

## Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota

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### Abstract

The chemosensory components shared by both lingual and intestinal epithelium play a critical role in food consumption and the regulation of intestinal functions. In addition to nutrient signals, other luminal contents, including micro-organisms, are important in signalling across the gastrointestinal mucosa and initiating changes in digestive functions. A potential role of gut microbiota in influencing food intake, energy homeostasis and weight gain has been suggested. However, whether gut microbiota modulates the expression of nutrient-responsive receptors and transporters, leading to altered food consumption, is unknown. Thus, we examined the preference for nutritive (sucrose) and non-nutritive (saccharin) sweet solutions in germ-free (GF, C57BL/6J) mice compared with conventional (CV, C57BL/6J) control mice using a two-bottle preference test. Then, we quantified mRNA and protein expression of the sweet signalling protein type 1 taste receptor 3 (T1R3) and  $\alpha$ -gustducin and Na glucose luminal transporter-1 (SGLT-1) of the intestinal epithelium of both CV and GF mice. Additionally, we measured gene expression of T1R2, T1R3 and  $\alpha$ -gustducin in the lingual epithelium. We found that, while the preference for sucrose was similar between the groups, GF mice consumed more of the high concentration (8%) of sucrose solution than CV mice. There was no difference in either the intake of or the preference for saccharin. GF mice expressed significantly more T1R3 and SGLT-1 mRNA and protein in the intestinal epithelium compared with CV mice; however, lingual taste receptor mRNA expression was similar between the groups. We conclude that the absence of intestinal microbiota alters the expression of sweet taste receptors and GLUT in the proximal small intestine, which is associated with increased consumption of nutritive sweet solutions.

**Key words:** Sucrose intake: Gut microflora: Intestinal adaptation

The gastrointestinal (GI) tract is a highly specialised sensory system capable of detecting and mounting appropriate responses to incoming luminal factors including nutrient- and non-nutrient-derived signals and micro-organisms. To achieve this, the GI tract is equipped with complex chemosensory machinery that acts in the interest of preserving its main functions including nutrient digestion, absorption, hormone secretion and metabolism while protecting itself against harmful substances. Although significant progress has been made recently in understanding the substrates and cellular events underlying chemosensory functions of the gut, the exact mechanisms involved in nutrient detection are not well understood. In addition to the presence of nutrients and other factors originating in the lumen, the intestinal epithelium comes in direct contact with trillions of micro-organisms that

interact in a bidirectional mode to affect gut functions, leading to shifts in metabolic parameters<sup>(1,2)</sup>. For example, entero-endocrine cells function as 'bacterial sensors' as they express bacterial recognition receptors (toll-like receptors)<sup>(3)</sup>, which are also present on the intestinal epithelium<sup>(4)</sup>. On the other hand, there is evidence that microbiota-generated by-products affect functional expression of intestinal nutrient-responsive G-protein receptors<sup>(5)</sup>, gastrointestinal hormones<sup>(6–8)</sup>, enzyme secretion<sup>(9,10)</sup> and cellular fatty acid metabolism<sup>(1)</sup>.

A variety of nutrient-responsive receptors are localised in the gut epithelium, with some implicated in the chemosensory detection of sweet stimuli<sup>(11)</sup>. For example, detection of sugars and sweeteners depends on the heterodimeric receptor T1R2/3, which comprises type 1 taste receptor subunits (T1R)<sup>(12,13)</sup> coupled to  $\alpha$ -gustducin, a transducin-like heterotrimeric

**Abbreviations:** CV, conventional; GF, germ free; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; PYY, peptide YY; SGLT-1, Na glucose luminal transporter-1.

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G-protein<sup>(14,15)</sup>. Rodent models lacking these receptors show severely diminished detection of nutritive and non-nutritive sweet solutions in short-term preference and lick tests<sup>(16)</sup>. T1R3 knockout mice display equal preferences following repeated exposures or during initial access to high concentrations<sup>(16,17)</sup>, but yet under-consume these solutions compared with control mice<sup>(16)</sup>. Ablation of T1R3 in the intestinal epithelium results in severely blunted postprandial glucagon-like peptide-1 (GLP-1) response to glucose<sup>(18,19)</sup> and a blunted up-regulation of Na glucose luminal transporter-1 (SGLT-1) in response to a high-carbohydrate diet<sup>(20)</sup>. Together, these findings, coupled with the ability of gut microbiota to modulate intestinal chemosensation and physiology, led us to hypothesise that the absence of gut microbiota could result in the modulation of the gut's capacity to detect and absorb sugars, similar to what has been observed in response to changes in dietary carbohydrates<sup>(21)</sup>. Specifically, this adaptive regulation due to changes in substrate (i.e. microbiota) may result in alterations in sweet taste receptors and associated proteins (T1R2, T1R3 and  $\alpha$ -gustducin) as well as sugar transporter (SGLT-1) expression, as a compensatory mechanism to facilitate uptake of sugars in the absence of microbiota. Thus, the increase in intestinal sugar detection and transport elements may lead to differential intake of sweet solutions due to altered intestinal feedback via a glucose absorptive mechanism.

Several factors drive consumption of sweet foods and fluids, involving both oral and post-oral signalling mechanisms (for a review, see Sclafani<sup>(22)</sup>). For example, real feeding of non-nutritive and sham feeding of nutrient sweet solutions results in heightened intake<sup>(23–25)</sup>. On the other hand, in the absence of taste, gastrointestinal infusions of sweet nutritive, but not non-nutritive solutions, stimulate intake of a non-energy flavoured solution through post-ingestive reinforcement<sup>(26,27)</sup>. Together, these findings illustrate the importance of both taste in the oral cavity and nutrient sensing in the intestinal tract as important factors controlling intake of sweet substances.

In addition to the modulation of GI parameters, a role of gut microbiota in the control of food intake and the regulation of body weight has recently emerged. For example, germ-free (GF) mice, devoid of gut microbiota, consume more food than conventional (CV) control mice, do not gain weight on a Western-style diet and rapidly gain body fat when colonised with microbiota<sup>(1,28)</sup>. Despite these advances in gut microbiota research, no studies have examined the behavioural effects of the gut microbiota as they relate to the consumption of highly preferred tastants. Interestingly, many of the taste receptors in the mouth also operate in the enteroendocrine cells of the GI tract<sup>(11)</sup>. Thus, gut microbiota may modulate expression of sweet receptors and transporters at both the level of the intestine and oral cavity through similar mechanisms. To determine the role of the gut microbiota on oral and post-oral signals controlling sweet solution intake, we first examined the preference for nutritive (sucrose) and non-nutritive (saccharin) sweet solutions in GF inbred C57BL/6J mice compared with CV inbred C57BL/6J control mice using a two-bottle preference test. Second, to determine the possible underlying mechanisms responsible for the microbiota's influence on

sweet acceptance and preference orally and post-orally, we quantitatively determined mRNA expression of taste receptors (T1R2 and T1R3) and the associated G-protein,  $\alpha$ -gustducin, in the lingual and intestinal epithelium and SGLT-1 in the intestinal epithelium of both CV and GF mice. To assess whether changes in gene expression result in alterations in protein expression, we also analysed intestinal protein levels of T1R3, SGLT-1 and  $\alpha$ -gustducin.

## Methods

### Animals

In the present study, 8-week-old male C57BL/6J GF mice from our in-house GF colonies (originally derived from Charles River colonies) and male C57BL/6J CV mice from Charles River Laboratories (Charles River Laboratories, L'Abresle, France; *n* 16, for each group), with similar body weights at the start of the experiments (GF 24.2 (SEM 0.5) g; CV 25.3 (SEM 0.6) g), were housed individually in polycarbonate cages with cedar bedding. Each group of mice was housed separately in two Trexler-type isolators (Igenia, Montreuil, France). Throughout the study, the GF isolator was verified as sterile through weekly analysis of mouse faecal samples. Both groups of mice were provided similar autoclaved, deionised sterile water and irradiated sterile standard rodent chow (Safe Diets, Augy, Belgium) *ad libitum*. Both groups were allowed a minimum of 1-week acclimatisation to their new cages and isolators before experimental manipulations begun. Procedures were carried out in accordance with the European Guidelines for the Care and Use of Laboratory Animals.

### 48 h two-bottle preference tests

GF and CV mice in each isolator were divided into two separate groups (*n* 8), with one group receiving saccharin (0.0125, 0.025, 0.1, 0.2, 0.4%, w/v) and the second group receiving sucrose (0.5, 2, 4, 8, 16%, w/v) solutions, in ascending concentration during two-bottle testing. Each two-bottle choice test was 48 h in duration. At the beginning of each test, mice were weighed, the water bottle was removed and replaced with two similar 250 ml plastic bottles with the spouts penetrating from the top floor of the cage at 2–4 cm distance from the floor and 5–6 cm apart. The positions of the two bottles were switched every 24 h to control for side preference. They were weighed at the beginning and at the end of each 24 h test. Spillage was estimated by placing two bottles of water in an empty cage and measuring fluid loss after 24 h on two consecutive days. In both groups, one concentration of each solution was tested per week, thus allowing a minimum of 4 d washout period during which mice had access to two bottles of deionised water.

### Intestinal and lingual epithelium collection

Approximately 12 weeks after two-bottle preference tests, five GF and five CV mice were killed for collection of lingual and intestinal epithelial samples. Under deep isoflurane

anaesthesia, the proximal portion of the small intestine, containing the duodenum and jejunum, was removed and placed into sterile physiological saline. Intestinal epithelial cells were collected using the everted sac method. Briefly, after excision, proximal intestines were flushed using 10 ml of ambient physiological saline followed by 10 ml oxygenated (95:5 O<sub>2</sub>:CO<sub>2</sub>) Ca<sup>2+</sup> and Mg<sup>2+</sup>-free Krebs–Henseleit buffer. After rinsing, intestines were everted, divided into three segments and placed into oxygenated Ca<sup>2+</sup>, Mg<sup>2+</sup>-free Krebs–Henseleit buffer with EDTA and dithiothreitol. Flasks containing sections were placed in a 37°C water-bath and shaken for 20 min to dissociate epithelial cells from the connective tissue. The suspension was collected, centrifuged and washed with Dulbecco's PBS without Ca<sup>2+</sup> or Mg<sup>2+</sup> (Lonza, Levallois-Perret, France). This process was repeated three times. After the last wash, solution D with β-mercaptoethanol was added to cell extract aliquots and snap-frozen and stored at –80°C until used for the study of gene expression. Separate aliquots of isolated intestinal cells were snap-frozen and stored at –80°C until used for the expression of proteins. For lingual epithelial tissue, the tongue was excised from anaesthetised mice and injected subdermally with 0.5 ml of 1 mg/ml of dispase and elastase dissolved in mammalian physiological saline containing 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetra-acetic acid. After 20 min incubation at room temperature, the posterior lingual epithelium containing the circumvallate papillae was dissected under a Zeiss stereoscope and placed into a 1.5 ml microfuge tube containing AllProtect Tissue Reagent (Qiagen, Courtaboeuf, France) and stored at 2°C until used for RNA extraction.

#### Quantitative real-time PCR

RNA was extracted from isolated epithelial cells using the phenol–chloroform method<sup>(29,30)</sup> adjusted for 300 mg of tissue. Lingual epithelium was lysed and homogenised using the TissueLyser (Qiagen) and RNA extracted using the RNeasy Fibrous Tissue Mini kit (Qiagen) according to the manufacturer's instructions. For cDNA synthesis, a total of 10 μg RNA from intestinal epithelial cells and 2 μg from the lingual epithelium were reverse transcribed in a total reaction volume of 100 and 60 μl, respectively, using the high-capacity cDNA kit (Applied Biosystems, Courtaboeuf, France). Subsequently, cDNA was diluted 5-fold for both intestinal and lingual samples. Quantitative real-time PCR was performed in a reaction volume of 20 μl using an ABI Prism 7700 (Applied Biosystems) thermal cycler. Samples were run in triplicate and transcription levels of T1R2, T1R3, α-gustducin and SGLT-1 were quantified using Taqman<sup>®</sup> Gene Expression Assays and Gene Expression Master Mix (Applied Biosystems). Relative mRNA expression was quantified with the 2<sup>–ΔCT</sup> method and β-actin served as the internal control.

#### Western blotting

Isolated intestinal epithelial cell aliquots were thawed on ice and suspended in 1 ml of radioimmunoprecipitation assay buffer containing protease inhibitors (Sigma, Saint-Quentin-Fallavier,

France). Cells were lysed and homogenised, and the resulting homogenate was centrifuged for 20 min at 13 000 rpm at 4°C. Protein concentration in the supernatant was determined with the NanoDrop system (Thermo Scientific, Illkirch, France). Soluble protein (100 μg) was then run on SDS-PAGE gels containing 8% acrylamide, transferred to nitrocellulose membranes, and probed with anti-T1R3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), SGLT-1 (AbCam, Cambridge, UK) and α-gustducin (Santa Cruz Biotechnology) antibodies. Immune complexes were detected by chemiluminescence (Thermo Scientific). Quantification was performed by scanning densitometry using ImageJ (NIH, Bethesda, MD, USA) against β-actin (Santa Cruz Biotechnology) as the internal control.

#### Statistical analyses

Body-weight comparisons between the groups at the beginning and end of the experiment were performed using two-way (group × nutrient) ANOVA. Differences between the groups for the preference for tastants were determined by the formula (48 h intake of tastant)/(48 h intake of total fluid) × 100, and subjected to two-way (group, concentration) repeated-measures ANOVA. Weights of solutions in grams were converted to millilitres using a conversion factor (weight of solution (g)/density of solution (g/ml)) and are presented as total 48 h intakes throughout the paper. Additionally, 48 h acceptance (raw intake solution) as well as total energy consumed from sucrose were subjected to two-way (group, concentration) repeated-measures ANOVA. To determine sensitivity to the tastant (concentration at which the animal first prefers tastant over water), we performed paired Student's *t* test for each concentration within the group. The resulting values from Western blotting and quantitative real-time PCR were analysed using Student's *t* test. For all statistical tests, differences were considered significant at α < 0.05.

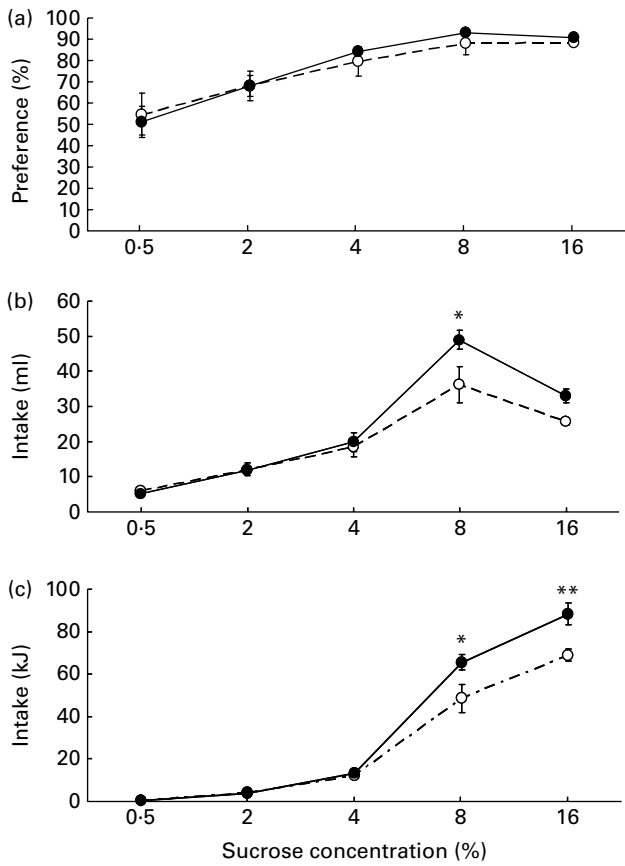
## Results

### Body weight

Body-weight gain of mice throughout the experiment was similar in both GF and CV groups, irrespective of the sweet solution to which they were exposed (GF 1.3 (SEM 0.4) g, CV 1.3 (SEM 0.5) g; *P* = 0.98). However, when body weight was analysed separately for each period of access to sucrose concentration, CV mice weighed more than GF mice during the access period to 16% sucrose solution (GF 23.8 (SEM 1.0) g, CV 27.3 (SEM 0.3) g; *P* < 0.01). There were no differences in body weight between GF and CV mice receiving saccharin solution (GF 28.2 (SEM 3.8) g, CV 26.7 (SEM 1.6) g; *P* = 0.81).

### Preferences for sucrose and saccharin solutions

When evaluating sucrose preference in GF and CV mice, there was a significant fixed effect of concentration ( $F(4,55) = 21.95$ , *P* < 0.0001), but not group ( $F(1,14) = 0.13$ , *P* = 0.72) or group × concentration interaction ( $F(4,55) = 0.30$ , *P* = 0.88) for sucrose preference. Thus, we did not find any significant



**Fig. 1.** (a) Percentage of sucrose preference, (b) sucrose solution intake and (c) energy intake in germ-free (GF, —●—) and conventional (CV, -○-) C57BL/6J mice during 48 h two-bottle sucrose *v.* water preference test (*n* 8). Sucrose concentrations were each presented for two consecutive days in ascending concentration. Tests were separated by 4 d of water only. (a) Sucrose preference was similar between GF and CV mice across all concentrations tested. (b) At the higher sucrose concentration (8%), GF mice consumed more solution than CV mice. (c) At 8 and 16% sucrose solution, GF mice consumed significantly more energy (kJ) from sucrose compared with CV controls. Values are means, with their standard errors represented by vertical bars. Mean values were significantly different from those of CV: \**P*<0.05, \*\**P*<0.01.

differences in sucrose preference between GF and CV mice at the concentrations tested (Fig. 1(a)). Additionally, the first concentration at which both GF (*P*<0.05) and CV (*P*<0.05) mice preferred sucrose to water was 2%. When examining 48 h intake of sucrose solutions, there were significant fixed effects of group ( $F(1,14) = 4.62$ , *P*<0.05), concentration ( $F(4,55) = 86.61$ , *P*<0.0001) and group × concentration interaction ( $F(4,55) = 3.48$ , *P*<0.05). GF mice consumed significantly more of the 8% sucrose solution (49.0 (SEM 2.7) ml) than CV mice (36.2 (SEM 5.1) ml), *P*<0.05; Fig. 1(b)). Furthermore, when expressed as energy (kJ) consumed from sucrose, GF mice consumed significantly more energy from 8% (65.6 (SEM 3.6) kJ, *P*<0.05) and 16% (88.4 (SEM 5.3) kJ, *P*<0.01) sucrose solutions compared with CV mice (8% 48.5 (SEM 6.8) kJ, 16% 68.9 (SEM 2.8) kJ; Fig. 1(c)).

Similar to sucrose preference, analysis of saccharin preference revealed significant fixed effects of concentration ( $F(4,55) = 3.43$ , *P*<0.0001), but not group ( $F(1,14) = 1.21$ , *P*=0.29) or group × concentration interaction ( $F(3,42) = 0.38$ ,

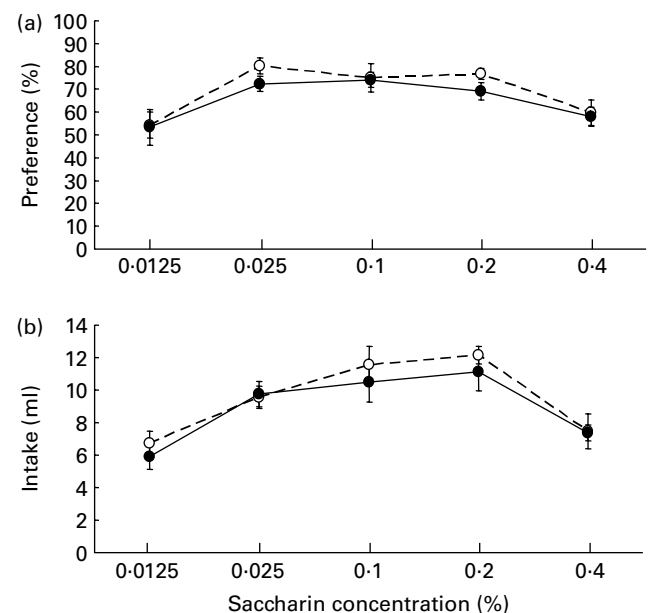
*P*=0.77). The concentration of saccharin at which both GF (*P*<0.0001) and CV (*P*<0.0001) mice preferred the tastant over water was 0.025%. In preference tests, both GF and CV mice similarly preferred all concentrations of saccharin (*P*=1.00 for all concentrations; Fig. 2(a)). When 48 h saccharin consumption was analysed, there was a significant fixed effect of concentration ( $F(4,55) = 19.09$ , *P*<0.0001), but not group ( $F(1,14) = 0.42$ , *P*=0.52) or group × concentration interaction ( $F(4,55) = 0.30$ , *P*=0.88). Furthermore, both GF and CV mice consumed similar volumes of saccharin at all concentrations tested (Fig. 2(b)).

### Lingual taste receptor expression

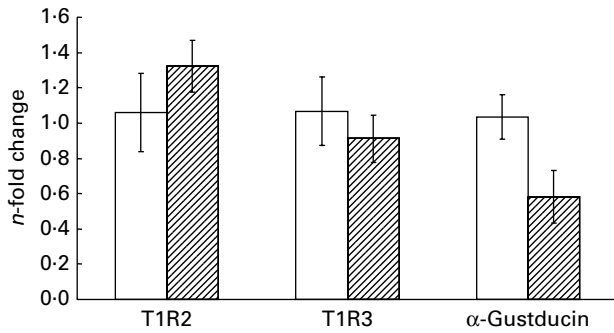
For both T1R2 and T1R3 in the lingual epithelium, we found no significant differences in mRNA expression between GF and CV mice (Fig. 3). However, α-gustducin expression appeared to be down-regulated in GF mice compared with controls, although it did not reach the level of significance (*P*=0.05).

### Intestinal nutrient receptor and transporter expression

When examining the expression of sweet taste receptors and GLUT mRNA in intestinal epithelial cells, we found a 6-fold increase in T1R3 expression in the small intestine of GF mice compared with CV counterparts (*P*<0.0001; Fig. 4(a)). This up-regulation of T1R3 was associated with a significantly increased expression of SGLT-1 that was 1.3-fold higher in



**Fig. 2.** (a) Percentage of saccharin preference and (b) saccharin solution intake in germ-free (GF, —●—) and conventional (CV, -○-) C57BL/6J mice during 48 h two-bottle saccharin *v.* water preference test (*n* 8). Saccharin concentrations were each presented for two consecutive days in ascending concentration. Tests were separated by 4 d of water only. (a) Preference between GF and CV mice was similar for all concentrations of saccharin tested. (b) Intake of saccharin solution across all concentrations was similar between GF and CV mice. Values are means, with their standard errors represented by vertical bars.



**Fig. 3.** Posterior lingual epithelial mRNA expression of sweet taste receptor components in germ-free (GF, ▨) and conventional (CV, □) C57BL/6J mice. Expression of the heterodimeric subunits type 1 taste receptor (T1R)2 and T1R3 and the G-protein-coupled subunit  $\alpha$ -gustducin was similar between GF and CV mice. Values are means of triplicate ( $n$  5), with standard errors represented by vertical bars, expressed relative to  $\beta$ -actin as the internal control.

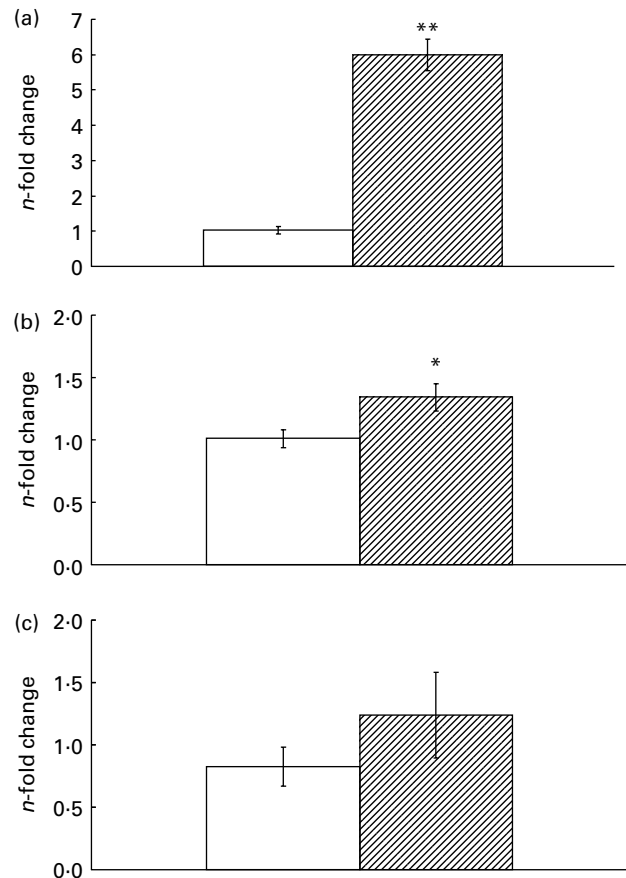
GF mice compared with CV controls ( $P < 0.05$ ; Fig. 4(b)).  $\alpha$ -Gustducin mRNA was slightly increased in GF mice, but the difference was not significant (Fig. 4(c)). Furthermore, protein expression was significantly increased in GF mice compared with controls, for T1R3, SGLT-1 ( $P < 0.001$  for each) as well as  $\alpha$ -gustducin ( $P < 0.05$ ; Fig. 5). We were not able to detect reliable expression of T1R2 from either GF or CV mice (data not shown).

## Discussion

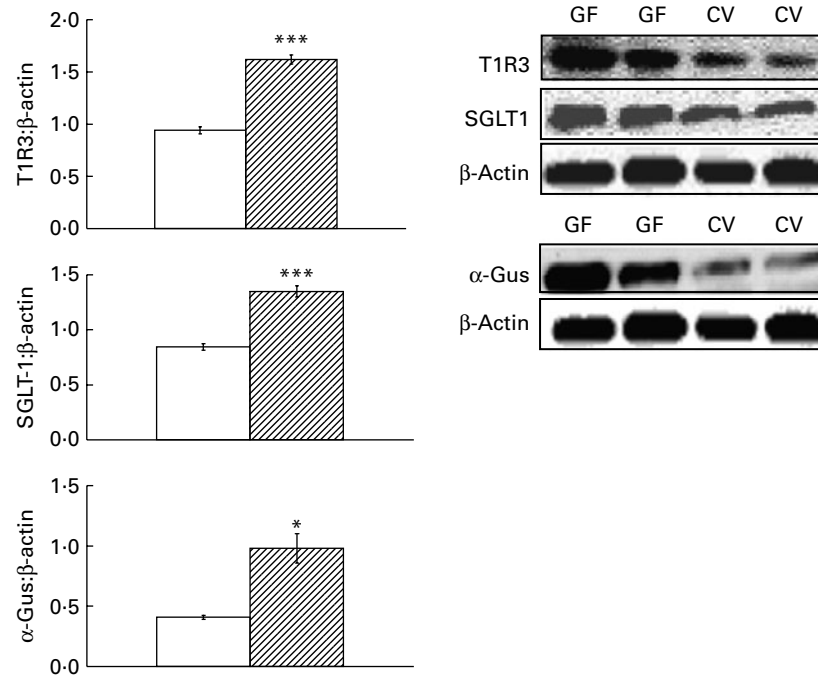
The results from the present study demonstrate that GF and CV mice display an equal preference for both nutritive (sucrose) and non-nutritive (saccharin) sweet solutions when tested in 48 h two-bottle preference tests. However, compared with CV mice, GF mice consumed more of the high concentration of sucrose solution, resulting in increased total energy intake from sucrose while the intake of saccharin remained similar between the groups. We also found that gene and protein expression of the intestinal sweet taste receptor, T1R3, and the luminal GLUT, SGLT-1, is significantly enhanced in GF mice compared with controls. In the lingual epithelium, the expression of the sweet taste heterodimer, T1R2/3, is unchanged in GF mice compared with control animals. Collectively, these results demonstrate, for the first time, that GF mice have increased avidity for concentrated sucrose solutions, with no apparent differences in sweet taste sensitivity. This behavioural effect may be due to adaptive changes in the intestinal sweet receptor and luminal GLUT that arise from a lack of an intact intestinal microbiota.

Both sweet acceptance (volume of intake) and preference (intake relative to water) are controlled in the short term by taste in the mouth while the development of the long-term acceptance of and the preference for nutritive sweet stimuli is governed primarily by post-oral nutrient feedback, reinforcing oral cues or taste associations, thus stimulating further consumption<sup>(31,32)</sup>. This supports the assertion that taste is a weak indicator of day-long sweet solution intake<sup>(33)</sup>. To examine whether the gut microbiota has an effect on either oral or

post-oral factors influencing sweet solution acceptance and preference, we employed 48 h two-bottle tests, which allow examination of both intake and preference of solutions. The present results revealed that GF mice have increased intake of 8% sucrose solution over 48 h compared with CV controls. Furthermore, the increased intake of higher concentration of sucrose solutions in GF mice compared with CV mice leads to an increased consumption of energy from 8 and 16% sucrose solutions during the 2 d period. This elevated sucrose intake may reflect their overeating response and may be an attempt to capture energy that is not available from the solid diet. However, compared with CV controls, GF mice were resistant to weight gain during access to 16% sucrose solution, supporting previous findings showing that GF animals do not gain weight and require a higher energy intake to maintain their weight than those with an intact microbiota<sup>(28)</sup>. Because sucrose is both sweet and nutritive, it is unclear based on these data alone whether GF mice consume more sucrose due to oral or post-oral factors. Therefore, we also presented mice with increasing concentrations of



**Fig. 4.** Small-intestinal mRNA expression of sweet taste receptor subunits and Na-glucose transporter 1 (SGLT-1) in germ-free (GF, ▨) and conventional (CV, □) C57BL/6J mice. (a) Type 1 taste receptor 3 expression was up-regulated 6-fold in GF mice compared with CV controls. (b) GF mice displayed a 1.4-fold increase in SGLT-1 mRNA expression in the small intestine compared with CV. (c)  $\alpha$ -Gustducin was up-regulated in GF mice but was not statistically different from CV controls. Values are means of triplicate ( $n$  5), with standard errors represented by vertical bars, normalised to the internal control and expressed relative to the CV control. Mean values were significantly different from those of CV: \* $P < 0.05$ , \*\* $P < 0.01$ .



**Fig. 5.** Western blot and analysis of the small-intestinal protein expression of sweet taste receptor subunits and Na-glucose transporter (SGLT-1) in germ-free (GF, ▨) and conventional (CV, □) C57BL/6J mice ( $n$  3). Protein expression of type 1 taste receptor (T1R3), SGLT-1 and  $\alpha$ -gustducin ( $\alpha$ -gus) was significantly higher in GF mice than in CV mice. Values are means, with standard errors represented by vertical bars, expressed relative to  $\beta$ -actin as the internal control. Mean values were significantly different: \* $P$ <0.05, \*\*\* $P$ <0.001.

non-nutritive saccharin solutions and found that acceptance of saccharin solutions was similar between the groups. Furthermore, we found that both preference and oral sensitivity (concentration at which each group preferred tastant to water) of saccharin and sucrose were similar between the groups. Together, these findings demonstrate that increased acceptance of sweet nutritive solutions in GF mice is due to the nutritive property of sucrose.

Accumulating evidence has demonstrated that intestinal T1R3 and other associated taste signalling proteins (e.g.  $\alpha$ -gustducin) located on enteroendocrine cells in the epithelium of the upper intestinal tract are responsible, at least in part, for the detection of sweet stimuli<sup>(16,20)</sup>. Specifically, short- and long-term exposure to dietary sugars<sup>(34)</sup> or a diet high in carbohydrates<sup>(20,35)</sup> significantly increases expression of intestinal T1R3 mRNA. The increase in T1R3 expression is accompanied by increased expression of luminal SGLT-1<sup>(20,36)</sup>. Similarly, in the present study, intestinal T1R3 as well as SGLT-1 mRNA and protein levels were significantly increased in GF mice compared with controls. There is as yet no direct evidence for a role of intestinal T1R3 in sugar ingestion. The finding that T1R3 KO mice under-consume concentrated Polycose solutions is consistent with this possibility<sup>(16)</sup>. On the other hand, the fact that T1R3 KO mice self-administer as much sucrose by intragastric infusion as do intact mice argues against a critical role of intestinal T1R3 in the control of sugar intake<sup>(37)</sup>. Nevertheless, based on these findings and the present results, we postulate that enhanced sucrose acceptance in GF mice might be due to an increased cellular capacity of enteroendocrine cells and

enterocytes to detect and/or transport more glucose from the intestinal lumen, an effect coordinated by T1R3 and SGLT-1. Thus, it appears that the small intestine adapts to a luminal environment devoid of microbiota by increasing sweet receptor and sugar transporter expression. This enhanced detection and absorption may further perpetuate increased consumption of sweet solutions. Similar phenomena have been reported in pathological conditions, such as diabetes<sup>(38,39)</sup>.

The sweet-responsive receptor in the proximal small intestine may be responsible, in part, for nutritive post-oral feedback from sucrose. For example, T1R3 KO mice, which are unresponsive to sugars in the mouth, consume less sucrose than control mice. Additionally, during 48 h two-bottle tests, T1R3 KO mice display both decreased acceptance of and preference for low concentrations of sucrose (0.5–8%)<sup>(16)</sup>. However, they exhibit strong preferences for high concentrations of sugars and are responsive to the post-oral conditioning effects of sugars<sup>(16,37)</sup>. Therefore, based on these data, it seems that the up-regulation of T1R3 expression in GF mice cannot be attributed to the post-oral reinforcement effect of sucrose. An interpretation of these findings is that intestinal T1R3 is associated with acceptance of, rather than preference for, sugars via increased absorption through SGLT-1 as a compensatory mechanism to facilitate sugar uptake in the absence of intestinal microbiota leading to increased intake. The exact mechanism(s) responsible for increased T1R3 and SGLT-1 expression in GF mice is not completely known; however, digestive, endocrine and morphological adaptations to the lack of gut microbiota are

the most plausible factors. Conducting microbiota inoculation studies in GF animals to determine whether these changes are reversible, ruling out a developmental difference, will shed more light on the direct role of the microbiota in glucose sensing. Previous work examining the effects of conventionalisation on metabolic, hormonal and morphological alterations in GF mice showed that conventionalisation reversed most, if not all, differences observed<sup>(5,28,40)</sup>.

A major role of microbiota is in energy metabolism by promoting intestinal monosaccharide absorption and energy extraction from normally undigested polysaccharides via release of SCFA<sup>(28)</sup>. For example, the absence of gut microbiota results in increased energy in faeces<sup>(28,41)</sup> and that conventionalisation of GF animals promotes increased monosaccharide uptake in the distal intestine<sup>(28)</sup>. Recently, Cresci *et al.*<sup>(40)</sup> have shown that SGLT-1 mRNA transcript levels were dramatically reduced in the terminal ileum of GF mice, and immediately restored after re-colonisation. This is not surprising since one of the main roles of microbiota is to process dietary carbohydrates via microbial glycosylhydrolases, thus increasing monosaccharide delivery in the distal intestine. However, the regulation of hexose transporter expression along the length of the intestinal epithelium is highly differential. In normal mice, SGLT-1 mRNA expression is the most intense in the first segments of the proximal small intestine, with nearly 4-fold higher levels than that of the distal small intestine<sup>(42)</sup>. Thus, it is conceivable that the GF state alters proximal intestinal transport differentially compared with the distal intestine. In addition, GF rodents have slower intestinal transit time<sup>(5)</sup> and increased duodenal brush-border disaccharidase levels<sup>(9,43–45)</sup>. Thus, by increasing the time of nutrient exposure to the intestine through decreased transit and increased disaccharide hydrolysis, GF mice may be able to better detect and transport sugar. Because the intestine modulates its glucose absorptive capacity through changes in intestinal SGLT-1 expression, it is reasonable to assume that increases in SGLT-1 mRNA expression in GF mice were associated with the increase in glucose absorption from 8 and 16% sucrose, which in turn might decrease luminal stimulation over a large area of the intestine, resulting in reduced satiation. Meyer *et al.*<sup>(46)</sup> demonstrated that the satiating effect of intestinal sugar is proportionate to the length of the intestine exposed.

Enteroendocrine cells act as primary chemoreceptors and respond to luminal constituents such as nutrients by releasing peptides known to regulate GI functions<sup>(47)</sup>. Indirect evidence suggests a role of gut microbiota in the secretion and function of some gastrointestinal peptides, such as 5-hydroxytryptamine, CCK, GLP-1, GLP-2, peptide YY (PYY) and ghrelin<sup>(3,5,7,18,19,48–50)</sup>. As such, increasing microbial fermentation results in lowered appetite, elevated plasma levels of GLP-1, GLP-2 and PYY<sup>(51)</sup> and decreased levels of ghrelin<sup>(50)</sup>. Furthermore, SCFA, a product of gut microflora, have been shown to induce enhancement in colonic motility via 5-hydroxytryptamine release<sup>(52)</sup>, as well as increase plasma PYY levels<sup>(5,53,54)</sup>. PYY inhibits food intake, gastric emptying, pancreatic and intestinal secretions and gut motility<sup>(55)</sup>. Although we did not measure plasma gut peptide levels in the present

study, based on the above evidence, it is tempting to speculate that decreased anorexigenic and increased orexigenic GI signalling in GF mice may also be responsible for increased sucrose acceptance. However, the intestinal epithelium undergoes profound morphological adaptive changes including changes in the content, density, distribution and function of the enteroendocrine cells. As a result, in the GF condition, for example, marked elevation in plasma enteroglucagon and PYY levels has also been reported<sup>(8)</sup>, similar to the findings observed following microbial manipulation through diet or prebiotics<sup>(51)</sup>. Thus, the role of the gut peptides in the control of food intake in GF animals, in general, or in increased sucrose acceptance, in particular, is not currently known. However, it is known that orally ingested sucrose initiates an incretin response, an effect mediated by GLP-1 from enteroendocrine cells that also express taste signalling elements such as T1R3 and gustducin<sup>(19)</sup>. It has been suggested that activation of T1R2, T1R3 and gustducin provokes GLP-1 release and enhanced SGLT-1 expression<sup>(20)</sup>. As such, T1R3 KO mice have decreased circulatory GLP-1 levels and a blunted up-regulation of SGLT-1 while maintained on a high-carbohydrate diet<sup>(19,20)</sup>. While we are unaware of studies examining GLP-1 in GF animals, based on previous findings demonstrating increased enteroglucagon in GF animals<sup>(6–8,56)</sup>, it is reasonable to stipulate that GLP-1 is also increased as these peptides are derived from the same transcription product of the proglucagon gene<sup>(57,58)</sup> and are co-released in response to nutrients. Despite these increased levels of inhibitory gut peptides, GF animals also have significantly up-regulated expression of dipeptidyl-peptidase 4, a ubiquitous enzyme that degrades GLP-1<sup>(44)</sup>. If T1R3 stimulates GLP-1 release, increasing nearby SGLT-1 expression, then increased degradation of GLP-1 via dipeptidyl-peptidase 4 may be one reason why we observed a much higher expression of T1R3 (6-fold) compared with SGLT-1 (1.6-fold) in GF animals. Taken together, endocrine and physiological GI alterations, due to the lack of microbiota and resulting impaired distal GI nutrient absorption, may be the predominant factors responsible for increasing expression of T1R3 and SGLT-1 and the associated increase in sucrose intake.

Although we found a difference in sucrose acceptance, threshold for detection and preference for both sucrose and saccharin relative to water was similar between the groups. However, there was no difference in the expression of the T1R2/T1R3 heterodimer in the lingual epithelium in GF mice. Several mechanisms may be responsible for this, including modulation of the sweet receptor by energy and nutritional status. For example, previous data have demonstrated that plasma leptin inversely correlates with T1R3 expression and responsiveness<sup>(59)</sup>. Specifically, dietary or genetically obese rodents have decreased T1R3 expression<sup>(60)</sup> and decreased activation of sweet-best taste cells in response to sweet stimuli<sup>(61)</sup>. Furthermore, taste cells express leptin receptors and are inhibited by leptin application<sup>(61,62)</sup>. In the present study, we did not measure leptin or analyse body adiposity. However, based on previous work as well as on our own observations, GF mice have a marked decrease in body fat content compared with controls, which results in

decreased circulating plasma leptin levels<sup>(5,28)</sup>. Thus, locally produced leptin acting through a paracrine or autocrine route may be responsible for taste cell responsiveness, similar to other taste transduction peptides<sup>(63–65)</sup>. Furthermore, we found a nearly significant down-regulation of  $\alpha$ -gustducin in the lingual epithelium of GF mice, which should result in decreased sensitivity and preference for sweet tastants. However,  $\alpha$ -gustducin is co-localised with bitter and umami taste receptors<sup>(66–69)</sup>, whose role is prominent in the posterior circumvallate papillae<sup>(70,71)</sup>. In our preliminary findings with the bitter tastant, quinine hydrochloride, however, we found no difference in preference or aversion between GF and CV mice (data not shown).

In summary, the present study shows that while GF and CV mice exhibit similar taste sensitivity to sweet solutions, intestinal nutrient detection and absorption are significantly increased in GF animals, which is associated with increased acceptance of high concentrations of sucrose solutions in this model. Specifically, intestinal but not lingual T1R3 mRNA and protein expression is markedly up-regulated in GF animals. This is accompanied by increased mRNA and protein expression of the luminal GLUT, SGLT-1. Collectively, these data provide strong evidence demonstrating, for the first time, that intestinal sweet receptors and transporters are adaptively regulated by the absence of microbiota, resulting in a compensatory mechanism allowing the proximal intestine to detect and absorb nutrients more readily to overcome distal GI tract deficits in absorption.

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