, the two endogenous lipases of human milk that facilitate lipids hydrolysis, lipoprotein lipase and bile salt stimulated lipase (BSSL), are completely inactivated during pasteurization (Blackberg & Hernell, 1981; Henderson et al., 1998). Secondly, some studies reported important losses of lipids and proteins during the nasogastric tube delivery of pasteurized human milk, which were attributed to increased adherence of the disrupted milk fat globules to flask and tube walls (Stocks, Davies, Allen, & Sewell, 1985; Vieira et al., 2011). Therefore, these two aspects may impact the energy and nutritional supply, and the ability of infant fed pasteurized human milk to digest lipids (Armand et al., 1996; Lindquist & Hernell, 2010).

Though the impact of bovine milk emulsion structure on its digestive behavior has been described *in vitro* (Bourlieu et al., 2015; Golding et al., 2011; Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009; Singh, Ye, & Horne, 2009) and *in vivo* (Armand et al., 1999; Golding et al., 2011), it remains unclear how the biochemical and structural consequences of Holder pasteurization impacts the digestion of the human milk. This understanding could represent a key step to optimize infant nutrition.

Despite its importance, several questions about infant digestion remain unsolved due to ethical and financial constraints of *in vivo* studies. In order to solve this problem, *in vitro* dynamic models are relevant tools which take into account the complexity of the physiological digestion and can mimic more realistically the digestive tract conditions and transient states of *in vivo* infant digestion (Ménard et al., 2014; Shani-Levi, Levi-Tal, & Lesmes, 2013; Zhang et al., 2014). Moreover, an exhaustive compilation of *in vivo* digestion data from the literature (Bourlieu et al., 2014) has allowed a pertinent adjustment of parameters of *in vitro* digestion systems.

The objective of this study was twofold: i) to compare the digestive behavior of raw *versus* pasteurized human milk having the same macronutrients composition; ii) to set up an *in vitro* dynamic model that simulates the gastrointestinal conditions of a term newborn. More specifically, this study aimed to determine the impact of Holder pasteurization on the kinetics of lipolysis, proteolysis and structural disintegration of human milk in the digestive tract of term newborns.

2. Materials and methods

2.1. Chemicals

Unless stated otherwise, chemicals were from commercial origin (Sigma-Aldrich, Saint-Quentin Fallavier, France).

2.2. Human milk samples

Mature human milk samples were obtained from a donor milk bank at the University Hospital Center in Rennes (France). Ethical approval for the study was granted by the Hospitals Ethics Committee (no. 13-12) and donors gave their informed written consent. Five lactating women collected their milk on average 11 weeks after term delivery (range: 6 to 14 weeks), following the recommendations of the milk bank. On average (±SD), donors were 32 ± 2 years old and gestational age was 39 ± 1.5 weeks. Milk was stored at −20 °C after collection.

Human milk was thawed in a 4 °C temperature controlled room over 16 h. Equal volumes of milk from each donor were pooled. Half of the pool, called raw human milk (RHM), went back to storage at −20 °C until digestion. The other half underwent Holder pasteurization (62.5 °C, 30 min) before going back to storage at −20 °C. The latter pool was called pasteurized human milk (PHM). Macronutrient composition of raw and pasteurized human milk was assessed by infrared spectrophotometry, using a Human Milk Analyzer (Miris AB, Uppsala, Sweden) previously validated (Billard et al., 2015). Raw and pasteurized human milk had the same macronutrients composition, which consisted of 26.8 ± 1.5 g/L of fat, 10.7 ± 0.5 g/L of proteins and 79.8 ± 1.5 g/L of carbohydrates.

2.3. In vitro dynamic digestion model simulating term newborn conditions

2.3.1. Digestion conditions

Gastrointestinal digestions of RHM and PHM were performed in an *in vitro* dynamic system (DIDGI®, INRA, Paris, France) using a digestion model for term newborns. This digestion system has been previously validated against *in vivo* digestion of infant formula in piglets (Ménard et al., 2014). Parameters for gastric and intestinal phases were based on an exhaustive literature review of *in vivo* digestive conditions of the infant's upper gastrointestinal tract (Bourlieu et al., 2014). The parameters were chosen to closely mimic the digestive conditions of term newborns fed human milk at the postnatal age of four weeks (Table 1). The *in vitro* dynamic system was controlled by the STORM® software, which allows regulating and monitoring the digestive parameters (Guillemin, Perret, Picque, Menard, & Cattenoz, 2010).

The pH acidification in the gastric compartment followed a linear regression obtained from *in vivo* data reported mainly for preterm newborns fed human milk or infant formula (Armand et al., 1996; Cavell, 1983; Mason, 1962; Mitchell, McClure, & Tubman, 2001; Omari & Davidson, 2003; Roman et al., 2007; Smith, Kaminsky, & D'Souza, 1986; Sondheimer, Clark, & Gervaise, 1985), since there is no abundant data from term newborns. The initial pH was set at the meal pH. Fasted conditions were based on 2 mL of simulated gastric fluid (SGF) adjusted at pH 2.7, as reported in fasted state for preterm newborn infants fed every 3 h (Armand et al., 1996; Mitchell et al., 2001; Omari & Davidson, 2003; Roman et al., 2007; Smith et al., 1986; Sondheimer et al., 1985). Concerning the intestinal compartment, the pH was constant and fixed at 6.2.

The transit time in the stomach and in the intestine followed an exponential pattern fitted by the mathematical model described by Elashoff, Reedy, and Meyer (1982). Gastric emptying of human milk was determined by fitting data from Billeaud, Guillet, and Sandler (1990), where 47 min corresponded to the estimated half-time of emptying ($t_{1/2}$) of term newborns fed human milk.

Gastric lipase and pepsin amounts were derived from data obtained in preterm newborns (Armand et al., 1996; Roman et al., 2007), assuming that their activities increased with body weight (which is linked to the digestive maturity). The mean body weight of term newborns at four weeks of age was considered 4.25 kg (WHO, 2006). A freeze-dried rabbit gastric extract (RGE) was employed in the gastric phase, since rabbit gastric lipase is reported to be a relevant option for *in vitro* gastric digestion studies, presenting 84% of homology with human gastric lipase (Moreau, Gargouri, Lecat, Junien, & Verger, 1988;

Please cite this article as: de Oliveira, S.C., et al., Holder pasteurization impacts the proteolysis, lipolysis and disintegration of human milk under *in vitro* dynamic term newborn digestion, *Food Research International* (2015), http://dx.doi.org/10.1016/j.foodres.2015.11.022