RESEARCH ARTICLE

Addition of sucralose enhances the release of satiety hormones in combination with pea protein

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Received: May 2, 2011
Revised: October 24, 2011
Accepted: November 6, 2011

Scope: Exposing the intestine to proteins or tastants, particularly sweet, affects satiety hormone release. There are indications that each sweetener has different effects on this release, and that combining sweeteners with other nutrients might exert synergistic effects on hormone release.
Methods and results: STC-1 cells were incubated with acesulfame-K, aspartame, saccharine, sucralose, sucrose, pea, and pea with each sweetener. After a 2-h incubation period, cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) concentrations were measured. Using Ussing chamber technology, the mucosal side of human duodenal biopsies was exposed to sucrose, sucralose, pea, and pea with each sweetener. CCK and GLP-1 levels were measured in basolateral secretions. In STC-1 cells, exposure to aspartame, sucralose, sucrose, pea, and pea with sucralose increased CCK levels, whereas GLP-1 levels increased after addition of all test products. Addition of sucrose and sucralose to human duodenal biopsies did not affect CCK and GLP-1 release; addition of pea stimulated CCK and GLP-1 secretion.
Conclusion: Combining pea with sucrose and sucralose induced even higher levels of CCK and GLP-1. Synchronous addition of pea and sucralose to enteroendocrine cells induced higher levels of CCK and GLP-1 than addition of each compound alone. This study shows that combinations of dietary compounds synergize to enhance satiety hormone release.

Keywords:
Cholecystokinin / Dietary protein / Gastrointestinal tract / Glucagon-like peptide 1 / Sweetener

1 Introduction

Nutrient-induced gut-to-brain signaling plays a major role in the control of the digestive function, appetite, and energy intake [1]. These effects are mediated by a number of interrelated factors, including the release of signaling peptides from enteroendocrine cells, such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1). Most of these hormones are secreted upon food intake, and contribute to the termination of the meal. Since overweight and obesity have become a major health problem [2], several types of diets have focused on favorable macronutrient compositions in order to stimulate the release of these satiety hormones [3–8].

Of all diets tested, high-protein diets seem to have the largest effects on food intake [9, 10]. The effects of several protein hydrolysates on the release of CCK from the enteroendocrine STC-1 cell line were determined previously [11]. It was shown that all hydrolysates were able to induce elevated levels of CCK, but there were no differences between the hydrolysates. In a study performed in our laboratory, it was demonstrated that intact proteins were the most potent in stimulating CCK and GLP-1 release versus hydrolysates and specific peptides, with intact pea protein being one of the most potent ones [12]. Pea hydrolysate was considered as most effective in suppressing hunger and stimulating satiety when compared to whole-milk protein [13], however, in a human study we demonstrated that intraduodenal infusion of an intact pea protein more effectively reduced food intake in both lean and obese subjects compared to the hydrolysate (submitted for publication) [14].

Over the past few years, not only food intake, but also consumption of soft drinks has increased [15]. This high

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Abbreviations: CCK, cholecystokinin; **GLP-1**, glucagon-like peptide 1; **HBSS**, Hank's balanced salt solution; **KRB**, Krebs-Ringer bicarbonate buffer; **PD**, potential difference; **TER**, transepithelial resistance

consumption of sugar-sweetened beverages has been linked with increased energy intake and obesity [16]. It was demonstrated that overweight subjects who consumed large amounts of caloric-sweetened beverages increased energy intake, body weight (BW), fat mass, and blood pressure after a 10-week intervention, whereas this was not observed in a similar group receiving artificial sweeteners [17]. However, literature is conflicting about the effects of artificial sweeteners. It has been suggested that intake of low-caloric sweetened beverages is linked to obesity, related to the potential mediating role of energy intake, e.g. that intake of noncaloric-sweetened beverages brings less satiation, causing a higher amount of calories consumed at a given meal and thereby a higher daily energy intake [18, 19]. It has also been suggested that the intake of noncaloric-sweetened beverages fail to trigger physiological satiety mechanisms, providing imprecise and incomplete energy compensation [20].

More recently, it has been demonstrated that the upper gut may be able to sense sweetness, resulting in the release of satiety hormones. In a human L cell line, glucose and sucralose were able to induce the release of GLP-1 [21]. However, in a previous study performed in our laboratory, we demonstrated that noncaloric or low-caloric sweeteners show increased levels of CCK and GLP-1, when compared to sucrose. Remarkably, Tagatesse, (a sweetener that contains inulin, tagatose, isomalt, sucralose, and oligo-saccharides) showed the strongest effect on the release of both CCK and GLP-1 [22]. These data lead us to believe that combining noncaloric sweeteners with specific macronutrients may induce stronger effects on hormone release than the sweeteners or macronutrients alone. Of all macronutrients, proteins are the most potent in stimulating satiety and inhibit food intake the strongest, with pea protein being one of the most potent proteins. Therefore, the hypothesis of the present study is that combining pea protein with specific sweeteners induces elevated effects on CCK and GLP-1 release, when compared to only sweeteners or the pea protein.

2 Materials and methods

The present study consisted of two parts. In the first part, all sweeteners and the combination of sweetener with pea protein were tested on their effects on satiety hormone release in vitro. The most potent results, with highest levels of hormone secretions, were then tested in an ex vivo study, using biopsy specimens from healthy male volunteers.

2.1 In vitro assay

2.1.1 Test products

All sweeteners were dissolved accordingly to match the sweetness of the sugar dosages, according to its known sweetness equivalent relative to sucrose [23]. Five different tastants were used, namely sucrose (6 g, Sigma-Aldrich, St. Louis, MO), aspartame (0.03 g), acesulfame K (0.04 g), saccharine (0.012 g) (all from Supelco, Bellefonte, PA), and sucralose (0.01 g, Tate&Lyle, London, UK) [22]. All sweeteners were dissolved in 300 mL of Hank's balanced salt solution (HBSS), since most sweeteners are consumed in approximately 200 mL as volume for one coffee or tea consumption. The fluid is then diluted by 100 mL of basal gastric juice volume before it enters the intestine. Pea protein (0.1 mg/mL, Dutch Protein Services, Tiel, The Netherlands) was added to the sweeteners.

2.1.2 Cell culture conditions

The STC-1 cell line is derived from an intestinal endocrine tumor that developed in a double-transgenic mouse expressing the rat insulin promotor linked to the simian virus 40 large T antigen and the polyoma small T antigen [24]. STC-1 cells (kindly provided by Dr. D. Hanahan, University of California, San Francisco) were maintained in Dulbecco's modified eagles medium (DMEM) with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin as additional supplements, at 37°C in 5% CO_2/air humidity. All products were obtained from Invitrogen, Carlsbad, CA, unless stated otherwise.

2.1.3 Secretion of CCK and GLP-1 from STC-1 cells

To determine the secretion of CCK and GLP-1 from STC-1 cells, suspensions of these cells were plated at 1.0×10^5 cells/well in 24-well plates (Costar, Sigma Aldrich, St. Louis, MO) and assays were performed on cultures that reached at least 80% confluency. Before treatment with the test products, culture medium was removed and dishes were rinsed with HBSS. Cells were then incubated with HBSS (negative control), the sweeteners, and the combination of sweeteners with pea protein, and were incubated at 37°C for 2 h. The supernatant was collected for the measurement of CCK and GLP-1. All analyses were performed in triplicate, using three biological replicate samples. The best results were then tested in the Ussing chambers.

2.2 Human ex vivo assay

2.2.1 Subjects

In this study, ten (five lean and five obese) healthy male subjects were recruited. Selection took place according to health criteria (no diabetes, no gastrointestinal diseases, and no medical treatment) and BW criteria (for lean subjects: body mass index (BMI) = 18–25 kg/m^2, and for obese subjects: BMI > 30 kg/m^2). Baseline characteristics of the subjects are presented in Table 1. The nature and risks of the experimental procedure were explained to the subjects, and all

Table 1. Subject characteristics

	Lean ($n = 5$)	Obese ($n = 5$)
Age (years)	27 ± 5	37 ± 10
BMI (kg/m^2)	24.2 ± 1	32.8 ± 2*
HbA1c (%)	4.6 ± 0.2	4.4 ± 0.2
Basal glucose (mmol/L)	4.9 ± 0.2	4.8 ± 0.2

All data are mean ± SEM.
*Difference between lean and obese subjects ($p < 0.05$).

subjects gave their written informed consent. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the Medical Ethical Committee of the University Hospital Maastricht approved all procedures involving human subjects (registration no. MEC 06–3-060).

2.2.2 Duodenal tissue sampling for *ex vivo* experiments

All subjects received a standardized meal (9 g protein, 39.5 g carbohydrates, 16 g fat) for the evening prior to the test day to standardize macronutrient intake. After an overnight fast, eight mucosal tissue samples from the horizontal part of the duodenum were obtained by flexible gastroduodenoscopy using standard biopsy forceps. During this procedure, no sedatives were given to the subjects. The diameter of the biopsies varied from 2.0 to 2.2 mm. After sampling, the biopsies were placed in ice-cold Krebs-Ringer bicarbonate buffer (KRB) and arrived at the laboratory within 15 min.

2.2.3 Ussing chamber experiments

Duodenal biopsies were mounted in modified Ussing chambers (Harvard Apparatus Inc., Holliston, MA) with a Ø 9-mm opening and reduced to an exposed tissue area of 1.76 mm^2, using a technique previously described by Wallon et al. [25, 26]. Mucosal compartments were filled with 1.5 mL 10 mM mannitol in KRB and the serosal compartments were filled with 10 mM glucose in KRB. The chambers were kept at 37°C and continuously oxygenated with 95% O_2/5% CO_2 and circulated by gas flow. Before the experiments were started, tissues equilibrated for 40 min in the chambers to achieve steady-state conditions in transepithelial potential difference (PD), with replacement of mannitol and glucose KRB at 20 min. A four-electrode system was used, as described previously [27]. One pair of Ag/Cl electrodes with 3 M NaCl/2% agar bridges was used for measurement of transepithelial PD and another pair of Ag/Cl electrodes was used to monitor current. The electrodes were coupled to an external six-channel electronic unit with a voltage-controlled current source. Data sampling was computer controlled via an A/D D/A board (Lab NB, National Instruments) by a program developed in LabVIEW (National Instruments, Austin, TX) by Wikman-Larhed et al. [28]. Every other minute, direct pulses of –3, 3, and 0 μA, with a duration of 2 s each, were sent across the tissue segments and the voltage response was measured. In each measurement, the mean voltage response of 2 s was calculated. A linear-squares fit was performed on the current (I) – voltage (U) pair relationship: $U = PD + TER \times I$. The transepithelial resistance (TER) was obtained from the slope of the I-U line and the PD from the intersection of the voltage.

After the equilibration period, the mucosal side of the biopsies was exposed to sucrose, sucralose, pea, pea with sucrose, and pea with sucralose. Serosal samples (1.25 mL) were collected the end of the experiment (after 2 h) for CCK- and GLP-1 analysis. Biopsies with PD less negative than –0.5 mV were excluded from all tested ($n = 3$) because of malfunction in the ability to uphold normal electrophysiology.

2.2.4 Hormone assays

CCK levels were determined using the RIA from Euria-CCK, Euro-Diagnostica AB, Malmö, Sweden. According to the manufacturers instructions, the detection limit of this kit was 0.3 pmol/L. The intra-assay variation ranges from 2.0 to 5.5% and the interassay variation from 4.1 to 13.7%. Cross-reaction with gastrin is ≤ 0.5%. Total GLP-1 levels were determined using the RIA from Linco Research, MO. The detection limit of this kit was 3 to 333 pM. The intra-assay variation ranges from 10 to 23% and the interassay variation from 22 to 38%. There is no cross-reaction with GLP-2 and glucagon (0.01 and 0.2%, respectively). GLP-1 samples were spiked with 100 pM of GLP-1 to be within range of the detection limit. Both RIAs can be used for the analysis of both rat and human samples.

2.2.5 Statistical analyses

The descriptive and statistical analyses were performed with SPSS, version 11.0. With regard to the cell culture results, the means of the secreted hormones were compared using the one-sample *t*-test. With regard to the Ussing chambers, the electrophysiological parameters were compared using the Wilcoxon signed rank test. Means of the secreted hormones between groups were compared using an unpaired Student's *t*-test. Means of the secreted hormones within a group were compared using a paired Student's *t*-test. All Student's *t*-tests were corrected for multiple testing using the Bonferroni correction. The means of the variables are presented with their standard error (mean ± SEM). A *p*-value of less than 0.05 was considered statistically significant.

3 Results

3.1 Hormone release from STC-1 cells

The release of CCK and GLP-1 from the enteroendocrine STC-1 cells is presented in Figure 1. As can be seen in Figure 1A, CCK release was significantly increased after

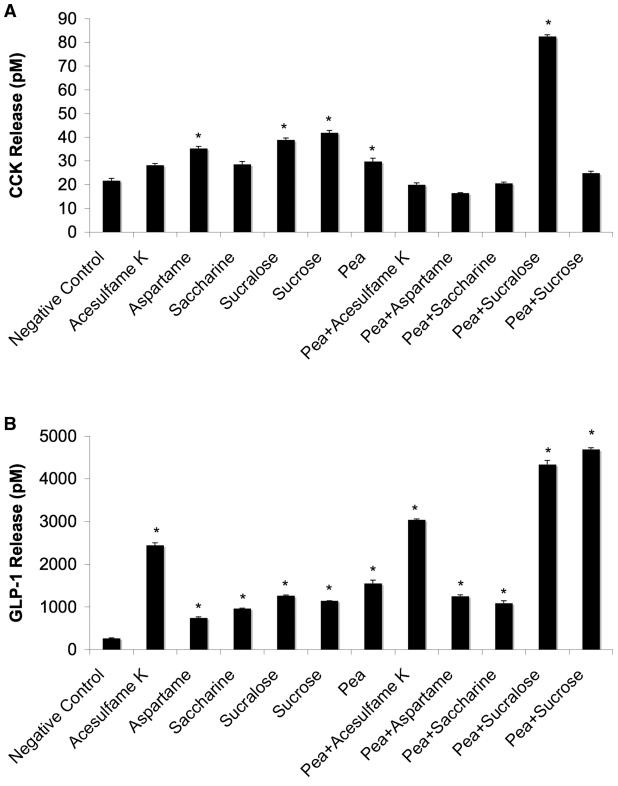

Figure 1. Hormone release from STC-1 cells after 2-h exposure to several sweeteners, pea protein, and combinations of pea protein with the sweeteners. STC-1 cells were exposed to different sweeteners, pea protein, and a combination of pea protein with sweeteners for 2 h. After the incubation period, cholecystokinin (CCK) (A) and glucagon-like peptide 1 (GLP-1) (B) levels were measured in the supernatant. Results are expressed as mean ± SEM. *Significantly different from negative control, $p < 0.05$).

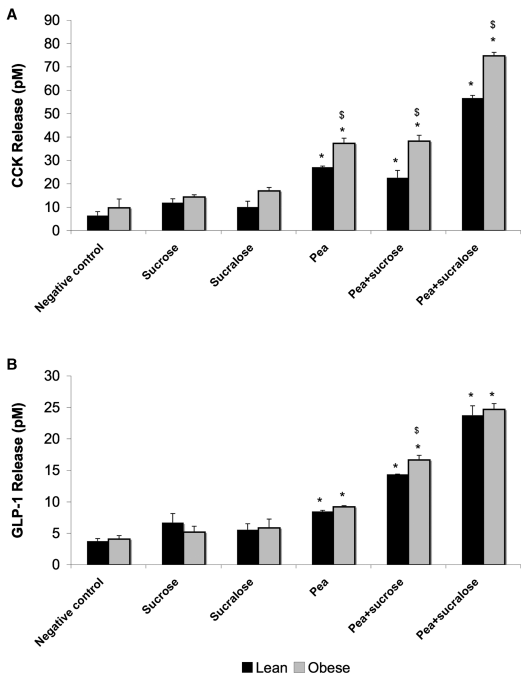

Figure 2. Hormone release from human duodenal biopsies in Ussing chambers after 2-h exposure to sucrose, sucralose, pea protein, and combinations of pea protein with the sucralose or sucrose. Duodenal biopsies of lean and obese subjects were exposed to either, sucrose, sucralose, pea protein, or a combination of pea protein with sucrose or sucralose. The levels of CCK (A) and GLP-1 (B) were measured in the supernatant of basolateral side of the biopsies in the Ussing chambers after being exposed to proteins for 2 h to the apical side. Results are expressed as mean ± SEM. *Significantly different from negative control, $p < 0.05$) $Significantly different from lean subjects.

addition of aspartame, sucralose, sucrose, pea, and pea with sucralose, when compared to the negative control. Highest levels of CCK release were found after addition of the combination of pea with sucralose (82.5 pM ± 0.7). As can be seen in Figure 1B, addition of all test products significantly increased the release of GLP-1 from the STC-1 cells when compared to the negative control (only HBSS buffer). Addition of acesulfame K, pea with acesulfame K, pea with sucrose, or pea with sucralose (2442 pM ± 60, 3040 pM ± 22, 4337 pM ± 93, and 4691 pM ± 38, respectively) induced the highest levels of GLP-1.

3.2 Hormone release from Ussing chamber experiments

In duodenal tissue of lean subjects, we observed basal CCK secretion levels of 6.4 pM ± 2 in lean subjects, whereas basal CCK levels in obese subjects were 9.7 pM ± 4 (Fig. 2A). After addition of pea, pea with sucrose, or pea with sucralose, the levels of CCK were significantly increased compared to the negative control, for both lean (27.1 pM ± 1, 22.6 pM ± 3, and 56.7 pM ± 1, respectively) and obese subjects (37.3 pM ± 2, 38.2 pM ± 3, and 74.7 pM ± 2, respectively). Also, addition of these compounds to the duodenal biopsies resulted in significantly increased CCK levels in obese subjects when compared to lean subjects. Addition of sucrose and sucralose alone did not affect CCK release when compared to the negative control.

GLP-1 secretion from duodenal biopsies is presented in Figure 2B. Basal GLP-1 secretions levels of 3.7 pM ± 0.4 from lean subjects and 4.1 pM ± 0.5 from obese subjects were observed. Addition of intact pea protein to the luminal side significantly increased GLP-1 levels (8.4 pM ± 0.2 in lean subjects, and 9.3 pM ± 0.2 in obese subjects) when compared to negative control and to addition of sucrose. Also, addition of intact pea protein induced significantly higher levels of GLP-1 when compared to lean subjects. Addition of the combination of pea with sucrose or pea with sucralose also induces significant elevated levels of GLP-1 (14.4 pM ± 0.1 and 23.8 pM ± 1, respectively, for lean subjects, 16.6 pM ± 0.7 and 24.7 pM ± 1, respectively, for obese subjects), when compared to the negative control and addition of only sucrose.

3.3 Electrical measurements

The electrical parameters PD, short circuit current (Isc), and TER were followed over time. Basal electrical properties of all biopsies were measured. After an equilibration period of 40 min, the mean PD of –1.4 mV ± 0.2 was observed. Overall, no changes in PD were observed in the following 120 min. A decrease in TER and an increase in Isc were observed. The TER was significantly less decreased after addition of pea with sucrose (–29.2 $\Omega.cm^2$ ± 1), when compared to the negative control (–51.4 $\Omega.cm^2$ ± 3) in obese subjects. All other products did not affect TER when compared to the negative control for both lean and obese subjects.

Addition of sucrose, sucralose, and pea with sucrose resulted in an increased Isc (243 $\mu A/cm^2$ ± 32, 247 $\mu A/cm^2$ ± 25, and 235 $\mu A/cm^2$ ± 26, respectively) when compared to the negative control (50 $\mu A/cm^2$ ± 19) in lean subjects. Also, addition of sucralose or pea with sucrose to biopsies from lean subjects resulted in increased Isc when compared to obese subjects. Addition of sucrose or pea with sucralose to the luminal side of biopsies from obese subjects resulted in an increased Isc (196 $\mu A/cm^2$ ± 34 and 387 $\mu A/cm^2$ ± 32, respectively) when compared to the negative control (32 $\mu A/cm^2$ ± 11). Even though the Isc values were significantly elevated compared to the negative control, there were no significant differences between the test compounds.

4 Discussion

In the present study, the effects of five sweeteners in the presence or absence of pea protein on satiety hormone release were investigated. We demonstrated that the combination of pea protein with sucrose and the artificial sweetener sucralose induce stronger effects on GLP-1 release compared to the compounds separately, both in vitro and ex vivo. It was also shown that addition of sucrose or sucralose to STC-1 cells stimulated the release of CCK and GLP-1, whereas addition of the sweeteners to human duodenal biopsies did not result in hormone release.

Carbohydrate is an adequate stimulus for secretion of GLP-1. Failure of non-nutritive sweeteners to elicit the release of such peptides could theoretically result in lower satiety and augment energy intake. Literature is contradictory about whether artificial sweeteners are able to induce hormone release from gastrointestinal cells. Studies describing positive effects on hormone release all use cell lines. GLP-1 release from human NCI-H716 cells was promoted by sugars and by the sweetener sucralose [21]. In contrast, aspartame did not stimulate GLP-1 secretion [29]. It was also demonstrated that the intestinal STC-1 cells respond to all five basic tastants by increasing intracellular calcium [30]. However, when the sweeteners are given orally, it was demonstrated that they do not enhance the release of incretin hormones in rodents [31, 32] or in humans [33–36]. The present data confirm this literature. Most artificial sweeteners were able to stimulate incretin release from the enteroendocrine STC-1 cells, whereas exposing human duodenal tissue to sucrose or sucralose, CCK and GLP-1 release was not affected. Surprisingly, combining sucrose and sucralose with pea protein, the release of CCK and GLP-1 was more increased then when exposing duodenal tissue to only pea protein. These results indicate that the sweeteners might synergize with a macronutrient, in this case pea protein, to enhance the incretin release, which is coherent with results described by Brown et al. [37]. However, the mechanism behind this possible synergism is unknown.

Taste buds signal the presence of chemical stimuli in the oral cavity to the central nervous system using both early transduction mechanisms, which allow single cells to be depolarized via receptor-mediated signaling pathways, and late transduction mechanisms, which involve extensive cell-to-cell communication among the cells in the buds. The latter mechanisms, which involve a large number of neurotransmitters and neuropeptides, are less well understood [38]. Heath et al. demonstrated that by increasing the levels of neurotransmitters, and in particular norepinephrine, the threshold to bitter and sour was reduced by 39 and 22%, respectively [39]. This might also be true in the GI tract. Exposing sucrose and sucralose to duodenal biopsies might not trigger hormone release from the enteroendocrine cells due to a very high threshold. Adding macronutrients to the mixture, in this case, pea protein might trigger the release of norepinephrine, which then results in a lower threshold for the sweeteners. However, if this is true for all macronutrients and sweeteners has to be investigated further. The opposite might also be true. Exposing STC-1 cells to a combination of pea protein with aspartame, saccharine, or sucrose showed no effect on CCK release compared to the negative control, whereas most compounds alone do have a significant effect on CCK release. The combination of these compounds appears to have an inhibitory effect. It might be due to the possibility that there is a competition for binding to receptors, or that sucrose might stick to the pea protein, preventing both compounds to bind to their receptors. However, this should be investigates futher before conclusions can be drawn.

In the present study, both lean and obese subjects were tested. There are indications that obese subjects are less sensitive for satiety signals compared to lean subjects. In rats, it was shown that the minimal effective dose of satiety hormones was three to four times greater in obese than in lean rats [40, 41]. Also, obese male subjects have higher blood levels of CCK after a protein infusion into the duodenum when compared to lean subjects (unpublished data). In the present study, we found that obese subjects have higher release of CCK after exposure to the pea protein compared to the lean subjects, which is in line with previous studies (unpublished data). Also the combination of pea protein with sucrose and sucralose resulted in higher levels of CCK in obese subjects. In a previous study, it was also demonstrated that after pea protein infusion into the duodenum, CCK levels were more

increased in obese subjects, but food intake decreased to the same extent in both lean and obese subjects. Obese subjects probably are less sensitive to CCK and GLP-1, and therefore need higher circulating levels to reach a similar satiety effect.

Although neither STC-1 cells nor Ussing chambers completely reflect the in vivo physiology, they are useful screening tools to select candidate food ingredients to determine whether these compounds are able to mediate food intake and weight management. It has previously been demonstrated that the STC-1 cells are suitable to screen macronutrients on their effects on release of several satiety hormones [11,42–44]; however, there are also some drawbacks related to using this cell line. It originates from mice, so the effects on hormone release may not necessarily be translatable to the effects in human duodenal tissue. Also, the concentrations used for the products may be different when testing the same product in an in vivo situation. By using the Ussing chambers as an intermediate model to study effects of dietary interventions on human physiological processes, the extrapolation from in vitro to in vivo findings might be easier. One major drawback of this method is that even though human tissue is used, the integrative physiological effects are lost. Also, in the present study, sucrose did not stimulate GLP-1 release from duodenal tissue samples, whereas when tested in vivo, plasma GLP-1 levels increase. GLP-1 is secreted in very low amounts from the duodenum, and most of the effects that are seen in vivo are due to GLP-1 secretion from jejunum and ileum. Therefore, it is reasonable that in the present study, no effects where found on GLP-1 secretion after stimulation with sucrose. These data demonstrate that the results from this technique might not always be perfectly predictive, however, previous data from our laboratory have shown that the results found in Ussing chambers are comparable to human in vivo studies [45].

Electrical parameters have also been measured in the present study. These parameters are widely accepted for monitoring the viability and integrity of tissue in the Ussing chambers. In general, PD reflects the voltage gradient generated by the tissue, TER reflects the tissue integrity, and Isc reflects the ionic fluxes across the epithelium [46–48]. In the present study, basal electrical parameters varied over a wide range. This variability has also been noticed in previous studies on human tissue samples from jejunum [49] and colon [50]. Moreover, the reported electrical parameters from investigators using different Ussing chambers on biopsy specimens from the same gastrointestinal region have been associated with a large variability. To correct for this variability, the areas under the curves have been calculated, after correcting for baseline values for each biopsy. The electrophysiology results from our study were comparable with those found in literature, and showed that all biopsies used in this study were viable throughout the experiments. Addition of sweeteners, pea protein, or a combination of both did not affect the resistance of the tissue, but the Isc was significantly increased compared to the negative control. The Isc is a marker for transepithelial ion transport. Once food compounds stimulate enteroendocrine cells, Ca^{2+} will be transported into the cell, resulting in release of satiety hormones [51]. This influx may have been the cause of the increase in Isc.

We conclude that the combination of food ingredients plays an important role in the regulation of the release of satiety hormones. There seems to be a difference in satiety hormone release after exposure to different sweeteners, and the combination of sweeteners with pea protein. Combining pea protein with sucralose induced the strongest effects on CCK and GLP-1 release by both STC-1 cells and human duodenal tissue samples. The true efficacy of a combination of pea protein and artificial sweeteners on food intake should be investigated in a human intervention study.

This research was funded by the Transnational University Limburg (TUL).

The authors have declared no conflict of interest.

5 References

[1] Read, N., French, S., Cunningham, K. The role of the gut in regulating food intake in man. *Nutr. Rev.* 1994, *52*, 1–10.

[2] Obesity: preventing and managing the global epidemic. Report of a WHO consultant. *World Health Organ. Tech. Rep. Ser.* 2000, *894*, 1–253.

[3] Adam, T. C., Lejeune, M. P., Westerterp-Plantenga, M. S., Nutrient-stimulated glucagon-like peptide 1 release after body-weight loss and weight maintenance in human subjects. *Br. J. Nutr.* 2006, *95*, 160–167.

[4] Anderson, G. H., Moore, S. E., Dietary proteins in the regulation of food intake and body weight in humans. *J. Nutr.* 2004, *134*, 974S–979S.

[5] Blom, W. A., Lluch, A., Stafleu, A., Vinoy S. et al., Effect of a high-protein breakfast on the postprandial ghrelin response. *Am. J. Clin. Nutr.* 2006, *83*, 211–220.

[6] Westerterp-Plantenga, M. S., Lejeune, M. P., Protein intake and body-weight regulation. *Appetite* 2005, *45*, p. 187–190.

[7] Bernard, C., Sutter, A., Vinson, C., Ratineau C. et al., Peptones stimulate intestinal cholecystokinin gene transcription via cyclic adenosine monophosphate response element-binding factors. *Endocrinology* 2001, *142*, 721–729.

[8] Hall, W. L., Millward, D. J., Long, S. J., Morgan, L. M., Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br. J. Nutr.* 2003, *89*, 239–248.

[9] Westerterp-Plantenga, M. S., The significance of protein in food intake and body weight regulation. *Curr. Opin. Clin. Nutr. Metab. Care* 2003, *6*, 635–638.

[10] Saris, W. H., Tarnopolsky, M. A., Controlling food intake and energy balance: which macronutrient should we select? *Curr. Opin. Clin. Nutr. Metab. Care* 2003, *6*, 609–613.

[11] Foltz, M., Ansems, P., Schwarz, J., Tasker, M. C. et al., Protein hydrolysates induce CCK release from enteroendocrine cells and act as partial agonists of the CCK1 receptor. *J. Agric. Food Chem.* 2008, *56*, 837–843.

[12] Geraedts, M. C., Troost, F. J., Fischer, M. A., Edens, L. et al., Direct induction of CCK and GLP-1 release from murine endocrine cells by intact dietary proteins. *Mol. Nutr. Food Res.* 2011, *55*, 476–484.

[13] Diepvens, K., Haberer, D., Westerterp-Plantenga, M., Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int. J. Obes. (Lond.)* 2008, *32*, 510–518.

[14] Geraedts, M. C. P., Troost, F. J., Munsters, M. J. M., Stegen, J. H. C. H., et al., Intraduodenal administration of intact pea protein effectively reduces food intake in both lean and obese male subjects. *PLoS ONE* 2011, *6*, e24878. DOI: 10.1371/journal.pone.0024878.

[15] Popkin, B. M., Nielsen, S. J., The sweetening of the world's diet. *Obes. Res.* 2003, *11*, 1325–1332.

[16] Ludwig, D. S., Peterson, K. E., Gortmaker, S. L., Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet* 2001, *357*, 505–508.

[17] Raben, A., Vasilaras, T. H., Moller, A. C., Astrup, A. et al., Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. *Am. J. Clin. Nutr.* 2002, *76*, 721–729.

[18] Bawa, S., The role of the consumption of beverages in the obesity epidemic. *J. R. Soc. Promot. Health* 2005, *125*, 124–128.

[19] Molgaard, C., Andersen, N. L., Barkholt, V., Grunnet, N. et al., The impact of sugar on health. *Ugeskr Laeger* 2003, *165*, 4207–4210.

[20] Almiron-Roig, E., Chen, Y., Drewnowski, A., Liquid calories and the failure of satiety: how good is the evidence? *Obes. Rev.* 2003, *4*, 201–212.

[21] Jang, H. J., Kokrashvili, Z., Theodorakis, M. J., Carlson, O. D. et al., Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc. Natl. Acad. Sci. USA* 2007, *104*, 15069–15074.

[22] Geraedts, M. C., Troost, F. J., Saris, W. H., Different tastants and low-caloric sweeteners induce differential effects on the release of satiety hormones. *Food Chem.* 2011, in press, DOI: 10.1016/j.foodchem.2011.05.013.

[23] Moskowitz, H., Ratio scales of sugar sweetness. *Percept. Psychophys.* 1970, *7*, 315–320.

[24] Rindi, G., Grant, S. G., Yiangou, Y., Ghatei, M. A. et al., Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression. *Am. J. Pathol.* 1990, *136*, 1349–1363.

[25] Wallon, C., Braaf, Y., Wolving, M., Olaison, G. et al., Endoscopic biopsies in Ussing chambers evaluated for studies of macromolecular permeability in the human colon. *Scand. J. Gastroenterol.* 2005, *40*, 586–595.

[26] Wallon, C., Yang, P. C., Keita, A. V., Ericson, A. C. et al., Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. *Gut* 2008, *57*, 50–58.

[27] Soderholm, J. D., Hedman, L., Artursson, P., Franzén, L. et al., Integrity and metabolism of human ileal mucosa in vitro in the Ussing chamber. *Acta. Physiol. Scand.* 1998, *162*, 47–56.

[28] Wikman-Larhed, A., Co-cultures of human intestinal goblet (HT29-H) and absorptive (Caco-2) cells for studies of drug and peptide absorption. *Eur. J. Pharm. Sci.* 1995, *3*, 171–183.

[29] Hall, W. L., Millward, D. J., Rogers, P. J., Morgan, L. M. et al., Physiological mechanisms mediating aspartame-induced satiety. *Physiol. Behav.* 2003, *78*, 557–562.

[30] Saitoh, O., Hirano, A., Nishimura, Y., Intestinal STC-1 cells respond to five basic taste stimuli. *Neuroreport* 2007, *18*, 1991–1995.

[31] Sclafani, A., Glass, D. S., Margolskee, R. F., Glendinning, J. I. et al., Gut T1R3 sweet taste receptors do not mediate sucrose-conditioned flavor preferences in mice. *Am. J. Physiol.* 2010, *299*, R1643–R1650.

[32] Fujita, Y., Wideman, R. D., Speck, M., Asadi, A. et al., Incretin release from gut is acutely enhanced by sugar but not by sweeteners in vivo. *Am. J. Physiol. Endocrinol. Metab.* 2009, *296*, E473–E479.

[33] Anton, S. D., Martin, C. K., Han, H., Coulon, S. et al., Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite* 2010, *55*, 37–43.

[34] Steinert, R. E., Frey, F., Töpfer, A., Drewe, J. et al., Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. *Br. J. Nutr.* 2011, *105*, 1320–1328.

[35] Ford, H. E., Peters, V., Martin, N. M., Sleeth, M. L. et al., Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. *Eur. J. Clin. Nutr.* 2011, *65*, 508–513.

[36] Ma, J., Bellon, M., Wishart, J. M., Young, R. et al., Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2009, *296*, G735–G739.

[37] Brown, R. J., Walter, M., Rother, K. I., Ingestion of diet soda before a glucose load augments glucagon-like peptide-1 secretion. *Diabetes Care* 2009, *32*, 2184–2186.

[38] Zhang, Y., Kolli, T., Hivley, R., Jaber, L. et al., Characterization of the expression pattern of adrenergic receptors in rat taste buds. *Neuroscience* 2010, *169*, 1421–1437.

[39] Heath, T. P., Melichar, J. K., Nutt, D. J., Donaldson, L. F., Human taste thresholds are modulated by serotonin and noradrenaline. *J. Neurosci.* 2006, *26*, 12664–12671.

[40] Stricker-Krongrad, A., Max, J. P., Musse, N., Nicolas, J. P. et al., Increased threshold concentrations of neuropeptide Y for a stimulatory effect on food intake in obese Zucker rats—changes in the microstructure of the feeding behavior. *Brain Res.* 1994, *660*, 162–166.

[41] Lieverse, R. J., Masclee, A. A., Jansen, J. B., Lam, W. F. et al., Obese women are less sensitive for the satiety effects of bombesin than lean women. *Eur. J. Clin. Nutr.* 1998, *52*, 207–212.

[42] Choi, S., Lee, M., Shiu, A. L., Yo, S. J. et al., GPR93 activation by protein hydrolysate induces CCK transcription and secretion in STC-1 cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2007, *292*, G1366–G1375.

[43] Cordier-Bussat, M., Bernard, C., Levenez, F., Klages, N. et al., Peptones stimulate both the secretion of the incretin hormone glucagon-like peptide 1 and the transcription of the proglucagon gene. *Diabetes* 1998, *47*, 1038–1045.

[44] Geraedts, M. C., Troost, F. J., Saris, W. H., Peptide-YY is released by the intestinal cell line STC-1. *J. Food Sci.* 2009, *74*, H79–H82.

[45] Geraedts, M. C., Troost, F. J., De Ridder, R. J., Bodelier, A. G. et al., Validation of Ussing chamber technology to study satiety hormone release from human duodenal specimens. *Obesity* 2011, in press, DOI: 10.1038/oby.2011.104.

[46] Larsen, R., Mertz-Nielsen, A., Hansen, M. B., Poulsen, S. S. et al., Novel modified Ussing chamber for the study of absorption and secretion in human endoscopic biopsies. *Acta. Physiol. Scand.* 2001, *173*, 213–222.

[47] Grass, G. M., Sweetana, S. A., In vitro measurement of gastrointestinal tissue permeability using a new diffusion cell. *Pharm. Res.* 1988, *5*, 372–376.

[48] Reims, A., Strandvik, B., Sjovall, H., Epithelial electrical resistance as a measure of permeability changes in pediatric duodenal biopsies. *J. Pediatr. Gastroenterol. Nutr.* 2006, *43*, 619–623.

[49] Taylor, C. J., Baxter, P. S., Hardcastle, J., Hardcastle, P. T. et al., Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. *Gut* 1988, *29*, 957–962.

[50] Tominaga, M., Tsukada, H., Hosokawa, M., Nakamura, H. et al., ONO-1078 antagonizes diarrhea-causing changes in ion transport and smooth muscle contraction induced by peptidoleukotrienes in rat and human colon in vitro. *J. Pharmacol. Exp. Ther.* 1996, *278*, 1058–1063.

[51] Sternini, C., Anselmi, L., Rozengurt, E., Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. *Curr. Opin. Endocrinol. Diabetes Obes.* 2008, *15*, 73–78.

