Disordered control of intestinal sweet taste receptor expression and glucose absorption in type 2 diabetes

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**Abbreviations:**

T1R2, T1R3 - G-protein-coupled taste receptor family 1

α-gustducin - alpha subunit of taste G-protein gustducin

TRPM5 - transient receptor potential ion channel M5

STR - sweet taste receptor

3-OMG - 3-O-methyl glucose

5-HT - 5-hydroxytryptamine

GLP-1 - glucagon-like peptide-1

GIP - glucose-dependent insulinotropic polypeptide-

BMI - body mass index

iAUC - incremental area under the curve

HbA1c - glycated hemoglobin

RT-PCR - real time reverse transcription polymerase chain reaction

PBS-T - phosphate-buffered saline + 0.2% Triton X-100
Abstract

We previously established that the intestinal sweet taste receptors (STRs), T1R2 and T1R3, were expressed in distinct epithelial cells in the human proximal intestine, and that their transcript levels varied with glycemic status in patients with type 2 diabetes. Here we determined whether STR expression was (i) acutely regulated by changes in luminal and systemic glucose levels, (ii) disordered in type 2 diabetes, and (iii) linked to glucose absorption. Fourteen healthy subjects and 13 patients with type 2 diabetes were studied twice, at euglycemia (5.2 ± 0.2 mmol/L) or hyperglycemia (12.3 ± 0.2 mmol/L). Endoscopic biopsies were collected from the duodenum at baseline and after a 30 min intraduodenal glucose infusion (30 g/150 ml water plus 3 g 3-O-methylglucose, 3-OMG). STR transcripts were quantified by RT-PCR and plasma assayed for 3-OMG concentration. Intestinal STR transcript levels at baseline were unaffected by acute variations in glycemia in healthy subjects and type 2 patients. T1R2 transcript levels increased after luminal glucose infusion in both groups during euglycemia (+5.8 × 10^4 and +5.8 × 10^4 copies, respectively), but decreased in healthy subjects during hyperglycemia (-1.4 × 10^4 copies). T1R2 levels increased significantly in type 2 patients under the same conditions (+6.9 × 10^5 copies). Plasma 3-OMG concentrations were significantly higher in type 2 patients than healthy controls during acute hyperglycemia. Intestinal T1R2 expression is reciprocally regulated by luminal glucose in health according to glycemic status, but is disordered in type 2 diabetes during acute hyperglycemia. This defect may enhance glucose absorption in type 2 patients and exacerbate postprandial hyperglycemia.
Introduction
Glucose in the small intestinal lumen induces feedback that regulates gastric emptying, absorptive function and energy intake (1-3), mediated both by vagal nerve pathways and secretion of gut peptides (4), including glucose-dependent insulinotropic polypeptide (GIP) from enteroendocrine K-cells, and glucagon-like peptide 1 (GLP-1) from L-cells. These ‘incretins’ substantially augment insulin secretion when glucose is given orally, when compared to an isoglycemic intravenous infusion (5). Both the rate of gastric emptying and the secretion and action of the incretin hormones are key determinants of postprandial glycemia. However, the precise mechanism of glucose detection in the small intestine remains unclear.

Lingual sweet taste cells possess two G-protein coupled receptors, T1R2 and T1R3, which form a heterodimeric sweet taste receptor (STR) for sugars, D-amino acids, sweet proteins and artificial sweeteners (6, 7). T1R2/R3 activation liberates the alpha-subunit of the G-protein gustducin (α-gustducin), leading to intracellular Ca$^{2+}$ release, gating of a taste-specific transient receptor potential ion channel TRPM5 (8) cellular depolarisation, and release of mediators that activate lingual afferent nerves.

We, and others, have shown that STRs, α-gustducin and TRPM5 are also expressed with cellular and regional specificity in the animal and human intestine where they may serve as glucose sensors (4, 9-13). In addition to expression in intestinal sweet taste cells, some of these taste components are also expressed in separate intestinal cell populations that detect umami (T1R3, α-gustducin, TRPM5), bitter and fats (α-gustducin, TRPM5) (4). STR activation may be linked to gut hormone secretion, since mice deficient in T1R3 or α-gustducin exhibit defective glucose-induced GLP-1 release (14) while the STR blocker, lactisole, decreases GLP-1 secretion and increases glycemic excursions after intragastric or intraduodenal glucose infusion in humans (15, 16). Animal studies also indicate that STR activation increases the availability and function of the primary intestinal glucose transporter, sodium-glucose cotransporter-1 (SGLT-1) (17, 18), although this link has not been assessed directly in humans.

Patients with type 2 diabetes frequently demonstrate disordered gastrointestinal responses to nutrients, with delayed gastric emptying in up to 30-50%, and abnormally rapid emptying in a few (19, 20) and a high prevalence of gastrointestinal symptoms (21). GLP-1 and GIP secretion has been inconsistently reported to be diminished in type 2 patients (22, 23), while intestinal levels of SGLT-1, and the capacity for glucose absorption, may be increased (24). Any of these
abnormalities could potentially relate to disordered intestinal sensing of glucose. We previously reported that duodenal expression of STRs during fasting was comparable in unselected patients with type 2 diabetes and non-diabetic controls, but was inversely related to the blood glucose concentration at the time of biopsy in type 2 patients (13). In rodents, we and others have also shown that intestinal STR transcript and protein levels are rapidly downregulated upon acute luminal exposure to glucose or artificial sweeteners (13, 25). Our current aims were, therefore, to evaluate the modulation of duodenal STR expression in response to acute changes in luminal and systemic glucose exposure in healthy humans, and to determine whether STR regulation is disordered in type 2 diabetes, and related to changes in glucose absorption and/or gut hormone secretion.
Methods

Subjects
Fourteen healthy subjects and 13 patients with type 2 diabetes were studied in randomised, cross-over fashion. The mean duration of known diabetes in the latter group was 5 ± 1 y, glycated haemoglobin (HbA1c) was 6.3 ± 0.2% (45 ± 2 mmol/mol) and all were free of significant comorbidities and managed by diet alone. The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital and conducted in accordance with the Declaration of Helsinki as revised in 2000. Each subject provided written informed consent.

Screening visit
Each subject attended the laboratory at 0830 h after an overnight fast (12 h for solids, 10 h for liquids). An intravenous cannula was inserted for blood sampling, and subjects consumed a glucose drink (75 g glucose dissolved in water to 300 mL, labelled with 150 mg $^{13}\text{C}$ acetate) within 5 min (T = -5 to 0 min). Blood was sampled at T = -5, 30, 60, 120, 180 min to measure blood glucose by glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA). Breath samples were collected before, and every 5 min after, oral glucose during the first hour, and every 15 min for a further 2 h to measure $^{13}\text{CO}_2$ concentrations by isotope ratio mass spectrometer (ABCA 2020; Europa Scientific, Crewe, UK). The gastric half-emptying time was calculated using the formula of Ghoos et al (26). Gastrointestinal symptoms were assessed by a standard questionnaire (maximum score 27), as previously (27). Autonomic nerve function was assessed in the type 2 patients using standardized cardiovascular reflex tests, with a score $\geq$ 3 (out of a maximum of 6) indicating autonomic dysfunction (28).

Endoscopy Protocol
After the screening visit each subject was studied twice, separated by at least a week, with female subjects studied exclusively during the follicular phrase of the menstrual cycle to limit variations in gut hormone concentrations (29). Subjects attended the laboratory at 0830 h following an overnight fast, and an insulin/glucose clamp was established to achieve euglycemia (~5 mmol/L) or hyperglycemia (~12 mmol/L) (30). A 50 mL iv bolus of 25% glucose (Baxter Healthcare, Old Toongabbie, NSW, Australia) was administered on the hyperglycemic day, and 0.9% saline (Baxter Healthcare) on the euglycemic day, over 1 min each, followed by continuous infusion of the same solution starting at 150 mL/h and adjusted according to blood glucose measurements every 5 minutes on the hyperglycemic day, or remaining at 150 mL/h on the euglycemic day. On the euglycemic day, 25% dextrose was infused iv if the blood glucose concentration fell below 5 mmol/L. In addition, 100 IU of insulin (Actrapid; Novo Nordisk, Baulkham Hills, NSW,
Australia), in 500 mL 4% succinylated gelatin solution (Gelofusine; B. Braun Australia, Bella Vista, NSW, Australia), was infused iv at a variable rate to maintain euglycemia. Once blood glucose concentrations were stable for 30 min (12.3 ± 0.2 mmol/L on the hyperglycemic day, 5.2 ± 0.2 mmol/L on the euglycemic day) a small diameter video endoscope (GIF-XP160, Olympus, Tokyo, Japan) was passed via an anesthetised nostril into the second part of the duodenum, from which mucosal biopsies were collected using standard biopsy forceps, and placed into either RNAlater (Qiagen, Sydney, NSW, Australia) or 4% paraformaldehyde for 2 h. At T = 0 an intraduodenal infusion containing 30 g glucose and 3 g of the glucose absorption marker 3-O-methyglucose (3-OMG, Sigma-Aldrich, St Louis, MO, USA) was commenced via the biopsy channel of the endoscope, and continued for 30 min (1 g/min; 4 kcal/min). At T = 10 and T = 30 min additional biopsies to be collected. Blood samples (20 mL) were taken every 10 min over 1 h to determine concentrations of 3-OMG, C-peptide, GLP-1 and GIP.

**Assays**

Plasma total GLP-1 concentrations were measured by radioimmunoassay (GLPIT-36HK; Millipore, Billerica, MA) with sensitivity of 3 pmol/L and intra- and inter-assay coefficients of variation (CV) of 4.2% and 10.5%. Total plasma GIP was measured by RIA as previously, with sensitivity of 2 pmol/L and intra- and inter-assay CV of 6.1% and 15.4%, respectively (31). Plasma C-peptide concentrations were measured by ELISA (10-1136-01, Mercodia, Uppsala, Sweden), with sensitivity of 15 pmol/L, and intra- and inter-assay CV of 3.6% and 3.3%. Serum 3-OMG concentrations were measured by liquid chromatography and mass spectrometry with sensitivity of 10 pmol/L (32).

**Quantification of gene expression by real time RT-PCR**

RNA was extracted from tissues using an RNeasy Mini kit (Qiagen) following manufacturer instructions, and RNA yield and quality determined using a NanoDrop (NanoDrop Technologies, Wilmington, DE). Quantitative real time reverse transcriptase PCR (RT-PCR) was then used to determine the absolute expression of sweet taste molecules. Validated human primers for T1R2, α-gustducin and TRPM5 were used as primer assays (QuantiTect, Qiagen), while T1R3 primers were designed using Primer 3.0 software (Applied Biosystems, Foster City, CA) based on target sequences obtained from the NCBI nucleotide database (Table 1). Absolute standard curves were generated by including known copy number standards in RT-PCR for each target (Table 2), as described (13). RT-PCR was performed on a Chromo4 (MJ Research, Waltham, MA, USA) real time instrument attached to a PTC-200 Peltierthermal cycler (MJ Research) using a QuantiTect SYBR Green one-step RT-PCR kit (Qiagen) according to the manufacturer’s specifications, as
previously (13). Each assay was performed in triplicate and included internal no-template and no-RT controls. All replicates were averaged for final mRNA copy number, which was expressed as copies per 50 ng of total RNA.

**Immunohistochemistry**

Fixed tissues were cryoprotected (30% sucrose in phosphate-buffered saline), embedded in cryomolds, and frozen, before sectioning at 6-10 µm (Cryocut 1800, Leica Biosystems, Nussloch, Germany) and thaw-mounting onto gelatin-coated slides. Immunoreactivity was detected using rabbit T1R2 primary (H90, 1:400, SC-50305, Santa Cruz Biotechnology, CA, USA), goat GLP-1 primary (1:400, SC-7782, Santa Cruz), and monoclonal 5-HT (1:1000, M0758, Dako Australia, Victoria, Australia) and GIP primary antibodies (1:800, AB30679, Abcam). All were visualised using species-specific secondary antibodies conjugated to Alexa Fluor dyes (1:200 in PBST) as previously described (12, 13). Antigen retrieval (S1700, Dako) was performed for T1R2 according to manufacturer instructions. Nucleated epithelial cells immunopositive for individual targets were counted per square millimetre of high power field and averaged over at least 10 intact transverse sections per subject.

**Data analysis**

The incremental area under the curve (iAUC) for 3-OMG, GLP-1 and GIP concentrations was calculated using the trapezoidal rule (33) and analysed by one-factor ANOVA using Prism software (version 6.0; Graphpad, La Jolla, CA, USA). These variables were also assessed using repeated-measures ANOVA, with treatment and time as factors. Post hoc comparisons, adjusted for multiple comparisons by Holm-Sidak’s correction, were performed if ANOVAs showed significant effects. One-way ANOVA, with Holm-Sidak’s post hoc test, was used to compare differences in duodenal levels of STR transcripts between healthy subjects and type 2 diabetic patients. Relationships between transcript expression and other factors were evaluated by Pearson correlation coefficient (r). Twelve subjects were calculated to have 80% power to detect a one third difference in duodenal T1R2 expression in paired studies (α = 0.05), compared to control (13). P values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM.
Results

All subjects tolerated the study well. The patients with type 2 diabetes were older than the healthy subjects, but gastrointestinal symptom scores, BMI and gastric emptying of glucose did not differ (Table 3). Five type 2 patients had autonomic dysfunction, but none had evidence of peripheral neuropathy, nephropathy, retinopathy, or macro-vascular complications. As expected, blood glucose concentrations were higher in type 2 patients during fasting and after oral glucose (P < 0.05, Figure 1A).

Baseline STR expression

Transcripts for T1R2, T1R3, α-gustducin and TRPM5 were readily detected in duodenal biopsies by quantitative RT-PCR. TRPM5 was the most abundant STR transcript in all subjects, with lower levels of α-gustducin and much lower levels of T1R2 and T1R3; T1R2 was the least expressed transcript (Figure 2A). TRPM5 transcript levels in healthy subjects during euglycemia were 34 ± 8 fold higher than those of T1R2 (P < 0.001), while α-gustducin levels were 22 ± 7 fold higher (P < 0.05) and T1R3 levels 12 ± 5 fold higher.

Effects of acute changes in glycemia on STR expression

Fasting expression of STR transcripts was unaffected by glycemic state in either health or type 2 diabetes, and did not differ between the groups (Figure 2B-E).

Effects of luminal glucose on duodenal STR expression

Due to inter-subject variability in STR expression, responses to luminal glucose were evaluated as changes from baseline. During euglycemia, T1R2 transcript levels increased in response to duodenal glucose infusion in health and type 2 diabetes after 30 min (+5.9 × 10^4 and +5.8 × 10^4 copies, Figure 3A). During hyperglycemia, T1R2 transcript levels decreased in healthy subjects after 30 min (-1.4 × 10^4 copies), but increased in type 2 patients (+6.9 × 10^5 copies), so that levels in health were lower at 30 min during hyperglycemia than euglycemia and lower than in type 2 patients during either glycemic state (subject × time interactions; P < 0.01 for each). Levels of T1R3, α-gustducin and TRPM5 transcript, in contrast, did not significantly change in response to luminal glucose under either glycemic condition (Figure 3B-D).

Plasma hormone concentrations

Fasting plasma GLP-1 concentrations did not differ between health and type 2 diabetes, and were not acutely affected by the glycemic state. Plasma GLP-1 increased in response to duodenal glucose infusion in all groups (Figure 1C; P < 0.001), with higher concentrations evident in type 2 patients
at 40 min irrespective of glycemic status (subject × time interactions; P < 0.01), and at 50 min during euglycemia, compared to healthy subjects (subject × time interactions; P < 0.05). The iAUC for GLP-1 was higher in type 2 patients during euglycemia and hyperglycemia, when compared to healthy subjects (Table 4; P < 0.05, each).

Fasting plasma GIP concentrations did not differ between healthy subjects and type 2 patients, and were not acutely affected by the glycemic state. Plasma GIP increased in response to duodenal glucose infusion in both groups (Figure 1D; P < 0.001), with higher GIP concentrations evident in type 2 diabetes patients at 40 min irrespective of glycemic status, and higher concentrations during euglycemia at 20 and 50 min, compared to healthy subjects (subject × time interaction; P < 0.05). The iAUC was higher in type 2 patients during euglycemia in comparison to healthy subjects (Table 4; P < 0.05).

Fasting C-peptide concentrations were higher during hyperglycemia than during euglycemia in healthy subjects (P < 0.001, Figure 1E), but not in type 2 patients. C-peptide concentrations increased in response to duodenal glucose infusion in both subject groups during hyperglycemia (subject × time interaction; P < 0.05 and iAUC P < 0.001), but not during euglycemia (Figure 1E, Table 4). C-peptide concentrations during hyperglycemia were higher in healthy subjects than in type 2 patients throughout the glucose infusion (subject × time interaction; P < 0.05 and iAUC P < 0.001).

**Serum 3-OMG concentrations**

Serum 3-OMG concentrations increased over time in all groups, but were higher at 60 min in type 2 patients during hyperglycemia than in any other group (subject × time interaction; P < 0.001, Figure 1F). The iAUC for 3-OMG was higher in both type 2 patients and healthy subjects during hyperglycemia than euglycemia (Table 4; P < 0.05).

**Phenotype of human intestinal sweet taste cells**

Immunolabelling for T1R2 was evident in single cells dispersed throughout the mucosal epithelium in healthy subjects and type 2 patients (Figure 4). Immunopositive cells showed a homogenous distribution of label throughout the cytoplasm, were largely open or ‘flask’ shaped and found with equal frequency within villi or crypts. In dual labelling experiments in healthy subjects, 19 ± 11% of T1R2 labelled duodenal cells co-expressed GLP-1, while 13 ± 8% of L-cells co-expressed T1R2 (Figure 4A). In a similar manner, 15 ± 10% of T1R2 labelled duodenal cells co-expressed GIP, while 12 ± 8% of K-cells co-expressed T1R2 (Figure 4B). Separate populations of T1R2 labelled
cells co-expressed 5-HT (31 ± 6%), while 5 ± 1% of EC-cells co-expressed T1R2 in healthy subjects (Figure 4C). During fasting, an equivalent number of T1R2 immunopositive cells were evident in healthy subjects and type 2 patients, under euglycemia or hyperglycemia, and the number did not change during duodenal glucose infusion. Similarly, the proportion of cells immunopositive for GLP-1, GIP and 5-HT did not differ between healthy subjects and type 2 patients, or with glycemic state or exposure to luminal glucose, although a trend for increased L-cells in fasting type 2 patients was evident (P = 0.07, data not shown).

Relationships between variables
Absolute copy numbers of STR transcripts during fasting, and after 30 min glucose infusion, did not correlate with age, gender, BMI, symptom score or gastric half-emptying time in either subject group, and in type 2 patients, they were not related to duration of diabetes, HbA1c, autonomic dysfunction, or symptom score. In contrast, the change in T1R2 transcript level after luminal glucose exposure correlated with the iAUC for 3-OMG in healthy subjects during euglycemia (r = 0.73, P < 0.05), and the change in TRPM5 transcript level with plasma GLP-1 concentrations at 30 min (r = 0.62, P < 0.05) in the same group. Changes in T1R2 (r = 0.78, P < 0.01) and T1R3 transcript levels (r = 0.59, P < 0.05) in type 2 patients during hyperglycemia also correlated with plasma GIP concentrations at 30 min, and the change in T1R2 correlated with the iAUC for GIP (r = 0.69, P = 0.03).

Discussion
This study is the first to define changes in expression of intestinal STR transcripts in healthy humans and patients with type 2 diabetes, in response to acute changes in systemic and luminal glucose. We have shown that absolute levels of STR transcripts are unaffected by acute variations in glycemia during fasting in either group, but that T1R2 expression increases upon exposure to luminal glucose during euglycemia. In contrast, T1R2 expression decreases markedly in response to luminal glucose during hyperglycemia in health, but in type 2 diabetes increases under the same conditions. Type 2 patients also exhibit increased glucose absorption during acute hyperglycemia compared to healthy subjects, suggesting that dysregulated expression of intestinal STRs can perpetuate postprandial hyperglycemia in this group.

We confirmed our previous observation that fasting STR transcript levels are similar in health and type 2 diabetes irrespective of age, gender or BMI (13). While we previously observed that levels of STR transcript were inversely related to fasting blood glucose concentrations in unselected type 2 patients presenting for endoscopy, we have now established unequivocally that acute changes in
glycemia do not influence fasting intestinal STR expression in either health or ‘well-controlled’ type 2 diabetes. The apparent discrepancy in these observations may reflect the effects of more longstanding hyperglycemia or differences in the duration of fasting in the earlier cross-sectional study. We have now shown that the intestinal STR system is, in contrast, highly responsive to the presence of luminal glucose, with rapid, and reciprocal, regulation of T1R2 transcripts in health, depending on the prevailing blood glucose concentration. Comparable changes were evident in T1R3 and TRPM5 transcript levels, although these were not statistically significant. Increased inter-subject variability seen for T1R3 and TRPM5 transcript levels may be due to their expression in additional populations of intestinal cells tuned to detect other taste modalities and, therefore, unresponsive to luminal and/or systemic glucose.

Healthy subjects that displayed the largest glucose-induced increase in duodenal T1R2 transcript levels during euglycemia had the highest plasma concentrations of the glucose absorption marker 3-OMG. As SGLT-1 is responsible for the active transport of luminal 3-OMG, our findings support a role of intestinal T1R2 signals in the regulation of glucose absorption via SGLT-1. Indeed, intestinal STR activation has been shown to upregulate SGLT-1 transcript, apical protein and function in a number of species (17, 18). Accordingly, reciprocal regulation of T1R2 in human health may increase SGLT-1 function at euglycemia to facilitate glucose absorption, and reduce SGLT-1 function during hyperglycemia to limit postprandial glycemic excursion. However, despite a reduction in T1R2 transcript after luminal glucose exposure during hyperglycemia, our healthy subjects still displayed greater rates of glucose absorption than during euglycemia, which might be accounted for by changes in SGLT-1 lagging behind those in T1R2. Our finding that plasma 3-OMG concentrations were elevated in type 2 patients during hyperglycemia is in keeping with the concept that SGLT-1 transporter capacity was maintained, or increased, in the presence of luminal glucose under these conditions. In fact, even small changes in SGLT-1 may increase this risk, as type 2 patients are reported to have up to 4-fold higher levels of transcript, protein and function of this transporter at baseline compared to healthy controls (24). It should be noted that an increased level of facilitated glucose transport via the basolateral glucose transporter GLUT2 may contribute to plasma levels of 3-OMG in the current study, however the role of STR signals to direct the apical insertion of GLUT2 in enterocytes appears to be limited to rodents (25, 34).

The link between STR stimulation and incretin hormone release in healthy humans is not clear. Most in vivo studies indicate that acute administration of non-nutritive sweeteners does not trigger incretin secretion in either humans or rodents (35-37). Nonetheless, we observed that subsets of duodenal L-cells, K-cells and EC-cells were immunopositive for T1R2, in accord with previous
reports (4, 12, 14). Together with positive associations between luminal glucose-induced changes in some STR transcripts and measures of GLP-1 and GIP secretion in the current study, it remains possible that STRs do have a regulatory role in gut hormone release. The inhibition of glucose-induced GLP-1 secretion in healthy humans by the STR blocker, lactisole (15), supports this concept. It must also be recognized that STR signals may serve autocrine and/or paracrine functions within the intestinal mucosa that are not reflected in circulating gut hormone concentrations; the latter appear to be a blunt marker for local concentrations of GLP-1 (38). There is also a large body of evidence indicating that the intestinotrophic gut peptide, glucagon-like peptide 2 (GLP-2), co-released from L-cells with GLP-1, is a powerful local stimulus to increase intestinal glucose transport via SGLT-1 and GLUT2 in rodents, and in patients with short bowel syndrome (39-41). Importantly, GLP-2 release has recently been revealed as STR-dependent in animals and a human enteroendocrine cell line (42, 43), highlighting an important link between STRs and GLP-2 in the regulation of intestinal glucose transport.

Reports concerning postprandial incretin hormone release in patients with type 2 diabetes have been inconsistent, with plasma GLP-1 concentrations after a mixed meal being either reduced (22) or intact (44), although such studies are potentially confounded by failure to control for differences in the rate of gastric emptying, which is frequently delayed in longstanding diabetes or during acute hyperglycemia (20). Our observation that GLP-1 and GIP responses to a standardized rate of duodenal glucose infusion were maintained, and indeed increased, in type 2 patients, supports our previous findings (45), and is in keeping with the trend for increased L-cell density in these patients in the current study and a report of an increased density of L-cells, and mixed L/K-cells, in the duodenum of well-controlled type 2 patients (46). There is now strong evidence that SGLT-1 transport is a key stimulus for release of GLP-1 and GIP, which occurs even after exposure to non-metabolized SGLT-1 substrates, and is inhibited by pharmacological blockade or genetic ablation of SGLT-1 in rodents (47-49). Therefore, increased SGLT-1 capacity could explain enhanced glucose-induced GLP-1 and GIP responses in our type 2 patients. Any deficiency in the incretin effect in type 2 diabetes is likely to be explained by impaired β-cell function, rather than deficient incretin hormone secretion (45, 50), and indeed, defective C-peptide responses in our type 2 patients during hyperglycemia support this assertion. Acute hyperglycemia had no effect on GLP-1 or GIP secretion, as noted previously (51, 52). While SGLT-1 transport appears a major determinant of GLP-1 and GIP release, other transporters (49, 53) or signalling pathways (54) may also be involved, so increased glucose absorptive capacity during hyperglycemia may not necessarily result in enhanced GLP-1 or GIP concentrations.
Our study had a number of limitations. While transcriptional regulation of intestinal T1R2 occurred rapidly in humans, we did not quantify changes in STR protein in parallel due to ethical considerations on the additional biopsies required. However, similarly rapid changes in these proteins following glucose or sucralose exposure are known to occur in apical membrane vesicles of rat jejunum (25). We have not assessed effects on SGLT-1 transcript or protein here, although measures of glucose absorption with 3-OMG reflect, in large part, SGLT-1 function as the primary intestinal glucose transporter in humans. There was considerable inter-individual variability in baseline expression of intestinal STR transcripts, so that our study was insufficiently powered to detect relationships between absolute transcript levels and concentrations of gut hormones and 3-OMG. Our 3-OMG measurements were limited to 60 min, and differences between groups or glycemic states may have become more marked after this point. The duodenal glucose infusion was also relatively brief, being limited by the tolerability of unsedated endoscopy. Our type 2 patients had relatively good glycemic control, and more marked differences from health might be observed in patients with a higher HbA1c. The type 2 patients were older than the healthy controls, although we have not previously shown any age-related differences in postprandial GLP-1 responses (55).

In conclusion, we have shown that the intestinal STR system is reciprocally regulated in the presence of luminal glucose according to glycemic status in health, but not in type 2 diabetes. In the latter, T1R2 dysregulation potentially increases the risk of postprandial hyperglycemia, but the intestinal STR system appears unlikely to be a major determinant of circulating GLP-1 or GIP concentrations in humans.

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The authors declare that there are no potential conflicts of interest relevant to this article.

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References

4. Young RL. Sensing via intestinal sweet taste pathways. Front Neurosci 2011 5:23
33. Wolever TM. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycemic index values. Br J Nutr 2004 91:295-301
### Table 1. Human primers used for absolute quantification of target genes in RT-PCR

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QT = QuantiTect primer assay (Qiagen)

### Table 2. Human primers used to generate RT-PCR product containing target amplicon to create absolute standard curves

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<td>T1R2</td>
<td>TACCTGCCTGGGGATTAC</td>
<td>AAATAGGGAGAGGAAGTTGG</td>
<td>390</td>
</tr>
<tr>
<td>T1R3</td>
<td>AGGGCTAATCACCACACAGA</td>
<td>CCGGTACAGGTCACAGT</td>
<td>953</td>
</tr>
<tr>
<td>Gαgust</td>
<td>GAGGACCAACGACAACCTTTA</td>
<td>ACAATGGAGGGTTGTGAAAA</td>
<td>491</td>
</tr>
<tr>
<td>TRPM5</td>
<td>CTTGCTGCCCTAGTGAC</td>
<td>CTGCAGGAAGTCCTTGAGTA</td>
<td>639</td>
</tr>
</tbody>
</table>

### Table 3. Demographic, anthropometric, metabolic and gastrointestinal parameters of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Type 2 Diabetic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>9M : 5F</td>
<td>4M : 9F</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>31 ± 3</td>
<td>66 ± 2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ± 1</td>
<td>27 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.3 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of known diabetes (yr)</td>
<td>5.0 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, at screening (mmol/L)</td>
<td>5.9 ± 0.2</td>
<td>7.4 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2h blood glucose after oral load (mmol/L)</td>
<td>6.3 ± 0.4</td>
<td>12.3 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gastrointestinal symptom score (maximum 27)</td>
<td>1.9 ± 0.6</td>
<td>1.2 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Autonomic function score (maximum 6)</td>
<td>-</td>
<td>2.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Gastric half-emptying (min)</td>
<td>123 ± 8</td>
<td>130 ± 12</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI – body mass index, NS – not significant; Data are mean ± SEM.
Table 4. Incremental area under the curve (iAUC) for glucagon-like peptide-1 (GLP-1), glucose-dependent insulinoctropic polypeptide (GIP), C-peptide and 3-O-methyl glucose (3-OMG) in healthy subjects and type 2 patients.

<table>
<thead>
<tr>
<th>iAUC&lt;sub&gt;60&lt;/sub&gt; (pmol/L.min)</th>
<th>Healthy Subjects</th>
<th>Type 2 Patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Euglycemia</td>
<td>Hyperglycemia</td>
<td>(1-factor ANOVA)</td>
</tr>
<tr>
<td>GLP-1</td>
<td>1530 ± 152</td>
<td>1403 ± 122</td>
<td>A, B &lt; 0.05</td>
</tr>
<tr>
<td>GIP</td>
<td>1308 ± 126</td>
<td>1261 ± 160</td>
<td>A, B &lt; 0.05</td>
</tr>
<tr>
<td>C-Peptide</td>
<td>2028 ± 178</td>
<td>7796 ± 715&lt;sup&gt;A&lt;/sup&gt;</td>
<td>A, B, C &lt; 0.001</td>
</tr>
<tr>
<td>3-OMG</td>
<td>542 ± 45</td>
<td>747 ± 55&lt;sup&gt;A&lt;/sup&gt;</td>
<td>A, B, C &lt; 0.05</td>
</tr>
</tbody>
</table>

|                               | Euglycemia       | Hyperglycemia  |                        |
| GLP-1                         | 2373 ± 219<sup>A,B</sup> | 2446 ± 354<sup>A,B</sup> |                     |
| GIP                           | 1978 ± 181<sup>A,B</sup> | 1849 ± 197     | A, B < 0.05 |
| C-Peptide                     | 1599 ± 184<sup>B</sup> | 4756 ± 405<sup>A,B,C</sup> | A, B, C < 0.001 |
| 3-OMG                         | 565 ± 48<sup>B</sup> | 715 ± 37<sup>A,C</sup> | A, B, C < 0.05 |

Significantly different from A. healthy euglycemia, B. healthy hyperglycemia, C. type 2 diabetes euglycemia. Data are mean ± SEM.
Figure legends

Figure 1. Effects of oral glucose or intraduodenal glucose infusion on blood glucose levels and plasma levels of hormones and the glucose absorption marker 3-OMG in healthy subjects and type 2 patients during euglycemia or hyperglycemia. (A) Blood glucose levels following a glucose drink in healthy control (HC) subjects and type 2 patients (T2D); *P < 0.05, #P < 0.01, δP < 0.001, T2D compared to HC. (B) Blood glucose levels following intraduodenal (ID) glucose infusion during glycemic clamp; δP < 0.001, HC euglycemic compared to hyperglycemic groups, also, T2D euglycemic compared to T2D hyperglycemic; *P < 0.05, T2D euglycemic compared to HC euglycemic; **P < 0.05, T2D hyperglycemic compared to HC hyperglycemic. (C) Plasma GLP-1, *P < 0.05, T2D groups compared to HC euglycemic; #P < 0.01, T2D groups compared to HC hyperglycemic. (D) Plasma GIP, *P < 0.05, T2D groups compared to HC euglycemic; **P < 0.05, T2D groups compared to HC hyperglycemic; ***P < 0.05, T2D euglycemic compared to HC groups. (E) C-peptide, δP < 0.001, HC hyperglycemic compared to euglycemic groups; #P < 0.05, T2D hyperglycemic compared to other groups. (F) 3-OMG, δP < 0.001, T2D hyperglycemic compared to other groups. Data are mean ± SEM; significance represents treatment × time interactions. GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic polypeptide; 3-OMG, 3-O-methy glucose.

Figure 2. Absolute transcript levels of STR in the duodenum of healthy subjects and type 2 patients at stable euglycemia and hyperglycemia. Absolute expression (copy number) of STR transcripts at baseline in the duodenum of healthy subjects (A) or patients with type 2 diabetes (B). (A) TRPM5 levels were 15-fold higher, α-gustducin 9-fold higher and T1R3 3-fold higher than T1R2 levels in healthy subjects. (B) TRPM5 levels were 29-fold higher, α-gustducin 11-fold higher and T1R3 5-fold higher than T1R2 levels in patients with type 2 diabetes. * P < 0.05, # P < 0.01 compared to T1R2. Duodenal levels of T1R2 (C), T1R3 (D), α-gustducin (E) and TRPM5 (F) transcript in healthy subjects and type 2 patients (T2D) at stable euglycemia or hyperglycemia. There were no significant differences in transcript levels detected at stable baseline. Data are mean ± SEM.
Figure 3. Effects of intraduodenal glucose infusion on sweet taste molecule transcript levels in healthy subjects and type 2 patients during euglycemia or hyperglycemia. (A) Change in absolute expression of T1R2 in human duodenum during ID glucose infusion under euglycemic or hyperglycemic clamp; \(^*P < 0.01,\) HC hyperglycemic compared to all other groups. Data are mean ± SEM. (B) T1R3, (C) \(\alpha\)-gustducin, (D) TRPM5.

Figure 4. Subsets of L-cells, K-cells and EC-cells express STR in healthy human duodenum. (A) Immunolabelling for GLP-1 was present in 19 ± 11% of T1R2 labelled duodenal cells in HC subjects at euglycemia, while 13 ± 8% of L-cells co-expressed T1R2. (B) GIP was present in 15 ± 10% of T1R2 labelled cells in HC subjects at euglycemia, while 12 ± 8% of K-cells co-expressed T1R2. (C) In similar manner, separate populations of T1R2 labelled cells co-expressed 5-HT (31 ± 6%), while 5 ± 1% of EC-cells co-expressed T1R2. Scale Bar (A-C) = 20 µm.
Figure 2

A Healthy
B Type 2 Diabetes

C T1R2
D T1R3

E α-gustducin
F TRPM5

Figure 2
113x135mm (600 x 600 DPI)
Figure 3

(A) T1R2

(B) T1R3

(C) α-gustducin

(D) TRPM5

Figure 3
99x103mm (600 x 600 DPI)
Figure 4

147×114mm (300 x 300 DPI)