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Processing of whey modulates proliferative and immune functions in intestinal epithelial cells

Duc Ninh Nguyen,*† Per T. Sangild,* Yanqi Li,*‡ Stine B. Bering,* and Dereck E. W. Chatterton*†[1]
*Comparative Pediatrics and Nutrition, Department of Veterinary Clinical and Animal Sciences, and
†Department of Food Science, University of Copenhagen, Frederiksberg C 1870, Denmark
‡Arla Foods Ingredients Group P/S, Viby 8260, Denmark

ABSTRACT

Whey protein concentrate (WPC) is often subjected to heat treatment during industrial processing, resulting in protein denaturation and loss of protein bioactivity. We hypothesized that WPC samples subjected to different degrees of thermal processing are associated with different levels of bioactive proteins and effects on proliferation and immune response in intestinal epithelial cells (IEC). The results showed that low-heat-treated WPC had elevated levels of lactoferrin and transforming growth factor-β2 compared with that of standard WPC. The level of aggregates depended on the source of whey, with the lowest level being found in WPC derived from acid whey. Following acid activation, WPC from acid whey enhanced IEC proliferation compared with WPC from sweet whey or nonactivated WPC. Low-heat-treated WPC from acid whey induced greater secretion of IL-8 in IEC than either standard WPC from acid whey or low-heat-treated WPC from sweet whey. Following acid activation (to activate growth factors), low-heat-treated WPC from sweet whey induced higher IL-8 levels in IEC compared with standard WPC from sweet whey. In conclusion, higher levels of bioactive proteins in low-heat-treated WPC, especially from acid whey, may enhance proliferation and cytokine responses of IEC. These considerations could be important to maintain optimal bioactivity of infant formulas, including their maturational and immunological effects on the developing intestine.
Key words: immunomodulation, milk protein, whey protein concentrate

INTRODUCTION

Mother's milk is generally accepted as the optimal diet for newborn infants and it is known to protect the intestine from infection and inflammation (Kelley, 2012; Chatterton et al., 2013). Breastfeeding is not always possible and infant formula based on bovine milk is often fed to a proportion of infants, including highly sensitive hospitalized preterm infants (Merewood et al., 2006). Unfortunately, intake of milk formula increases the incidence of severe intestinal complications, including necrotizing enterocolitis (**NEC**), in preterm infants (Donovan, 2006; Boyd et al., 2007). The fact that formula feeding is inferior to breastfeeding in both preterm and term infants may be explained in part by a lower intake of bioactive proteins, resulting from the necessary processing technology applied during the manufacture of formula products.

Whey protein concentrate (**WPC**) is a common protein source used in infant formula manufacture (de Wit, 1998). It is commonly produced from sweet whey, which is a byproduct of casein coagulation by addition of chymosin to bovine milk (Li et al., 2013). However, due to the presence of lactic acid bacteria and calcium ions, industrial sweet whey is often pasteurized to reduce bacterial content, which may lead to a higher degree of electrostatic interaction between proteins due to the presence of divalent calcium ions (Havea et al., 2002). In contrast, caseins from bovine milk can be precipitated by adjusting the pH to 4.6, which results in a soluble fraction called acid whey. Both whey types can be further processed by pasteurization and various filtration steps to increase the protein content before spray-drying to obtain the final WPC product in a powdered form (Li et al., 2013). Whey protein concentrate consists of major whey proteins, including α-LA, β-LG, and BSA; several immunologically active proteins such as lactoferrin (**LF**), lactoperoxidase (**LP**), and immunoglobulins (Havea et al., 2002; Chatterton et al., 2004); and important growth factors, including transforming growth factor β (**TGF-β**) and insulin-like growth factor (Hering et al., 2011; Chatterton et al., 2013). Different TGF-β types in milk and dairy products are partly present in the latent forms, which are activated under acidic conditions or by di-

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[1]Corresponding author: dcha@food.ku.dk

gestive enzymes, thereby releasing the bioactive form. In addition, TGF-βs are stable under intestinal digestion and able to exert protective effects in the small intestine (Khalil, 1999; Nakamura et al., 2009; Nabil et al., 2011). Supplementation of LF and TGF-β2 to infant formulas has shown benefits in preventing intestinal inflammation in several animal models of newborn infants (Togawa et al., 2002; Maheshwari et al., 2011). In preterm infants, LF alone reduces late-onset sepsis, and the combination of LF and probiotics decreases both late-onset sepsis and incidence of NEC (Manzoni et al., 2009). Transforming growth factor-β2 suppresses proinflammatory cytokine production in macrophages of the preterm intestine, and oral administration of TGF-β2 protects mice from experimental NEC (Maheshwari et al., 2011). On the other hand, moderate levels of the proinflammatory cytokine IL-8 secreted by the immature intestine may be beneficial in terms of triggering intestinal repair and immune response toward the presence of bacteria (Nguyen et al., 2014a).

Reduced thermal processing of infant formula has been shown to enhance intestinal maturation and maintain moderate levels of the proinflammatory cytokine IL-8 during the first days of life in preterm pigs (Li et al., 2013), and these effects may be explained by the presence of higher amounts of bioactive proteins in the diet. To a variable degree, protein bioactivity is destroyed during thermal processing of bovine milk such as pasteurization and spray-drying, which facilitate protein denaturation and aggregation (Elfstrand et al., 2002; Anandharamakrishnan et al., 2008; Ewaschuk et al., 2011). Such effects have been reported to occur for immunoglobulins (Elfstrand et al., 2002), LF (Brisson et al., 2007), and TGF-β (Elfstrand et al., 2002; Akbache et al., 2011).

We hypothesized that gentle thermal processing of WPC preserves high levels of bioactive components, such as LF and TGF-β2, and that this enhances the proliferative and immunomodulatory effects of WPC in intestinal epithelial cells (**IEC**). We investigated the protein composition of WPC products prepared from 4 different types of thermal processing. The effects of WPC products on cell proliferation and proinflammatory cytokine secretion were investigated in porcine IEC to elucidate the role of different thermal processing on milk protein bioactivity.

MATERIALS AND METHODS

Materials and Reagents

Four WPC (Table 1) produced under different thermal processing conditions and whey sources were tested, such that the effects of more gentle thermal processing conditions could be compared with standard conditions. The total protein content in all WPC was analyzed by Eurofins Steins Laboratorium (Vejen, Denmark). Standard WPC from acid whey (**A-WPC**) and sweet whey (**S-WPC**) were produced at Arla Foods Ingredients (**AFI**, Viby, Denmark) using standard pasteurization (72–75°C, 15 s) and spray drying (inlet and outlet temperatures of 180–220°C and 83–90°C, respectively). Low-heat-treated WPC from acid whey (**LA-WPC**) was also produced at AFI using similar processing conditions as A-WPC but with fewer steps of pasteurization and low-temperature spray-drying (Table 1). Low-heat-treated WPC from sweet whey (**LS-WPC**) was produced in the dairy pilot plant of the University of Copenhagen (Denmark) under minimal processing conditions (temperature <40°C), and freeze-drying was applied instead of spray-drying before sterilization by γ-irradiation (at 50 Gy).

Advanced Dulbecco's modified Eagle medium (**DMEM**), reagents for cell culture, and polyvinylidene

Table 1. Whey protein concentrate (WPC) powders used in this study and their specifications

Material	Abbreviation	Whey source	Protein content (% wt/wt)	Heat treatment characteristics[1]
Bioactive WPC	LA-WPC	Acid whey	61	Standard milk pasteurization; no pasteurization of acid whey, low inlet (160°C) and outlet temperature (78°C) during spray-drying.
Low-heat-treated WPC	LS-WPC	Sweet whey	80	No pasteurization of milk or sweet whey but γ-irradiated (50 Gy) after freeze-drying.
Lacprodan DI-8090[2]	S-WPC	Sweet whey	78	Standard pasteurization of milk and whey; standard spray-drying.
Lacprodan DI-8095[2]	A-WPC	Acid whey	78	Standard pasteurization of milk and whey; standard spray-drying.

[1]Standard pasteurization = 72–75°C for 15 s; standard spray-drying = an inlet temperature of between 180 and 220°C and an outlet temperature of between 83 and 90°C.

[2]Arla Foods Ingredients (Viby, Denmark).