International Journal of Food Science +Technology

Original article

Effect of food ingredients on glucagon-like peptide-1 secretion in STC-1 and HuTu-80 cells

Yuan Yue,[1†] Sidsel Madsen,[1†] Mette Skou Hedemann,[1] Knud Erik Bach Knudsen,[1] Flemming Vang Sparsø,[2] Anne Laursen,[2] Henrik Max Jensen,[2] Tine Ahrendt Knudsen[2] & Stig Purup[1*]

1 Department of Animal Science, Aarhus University, Blichers Allé 20, Foulum, Tjele DK-8830,Denmark
2 DuPont Nutrition Biosciences Aps, Edwin Rahrs Vej 38, Brabrand DK-8220,Denmark

(Received 22 January 2019; Accepted in revised form 15 May 2019)

Summary This study aims at identifying food ingredients that have potential to enhance satiety and therefore potentially can be used for preventing and treating obesity. To do so, effects of thirteen food ingredients on the secretion of gut satiety hormone glucagon-like peptide-1 (GLP-1) in enteroendocrine STC-1 and HuTu-80 cells were investigated. First, the effects of food ingredients on cell viability were investigated. This was done to determine if the individual ingredient affected cell viability, and in case so, to determine the ingredient concentration below which, the cell viability was unaffected. Enzyme-treated whey protein, palmitic acid, stearic acid, green tea extract, and hesperidin did not affect GLP-1 secretion. Functional soy protein, fractionated functional soy protein, acetylated monoglyceride, N-oleoylethanolamine, epigallocatechin-3-gallate, hesperetin, rosemary extract, and kale extract increased GLP-1 secretion in at least one cell line. However, the effects of the eight potent ingredients on GLP-1 secretion need to be further investigated *in vivo*.

Keywords Enteroendocrine cells, food ingredients, glucagon-like peptide-1.

Introduction

Obesity is one of the major health problems with high prevalence worldwide. Yet, the current pharmacological anti-obesity treatment has not been satisfying due to poor efficacy, high cost and considerable side effects (Srivastava & Apovian, 2018). During the last decade, many natural bioactive components in food are found to have anti-obesity properties and this possibility of controlling body weight by food ingredients have been highlighted (Yun, 2010). Previous studies indicate that food components are able to interact with enteroendocrine cells in the gastrointestinal tract and stimulate gut hormone glucagon-like peptide-1 (GLP-1) secretion, and thereby enhance satiety and reduce food intake (Tian & Jin, 2016; Steinert *et al.*, 2017).

GLP-1 is one of the most important satiety hormones, and is secreted by enteroendocrine L-cells that are distributed throughout the small and large intestine (Steinert *et al.*, 2017). However, because of the rapid degradation of active GLP-1, its secretion is often measured by total GLP-1, which is the sum of active and inactive GLP-1 (Steinert *et al.*, 2017). *In vivo*, GLP-1 regulates glucose homeostasis by stimulating insulin secretion, inhibiting glucagon secretion, slowing gastric emptying, which will in turn enhance satiety, decrease food intake and lead to subsequent weight loss (Steinert *et al.*, 2017). Previous studies suggest that impaired GLP-1 function is a crucial contributor in obesity, and synthetic GLP-1 agonists are already used in obesity therapy (Steinert *et al.*, 2017). GLP-1 secretion can be stimulated by many nutrients and bioactive components like carbohydrates, proteins (Steinert *et al.*, 2017), fatty acids and polyphenols from plants (Tian & Jin, 2016).

The research presented here is part of a larger project focusing on identifying novel satiety hormone-stimulating bioactives/food ingredients and development of an advanced encapsulation system, enabling enteric protection and targeted intestinal release of these (Innovation Fund Denmark, 2019). The aim of this study was to identify which bioactive/food ingredient to encapsulate. This evaluation was done by investigating various food ingredients effect on the secretion of satiety hormone GLP-1 *in vitro*. Unfortunately, cultures of primary enteroendocrine cells have not been established yet (Kuhre *et al.*, 2016), thus this study

*Correspondent: E-mail: stig.purup@anis.au.dk
†Yuan Yue and Sidsel Madsen contributed equally as first authors.

doi:10.1111/ijfs.14247

was performed on two enteroendocrine cell lines, STC-1 and HuTu-80. STC-1 is originated from mouse duodenal tumours, while HuTu-80 is originated from a human duodenal adenocarcinoma. Both STC-1 and HuTu-80 cells are able to secrete GLP-1 upon nutrient stimulation, and are often used as cell models for studying GLP-1 secretion (Geraedts *et al*., 2011; Pham *et al*., 2016). However, GLP-1 secretion is not always similar among species (Kuhre *et al*., 2014) and cell lines (Kuhre *et al*., 2016). To ensure that the identified potential ingredients can be used in human functional food products, both murine STC-1 and human HuTu-80 cells were used for screening GLP-1 secretion.

Materials and methods

Materials

The non-cancerous human small intestinal epithelial cell line FHs 74 Int. was purchased from ATCC (LGC Standards AB, Borås, Sweden; Purup *et al*., 2007). The mouse enteroendocrine cell line STC-1 was a kind gift from Prof. Douglas Hanahan (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland). The human enteroendocrine cell line HuTu-80 was purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany). Krebs-Henseleit buffer (KHB) was purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.) and prepared according to manufacturer's instructions. The food ingredients tested in this study were chosen based on their potential ability to stimulate GLP-1 secretion according to the literature (Hirasawa *et al*., 2005; Song *et al*., 2015; Power-Grant *et al*., 2015). All ingredients were provided by Dupont Nutrition Biosciences ApS (Brabrand, Denmark). However, the raw material for the kale extract (KE) was provided by Green Gourmet A/S (Randers, Denmark). Among the ingredients, functional soy protein (FSP; functionalised to increased water solubility and lower the molecular weight), fractionated functional soy protein (FFSP), enzyme-treated whey protein (ETWP), green tea extract (GTE), epigallocatechin-3-gallate (EGCG), and KE were water soluble and the solutions were freshly made prior to each experiment. Stock solutions in DMSO were prepared for palmitic acid (PA, 2 mg mL^{-1}), stearic acid (SA, 2 mg mL^{-1}), hesperidin (HD, 20 mg mL^{-1}), hesperetin (HT, 10 mg mL^{-1}), and rosemary extract (RE, 100 mg mL^{-1}) and were kept at −20 °C protected from light. N-oleoylethanolamine (N-OEA) was dissolved in 96% ethanol (0.32 mg mL^{-1}) and kept at −20 °C. Acetylated monoglyceride (AMG) was dispersed evenly into cell culture media or KHB (v/v 0.5%) using an 'Ultra-turrax' disperser (IKA, Staufen, Germany) immediately before each assay.

Cell culture

FHs 74 Int. cells were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco 42430-025, Thermo Fisher Scientific, MA, U.S.A.), supplemented with 10% fetal bovine serum (FBS, Gibco), 6 μg mL^{-1} bovine insulin (Sigma–Aldrich), 30 ng mL^{-1} human recombinant EGF (Austral Biologicals, San Ramon, CA, U.S.A.), 8% NCTC-135 medium (Gibco 41350-026), 1% nonessential amino acids (Gibco), 0.4 mM sodium pyruvate (Gibco), 0.1 mg mL^{-1} oxaloacetate (Sigma–Aldrich), 2 mM Glutamax (Gibco), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Sigma–Aldrich; Purup *et al*., 2009). STC-1 cells were cultured in DMEM (Gibco 11960-044) supplemented with 10% FBS, 4 mM Glutamax (Gibco), 10 mM HEPES (Gibco), 0.5 mM sodium pyruvate (Gibco), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Sigma–Aldrich). HuTu-80 cells were cultured in DMEM (Gibco 11960-044) supplemented with 10% FBS, 4 mM Glutamax (Gibco), 10 mM HEPES buffer solution (Gibco), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Sigma–Aldrich). All cells were maintained in 5% CO_2 humidified atmosphere at 37 °C. FHs 74 Int. cells between passages 6-11, STC-1 cells between passages 42-51, and HuTu-80 cells between passages 5-16 were used for all assays.

Cell viability assays

To ensure the validity of the results, ingredient concentrations used in GLP-1 secretion assays should not lead to dramatic decrease of cell viability. To guarantee that cell viability was maintained in the GLP-1 secretion assay (2 h), cell viability assays were performed first on STC-1 and HuTu-80 cells with a longer treatment period (4 h). However, STC-1 and HuTu-80 are cancer cell lines, thus may respond differently than non-cancerous cells. To ensure that the identified potential ingredients can be used in human functional food products, the concentrations used in GLP-1 secretion assays should not affect the viability of non-cancerous intestinal cells. Thus, cell viability was also evaluated in a non-cancerous human intestinal cell line, FHs 74 Int. for a treatment period of 12 h.

FHs 74 Int., STC-1 and HuTu-80 cells were seeded in 96-well plates at a density of 1000, 13 000 and 3000 cells/well, respectively. At 80–90% confluence (4 day for FHs 74 Int. and HuTu-80 cells, 5 day for STC-1 cells), cells were washed twice with phosphate buffered saline (PBS, Gibco), followed by addition of different ingredient dilutions. The concentrations used in cell viability assays were determined based on the solubility of the food ingredients in aqueous solution. For FHs 74 Int. cells, ingredients were diluted in cell culture medium containing 0.3% FBS and low

concentrations of insulin (10 ng mL^{-1}) and EGF (0.5 ng mL^{-1}) to simulate responses of non-cancerous intestinal cells in vivo, and cells were incubated at 37 °C for 12 h. For STC-1 and HuTu-80 cells, dilutions were made in KHB, and cells were incubated for 4 h. For all three cell lines, cell culture medium with 10% FBS was used as a positive control, and medium or KHB alone was used as a negative control (without solvent) and another negative control that included vehicle (either ethanol or DMSO) at the same concentration as in the test solutions in each assay. After incubation, cell viability was accessed with 2 h incubation of alamarBlue® cell viability reagent (Invitrogen, Thermo Fisher Scientific, MA, U.S.A.) as previously described (Purup *et al*., 2009; Yue *et al*., 2017). The relative cell viability (%) was expressed as a percentage relative to the corresponding vehicle control, and cells without treatments (0 μg mL^{-1}, medium or KHB alone) were considered as 100%.

GLP-1 secretion assays

GLP-1 secretion in response to the tested food ingredients were measured in STC-1 and HuTu-80 cells (Geraedts *et al*., 2011). STC-1 cells were grown in 12-well plates (1.2×10^5 cells/well) and HuTu-80 cells were grown in 48-well plates (1×10^4 cells/well) until confluence. Thereafter, the cells were washed twice with KHB and exposed to vehicle or ingredient dilutions in the same buffer for 2 h. One of the ingredients, FSP, was found to stably induce GLP-1 secretion in both STC-1 and HuTu-80 cells in preliminary experiments. Therefore, 500 μg mL^{-1} FSP was used as a positive control for GLP-1 secretion in this study. KHB alone was used as the negative control. Cell supernatants were collected on ice after 2 h incubation, centrifuged to remove cells, and stored at −80 °C until further analysis.

The GLP-1 sequence is highly conserved in mammals, thus the total GLP-1 (both active and inactive GLP-1) concentration in cell culture supernatants were measured using a commercial total GLP-1 ELISA kit (EMD Millipore, Billerica, MA, U.S.A.) that is suitable for human, mouse and rat samples. All measurements were performed according to the manufacturer's recommendations. Data are presented as fold changes of the corresponding vehicle controls, and cells without treatments (0 μg mL^{-1}, KHB alone) were considered as 1 (baseline, no change).

Statistical analysis

All assays were performed three times with at least three replicates, and results are presented as mean ± SEM. To investigate the effect of each ingredient on each cell line, statistical analyses were performed with a statistical software R version 3.4.2 (R core team) using mixed linear model with a fixed effect of concentration and a random effect of assay number ($n = 3$; Hothorn & Everitt, 2014). The effect of concentration was analysed by one-way ANOVA. For the analysis of cell viability, the differences between indicated concentrations and controls were analysed by Dunnett test. For the analysis of GLP-1 secretion, multiple comparisons were carried out using Tukey test. P values < 0.05 were considered significant.

Results

Effects of food ingredients on cell viability

Positive control (10% FCS) maintained cell viability of FHs 74 Int., STC-1, and HuTu-80 at 99.1 ± 0.4%, 109.9 ± 1.1%, 104.1 ± 1.2% (mean ± SEM), respectively. The viability of all three cell lines decreased significantly with the addition of N-OEA, GTE, EGCG, RE at high concentrations (Fig. 1). The other ingredients did not affect cell viability or only showed a small decrease (<20%) at the tested concentrations (Fig. S1). Thus, in this study, the maximal concentrations used in secretion assays were decided as the highest concentration that maintain cell viability above 80%, except for KE, where HuTu-80 cell viability at 5000 μg mL^{-1} was 77%. However, 5000 μg mL^{-1} KE increased GLP-1 secretion in preliminary experiments, thus 5000 μg mL^{-1} was set as the maximal concentration on HuTu-80 cells. The maximal concentrations of food ingredients that were used in GLP-1 secretion assays are shown in Table 1.

GLP-1 secretion in response to food ingredients

To ensure the validity of the results, and ensure that the identified potential ingredients can be used in human functional food products, the concentrations of food ingredients for GLP-1 secretion assays were selected based on the results of the cell viability assay. For STC-1 cells, the test concentrations of food ingredients were equal or lower than the maximal concentrations determined for STC-1 and FHs 74 Int. shown in Table 1. For HuTu-80 cells, the tested concentrations of food ingredients were equal or lower than the maximal concentrations determined for HuTu-80 and FHs 74 Int. cells. In general, HuTu-80 cells showed no response or a smaller response (fold change) compared with STC-1 cells when stimulated with the same ingredient (Fig. 2). Positive control (500 μg mL^{-1} FSP) induced GLP-1 secretion in STC-1 and HuTu-80 cells to 3.3 ± 0.1, 1.6 ± 0.1 fold (mean ± SEM), respectively. Because each ingredient and each cell line has its own secretion curve, a high dose may in turn inhibit GLP-1 secretion. To avoid this interference,

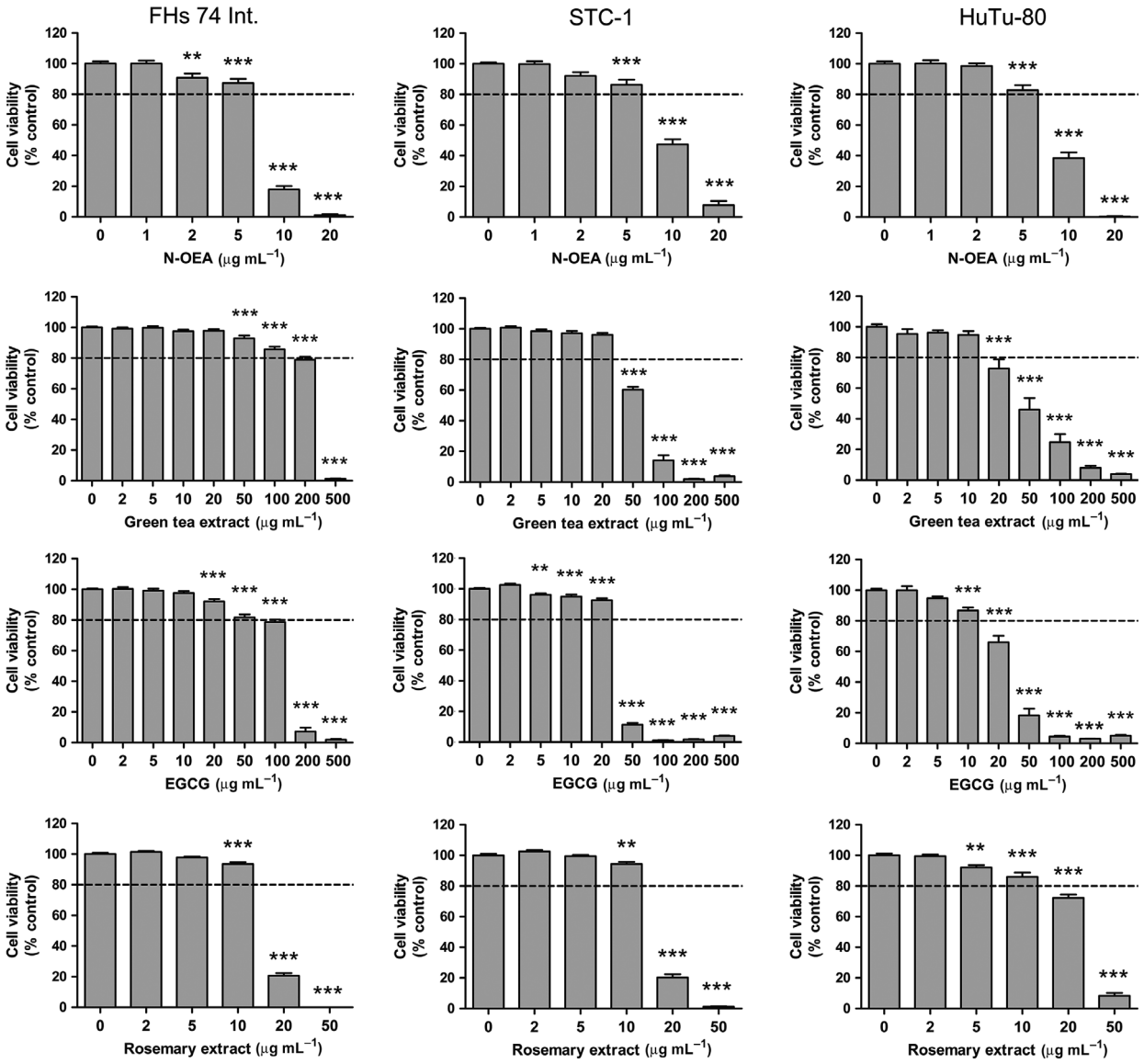

Figure 1 Four food ingredients affected viability of FHs 74 Int., STC-1, and HuTu-80 cells. The three cell lines were incubated with different concentrations of the indicated ingredients for 4 h (STC-1 and HuTu-80) or 12 h (FHs 74 Int.). Cell viability was determined by the alamarBlue® assay and presented as a percentage of vehicle control, and cells without treatments (0 μg mL^{-1}) were considered as 100%. N-OEA, N-oleoylethanolamine; EGCG, epigallocatechin-3-gallate. Assays were performed three times with at least three replicates. Data are presented as means ± SEM of three independent assays and are compared with control. "**", $P < 0.01$; "***", $P < 0.001$.

different concentrations, from high to low, were tested for each ingredient. However, for ETWP, PA, SA, AMG, GTE, EGCG and RE, the concentrations higher than those shown in Fig. 2 did not induce GLP-1 secretion in preliminary experiments.

As shown in Fig. 2, FSP, FFSP, AMG, N-OEA, HT, RE, and KE induced GLP-1 secretion in STC-1 cells in a dose-dependent manner, and resulted in up to 4.9-fold increase (5 μg mL^{-1} N-OEA). Similarly, FSP and FFSP also dose-dependently increased GLP-1 secretion in HuTu-80 cells, and both lead to equally 1.6-fold increase at the highest concentration. KE at all tested concentrations stimulated GLP-1 secretion in HuTu-80 cells despite the small decrease in viability, but there was no difference between concentrations. Notably, 5000 μg mL^{-1} KE induced highest GLP-1 secretion in HuTu-80 cells (1.8-fold), as well as the second highest secretion in STC-1 cells (4.4-fold).

Table 1 Maximal concentrations of food ingredients (μg mL^{-1}) used in GLP-1 secretion assays[a]

	Cell lines		
Ingredients	**STC-1**	**HuTu-80**	**FHs 74 Int.[d]**
Food ingredients that maintain cell viability above 80%[b]			
Functional soy protein (FSP)	500	500	500
Fractionated functional soy protein (FFSP)	2000	2000	2000
Enzyme-treated whey protein (ETWP)	5000	5000	5000
Palmitic acid (PA)	20	20	20
Stearic acid (SA)	20	20	20
Acetylated monoglyceride (AMG, v/v %)	0.5	0.5	0.5
Hesperidin (HD)	200	200	200
Hesperetin (HT)	100	100	100
Kale extract (KE)	5000	5000[c]	5000
Food ingredients that lead to cell viability decrease to <80%			
N-oleoylethanolamine (N-OEA)	5	5	5
Green tea extract (GTE)	20	10	100
Epigallocatechin-3-gallate (EGCG)	20	10	50
Rosemary extract (RE)	10	10	10

[a]The maximal concentrations used in GLP-1 secretion assays were set based on the results of cell viability assay.
[b]Ingredients that did not affect cell viability or only showed a small decrease (<20%) at the tested concentrations. Concentrations shown in the table were the maximal concentrations tested in cell viability assays, based on the solubility of the food ingredients in aqueous solutions.
[c]Cell viability at 5000 μg mL^{-1} was 76.79%. Kale extract at 5000 μg mL^{-1} increased GLP-1 secretion in preliminary experiment, thus 5000 μg mL^{-1} was set as the maximal concentration for kale extract on HuTu-80 cells.
[d]Maximal concentrations that maintain cell viability of FHs 74 Int. cells above 80% given as references.

Different from STC-1 cells, GLP-1 secretion in HuTu-80 cells was only stimulated by 100 μg mL^{-1} HT (1.4-fold), 0.001 and 0.1 μg mL^{-1} RE (1.2-fold), and did not increase in response to AMG and N-OEA. EGCG at 0.001, 0.005, 0.01 μg mL^{-1} increased GLP-1 secretion in HuTu-80 cells (~1.5-fold), but only 0.001 μg mL^{-1} EGCG stimulated GLP-1 secretion in STC-1 cells (1.2-fold). ETWP, PA, SA, GTE, HD did not lead to any significant GLP-1 secretion increase in either of the cell lines (Fig. S2).

Discussion

In the past decade, it has been clear that the secretion of GLP-1 can be stimulated by many food components. Proteins are among the well-known GLP-1 stimulants (Steinert *et al.*, 2017), thus it is not surprising that the two soy proteins, FSP and FFSP, significantly increased GLP-1 secretion in both cell lines. However, ETWP did not show any stimulatory effect in our study. This is in agreement with previous studies reporting GLP-1 secretion enhanced only by intact whey proteins (Geraedts *et al.*, 2011; Power-Grant *et al.*, 2015). It appears that enzyme treatment may change the performance of a protein, and the integrity of whey proteins is important to maintain its GLP-1 stimulatory property (Power-Grant *et al.*, 2015).

The effect of fatty acids on GLP-1 secretion depends on their chain length and degree of saturation (Hirasawa *et al.*, 2005). Hirasawa *et al.* (2005) found that saturated long-chain fatty acids did not influence GLP-1 secretion in STC-1 cells, which is in line with our results, and implicate that saturated long-chain fatty acids may not directly stimulate GLP-1 secretion *in vitro*. Besides fatty acid itself, some fatty acid derivatives, for instance, monoglycerides and N-OEA, are also reported to induce GLP-1 secretion *in vitro* (Lauffer *et al.*, 2009; Hansen *et al.*, 2011). Our findings of AMG and N-OEA support these previous reports and suggest that fatty acid derivatives are also capable of inducing GLP-1 secretion. In our study, GLP-1 secretion was stimulated by N-OEA in mouse STC-1 cells but not in human HuTu-80 cells, but Lauffer *et al.* (2009) found that N-OEA stimulates GLP-1 secretion in both mouse GLUTag cells and human NCI-H716 cells. This discrepancy may be caused by the origin and properties of the cells. NCI-H716 cells originate from colon (Kuhre *et al.*, 2016), while HuTu-80 cells originate from duodenum, thus different hormone responses could be expected.

Polyphenols are a class of phenolic components that are mostly found in tea, coffee, vegetables, fruits, etc. EGCG is one of the most abundant polyphenols in green tea, and it was recently found to stimulate GLP-1 secretion in Caco-2 human colonic epithelial cells *in vitro* (Song *et al.*, 2015). Our results are in line with this finding, implicating that EGCG may contribute to food intake reduction by inducing GLP-1 secretion. In contrast, GTE did not have any effect on GLP-1 secretion in either of the cell lines, suggesting that GTE may not as effective as the pure component EGCG. HT and its glycoside HD (hesperetin 7-rhamnoglucoside) are another two potent polyphenols. Kim *et al.* (2013) found that HT but not HD, induced gut hormone cholecystokinin secretion in STC-1 cells through increasing intracellular Ca^{2+} influx. The increases in intracellular Ca^{2+} appears to be the primary trigger of GLP-1 secretion (Tian & Jin, 2016), thus it is predictable that HT also stimulates GLP-1 secretion. Thus, our results implicate that only HT but not its glycoside HD may stimulate GLP-1 secretion.

Rosemary is a rich source of bioactive components such as carnosic acid, carnosol and rosmarinic acid, and kale also has a high content of

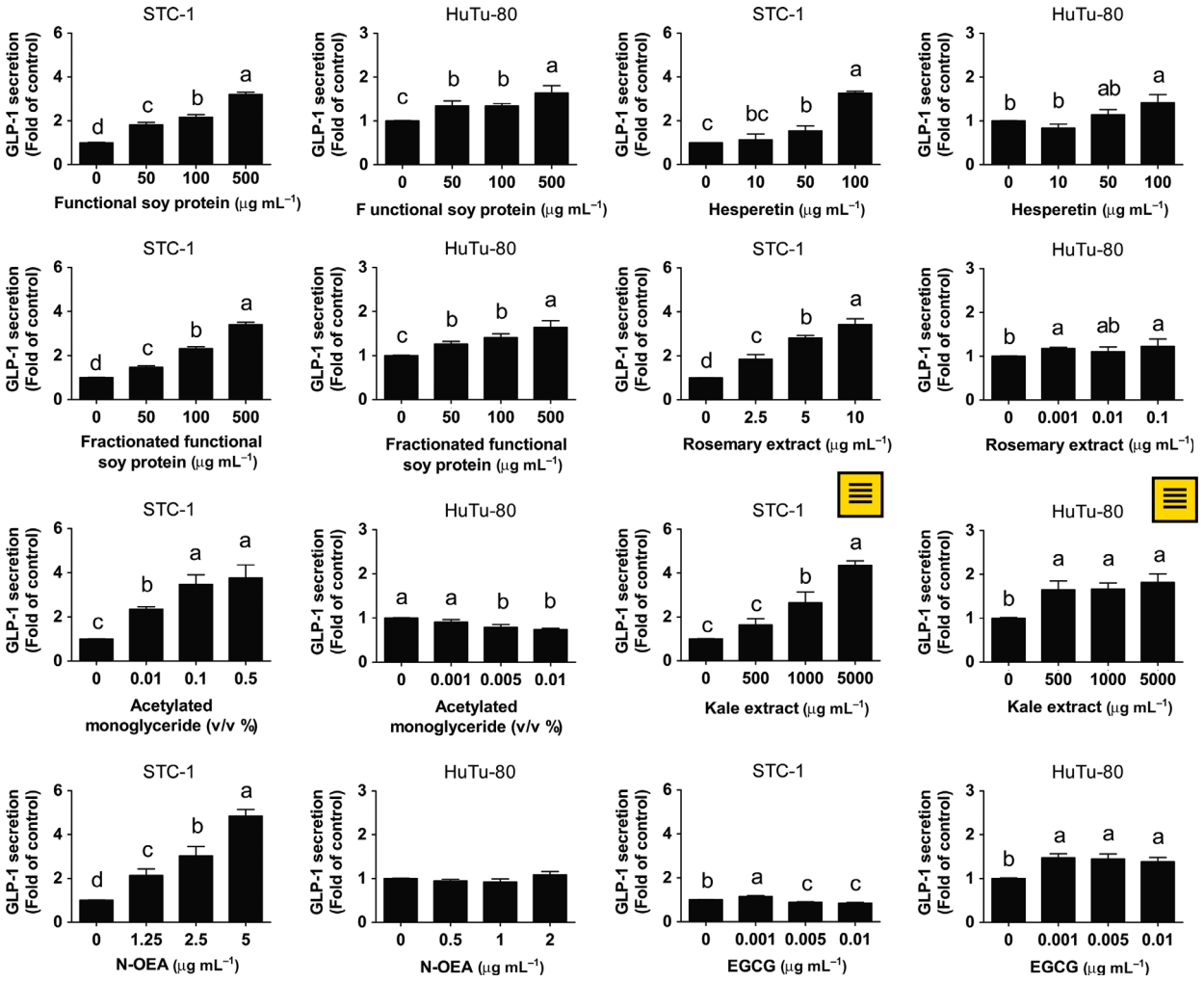

Figure 2 Eight food ingredients increased GLP-1 secretion in STC-1 and HuTu-80 cells. STC-1 and HuTu-80 cells were incubated with three concentrations of the indicated ingredients for 2 h, and GLP-1 concentrations in supernatants were measured by ELISA. Data are presented as fold change of corresponding vehicle controls, and cells without treatments (0 μg mL^{-1}) were considered as 1. N-OEA, N-oleoylethanolamine; EGCG, epigallocatechin-3-gallate. Assays were performed three times with at least three replicates. Data are presented as means ± SEM. Means without common letters differ significantly ($P < 0.05$).

polyphenols, especially quercetin and kaempferol. Both rosemary and kale have been reported to have anti-obesity properties (Williams *et al.*, 2013; Hassani *et al.*, 2016). Nevertheless, so far there is no investigation on the effects of rosemary and kale on GLP-1 secretion. To our knowledge, this is the first report showing that RE and KE can stimulate GLP-1 secretion from both mouse STC-1 cells and human HuTu-80 cells. This finding may provide a new perspective to the anti-obesity properties of rosemary and kale.

Although there are few studies that have investigated GLP-1 secretion in HuTu-80 cells, our results indicate that HuTu-80 cells are responsive to various food ingredients, as other enteroendocrine cells. However, HuTu-80 cells showed no response or a smaller response (fold change) compared with STC-1 cells, which supports the findings that GLP-1 secretion differs between species (Kuhre *et al.*, 2014; Gribble & Reimann, 2017) and cell lines (Kuhre *et al.*, 2016). In addition, nutrients and bioactive components induce GLP-1 secretion through activating various G protein-coupled receptors (GPCR) (Gribble & Reimann, 2017), and mice have been reported to have larger GPCR repertoires (Vassilatis *et al.*, 2003) and higher diversification than humans (Andres-Barquin & Conte,

2004). This may explain the difference between mouse STC-1 and human HuTu-80 cells found in our study, but further studies are needed.

In conclusion, this study investigated the effects of thirteen food ingredients on GLP-1 secretion in STC-1 and HuTu-80 cells, and found that FSP, FFSP, AMG, N-OEA, EGCG, HT, RE and KE increased GLP-1 secretion in at least one cell line. The results implicate that these ingredients have the potential to enhance satiety, thus provide a new possibility of using them as functional food ingredients for the management of obesity in future. However, STC-1 and HuTu-80 cells are derived from carcinomas, and they may respond differently than enteroendocrine cells *in vivo* (Kuhre *et al*., 2016). Thus, the *in vitro* effects on hormone secretion may not be directly translated to the effects *in vivo*. The effects of these potent food ingredients need to be further examined in an *in vivo* situation in future studies.

Acknowledgment

The work was part of the project Delivery of Intestinal Satiety inducing Ingredients (DISI) supported by Innovation Fund Denmark.

Conflict of interests

The authors declare no conflict of interests.

References

Andres-Barquin, P.J. & Conte, C. (2004). Molecular basis of bitter taste: the T2R family of G protein-coupled receptors. *Cell Biochemistry and Biophysics*, **41**, 99–112.

Geraedts, M.C., Troost, F.J., Fischer, M.A., Edens, L. & Saris, W.H. (2011). Direct induction of CCK and GLP-1 release from murine endocrine cells by intact dietary proteins. *Molecular Nutrition & Food Research*, **55**, 476–484.

Gribble, F.M. & Reimann, F. (2017). Signalling in the gut endocrine axis. *Physiology & Behavior*, **176**, 183–188.

Hansen, K.B., Rosenkilde, M.M., Knop, F.K. *et al*. (2011). 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. *Journal of Clinical Endocrinology and Metabolism*, **96**, E1409–E1417.

Hassani, F.V., Shirani, K. & Hosseinzadeh, H. (2016). Rosemary (*Rosmarinus officinalis*) as a potential therapeutic plant in metabolic syndrome: a review. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **389**, 931–949.

Hirasawa, A., Tsumaya, K., Awaji, T. *et al*. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nature Medicine*, **11**, 90–94.

Hothorn, T. & Everitt, B.S. (2014). *A Handbook of Statistical Analyses Using R*. 3rd edn. Boca Raton, FL: CRC Press.

Innovation Fund Denmark (2019). Investments overview. Online reference included in article [internet document]. https://innovationsfonden.dk/da/investeringer/investeringsoversigt?field_program_target_xml:id = 73&field_start_year_value = 2016&field_region_target_xml:id = All&field_area_target_xml:id = All. Accessed, 30/04/2019.

Kim, H.Y., Park, M., Kim, K., Lee, Y.M. & Rhyu, M.R. (2013). Hesperetin stimulates cholecystokinin secretion in enteroendocrine STC-1 cells. *Biomolecules and Therapeutics (Seoul)*, **21**, 121–125.

Kuhre, R.E., Albrechtsen, N.W., Windelov, J.A., Svendsen, B., Hartmann, B. & Holst, J.J. (2014). GLP-1 amidation efficiency along the length of the intestine in mice, rats and pigs and in GLP-1 secreting cell lines. *Peptides*, **55**, 52–57.

Kuhre, R.E., Wewer Albrechtsen, N.J., Deacon, C.F. *et al*. (2016). Peptide production and secretion in GLUTag, NCI-H716, and STC-1 cells: a comparison to native L-cells. *Journal of Molecular Endocrinology*, **56**, 201–211.

Lauffer, L.M., Iakoubov, R. & Brubaker, P.L. (2009). GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. *Diabetes*, **58**, 1058–1066.

Pham, H., Hui, H., Morvaridi, S. *et al*. (2016). A bitter pill for type 2 diabetes? The activation of bitter taste receptor TAS2R38 can stimulate GLP-1 release from enteroendocrine L-cells. *Biochemical and Biophysical Research Communications*, **475**, 295–300.

Power-Grant, O., Bruen, C., Brennan, L., Giblin, L., Jakeman, P. & FitzGerald, R.J. (2015). *In vitro* bioactive properties of intact and enzymatically hydrolysed whey protein: targeting the enteroinsular axis. *Food & Function*, **6**, 972–980.

Purup, S., Vestergaard, M., O Pedersen, L. & Sejrsen, K. (2007). Biological activity of bovine milk on proliferation of human intestinal cells. *Journal of Dairy Research*, **74**, 58–65.

Purup, S., Larsen, E. & Christensen, L.P. (2009). Differential effects of falcarinol and related aliphatic C(17)-polyacetylenes on intestinal cell proliferation. *Journal of Agriculture and Food Chemistry*, **57**, 8290–8296.

Song, W.Y., Aihara, Y., Hashimoto, T., Kanazawa, K. & Mizuno, M. (2015). (-)-Epigallocatechin-3-gallate induces secretion of anorexigenic gut hormones. *Journal of Clinical Biochemistry and Nutrition*, **57**, 164–169.

Srivastava, G. & Apovian, C.M. (2018). Current pharmacotherapy for obesity. *Nature Reviews Endocrinology*, **14**, 12–24.

Steinert, R.E., Feinle-Bisset, C., Asarian, L., Horowitz, M., Beglinger, C. & Geary, N. (2017). Ghrelin, CCK, GLP-1, and PYY(3–36): Secretory controls and physiological roles in eating and glycemia in health, obesity, and after RYGB. *Physiological Reviews*, **97**, 411–463.

Tian, L. & Jin, T. (2016). The incretin hormone GLP-1 and mechanisms underlying its secretion. *Journal of Diabetes*, **8**, 753–765.

Vassilatis, D.K., Hohmann, J.G., Zeng, H. *et al*. (2003). The G protein-coupled receptor repertoires of human and mouse. *Proceedings of the National Academy of Sciences United States of America*, **100**, 4903–4908.

Williams, D.J., Edwards, D., Hamernig, I. *et al*. (2013). Vegetables containing phytochemicals with potential anti-obesity properties: a review. *Food Research International*, **52**, 323–333.

Yue, Y., Hymøller, L., Jensen, S.K., Lauridsen, C. & Purup, S. (2017). Effects of vitamin D and its metabolites on cell viability and *Staphylococcus aureus* invasion into bovine mammary epithelial cells. *Veterinary Microbiology*, **203**, 245–251.

Yun, J.W. (2010). Possible anti-obesity therapeutics from nature – a review. *Phytochemistry*, **71**, 1625–1641.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Effects of nine food ingredients on viability of FHs 74 Int., STC-1 and HuTu-80 cells.

Figure S2. Effects of five food ingredients on GLP-1 secretion in STC-1 and HuTu-80 cells.