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Intestinal health benefits of bovine whey proteins after simulated gastrointestinal digestion

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ABSTRACT

Bovine whey proteins are widely incorporated in foods for their nutritional, health promoting and functional value. However, whey proteins are readily digested in the upper gut. The objective of this study was to determine the fate and bioactivity of bovine whey proteins post simulated gastrointestinal digestion (GID). Our results demonstrated that β-lactoglobulin and α-lactalbumin post GID protect human intestinal cells from free radical formation. Post GID, lactoferrin significantly increased the amount of the intracellular antioxidant enzymes superoxide dismutase 1, 2 and thioredoxin. In addition, all whey samples post GID inhibited the activity of the dipeptidyl peptidase IV. However, the conditions of the gut destroyed the ability of whey proteins to act as glucagon-like peptide-1 secretagogues. The peptide profiles of GID whey protein isolate, β-lactoglobulin, α-lactalbumin, bovine serum albumin and lactoferrin revealed several peptides with bioactive potential.

1. Introduction

Bovine whey proteins are rich in essential amino acids, have a high nutritional value and contain bioactive peptides encrypted in their sequences (Corrochano, Buckin, Kelly, & Giblin, 2018). It has been suggested that these bioactive peptides have several health benefits including antidiabetic (Nongonierma & FitzGerald, 2013), weight management (Chaudhari et al., 2017) and reduction of cellular oxidative stress (Corrochano et al., 2018). Whey peptides can also give rise to food intolerances particularly notable in the infant gut (Brill, 2008). The protein component of bovine whey is composed of β-lactoglobulin (β-LG, 50–60%), α-lactalbumin (α-LA, 15–25%), bovine serum albumin (BSA, 6%), immunoglobulins (10%) and lactoferrin (LF, < 3%) (Corrochano et al., 2018). Commercial whey products differ in their protein content from 95% (whey protein isolate, WPI) to 34% (whey protein concentrate) (Corrochano et al., 2018) and are commonly used as food ingredients especially in the sports nutrition sector. Once ingested, intestinal cells are the first point of contact and where whey proteins are most likely to exert their greatest effect.

The mechanism by which whey proteins may have a positive effect on ameliorating type 2 diabetes or aiding weight management is in their ability to increase the enteroendocrine incretin hormone glucagon-like peptide-1 (GLP-1) (Geraedts, Troost, Fischer, Edens, & Saris, 2011) and inhibit the ubiquitous dipeptidyl peptidase IV (DPP-IV) (Lacroix & Li-Chan, 2014). GLP-1 is produced by L-cells in the gut and functions to stimulate insulin production, increase satiety, influence appetite and regulate gastric emptying and ileal brake. There is conflicting data on whether consumption of whey can promote postprandial GLP-1 levels. Whey protein supplementation (25% energy intake) significantly increased postprandial plasma GLP-1 (425 ± 135 pmol/L h) compared to casein supplementation (161 ± 90 pmol/L h) in 25 healthy individuals (Veldhorst et al., 2009).

Abbreviations: α-LA, α-lactalbumin; β-LG, β-lactoglobulin; ABAP, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; AMC, H-Gly-Pro-7-amino-4-methylcoumarin; BSA, bovine serum albumin; CAT, catalase; DCFH-DA, 2′,7′-dichlorofluorescin di-acetate; DMEM, Dulbecco's modified Eagle medium; DPP-IV, dipeptidyl peptidase IV; FBS, foetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GID, gastrointestinal digestion; GLP-1, glucagon-like peptide-1; GPx, glutathione peroxidase; HBSS, Hank's Balanced Salt Solution; IC_{50}, half maximal inhibitory concentration; LF, lactoferrin; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PBS, phosphate buffered saline; PRX2, peroxiredoxin; RFU, relative fluorescence units; SOD, superoxide dismutase; TRX1, thioredoxin; USP, United States pharmacopeia; WPI, whey protein isolate

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In contrast, 14 type 2 diabetic subjects who consumed 36.4 g whey protein per day did not show increases in postprandial serum GLP-1 levels compared to those who consumed a reference diet without whey (Frid, Nilsson, Holst, & Björck, 2005). Certainly, 10 mg/mL of intact whey protein concentrate can stimulate GLP-1 secretion 1.3 fold from the enteroendocrine cells line STC-1 (Power-Grant et al., 2015). Whether whey proteins are pre hydrolysed or intact also appears to influence GLP-1 levels in vitro and in vivo (Gillespie, Calderwood, Hobson, & Green, 2015).

The enzyme DPP-IV is a ubiquitous protease, which is produced and secreted by intestinal cells (Gu et al., 2008) and inactivates GLP-1 by cleavage of N-terminal proline and alanine (Gu et al., 2008). To prolong the antidiabetogenic effect of GLP-1 and reduce diabetes progression, the treatment with DPP-IV inhibitors is being used as antidiabetic therapy (Ahren et al., 2004). Power-Grant et al. (2015) showed that 50% inhibition of the DPP-IV enzyme can be achieved with commercial whey hydrolysates at concentrations 1.5 and 1.1 mg/mL.

Intestinal cells are routinely exposed to exogenous molecules, which can trigger the formation of free radicals and damage the intestinal epithelium and mucus (Bhattacharyya, Chattopadhyay, Mitra, & Crowe, 2014). Bovine whey products and their hydrolysates have demonstrated antioxidant activity in vitro by chelating metals (Gad et al., 2011; Peng, Kong, Xia, & Liu, 2010), decreasing lipid peroxidation (de Castro & Sato, 2014), reducing ferric ion (Lin, Tian, Li, Cao, & Jiang, 2012), scavenging radicals (peroxyls (Adjonu, Doran, Torley, & Agboola, 2013), hydroxyls (Kerasioti et al., 2014) and superoxides (Zhang et al., 2012)) and also neutralizing synthetic radicals (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Torkova et al., 2016) and 1,1-diphenyl-2-picrylhydrazyl (Mohammadian & Madadlou, 2016)). Whey products (0.02–2.00 mg/mL) can protect against cellular oxidation and boost intracellular antioxidant markers such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in lung fibroblasts, hepatocytes and endothelial cells (Kong, Peng, Xiong, & Zhao, 2012; O'Keeffe & FitzGerald, 2014; Pyo, Yang, Chun, Oh, & Lee, 2016). In the human epithelial colorectal adenocarcinoma cell line, Caco-2, Piccolomini, Iskandar, Lands, & Kubow (2012) reported that a 23 h treatment with digested WPI (0–2 mg/mL) protected H_2O_2-stressed cells against free radical formation and increased the ferric reducing antioxidant power of cellular supernatants.

However, when considering the physiological benefits of whey, it is important to be cognizant that whey proteins do not reach the intestine in their intact form. As they transit the conditions of the upper gut, they will be extensively hydrolysed with a digestibility score of 1.09 (Rutherfurd, Fanning, Miller, & Moughan, 2015).

We therefore pose the question of what happens to the bioactivities of commercial WPI and individual whey proteins after simulated upper gastrointestinal digestion (GID).

2. Materials and methods

2.1. Materials

Bovine WPI from pasteurized milk (≥72 °C, 26 s) used in cheese manufacturing (Isolac, 91.4% protein content) was purchased from Carbery Food Ingredients (Cork, Ireland). The proteins β-LG (92.1% β-LG content) and α-LA (93% α-LA content) were obtained from Davisco Foods International, Inc. (Minnesota, USA). The BSA (98% protein content) was purchased from Sigma-Aldrich (Dublin, Ireland) and LF (Bioferrin 2000, which contains 95% of LF and 0.02% of iron) was donated by Glanbia Nutritionals, Inc. (Wisconsin, USA). Porcine pepsin (4400 U/mg), porcine pancreatin (8 × USP, United States Pharmacopeia) and bovine bile extract were purchased from Sigma-Aldrich (Dublin, Ireland). DPP-IV Drug discovery kit was purchased from Enzo Life Sciences Ltd. (Exeter, UK). Vivaspin Turbo 4 (10 kDa) was purchased from Sartorius Stedim Ltd. (Dublin, Ireland). The Caco-2 cell line was purchased from the European Collection of Cell Cultures (collection reference: ECACC 86010202). The murine enteroendocrine STC-1 cell line (ATCC SD5482) and the human colon adenocarcinoma HT-29 cell line (ATCC HTB-38) were obtained from the American Type Culture Collection. Tissue culture plastic material was from Sarstedt Ltd. (Wexford, Ireland). CellTiter 96 AQueus One Solution reagent was from Promega (MyBio, Kilkenny, Ireland). The mouse metabolic magnetic bead panel for active GLP-1 (#MMHMAG-44K) and the human oxidative stress kit (#H0XSTMAG-18K) were obtained from Merck Millipore (Dublin, Ireland). Halt Protease and Phosphatase inhibitor (100×) and Pierce BCA Protein Assay Kit were purchased from Thermo Fisher Scientific (Dublin, Ireland). RNeasy RNA extraction kit and on-column DNase digestion kit were obtained from Qiagen (Manchester, UK). Tetro cDNA synthesis kit was from Bioline supplied by Medical Supply Company Ltd. (Dublin, Ireland). Oligonucleotide primers were synthesized by Integrated DNA Technologies (Leuven, Belgium). LightCycler 480 SYBR Green I Master was from Roche Diagnostics Ltd. (West Sussex, UK). All other reagents were purchased from Sigma Aldrich (Dublin, Ireland).

2.2. Static simulated upper gastrointestinal digestion

Protein powders (WPI, β-LG, α-LA, BSA and LF) were reconstituted in water, exhibited high solubility, and were subjected to a simulated in vitro GID following Minekus et al. (2014) method. Oral phase was not performed on these liquid formulations. Briefly, protein powder was reconstituted following supplier recommendations to the consumer i.e. 1 g in 5 mL Milli-Q H_2O. This solution was then mixed with 2 mL of simulated gastric fluid, which contained KCl (6.9 mM), KH_2PO_4 (0.9 mM), $NaHCO_3$ (25 mM), NaCl (47.2 mM), $MgCl_2$ (0.1 mM), $(NH_4)_2CO_3$ (0.5 mM) and $CaCl_2$ (0.15 mM). A stock solution (8000 U/mL) of porcine pepsin was prepared in simulated gastric fluid and 2.5 mL was then added to the test samples to reach 2000 U/mL. The gastric phase at pH 3 was performed for 2 h at 37 °C with continuous shaking. Pepsin was inactivated by increasing the pH to 6.5 with NaOH (1 M). For the intestinal phase, bile extract and pancreatin were dissolved in simulated intestinal fluid and added to the gastric chyme (concentrations 10 mM and 100 U/mL, respectively), pH was increased to 7.0 and the volume adjusted to 20 mL with Milli-Q H_2O. The mixture was incubated with continuous shaking at 37 °C for 2 h. The protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (final concentration: 1 mM) was then added to stop the digestion. Samples were aliquoted, snap frozen in liquid nitrogen and stored at −80 °C prior cell exposure. Simulated GID of powder proteins was performed at least in duplicate. Prior experiments, GID samples were diluted to assay concentrations. Before cell treatment, digested samples were ultrafiltered in Vivaspin Turbo 4 tubes (10 kDa cut-off, 5000*g*, 20 min, 4 °C) to remove trypsin (23.3 kDa) and therefore avoid cell detachment during exposure.

A GID control with gut enzymes, electrolytes and bile salts without whey proteins was also performed.

2.3. Ultra-performance liquid chromatography/electrospray ionisation-high resolution tandem mass spectrometry (UPLC/ESI-HR-MS/MS)

Samples were separated by UPLC, and the peptides were identified by HR-MS(/MS). The UPLC/ESI-HR-MS(/MS) analyses were carried by coupling an Acquity UPLC module (Waters, Milford, MA, USA) to a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA). The samples were separated on an Aeris PEPTIDE XB–C18 column (150 × 2.1 mm, 1.7 µm, 100 Å) equipped with a SecurityGuard ULTRA cartridge (Phenomenex, Torrance, CA, USA) kept at 50 °C, using 0.1% formic acid (FA) in MilliQ-treated water (solvent A) and 0.1% FA in acetonitrile (solvent B). The linear elution gradient expressed as the solvent B proportion was as follows: 0 min, 2%; 0–5 min, 2%; 5–30 min, 2–35%; 30–35 min, 35–95%; 35–36 min, 95%; 36–37 min, 95–2% (run-to-run time, 47 min). Peptides were