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Satiating effect of a sodium caseinate hydrolysate and its fate in the upper gastrointestinal tract

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ABSTRACT

Previously we identified a sodium caseinate (NaCas) hydrolysate, LFC25, which significantly increased secretion of satiety hormone glucagon-like peptide-1 (GLP-1) *in vitro* and reduced food intake in mice when administered intraperitoneally. This study investigates whether LFC25, administered orally, promotes GLP-1 secretion and/or reduces food intake *in vivo*. Over an 8-hour period, mice received LFC25 by oral gavage had similar food intake to mice received NaCas. Postprandial blood glucose, plasma active GLP-1, amino acids, insulin and food consumed at the next meal were not significantly different in pigs that consumed LFC25 compared to NaCas in a dairy beverage, at a dosage relevant for human consumption. Simulated *in vitro* gastro-duodenal digestion of LFC25 revealed a significant reduction in bioactivity. In contrast, the harsh conditions of the upper gut appear to functionalize intact NaCas as a GLP-1 secretagogue. In conclusion, LFC25 will need enteric protection to be used as a food ingredient for satiety.

1. Introduction

The intestinal epithelium produces a variety of satiety-regulating hormones that not only influence intestinal function but also play a wider role in controlling food intake and metabolism (Tolhurst, Reimann, & Gribble, 2012). Prominent amongst these is glucagon-like peptide-1 (GLP-1), which once activated, functions to delay gastric emptying, reduce gut mobility, enhance glucose-dependent insulin secretion and reduce food intake *via* the vagus nerve, hindbrain and hypothalamus signalling pathways (McDermott et al., 2016). GLP-1 is produced and secreted by L cells in response to food (Tolhurst et al., 2012). L cells are scattered in the gut epithelium and account for around 1% of total cell number, corresponding to 30×10^6 cells in human jejunum, 23×10^7 in the ileum and 21×10^7 in the colon (Sjölund, Sanden, Håkanson, & Sundler, 1983). Analogues of GLP-1, including liraglutide (Victoza®) and exenatide (Byetta®), have been successfully used in the treatment of type 2 diabetes mellitus and have demonstrated good potential to treat obesity (Buse et al., 2009; DeFronzo et al., 2005). Food components that increase GLP-1 secretion or deliver GLP-1 analogues (Ryan et al., 2017) could potentially be used as ingredients in food snacks to delay hunger and thereby aid weight management over time.

A number of intact proteins, protein hydrolysates and hydrolysate fractions from a wide variety of food sources have been found to induce GLP-1 signalling *in vitro* (Cudennec, Fouchereau-Peron, Ferry, Duclos, & Ravallec, 2012; Geraedts, Troost, Fischer, Edens, & Saris, 2011; R. A. Reimer, 2006) but to date little is known of their efficacy during gut transit. Dairy proteins have received considerable attention as satiety hormone modulators. This in part stems from the correlation between increased consumption of dairy products and the positive effects on satiety, body weight and blood glucose levels (Anderson, Luhovyy, Akhavan, & Panahi, 2011). In addition, bovine whey proteins, especially α-lactalbumin, have been shown to increase GLP-1 production and secretion from the murine enteroendocrine cell line, STC-1 (Gillespie, Calderwood, Hobson, & Green, 2015). Bovine β- and α-casein also significantly increased GLP-1 secretion from STC-1 cells

Abbreviations: AA, amino acid; ANOVA, one-way analysis of variance; CCK, cholecystokinin; DPP-IV, dipeptidyl peptidase-IV; GI, gastrointestinal; GLP-1, glucagon-like-peptide-1; I.p., intra-peritoneal; NaCas, sodium caseinate; SMP, skim milk powder; PICC, peripherally inserted central catheter; VAC, venous access cannula

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(Gillespie & Green, 2016; Kondrashina, Papkovsky, & Giblin, 2017; Rafferty et al., 2011). Sodium caseinate (NaCas) is produced commercially in large quantities and used as a common ingredient in dairy products. NaCas-derived hydrolysates produced by the action of chymosin have been patented for their ability to dose-dependently stimulate GLP-1 secretion from the human colorectal cell line NCI-H716 (R. Reimer, Darimont-Nicolau, Mace, Gremlich, & Neeser, 2009). Previously, we have reported the identification of a NaCas hydrolysate produced by chymosin, LFC25, from a screening effort of 765 individual unique dairy hydrolysates (O'Halloran et al., 2018). LFC25 significantly increased total GLP-1 secretion from STC-1 cells compared to unhydrolysed NaCas and reduced cumulative food intake in adult male mice when administered by intra-peritoneal (I.p.) injection (O'Halloran et al., 2018). In a subsequent human trial, delivery of LFC25 (15 g) by naso-jejunal tube directly into the jejunum of 13 participants resulted in plasma total GLP-1 levels of 36.4 ± 3.8 pg/mL at 25 min which was significantly increased from baseline and, surprisingly, could not be further elevated upon consumption of an *ad-libitum* meal of 459 ± 49 kcal (Giblin et al., 2016; le Roux, Björnfot, Fändriks, & Docherty, 2016).

Based on its digestibility and adsorption rates, casein is classified as a “slow” protein (Boirie et al., 1997), with plasma amino acid (AA) concentrations reaching their plateau within 1 h after casein consumption and remaining at these levels for up to 2–3 h (Boutrou et al., 2013). Several casein-derived bioactive peptides have been identified in the gastric, duodenal and proximal jejunal phases of digestion using *in vitro* models and nasogastric sampling, however, none of these have been associated with GLP-1 bioactivity (Boutrou et al., 2013; Boutrou, Henry, & Sanchez-Rivera, 2015; Kopf-Bolanz et al., 2014).

The objective of this study was to investigate whether LFC25 alters food intake when administered to mice orally. Successful scale-up of LFC25 to 10 L without loss of bioactivity (O'Halloran et al., 2018) allowed for a pig acute feeding study with a LFC25 dairy beverage. Postprandial active GLP-1 levels and associated blood markers were measured at various time points to ascertain whether LFC25 modulates circulating GLP-1 compared to its macronutrient and isocaloric control, NaCas, at a dosage and within a food matrix relevant for human consumption. A simulated duodenal digestion protocol was also employed to track LFC25 bioactivity as it transited the upper gut.

2. Materials and methods

2.1. Materials

Murine enteroendocrine cell line STC-1 was supplied by American Tissue Culture Collection (ATCC, SD5482, USA). Halt Protease and Phosphatase Inhibitor was from Thermo Fisher Scientific, USA. Dipeptidyl peptidase-IV (DPP-IV) inhibitor was purchased from Millipore Ltd., UK. GLP-1 metabolic assay kit (#K150JVC-2) and twoplex MSD® human assay for active GLP-1 and insulin were supplied by Meso Scale Discovery, USA. Detomidine, Butorphanol, Ketamine, isotonic saline and heparin solution were purchased from Ark Animal Care, Ireland. Peripherally inserted central catheters (PICC, 3FR 55 cm) were purchased from Arrow International Inc., USA. Vacutainers containing 18.0 mg K_2EDTA were supplied by BD Biosciences, UK. NaCas (protein 90%, moisture 5% , ash (825 °C) 4%, Fat 0.8%, Lactose 0.2%, pH(10% solution at 20 °C) 6.8, scorched particles (ADPI) Disc A) and skim milk powder (SMP) were purchased from Kerry Group, Ireland. All other reagents were from Sigma Aldrich, Ireland. Standard lab chow (2018S Teklad Global 18% Protein Rodent Diet) was from Harlan, UK. Plastic ware was from Sarstedt, Ireland.

2.2. Preparation of LFC25

Briefly, to produce LFC25 at pilot scale, 100 kg of NaCas was dispersed in 900 L of reverse-osmosis water and hydrolysed for 220 min at 37 °C using chymosin (Trade name: Maxiren 180, Carbon Group, Ireland; enzymatic activity 180 U/mL) at an enzyme:substrate ratio of 0.05:1 (O'Halloran et al., 2018). Chymosin inactivation was then achieved by heating to 85 °C for 30 s. The heated hydrolysate was withdrawn, evaporated (to 40% total solids) on a Tetra Scheffers falling-film single-stage evaporator (Tetra Pak, Gorredijk, The Netherlands) and spray-dried using a pilot-scale Anhydro Lab 3 spray-drier (SPX Flow Technology A/S, Soeborg, Denmark). Inlet and outlet temperatures during spray drying ranged from 185 to 190 and 85 to 90 °C, respectively. The final product contained 90% protein, 5% moisture and ash. The unhydrolysed NaCas control was prepared by the same protocol, however, chymosin was heat inactivated prior to use.

2.3. Simulated in vitro gastro-duodenal digestion

In vitro gastric and duodenal digestions were performed according to the method described by Dupont et al. (2010) with some modifications (Dupont et al., 2010). Briefly, NaCas and LFC25 were dissolved at a concentration of 10 mg/mL in simulated gastric fluid (0.15 M NaCl; pH = 2.5) and the pH was adjusted to 2.5 with 0.5 M HCl solution. A Krebs control was prepared with identical chemicals, where sample was substituted with the same volume of Krebs buffer, and underwent the same conditions as the samples. Porcine gastric mucosa pepsin was added to give 182 U per mg of NaCas or LFC25 and the mixture was incubated for 1 h at 37 °C with mixing. During the gastric digestion, aliquots were removed at times T0, T1, T2, T5, T10, T20, T40 and T60 minutes and the reaction was stopped by adding 200 µL of 0.1 M NaOH to every 5 mL aliquot. Gastric proteolysis was terminated by adjusting the pH to 7.0 using 0.5 M NaOH. For the duodenal phase, pH was adjusted to 6.5, followed by adding Bis-Tris buffer (26.1 mM, pH 6.5) and bile salt mixture, containing equimolar quantities (4 mM) of sodium glycodeoxycholate and sodium taurocholate. After a 5-min incubation period, trypsin and α-chymotrypsin were added to the intestinal mix at ratios of 0.4 U and 34.5 U per mg of sample, respectively. Aliquots were taken at T0, T1, T2, T5, T10, T15 and T30 minutes of intestinal digestion. The process was terminated by the addition of 100 µL of 5 g/L Bowman–Birk trypsin–chymotrypsin inhibitor to every 5 mL of digesta. In all aliquots, pH was adjusted to 7.5 and they were stored at -20 °C until further analysis.

2.4. In vitro GLP-1 secretion

STC-1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L glucose and L-glutamine (Sigma Aldrich, Ireland). Media was supplemented with 10% foetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were passaged upon reaching confluency and all cells used in this study were between passages 15–25. STC-1 cells were seeded into 6-well plates at a density of 1.5×10^6 cells/well and incubated at 37 °C, 5% CO_2, for 18 h prior to test sample addition. Cell monolayers in each well were washed with 1 mL of Krebs-1% BSA solution and pre-incubated for 1 h in the same solution. Krebs-1% BSA was aspirated off and replaced with 1 mL of Krebs-1% BSA buffer alone (vehicle control), LFC25 or NaCas (10 mg/mL prepared in Krebs-1% BSA). Plates were incubated for 4 h at 37 °C, 5%CO_2. Test samples were assayed in duplicate on separate days. Following the 4 h incubation period, 10 µL of 100 × Halt Protease and Phosphatase Inhibitor (Thermo Fisher Scientific, USA) was added to each well to inactivate endogenous DPP-IV activity. Cellular supernatants were collected by aspiration and stored at −80 °C prior to analysis. Total GLP-1 was quantified with MSD murine total GLP-1 assay kit (K150JVC-2) as per the manufactures instructions, using a Sector Imager 2400 instrument (Meso Scale Discovery, MD, USA).

2.5 Mouse studies

Experiments were conducted in accordance with European