Short Communication

Effect of the artificial sweetener, sucralose, on small intestinal glucose absorption in healthy human subjects

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It has been reported that the artificial sweetener, sucralose, stimulates glucose absorption in rodents by enhancing apical availability of the transporter GLUT2. We evaluated whether exposure of the proximal small intestine to sucralose affects glucose absorption and/or the glycaemic response to an intraduodenal (ID) glucose infusion in healthy human subjects. Ten healthy subjects were studied on two separate occasions in a single-blind, randomised order. Each subject received an ID infusion of sucralose (4 mM in 0·9 % saline) or control (0·9 % saline) at 4 ml/min for 150 min ($T = -30$ to 120 min). After 30 min ($T = 0$), glucose (25 %) and its non-metabolised analogue, 3-O-methylglucose (3-OMG; 2·5 %), were co-infused intraduodenally ($T = 0$–120 min; 4·2 kJ/min (1 kcal/min)). Blood was sampled at frequent intervals. Blood glucose, plasma glucagon-like peptide-1 (GLP-1) and serum 3-OMG concentrations increased during ID glucose/3-OMG infusion ($P < 0.005$ for each). However, there were no differences in blood glucose, plasma GLP-1 or serum 3-OMG concentrations between sucralose and control infusions. In conclusion, sucralose does not appear to modify the rate of glucose absorption or the glycaemic or incretin response to ID glucose infusion when given acutely in healthy human subjects.

3-O-methylglucose: Sodium-dependent GLUT 1: GLUT 2: Glucagon-like peptide-1

The mechanisms by which the gut senses nutrients are unclear, and the 'receptor' for detecting luminal carbohydrates has, until recently, been elusive. Recent studies indicate the presence of G-protein-coupled taste receptors, T1R2 and T1R3, and their taste signal transduction partners, the G-protein gustducin and the transient receptor potential ion channel TRPM5, in the mucosa of the mouse and human gastrointestinal tract[1,2]. These receptors, analogous to sweet taste receptors on the tongue, broadly respond to sugars and artificial sweeteners, and among several cell types, they appear to co-localise with glucagon-like peptide-1 (GLP-1)-secreting L cells[3].

It has been reported that the artificial sweetener, sucralose, stimulates the secretion of both GLP-1 and glucose-dependent insulinotrophic polypeptide from the mouse enteroendocrine cell line GLUTag[4], and it stimulates GLP-1 secretion from the human L cell line NCI-H716[3], a response that is blocked by the sweet receptor antagonist, lactisole, and siRNA for α-gustducin[3]. However, we recently demonstrated that sucralose, in two different loads, had no effect on GLP-1, glucose-dependent insulinotrophic polypeptide or insulin secretion, and that it did not elicit any feedback response on gastric emptying in healthy human subjects[5]. While this implies that artificial sweeteners may have no therapeutic benefit in the dietary management of diabetes, other than as a substitute for carbohydrates, it remains possible that sucralose affects small intestinal carbohydrate absorption as a result of its interaction with the sweet taste receptors.

Glucose is absorbed from the small intestine through both the Na-dependent GLUT 1 (SGLT1) and the facilitative transporter GLUT2[6]. Supplementation of the diet with sucralose increases the expression of SGLT1 in the enterocytes of wild-type mice, but not in mice deficient in T1R3 or α-gustducin[4]. The presence of sucralose enhances insertion of GLUT2 into the apical region of the enterocytes, and thus, stimulates glucose absorption in rats[7,8]. For example, sucralose administration doubled the level of GLUT2 protein

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Abbreviations: 3-OMG, 3-O-methylglucose; GLP-1, glucagon-like peptide-1; ID, intraduodenal; SGLT1, Na-dependent GLUT 1.

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detected in apical membrane vesicles in response to low luminal glucose concentrations (20 mM)[7], and the maximum rate of glucose absorption was reached after 20 min of exposure to sucralose *in vivo* [8]. This raises the question as to whether the combination of an artificial sweetener with a carbohydrate could have a synergistic effect on glucose absorption in human subjects. The notion that consuming artificial sweeteners together with carbohydrates could enhance glucose absorption and therefore elevate postprandial blood glucose concentrations is of fundamental clinical importance.

The aim of the present study was to evaluate whether exposure of the proximal small intestine to sucralose affects the subsequent response to glucose in terms of the rate of glucose absorption and the glycaemic response.

Study plan and design

Subjects

Ten healthy subjects (eight males and two females; age 27 (SD 2) years and BMI 23·4 (SD 0·8) kg/m^2) were studied twice in a randomised, single-blind, cross-over design. None of them had a history of gastrointestinal disease, upper or lower gastrointestinal symptoms, or significant previous surgery. Each subject provided written informed consent before participating, and the study was approved by the Royal Adelaide Hospital Research Ethics Committee.

Protocol

All the participants attended the Discipline of Medicine at the Royal Adelaide Hospital at approximately 08.30 hours after an overnight fast (14 h for solids and 12 h for liquids) on two occasions, which were separated by at least 3 d. The women were studied in the follicular phase of the menstrual cycle to avoid any potential effects of the menstrual cycle on the upper gastrointestinal function[9].

On each study day, a multilumen silicone catheter (external diameter 4 mm) was introduced into the stomach through an anaesthetised nostril, and was allowed to pass into the duodenum by peristalsis. This catheter incorporated a side hole to deliver infusions into the proximal duodenum; its position was monitored continuously during the study by measurement of the transmucosal potential difference, from two other saline-perfused side holes on either side of the pylorus, using established criteria (antral potential < -20 mV, duodenal potential > -15 mV and difference > 15 mV)[10]. This required the insertion of a 20G saline-filled cannula subcutaneously into the forearm as a reference. When the catheter was positioned correctly, an intravenous cannula was inserted into a forearm vein for subsequent blood sampling.

After the baseline blood samples were collected ($T = -30$), an intraduodenal (ID) infusion of sucralose (960 mg made up to 600 ml with 0·9 % saline (sucralose concentration 4 mM)) or 600 ml of 0·9 % saline alone as a control was commenced and continued at a rate of 4 ml/min for 150 min ($T = -30$ to 120 min). At $T = 0$ min, an ID infusion of glucose solution (30 g glucose together with 3 g 3-*O*-methylglucose (3-OMG), Sigma-Aldrich, St Louis, MO, USA) made up to a total volume of 120 ml with water (approximately 1390 mM glucose) was also commenced via the same channel in the catheter, and was continued at a rate of 1 ml/min (= 4·2 kJ/min (1 kcal/min)) for 120 min ($T = 0$–120 min). The final concentration of glucose that was infused was approximately 280 mM due to dilution with co-infused saline/sucralose.

Venous blood samples (approximately 20 ml) were collected immediately before the ID infusion was commenced, and at $T = -15$, 0, 5, 10, 20, 30, 40, 60, 90 and 120 min. Blood glucose concentrations were measured immediately using a glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA). The remainder of each blood sample was distributed into a serum tube and a tube containing EDTA and 0·056 mg aprotinin (Trasylol; Bayer Australia, Pymble, NSW, Australia) per litre of blood, stored on ice and centrifuged at 3200 rpm for 15 min. Serum and plasma were separated, and the samples were stored at -70°C for subsequent analysis. 3-OMG was extracted from serum by protein precipitation extraction with methanol under monitoring using an API3000TM LC/MS/MS mass spectrometer (Applied Biosystems, Foster City, CA, USA; MDS Sciex, ON, Canada). The range of detection was 2000–200 000 ng/ml when using a sample volume of 100 μl. 3-OMG is an analogue of glucose that is absorbed from the gut in the same way as glucose but not metabolised; therefore, serum concentrations represent an index of glucose absorption[11]. Plasma total GLP-1 concentrations were measured by RIA (GLPIT-36HK; Linco Research, St Charles, MO, USA); the intra-assay CV was 6·7 % and inter-assay CV was 7·8 %, and the sensitivity was 3 pmol/l.

Statistical analysis

Repeated measures ANOVA (SuperANOVA; Abacus Concepts, Berkeley, CA, USA) was used to analyse the blood glucose, plasma GLP-1 and serum 3-OMG concentrations with both treatment and time as factors. Statistical significance was accepted as $P < 0.05$, and data are presented as mean values with their standard errors.

Results

All the subjects tolerated the study well.

Blood glucose concentrations

There was no difference in baseline glucose concentrations between the two study days. Blood glucose concentrations also did not differ over $T = -30$ to 0 min during infusions of sucralose or saline. There was a rise in blood glucose concentration after the ID glucose infusion was begun, which was evident from $T = 20$ min ($P < 0.001$) on both the days. There were no differences between blood glucose concentrations in the subjects receiving sucralose or saline infusions (Fig. 1(a)).

Plasma glucagon-like peptide-1 concentrations

There was no difference in baseline GLP-1 concentrations between the two study days. GLP-1 concentrations did not differ over $T = -30$ to 0 min during infusions of sucralose or saline, but they increased transiently on both the days
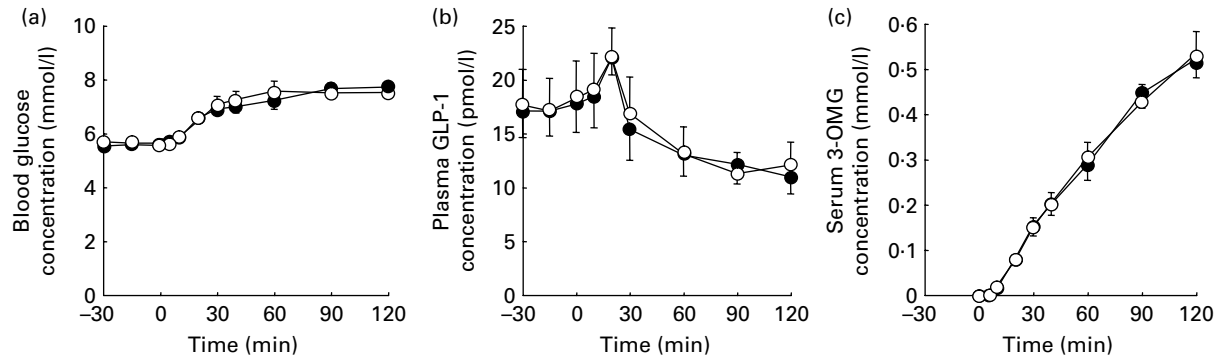
Fig. 1. Blood glucose (a), plasma glucagon-like peptide-1 (GLP-1) (b) and serum 3-O-methylglucose (3-OMG) (c) concentrations in ten healthy human subjects in response to an intraduodenal infusion of sucralose (●; 4 mM in 0·9 % saline) or 0·9 % saline (○) control at 4 ml/min for 150 min ($T = -30$ to 120 min) with co-infusion of glucose (25 %) and 3-OMG (2·5 %) between $T = 0$ and 120 min (4·2 kJ/min (1 kcal/min)). Data are presented as mean values with their standard errors. There were no differences in blood glucose, plasma GLP-1 or serum 3-OMG concentrations between sucralose and saline infusions.

($P < 0.005$) after the ID glucose infusion was begun, before subsequently declining. There were no differences between plasma GLP-1 concentrations in the subjects receiving sucralose or saline infusions (Fig. 1(b)).

Serum 3-O-methylglucose concentrations

Serum 3-OMG concentrations increased from 20 min after the ID glucose infusion was begun ($P < 0.001$) on both the study days. There were no differences between serum 3-OMG concentrations in the subjects receiving sucralose or saline infusions (Fig. 1(c)).

Discussion

Our study indicates that ID administration of sucralose has no effect on the rate of glucose absorption from the lumen of the small intestine, and that it does not elevate postprandial blood glucose concentrations or influence GLP-1 secretion in healthy human subjects.

Sucralose is used as a non-energetic sweetener in the food industry, and is widely consumed by individuals who are obese or have diabetes. The recent identification of elements of the sweet taste receptors in the rodent and human small intestine, which are linked to peptide hormone release and modulation of glucose transport, suggests that artificial sweeteners could potentially be metabolically active. However, *in vivo* studies in mice[12] and human subjects[5] have failed to support any effect of sucralose on insulin, GLP-1 or glucose-dependent insulinotrophic polypeptide release. Similarly, in the present study, sucralose had no effect on GLP-1 secretion when given alone, and it did not enhance the modest rise in GLP-1 observed with ID glucose infusion. Recently, however, it has been reported in rats that sucralose acted synergistically with glucose to activate the T1R2 + T1R3 heterodimer and increase the rate of small intestinal glucose absorption by inserting GLUT2 into the apical membrane[7]. Hence, consuming artificial sweeteners in conjunction with carbohydrates could raise concerns about increasing the postprandial glycaemic response, particularly as there is already overexpression of SGLT1 and GLUT2 in the small intestine in animal models of diabetes[13,14] and in diabetic human subjects[15]. Our study is the first to examine the potential acute interaction of intraluminal sucralose with glucose in relation to small intestinal glucose absorption and blood glucose concentrations in apparently healthy human subjects. The negative outcome is consistent with previous studies that showed no effect of sucralose supplementation on glycaemic response in patients with diabetes[16,17].

In the present study, we infused sucralose and glucose directly into the duodenum, rather than administering them orally, in order to regulate precisely the exposure of the small intestine to these substances. In studies where sucralose stimulated T1R2 + T1R3, resulting in apical insertion of GLUT2, the concentration of sucralose was 1 mM[7]. Thus, 4 mM sucralose should have been sufficient to obtain a response. We used a glucose concentration (approximately 280 mM) which would itself be expected to induce apical GLUT2 insertion maximally[7], but this process would have taken about 20 min[8]. If prior exposure to sucralose did indeed modulate apical GLUT2, a difference in glucose absorption should have been evident early in the ID glucose infusion.

Species differences are likely to account for the lack of effect of sucralose in human subjects. It has been reported that there is much lower duodenal expression of GLUT2 in human subjects than in rats and mice, while expression of SGLT1 is much greater in human subjects[18]. Therefore, SGLT1 is likely to play a dominant role in glucose absorption in the human small intestine. Mutation of SGLT1 in human subjects results in glucose–galactose malabsorption, whereas absorption of these sugars is not disrupted by mutations of GLUT2[19]. Moreover, if apical GLUT2 insertion occurred in human subjects, one might expect this to ameliorate the effects of SGLT1 mutation and allow glucose to be tolerated in this disorder, but this appears not to be the case[15]. Supplementation of a low-sugar chow with sucralose for 2 weeks in mice has been reported to increase SGLT1 protein and mRNA expression[4]. Thus, it is still possible that prolonged exposure to sucralose (substantially for more than 20 min) could increase small intestinal glucose absorption in human subjects, although this would need to be evaluated in a separate study.

In conclusion, we have demonstrated that acute ID administration of sucralose does not enhance the absorption of glucose from the small intestine or increase blood glucose or plasma GLP-1 concentrations in healthy human subjects.

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