Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects

Steve Bloom, Heather Ford, Veronique Peters, Niamh Martin, Michelle Sleeth, Mohammad Ghatei, Gary Frost

Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects

Heather E. Ford BSc*, Veronique Peters PhD*, Niamh M. Martin PhD, Michelle L. Sleeth BSc, Mohammad A. Ghatei PhD, Gary S. Frost PhD, Steve R. Bloom MD

Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Hammersmith Campus, Imperial College London, UK.

Running head (38 characters): sweet taste receptors and gut hormones

* These authors contributed equally to the project.

Correspondence and request for reprints to Stephen R Bloom, Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Faculty of Medicine, Hammersmith Hospital Campus, Imperial College London, 6th Floor, Commonwealth Building, Du Cane Road, London, W12 0NN

Telephone number: +44(0)208 383 3242

Fax number: +44(0)208 383 8320

Email address: s.bloom@imperial.ac.uk

This research is funded by program grants from the MRC (G7811974) and Wellcome Trust (072643/Z/03/Z) and by an EU FP6 Integrated Project Grant LSHM-CT-2003-503041. We are also grateful for support from the NIHR Biomedical Research Centre funding scheme. We thank Tate and Lyle for the provision of sucralose.

Abstract (234 words)

Background: The sweet taste receptor (T1r2+T1r3) is expressed by enteroendocrine L-cells throughout the gastrointestinal tract. Application of sucralose (a non-calorific, non-metabolisable sweetener) to L-cells *in vitro* stimulates glucagon-like peptide (GLP)-1 secretion, an effect that is inhibited with co-administration of a T1r2+T1r3 inhibitor.

Objective: We conducted a randomised, single-blinded, cross-over study in eight healthy subjects to investigate whether oral ingestion of sucralose could stimulate L-cell derived GLP-1 and peptide YY (PYY) release *in vivo*.

Methods: Fasted subjects were studied on four study days in random order. Subjects consumed 50ml of either water, sucralose (0.083% w/v), a non-sweet, glucose-polymer matched for sweetness with sucralose addition (50% w/v maltodextrin + 0.083% sucralose) or a modified sham feeding protocol (MSF = oral stimulation) of sucralose (0.083% w/v). Appetite ratings and plasma GLP-1, PYY, insulin and glucose were measured at regular time points for 120 minutes. At 120 minutes, energy intake at a buffet meal was measured.

Results: Sucralose ingestion did not increase plasma GLP-1 or PYY. MSF of sucralose did not elicit a cephalic phase response for insulin or GLP-1. Maltodextrin ingestion significantly increased insulin and glucose compared to water ($p<0.001$). Appetite ratings and energy intake were similar for all groups.

Conclusions: At this dose, oral ingestion of sucralose does not increase plasma GLP-1 or PYY concentrations and hence, does not reduce appetite in healthy subjects. Oral stimulation with sucralose had no effect on GLP-1, insulin, or appetite.

Keywords: obesity, sucralose, sweetener, gut hormone, appetite

Introduction

Recently, there have been significant advances in our understanding of how hormonal signals released from the gastrointestinal (GI) tract interact with circuits within the central nervous system to control appetite and energy intake (Murphy and Bloom, 2006). The gut hormones peptide YY (PYY) and glucagon-like peptide (GLP)-1 are co-secreted from intestinal enteroendocrine L-cells and released post-prandially in proportion to the amount of energy ingested (Adrian et al., 1985; Ghatei et al., 1983; Le Roux et al., 2006). PYY and GLP-1 have both been shown to be satiety factors, reducing food intake when administered to rodents (Batterham et al., 2002; Challis et al., 2003; Chelikani et al., 2005a; Chelikani et al., 2005b; Chelikani et al., 2006; Halatchev et al., 2004; Talsania et al., 2005) and to humans (Batterham et al., 2002; Degen et al., 2005; Flint et al., 1998; Gutzwiller et al., 1999; Le Roux et al., 2006). The incretin effect of GLP-1, augmentation of insulin secretion in response to an oral glucose load, has been well characterised (Elrick et al., 1964). Secretion of PYY and GLP-1 is regulated by a complex neuro-humoral system in addition to direct nutrient contact with specific receptors expressed by intestinal L-cells. However, the mechanisms by which luminal nutrients stimulate the release of GLP-1 and PYY from L-cells remain poorly understood.

The two proteins T1r2 and T1r3 form a heterodimer and function together as a general sweet-taste receptor (Li et al., 2002; Nelson et al., 2001). T1r2+T1r3 is coupled to the G-protein gustducin, which mediates transduction of sweet taste signals (Wong et al., 1996). T1r, and the alpha subunit of gustducin (α-gust), are co-localised with GLP-1 and PYY in enteroendocrine L-cells of the intestinal brush border membranes (Jang et al., 2007; Rozengurt et al., 2006; Sutherland et al., 2007). Recently, a key role for α-gust and a functioning sweet-taste receptor in glucose stimulated GLP-1 secretion from the L-cell has

been demonstrated (Jang et al., 2007). Application of sucralose (a non-calorific, non-metabolisable sweetener) to human L-cells *in vitro* stimulated GLP-1 secretion and this effect was inhibited with co-administration of a T1r3 inhibitor. This evidence supports a new signaling mechanism which regulates gut hormone secretion via the sweet-taste receptor T1r2+T1r3 in the GI tract.

One proposed factor in the increasing prevalence of obesity and type 2 diabetes is an increased consumption of processed foods containing high levels of sucrose and fructose (Bray et al., 2004; Elliott et al., 2002; Raben et al., 2002). To offset this, the food industry has attempted to replace sugars with artificial sweeteners. The ability of non-calorific sweeteners to enhance endogenous gut hormone release would represent a potentially exciting opportunity for their addition to foods as agents to control glucose homeostasis and appetite regulation in populations at risk of type 2 diabetes and obesity.

The aim of this study is to investigate whether oral ingestion of sucralose, at a dose that would be consumed in a normal diet, increases circulating GLP-1 or PYY concentrations in man.

Subjects and Methods

Subjects

Eight normal-weight, healthy volunteers were locally recruited. All were non-smokers, aged 22-27y (seven females and one male) with a stable body weight and a body mass index ranging from 18.8 to 23.9 kg/m^2. Persons who disliked the study food, who had food allergies or food restrictions, who were taking medication that was likely to affect taste, smell or appetite or who reported recent weight loss or weight cycling were excluded. Subjects were

70 screened using the standard Dutch Eating Behaviour questionnaire (Van Strien et al., 1986)
71 and SCOFF questionnaire (Morgan et al., 2000) and were excluded if they demonstrated
72 abnormal eating behaviour. Female volunteers attended all study days within the follicular
73 stage of the menstrual cycle.

74 The study was conducted with local ethical approval (project registration number:
75 07/Q0406/62). Written informed consent was obtained from all volunteers and the study was
76 performed in accordance with the Declaration of Helsinki.

77

78 *Study design*

79 The study had a randomised, single-blinded, cross-over design. Subjects were randomly
80 assigned to receive one of four solutions on four separate study sessions. Study sessions
81 lasted from 0830h until 1230h with at least three days between sessions. Subjects were asked
82 to refrain from drinking alcohol and to keep evening meals and activity levels as similar as
83 possible the day before each test session and to fast from 2100h, consuming only water.

84 On arrival at the study centre subjects were asked to be seated and to relax for 30 minutes
85 following placement of the intravenous cannula. After two baseline blood samples subjects
86 completed one of four experimental manipulations. Subjects ingested, in a single swallow,
87 50mL of either water (W), sucralose (S; 0.083% w/v, 2mmol/L Splenda®, Tate and Lyle
88 PLC, Southampton, UK) or the positive control maltodextrin (MD) which was matched for
89 sweetness with sucralose (50% w/v Polycose®, Abbott Laboratories Ltd, US, plus 0.083%
90 sucralose). Each was followed by a one minute period of modified-sham-feeding (MSF)
91 protocol of the same solution that was swallowed. The fourth experimental manipulation was
92 designed to ascertain the involvement of stimulation of the sweet-taste receptors within the

oral cavity independently of the sweet-taste receptors throughout the GI tract. In this instance subjects consumed 50mL water followed by the one-minute MSF of the sucralose solution (WS; 0.083% w/v sucralose). The test solutions used are described in Table 1. The MSF protocol involved drawing the solution up into the mouth through a straw, moving it around in the mouth and then spitting it out, doing so repeatedly until the entire volume of 200mL was finished and the one minute time limit was up. In order to investigate the cephalic effects of sucralose, the MSF was performed after ingestion of the test solution to ensure that no residual sucralose would be swallowed with the subsequent ingestion of water in the WS manipulation.

The dose of sucralose was chosen to represent a normal dietary load and the total volume ingested was kept to a minimum, as it is known that ingestion of large volumes of water alone can induce a gut hormone response (Christofides et al., 1979). Maltodextrin (a five polymer chain of glucose) was used to assess the effect of glucose on gut hormone release without the potential confounder of high concentrations of glucose effecting gastric emptying. The expectorate from the MSF was weighed to ensure compliance with the protocol. Blood samples and visual analogue scores (VAS) pertaining to subjective feelings of appetite, were taken for a further two hours. After the last blood sample at 120 minutes, a test meal of known energy content was given in excess and subjects were asked to eat until comfortably full. Energy intake was calculated from the weight of food eaten.

Blood samples

Two baseline blood samples were taken at -15 and 0 minutes before consumption of test solutions, then further samples were taken at 15, 30, 45, 60, 90 and 120 minutes after

consumption of test solutions. For analysis of the cephalic phase insulin release (CPIR) and the cephalic phase GLP-1 response, blood samples were also taken at 2, 4, 6, 8 and 10 minutes for insulin and GLP-1 analysis only. Blood was collected in lithium heparin tubes containing 5000 kallikrein units of aprotinin (200μL; Trasylol; Bayer) and immediately centrifuged at 4ºC. Plasma was separated and stored at -20ºC until analysis.

Appetite ratings

Subjective feelings of appetite were assessed at -15, 0, 15, 30, 45, 60, 90 and 120 minutes using VAS (Flint et al., 2000) with questions pertaining to desire to eat, hunger and prospective food consumption. Subjects were also asked to score the palatability and sweetness of the test solutions. Subjects marked their answers to the questions on scales of 100mm in length anchored at either end with the most positive and the most negative response. The distance along the scale that the subjects placed their mark was measured from one end and the reading in millimeters was recorded.

Biochemical analyses

All samples were assayed in duplicate and in a single assay to eliminate inter assay variation. Plasma PYY and GLP-1 were assessed using an established in-house radioimmunoassay (RIA) described previously (Adrian et al., 1985; Kreymann et al., 1987). The detection limit of the PYY and GLP-1 assays was 2.5pmol/L and 7.5pmol/L with an intra-assay coefficient variation (CV) of 5.8% and 5.4% respectively. Insulin was measured using Axsym analyser (Abbott Diagnostics, Maidenhead, UK). Sensitivity was 7 pmol/L with an intra-assay CV of

2.6%. Plasma glucose was measured using an Abbott Architect ci8200 analyser (Abbott Diagnostics, Maidenhead, UK). Sensitivity was 0.3mmol/L and intra-assay CV was 1%.

Statistics

All data are represented as mean values ± SEM. Plasma hormone and glucose concentrations were adjusted from baseline and represented as time course from change from baseline. Incremental area under the curve (iAUC) was calculated over baseline by the trapezoidal rule. Data for energy intake and iAUC were tested for normality and analysed using repeated measures one-way ANOVA with Bonferroni's test for *post hoc* comparisons (GraphPad Prism 4.03 Software, San Diego, USA). In all cases $P<0.05$ was considered to be statistically significant.

Results

Validation of MSF

After all test solutions, the expectorate weight was greater than the weight of the MSF solutions sipped due to the addition of saliva indicating a successful MSF with minimum swallowing of test solutions.

The water solution was significantly less sweet than the remaining three test solutions (70.9 ± 3 mm [WS], $P<0.001$; 65.9 ± 10.8 mm [S], $P<0.01$; 73.1 ± 8 mm [MD], $P<0.001$ *vs.* 5.9 ± 2 mm [W]; n=8). The WS, S and MD solutions were rated as having the same sweetness and palatability (42.8 ± 8.9 mm [WS]; 36.8 ± 10 mm [S]; 38.1 ± 8.5 mm [MD], 26.1 ± 6 mm [W]; n=8).

Appetite and food intake

For the two hour period following administration of the test solutions, there was no significant difference in the $iAUC_{(0\text{-}120min)}$ of subjective feelings of appetite (Table 2). Two hours after consumption of test solutions, there was no significant difference in energy intake or water intake at the buffet meal (Table 2).

Hormones and glucose

Plasma insulin and GLP-1 did not show any significant change during the first 10 min after the MSF of any solution (Table 2). $iAUC_{(0\text{-}120min)}$ for plasma GLP-1 and PYY concentrations were similar in all four groups. The MD group had a significantly higher $iAUC_{(0\text{-}120min)}$ of insulin and glucose concentrations compared with water, but there was no difference between any other solution tested (Figure 1 and Table 2).

Discussion

In the present study, we show that oral ingestion of a common dietary dose of the non-calorific, artificial sweetener sucralose does not increase plasma GLP-1 or PYY concentrations nor does it affect subjective feelings of appetite or energy intake at the next meal in healthy volunteers. This study mimics the physiological intake of a sweetened solution. Our data are in accord with recently published human data (Ma et al., 2009) and *in vivo* rat data (Fujita et al., 2009) in which sucralose ingestion failed to stimulate a rise in two circulating incretin hormones, GLP-1 and the K cell derived glucose-dependent

insulinotropic polypeptide (GIP). Ma *et al*. administered sucralose nasogastrically to healthy, normal weight volunteers and observed no effect on plasma GLP-1 or GIP concentrations (Ma et al., 2009). Similarly, Fujita *et al.* demonstrated in rats that in contrast to sucrose gavage, oral gavage of sucralose did not induce a rise in plasma GLP-1 (Fujita et al., 2009). The only other published study to investigate the acute effect of oral sweetener ingestion on gut hormone release in humans used the sweetener aspartame. Although, ingestion of encapsulated aspartame was associated with a reduction in subsequent food intake, this effect did not seem to be mediated by GLP-1 release (Hall et al., 2003). We chose not to encapsulate sucralose in our study, as it remains intact throughout the GI tract and very little is absorbed (Grice and Goldsmith, 2000). Therefore, sucralose may stimulate receptors on more distal L cells throughout the GI tract.

We did not observe a plasma GLP-1 response following ingestion of maltodextrin plus sucralose. GLP-1 response to glucose seems to be dependent on glucose been present in the distal duodenum where L-cells are present (Parker et al., 2010). In this experiment we used relatively low amount of a glucose polymer which is cleared efficiently in the proximal duodenum before it can elicit a gut hormone response.

Oral ingestion of two non-calorific sweeteners, sucralose plus acesulfame K, followed by an oral glucose tolerance test, produces higher plasma peak GLP-1 concentrations compared to ingestion of water followed by an oral glucose tolerance test in healthy normal weight subjects (Brown et al., 2009). Consistent with this sucralose plus glucose has an additive stimulatory effect on GLP-1 secretion from murine primary L-cells compared to sucralose or glucose alone (Reimann et al., 2008). Taken together these studies suggest that non-calorific sweeteners and sugars may act synergistically to stimulate GLP-1 release from L-cells. In the current study, the maltodextrin solution was matched for sweetness by addition of sucralose.

Therefore we cannot exclude a synergistic effect of maltodextrin and sucralose on plasma gut hormone concentrations, if a gut hormone response had occurred. It would be interesting to compare the effects of maltodextrin alone, without any match for sweetness, to the combination of maltodextrin plus sucralose to assess any additive effect of sucralose and maltodextrin on GLP-1 release.

Furthermore, we show that sucralose ingestion does not affect plasma glucose and insulin. This is consistent with previous human studies in which no effect on plasma glucose and insulin was observed following ingestion of encapsulated sucralose in diabetic patients (Grotz et al., 2003) or following intragastric infusion of sucralose in healthy subjects (Ma et al., 2009). Similarly, oral gavage of sucralose in rats did not improve glucose homeostasis following an intraperitoneal glucose tolerance test (Fujita et al., 2009), suggesting that there was no incretin effect mediated by the sucralose gavage.

Our study is the first to investigate the cephalic phase GLP-1 and insulin responses to sucralose. We demonstrate that stimulation of the oral cavity with a sucralose-sweetened solution does not lead to an early (0-10 min) increase in plasma GLP-1 and does not affect subsequent food intake. This is in keeping with previous studies which have not demonstrated a cephalic phase GLP-1 response to either ingestion of a mixed meal (Ahren and Holst, 2001) or to a sham fed meal (Luscombe-Marsh et al., 2009). Furthermore, we show that sucralose does not elicit a preabsorptive insulin response. This is consistent with previous studies using a similar MSF protocol to assess the ability of non-calorific sweeteners such as aspartame and saccharin to induce a CPIR in humans (Abdallah et al., 1997; Teff et al., 1995).

The concentration of sucralose used in the present study (2mmol/L) was chosen to be both palatable and within the dose range (1-5mmol/L) previously shown to trigger GLP-1 release from intestinal L-cells *in vitro* (Jang et al., 2007). However, with ensuing dilution in the gut

lumen post-ingestion, it is possible that the concentration of sucralose reaching the small intestine was below 2mmol/l, which may have been insufficient to stimulate GLP-1 secretion.

An alternative explanation for our findings is that sucralose does not stimulate GLP-1 release from intestinal enteroendocrine cells. In support of this, recent *in vitro* studies failed to demonstrate an effect of sucralose on GLP-1 (Reimann et al., 2008) from enteroendocrine cells using a similar sucralose concentration to that used in the current study. Furthermore, a recent *in vivo* study has shown that intragastric infusion of up to 40mmol/L sucralose does not induce GLP-1 secretion in humans (Ma et al., 2009). Together with our data, these studies suggest that there is no measurable acute enhancement of GLP-1 or PYY release *in vivo* following oral ingestion of sucralose. The reason for the apparent disparity of the effect of sucralose on gut hormone release *in vitro* and *in vivo* is not clear and requires further investigation.

In summary, we have shown that oral ingestion of sucralose does not elicit a cephalic phase GLP-1 or insulin response nor increase post-ingestive plasma GLP-1 or PYY concentrations, and therefore does not subsequently affect appetite. Our findings, using a dietary dose of sucralose, do not support the proposal that stimulation of the sweet taste receptor in the GI tract can stimulate release of GLP-1 and PYY from enteroendocrine L-cells.

Acknowledgements

We thank Mandy Donaldson and John Meek for glucose and insulin assays, the Sir John McMichael research centre for Clinical Investigation and Research, Hammersmith Hospital and the volunteers. V.P. is funded through a European Union framework 6 Marie Curie fellowship (NuSISCO). N.M.M. is funded by a HEFCE Clinical Senior Lecturer Award.

Contributions of the authors:

H.E.F. and V.P. designed the experiment, collected and analysed data and wrote the manuscript. N.M.M. helped with the writing of the manuscript. M.S. contributed to the data analysis. M.A.G, G.S.F and S.R.B. provided significant advice.

Conflict of interest:

The authors do not declare any conflict of interest.

Reference List

Abdallah,L., Chabert,M., and Louis-Sylvestre,J. (1997). Cephalic phase responses to sweet taste. Am. J. Clin. Nutr. *65*, 737-743.

Adrian,T.E., Ferri,G.L., Bacarese-Hamilton,A.J., Fuessl,H.S., Polak,J.M., and Bloom,S.R. (1985). Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology *89*, 1070-1077.

Ahren,B. and Holst,J.J. (2001). The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. Diabetes *50*, 1030-1038.

Batterham,R.L., Cowley,M.A., Small,C.J., Herzog,H., Cohen,M.A., Dakin,C.L., Wren,A.M., Brynes,A.E., Low,M.J., Ghatei,M.A., Cone,R.D., and Bloom,S.R. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. Nature *418*, 650-654.

Bray,G.A., Nielsen,S.J., and Popkin,B.M. (2004). Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am. J. Clin. Nutr. *79*, 537-543.

Brown,R.J., Walter,M., and Rother,K.I. (2009). Ingestion of Diet Soda before a Glucose Load Augments GLP-1 Secretion. Diabetes Care.

Challis,B.G., Pinnock,S.B., Coll,A.P., Carter,R.N., Dickson,S.L., and O'rahilly,S. (2003). Acute effects of PYY3-36 on food intake and hypothalamic neuropeptide expression in the mouse. Biochem. Biophys. Res. Commun. *311*, 915-919.

Chelikani,P.K., Haver,A.C., Reeve,J.R., Jr., Keire,D.A., and Reidelberger,R.D. (2006). Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats. Am. J. Physiol Regul. Integr. Comp Physiol *290*, R298-R305.

Chelikani,P.K., Haver,A.C., and Reidelberger,R.D. (2005a). Intravenous infusion of peptide YY(3-36) potently inhibits food intake in rats. Endocrinology *146*, 879-888.

Chelikani,P.K., Haver,A.C., and Reidelberger,R.D. (2005b). Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. Am. J. Physiol Regul. Integr. Comp Physiol *288*, R1695-R1706.

Christofides,N.D., Sarson,D.L., Albuquerque,R.H., Adrian,T.E., Ghatei,M.A., Modlin,I.M., and Bloom,S.R. (1979). Release of gastrointestinal hormones following an oral water load. Experientia *35*, 1521-1523.

Degen,L., Oesch,S., Casanova,M., Graf,S., Ketterer,S., Drewe,J., and Beglinger,C. (2005). Effect of peptide YY3-36 on food intake in humans. Gastroenterology *129*, 1430-1436.

Elliott,S.S., Keim,N.L., Stern,J.S., Teff,K., and Havel,P.J. (2002). Fructose, weight gain, and the insulin resistance syndrome. Am. J. Clin. Nutr. *76*, 911-922.

Elrick,H., Stimmler,L., Hlad,C.J., Jr., and Rai,Y. (1964). Plasma insulin response to oral and intravenous glucose administration. J. Clin. Endocrinol. Metab *24*, 1076-1082.

Flint,A., Raben,A., Astrup,A., and Holst,J.J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J. Clin. Invest *101*, 515-520.

Flint,A., Raben,A., Blundell,J.E., and Astrup,A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int. J. Obes. Relat Metab Disord. *24*, 38-48.

Fujita,Y., Wideman,R.D., Speck,M., Asadi,A., King,D.S., Webber,T.D., Haneda,M., and Kieffer,T.J. (2009). Incretin release from gut is acutely enhanced by sugar but not by sweeteners in vivo. Am. J. Physiol Endocrinol. Metab *296*, E473-E479.

Ghatei,M.A., Uttenthal,L.O., Christofides,N.D., Bryant,M.G., and Bloom,S.R. (1983). Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. J. Clin. Endocrinol. Metab *57*, 488-495.

Grice,H.C. and Goldsmith,L.A. (2000). Sucralose--an overview of the toxicity data. Food Chem. Toxicol. *38 Suppl 2*, S1-S6.

Grotz,V.L., Henry,R.R., McGill,J.B., Prince,M.J., Shamoon,H., Trout,J.R., and Pi-Sunyer,F.X. (2003). Lack of effect of sucralose on glucose homeostasis in subjects with type 2 diabetes. J. Am. Diet. Assoc. *103*, 1607-1612.

Gutzwiller,J.P., Goke,B., Drewe,J., Hildebrand,P., Ketterer,S., Handschin,D., Winterhalder,R., Conen,D., and Beglinger,C. (1999). Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut *44*, 81-86.

Halatchev,I.G., Ellacott,K.L., Fan,W., and Cone,R.D. (2004). Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. Endocrinology *145*, 2585-2590.

Hall,W.L., Millward,D.J., Rogers,P.J., and Morgan,L.M. (2003). Physiological mechanisms mediating aspartame-induced satiety. Physiol Behav. *78*, 557-562.

Jang,H.J., Kokrashvili,Z., Theodorakis,M.J., Carlson,O.D., Kim,B.J., Zhou,J., Kim,H.H., Xu,X., Chan,S.L., Juhaszova,M., Bernier,M., Mosinger,B., Margolskee,R.F., and Egan,J.M. (2007). Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proc. Natl. Acad. Sci. U. S. A *104*, 15069-15074.

Kreymann,B., Williams,G., Ghatei,M.A., and Bloom,S.R. (1987). Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet *2*, 1300-1304.

Le Roux,C.W., Batterham,R.L., Aylwin,S.J., Patterson,M., Borg,C.M., Wynne,K.J., Kent,A., Vincent,R.P., Gardiner,J., Ghatei,M.A., and Bloom,S.R. (2006). Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology *147*, 3-8.

Li,X., Staszewski,L., Xu,H., Durick,K., Zoller,M., and Adler,E. (2002). Human receptors for sweet and umami taste. Proc. Natl. Acad. Sci. U. S. A *99*, 4692-4696.

Luscombe-Marsh,N.D., Smeets,A.J., and Westerterp-Plantenga,M.S. (2009). The addition of monosodium glutamate and inosine monophosphate-5 to high-protein meals: effects on satiety, and energy and macronutrient intakes. Br. J. Nutr. *102*, 929-937.

Ma,J., Bellon,M., Wishart,J.M., Young,R., Blackshaw,L.A., Jones,K.L., Horowitz,M., and Rayner,C.K. (2009). Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. Am. J. Physiol Gastrointest. Liver Physiol *296*, G735-G739.

Morgan,J.F., Reid,F., and Lacey,J.H. (2000). The SCOFF questionnaire: a new screening tool for eating disorders. West J. Med. *172*, 164-165.

Murphy,K.G. and Bloom,S.R. (2006). Gut hormones and the regulation of energy homeostasis. Nature *444*, 854-859.

Nelson,G., Hoon,M.A., Chandrashekar,J., Zhang,Y., Ryba,N.J., and Zuker,C.S. (2001). Mammalian sweet taste receptors. Cell *106*, 381-390.

Parker,H.E., Reimann,F., and Gribble,F.M. (2010). Molecular mechanisms underlying nutrient-stimulated incretin secretion. Expert. Rev. Mol. Med. *12*, e1.

Raben,A., Vasilaras,T.H., Moller,A.C., and Astrup,A. (2002). 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. *76*, 721-729.

Reimann,F., Habib,A.M., Tolhurst,G., Parker,H.E., Rogers,G.J., and Gribble,F.M. (2008). Glucose sensing in L cells: a primary cell study. Cell Metab *8*, 532-539.

Rozengurt,N., Wu,S.V., Chen,M.C., Huang,C., Sternini,C., and Rozengurt,E. (2006). Colocalization of the alpha-subunit of gustducin with PYY and GLP-1 in L cells of human colon. Am. J. Physiol Gastrointest. Liver Physiol *291*, G792-G802.

Sutherland,K., Young,R.L., Cooper,N.J., Horowitz,M., and Blackshaw,L.A. (2007). Phenotypic characterization of taste cells of the mouse small intestine. Am. J. Physiol Gastrointest. Liver Physiol *292*, G1420-G1428.

Talsania,T., Anini,Y., Siu,S., Drucker,D.J., and Brubaker,P.L. (2005). Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. Endocrinology *146*, 3748-3756.

Teff,K.L., Devine,J., and Engelman,K. (1995). Sweet taste: effect on cephalic phase insulin release in men. Physiol Behav. *57*, 1089-1095.

Van Strien,T., Rookus,M.A., Bergers,G.P., Frijters,J.E., and Defares,P.B. (1986). Life events, emotional eating and change in body mass index. Int. J. Obes. *10*, 29-35.

Wong,G.T., Gannon,K.S., and Margolskee,R.F. (1996). Transduction of bitter and sweet taste by gustducin. Nature *381*, 796-800.

Table 1. Description of the test solutions used on the four study days.

Study day	**Test solution ingested** (Volume=50ml)	**Test solution used for the one-minute MSF** (Volume=200ml)
water (W)	water	water
cephalic sucralose (WS)	water	sucralose dissolved in water
sucralose (S)	sucralose dissolved in water	sucralose dissolved in water
maltodextrin+sucralose (MD)	maltodextrin plus sucralose dissolved in water	maltodextrin plus sucralose dissolved in water

Table 2. Incremental AUC data for plasma hormones, glucose and appetite scores measured between 0 and 120 minutes (unless specified) and energy and water intake at the buffet meal.

	W	**WS**	**S**	**MD**
Insulin$_{(0\text{-}10min)}$ (pmol.min/L)	171 ± 60	235 ± 83	70 ± 25	68 ± 24
GLP-1$_{(0\text{-}10min)}$ (pmol.min/L)	882 ± 113	948 ± 171	922 ± 132	658 ± 78
Glucose (mmol.min/L)	33.5 ± 6.9**a**	51 ± 12.5**b**	25.3 ± 20.4**c**	122.3 ± 17.5
Insulin (pmol.min/L)	287 ± 331**c**	-471 ± 132**c**	-459 ± 352**c**	5669 ± 519
GLP-1 (pmol.min/L)	-675 ± 1610	-248 ± 784	-359 ± 401	415 ± 610
PYY (pmol.min/L)	-179 ± 119	-128 ± 119	-56 ± 192	283 ± 185
Hunger (mm.min)	1724 ± 322	1641 ± 336	1993 ± 199	2017 ± 472
Desire to eat (mm.min)	1376 ± 216	1128 ± 275	1330 ± 458	1441 ± 461
Prospective food consumption (mm.min)	1318 ± 305	1623 ± 266	2002 ± 247	1676 ± 441
Energy intake (kJ)	2355±227	2417±222	2597±277	2460±167
Water intake (mL)	267.0±69.0	250.7±45.1	291.8±49.8	305.0±49.6

W=water, WS= cephalic sucralose, S=sucralose, MD=maltodextrin+sucralose. Data are represented as mean ± SEM. **a** = $p<0.05$ compared to MD, **b** = $p<0.01$ compared to MD, **c** = $p<0.001$ compared to MD. n=8.

Figure legends

Figure 1. Change in plasma A) GLP-1, B) PYY, C) insulin and D) glucose from baseline following administration of test solutions (n=8). ●=water, ○=cephalic sucralose, ■=sucralose, □=maltodextrin+sucralose. Data are represented as mean ± SEM.

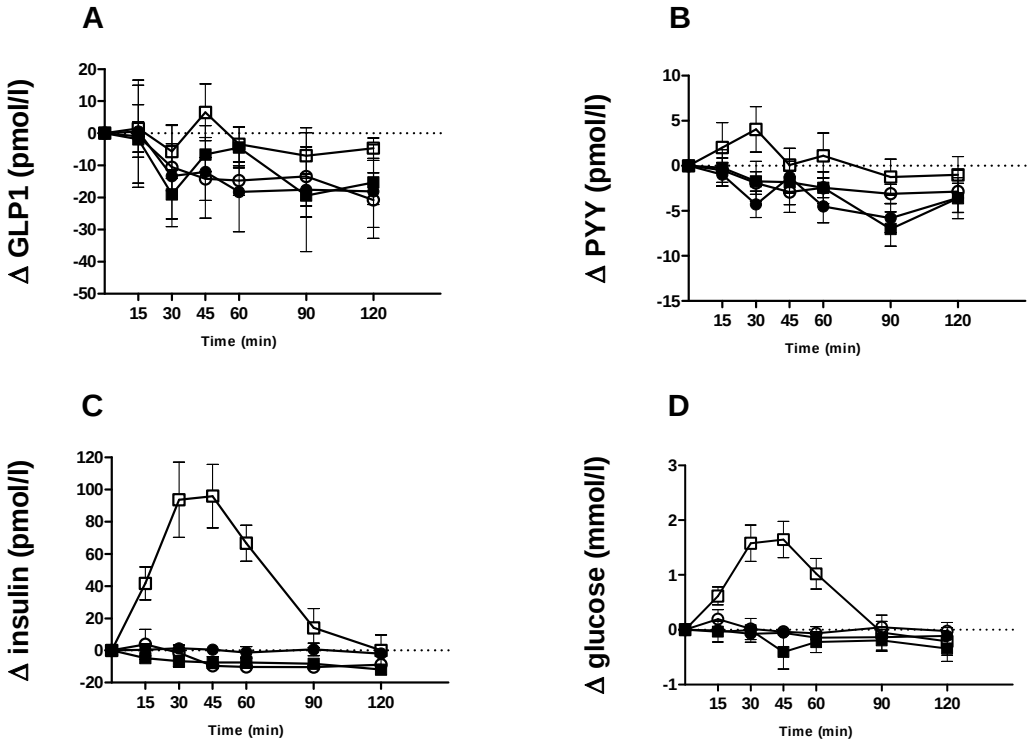
A
Δ GLP1 (pmol/l)
20
10
0
-10
-20
-30
-40
-50
15 30 45 60 90 120
Time (min)
B
Δ PYY (pmol/l)
10
5
0
-5
-10
-15
15 30 45 60 90 120
Time (min)
C
Δ insulin (pmol/l)
120
100
80
60
40
20
0
-20
15 30 45 60 90 120
Time (min)
D
Δ glucose (mmol/l)
3
2
1
0
-1
15 30 45 60 90 120
Time (min)

Figure 1