



Nutritive/non-nutritive sweeteners and high fat diet contribute to dysregulation of sweet taste receptors and metabolic derangements in oral, intestinal and central nervous tissues

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Abstract

Objectives Overconsumption of non-nutritive sweeteners is associated with obesity, whereas the underlying mechanisms remain controversial. This study aimed to investigate the effects of long-term consumption of nutritive or non-nutritive sweeteners with or without high fat diet on sweet taste receptor expression in nutrient-sensing tissues and energy regulation dependent on sweet-sensing.

Methods 50 Male Sprague–Dawley rats (140–160 g) were assigned to 10 groups (n = 5/group). All received fructose at 2.5% or 10%, sucralose at 0.01% or 0.015% or water with a normal chow diet or high fat diet for 12 weeks. Food and drink intake were monitored daily. Oral glucose tolerance test and intraperitoneal glucose tolerance test were performed at week 10 and 11 respectively. Serum was obtained for measurement of biochemical parameters. Tongue, duodenum, jejunum, ileum, colon and hypothalamus were rapidly removed to assess gene expression.

Results Long-term consumption of sweeteners impaired glucose tolerance, increased calorie intake and body weight. A significant upregulation of sweet taste receptor expression was observed in all the four intestinal segments in groups fed 0.01% sucralose or 0.015% sucralose, most strikingly in the ileum, accompanied by elevated serum glucagon-like peptide-1 levels and up-regulated expression of sodium-dependent glucose cotransporter 1 and glucose transporter 2. A significant down-regulation in the tongue and hypothalamus was observed in groups fed 10% fructose or 0.015% sucralose, with alterations in hypothalamic appetite signals. The presence of high fat diet differentially modulates sweet taste perception in nutrient-sensing tissues.

Conclusions Long-term consumption of whether nutritive sweeteners or non-nutritive sweeteners combined with high fat diet contribute to dysregulation of sweet taste receptor expression in oral, intestinal and central nervous tissues.

Keywords Non-nutritive sweeteners · High fat diet · Sweet taste receptor · Glycemic control · Obesity

Abbreviations

AGRP	Agouti-related peptide
AUC	Area under the curve
GLP-1	Glucagon-like peptide-1
GLUT2	Glucose transporter 2
HFD	High-fat diet
IPGTT	Intraperitoneal glucose tolerance test
NCD	Normal control diet
NNS	Non-nutritive sweetener
NPY	Neuropeptide Y

OGTT	Oral glucose tolerance test
SGLT-1	Sodium-dependent glucose cotransporter 1
STR	Sweet taste receptor
T1R2	Taste receptor type 1 member 2
T1R3	Taste receptor type 1 member 3

Introduction

In the last few decades obesity has become a major public health problem that places patients at a greater risk of obesity-related diseases and significantly decreases the quality of life [1]. Excessive sugar sweetened beverages consumption, high-fructose corn syrup in particular, is increasingly considered as a major contributor to the obesity epidemic worldwide [2]. Therefore, non-nutritive sweeteners

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(NNSs) emerged as sugar substitutes to assist in weight control because they provide sweet taste with negligible energy [3]. However, there is a growing body of evidence suggesting that the consumption of NNSs may be closely associated with a greater risk of the metabolic syndrome and obesity epidemic, whereas the underlying mechanisms remain elusive [4].

Sweet taste performs a critical function in nutrient sensing as well as the control of food intake [5] and is initially sensed in gustatory tissues such as the anterior and posterior tongue and palate via sweet G protein-coupled taste receptors, the heterodimeric receptor of taste receptor type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3) [6]. Of note, sweet taste receptors (STRs) have also been found in chemosensory cells located in extra-oral tissues, most of which play an essential role in regulating glucose homeostasis and maintaining energy balance [7], such as gastrointestinal tract [8–10], hypothalamus [11], pancreas [12], and adipose tissue [13]. Hypothalamus is a nodal brain structure where information on systemic energy levels as well as metabolic status is detected via hormones or nutrients and integrated for homeostatic regulation, including the stimulation or inhibition of feeding behaviors and the control of energy expenditure [14]. Recent studies have indicated that STRs may play a role in nutrient sensing, especially glucose sensing in the hypothalamic feeding center via activating the arcuate nucleus neurons [15]. Similar to the response in hypothalamus, the presence of STRs in the gastrointestinal tract could also be functionally involved in the glucose responsiveness and multiple regulatory effects independently of carbohydrate metabolism, such as the modulation of hormone secretion and glucose absorption [16].

Accumulating evidence indicates that not only nutritive sweeteners, such as sucrose and fructose, but also NNSs could selectively bind to different domains of T1R2/T1R3 [17], raising the possibilities that sweeteners may disturb sweet taste perception in multiple tissues via long-term activation of STRs and further lead to potential health risks [18]. However, little is known about the long-term effects of sweeteners on the expression levels of STRs in different tissues. Besides, while most of the published findings on sweeteners have been short-term or single-dose [19, 20], it is worthwhile to investigate the long-term effects of sweeteners at various concentrations. Further, considering that the modern diets most often comprise of a mixture of various nutrient components, what also interests us is that how the joint use of different sweeteners and a high fat diet (HFD) contribute to the dysregulation of STRs [21]. Thus, the aim of the present work was to investigate how long-term consumption of nutritive sweeteners or NNSs in the presence or absence of HFD alter mRNA expression of STRs and affect hormone secretion, glucose transport and ingestive

behavior. We chose fructose and sucralose as our target sweeteners, in view of their increasing popularity worldwide and the continuing controversy over their associations with obesity and type 2 diabetes [22–24].

Methods

Animals and diets

A total of 50 Male Sprague–Dawley rats (140–160 g) were purchased (SJA Laboratory Animal, Hunan, China) and housed in individual cages with constant temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$), relative humidity ($60\% \pm 5\%$), a 12 h light–dark cycle and free access to water and food. After one-week quarantine and acclimation period, rats were randomized into 10 groups ($n=5$ per group), which were fed a normal control diet (NCD) or HFD (45% of energy as fat; Xietong Shengwu, Jiangsu, China) and received water or different sweeteners dissolved in water at the following concentration: fructose at 2.5% or 10% [25]; sucralose at 0.01% or 0.015% [26], and weighed daily (For more information about the composition of different diets, please see Supplementary Tables 1, 2, 3). Pre-weighed portions of food and premeasured volume of either water or the sweet taste solutions were offered to the rats. Food intake and drink consumption were monitored daily from week 1 to week 10. At the end of the 12 weeks experiment, the animals were fasted for 12 h and sacrificed by cervical dislocation after anesthetized with intraperitoneal injection of sodium pentobarbital. Blood samples were collected for serum biochemical analysis. Tongue, duodenum, jejunum, ileum, colon and hypothalamus were immediately frozen after dissection in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$. Animal use in this study was approved by the Institutional Animal Care and Use Committee, Wuhan University Center for Animal Experiment, Wuhan, China (AUP No. WP20210533).

Oral glucose tolerance test (OGTT) and intraperitoneal glucose tolerance test (IPGTT)

After feeding for 10 weeks, an oral glucose tolerance test was performed in all groups. After fasting for 14 h, all rats received 50% glucose solution via oral gavage (2 g/kg BW). Blood samples were adopted by rat tail snipping method and blood glucose levels were measured at 0 min (before gavage), 15 min, 30 min, 60 min, 90 min and 120 min via a glucometer and glucose test strips [27] (Roche, Shanghai, China).

For IPGTT, rats were fasted for 14 h after 11 weeks of diet administration and injected with a bolus of 50% glucose (2 g/kg BW) [28–30]. Blood glucose levels were measured

at 0 min (before injection), 15 min, 30 min, 60 min, 90 min and 120 min by a glucometer.

Biochemical parameters

Serum glucose concentrations were analyzed using standard kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and serum insulin, glucagon-like peptide-1 (GLP-1) and leptin were determined using commercial ELISA kits (Ruixin Biotechnology, Quanzhou, China) following the manufacturers' instructions.

Quantitative real-time PCR

Total RNA was extracted from tongue, duodenum, jejunum, ileum, colon and hypothalamus of individual rats using Biomarker Cell/Tissue Total RNA Isolation Kit (Biomarker Technologies, Beijing, China). cDNA was synthesized using BiomarkerScript II 1st Strand cDNA Synthesis Kit (Biomarker Technologies, Beijing, China). Real-time PCR was performed using the Biomarker 2X SYBR Green Fast qPCR Mix (Biomarker Technologies, Beijing, China) and Bio-Rad CFX maestro system. All manipulations were carried out according to the manufacturer's instructions. (All primer sequences for gene expression determination are shown in Supplementary Table 4). The β -actin gene was used as the internal control.

Statistical analysis

Shapiro–Wilk normal distribution test was used for assessing the compliance of data with the normal distribution. All data comply with normal distribution and are presented as mean \pm standard deviation. Statistical analysis between multiple groups was performed using one-way ANOVA followed by Tukey's post-hoc test; statistical analysis between two groups was performed using Student's *t* test. All the data analysis in this study were performed in GraphPad Prism 8.0 (GraphPad Software, USA); $p < 0.05$ was considered to be statistically significant.

Results

Body weight and changes in food intake, drink intake and calorie intake

Rats fed 2.5% fructose or 10% fructose plus NCD consumed significantly less food than the control group (NCD + water). On the contrary, an evident increase in food consumption was observed in groups fed 0.015% sucralose. Food intake was significantly reduced in all groups by the presence of HFD (Fig. 1a and b). The addition of sweetener in the

water significantly increased drink consumption, which was evidently reduced by the presence of HFD in all groups. Notably, rats fed 10% fructose exhibited the highest drink consumption whether in the presence of HFD or not (Fig. 1c and d). For total calorie intake, rats fed 0.015% sucralose unexpectedly showed the highest calorie intake among groups fed NCD, even higher than those fed 10% fructose. On the contrary, rats fed 2.5% fructose maintained a normal level of calorie intake. The presence of HFD significantly increased calorie intake in groups fed 2.5% fructose or 0.01% sucralose (Fig. 2a and b). Among groups fed NCD, rats fed with 10% fructose or 0.015% sucralose gained significantly more weight than those fed water, while the addition of HFD further increased body weight in all groups (Fig. 2c).

Long-term supplementation of sweeteners and HFD impaired glucose tolerance

We performed OGTT and IPGTT to test whether challenges of long-time dietary intervention were accompanied by changes in glucose homeostasis. The IPGTT bypasses the oral cavity as well as intestine and assesses peripheral glucose tolerance which depends on pancreas, muscle, liver, adipose tissue [31]. Among groups fed NCD, AUC_{OGTT} was significantly increased in rats fed 2.5% fructose or 10% fructose (Fig. 3a and b), while rats fed 0.015% sucralose had the highest AUC_{IPGTT} , followed by those fed 0.01% sucralose (Fig. 3c and d). The presence of HFD increased both AUC_{OGTT} (Fig. 3a and b) and AUC_{IPGTT} (Fig. 3c and d) in all groups, while the statistical significance was reached in rats fed 10% fructose, 0.015% sucralose or water in OGTT and rats fed water in IPGTT compared with the corresponding group fed NCD.

Biochemical parameters

Rats fed with 2.5% fructose, 10% fructose, 0.01% sucralose or 0.015% sucralose plus NCD demonstrated significantly increased fasting blood glucose levels, whereas only those fed with 2.5% fructose, 10% fructose or 0.015% sucralose developed hyperinsulinemia. The presence of HFD evidently raised fasting blood glucose in rats fed 10% fructose or water and insulin levels in groups fed 10% fructose, 0.01% sucralose, 0.015% sucralose or water (Fig. 4a and b). Fasting serum GLP-1 levels were significantly increased in all groups, while the presence of HFD further elevated serum GLP-1 levels in those fed 2.5% fructose, 10% fructose, 0.015% sucralose or water (Fig. 4c). Among groups fed NCD, rats fed 10% fructose, 0.01% sucralose or 0.015% sucralose developed hyperleptinemia. Rats fed water plus HFD also showed significantly increased serum leptin levels, while the presence of HFD further significantly increased

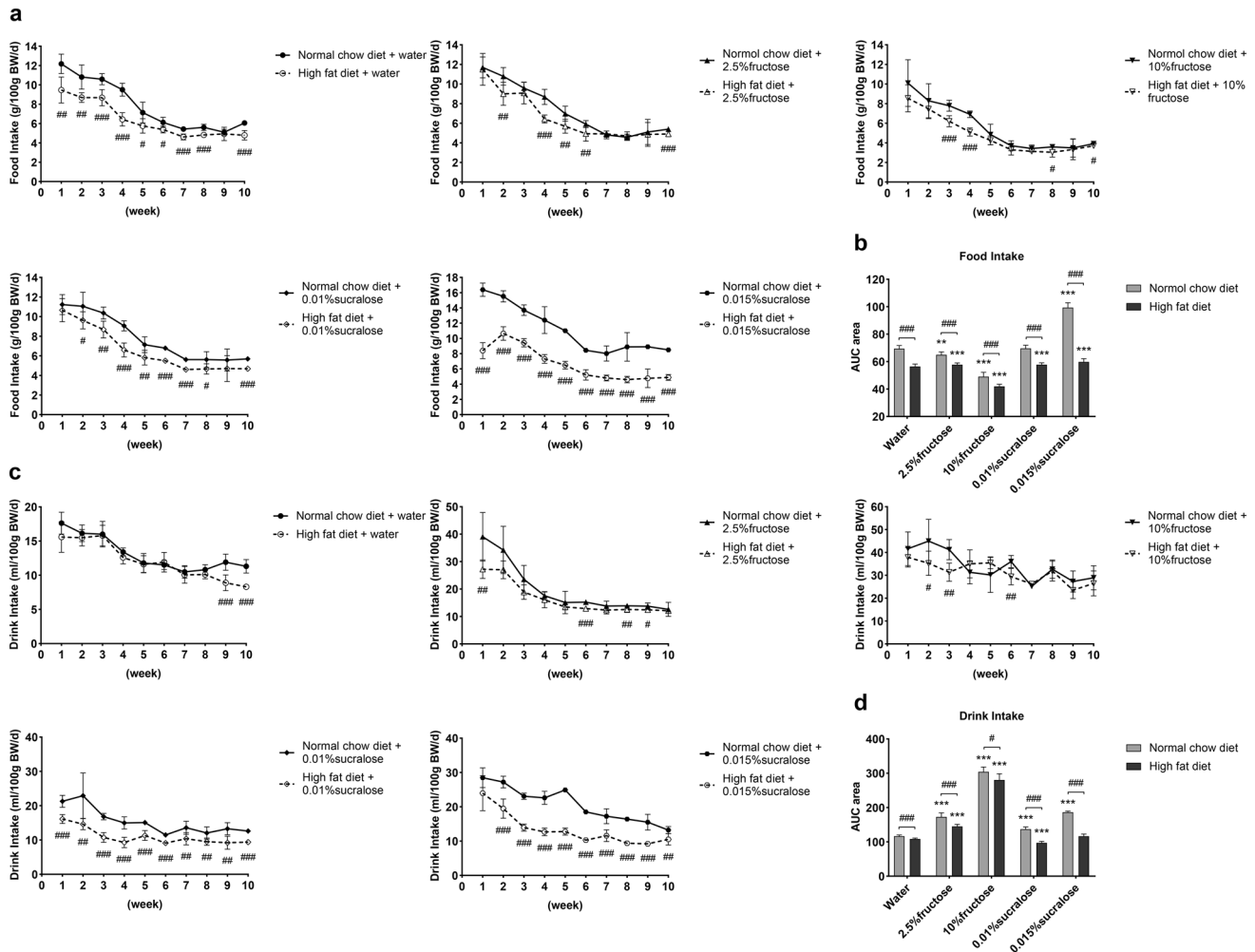


Fig. 1 Changes in food intake and drink intake. **a** Food intake from week 1 to week 10; **b** AUC for food intake; **c** Drink intake from week 1 to week 10; **d** AUC for drink intake. Data are presented as mean \pm SD, $n = 5$ per group. Results are considered significant at $p < 0.05$.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

serum leptin levels in those fed 2.5% fructose, 0.01% sucralose, 0.015% sucralose (Fig. 4d).

STR expression along the alimentary canal

In the tongue, among groups fed NCD, rats fed 10% fructose or 0.015% sucralose showed significantly down-regulated T1R2 and T1R3 expression, while the presence of HFD evidently up-regulated T1R2 and T1R3 expression in those fed 0.01% sucralose, 0.015% sucralose or water (Fig. 5, a and b). In the duodenum, T1R2 was evidently induced by all sweeteners in groups fed NCD, while 0.015% sucralose stimulated the expression of T1R3 to a higher extent than the rest of the sweeteners, followed by 0.01% sucralose and 10% fructose (Fig. 5c and d).

An increase in the expression of T1R2 and T1R3 in jejunum (Fig. 6a and b) and ileum (Fig. 6c and d) was also

observed among groups fed NCD. By contrast, colonic expression of T1R2 and T1R3 was significantly down-regulated in rats fed 2.5% fructose plus NCD (Fig. 6e and f). In the presence of HFD, mRNA expression of T1R2 and T1R3 was significantly down-regulated in groups fed 2.5% fructose or 0.01% sucralose, but up-regulated in groups fed 0.015% sucralose or water in the jejunum (Fig. 6a and b). By contrast, in the ileum, the presence of HFD further significantly increased the expression of T1R2 and T1R3 in most of the groups with the exception of those fed 10% fructose (Fig. 6c and d). For the colon, the presence of HFD evidently up-regulated T1R2 expression in groups fed 2.5% fructose, 10% fructose or water (Fig. 6e and f).

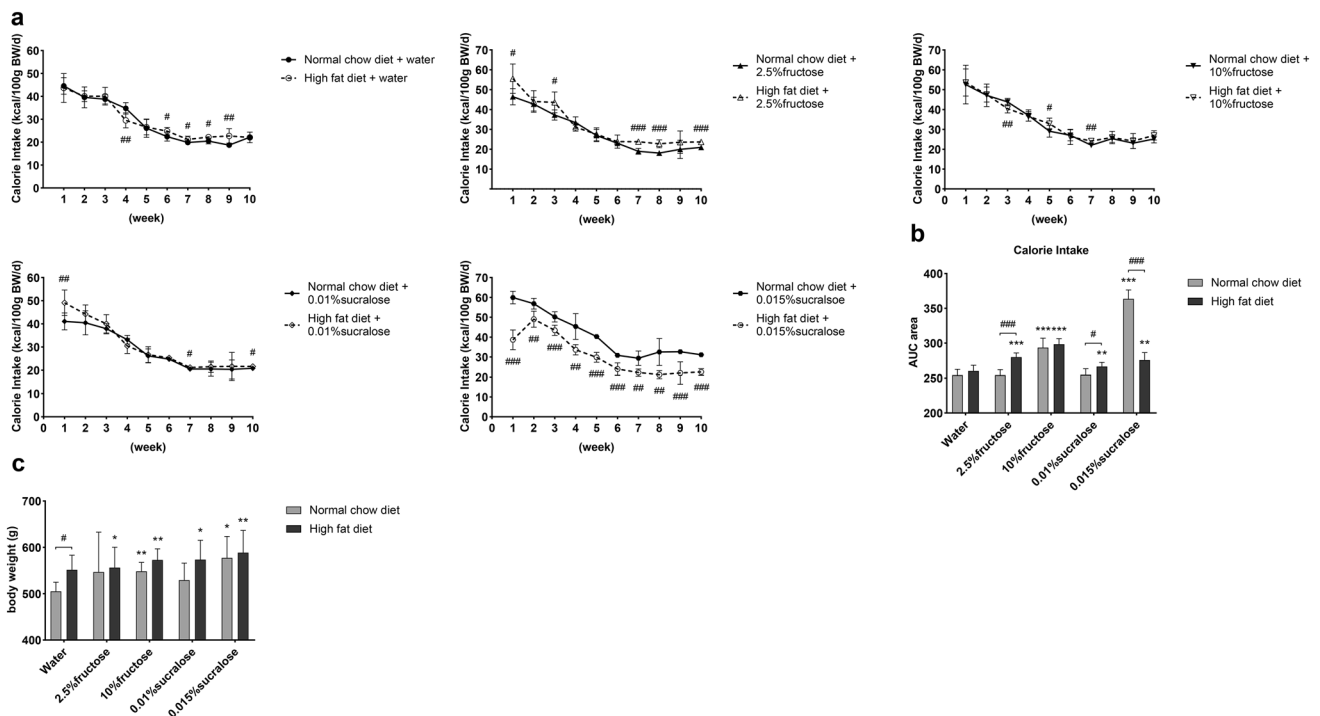


Fig. 2 Changes in calorie intake and body weight. **a** Calorie intake from week 1 to week 10; **b** AUC for calorie intake; **c** Body weight. Data are presented as mean ± SD, n = 5 per group. Results are

considered significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

Changes in expression of glucose transporters in the duodenum, jejunum and ileum

Upon activation by the presence of sweeteners in the intestinal lumen, STRs could facilitate glucose transport capacity via sodium-dependent glucose cotransporter 1 (SGLT-1) and glucose transporter 2 (GLUT2), both of which are critical transporters for glucose absorption [32]. In the duodenum, SGLT-1 expression was significantly up-regulated in rats fed 2.5% fructose or 10% fructose among groups fed NCD. The presence of HFD up-regulated SGLT-1 expression in rats fed 0.015% sucralose or water but exhibited an opposite effect in groups fed 2.5% fructose, 10% fructose or 0.01% sucralose (Fig. 7a). SGLT-1 expression in the jejunum (Fig. 7c) followed a similar pattern. In the ileum, rats fed 2.5% fructose or 0.015% sucralose showed significantly up-regulated SGLT-1 expression, which was further evidently induced by the presence of HFD in groups fed 0.01% sucralose or water (Fig. 7e).

The stimulating effect on jejunal and ileal GLUT2 expression by the long-term consumption of sweeteners was more evident than that on duodenal GLUT2 expression (Fig. 7b), while rats fed 2.5% fructose, 10% fructose, 0.01% sucralose or 0.015% sucralose showed significantly up-regulated GLUT2 expression both in the jejunum (Fig. 7d) and ileum among groups fed NCD. Of note, the

presence of HFD also remarkably up-regulated ileal GLUT2 expression in groups fed 2.5% fructose, 0.01% sucralose or 0.015% sucralose (Fig. 7f).

Changes in expression of STR and appetite signals in the hypothalamus

Among groups fed NCD, rats fed 10% fructose or 0.015% sucralose showed significantly down-regulated T1R2 and T1R3 expression in the hypothalamus, whereas only 2.5% fructose significantly up-regulated hypothalamic STR expression compared to the control group (NCD + water). Of note, the presence of HFD down-regulated both T1R2 and T1R3 expression in all groups, while the statistical significance was reached in groups fed 0.01% sucralose, 0.015% sucralose or water (Fig. 8a and b). Agouti-related peptide (AGRP) and neuropeptide Y (NPY) were significantly down-regulated in groups fed 10% fructose, 0.01% sucralose or 0.015% sucralose, and this effect was further exacerbated by the presence of HFD (Fig. 8c and d). On the contrary, proopiomelanocortin expression was significantly up-regulated in rats fed 2.5% fructose, 10% fructose or 0.015% sucralose, while HFD further increased proopiomelanocortin expression in groups fed 0.01% sucralose, 0.015% sucralose or water (Fig. 8e).

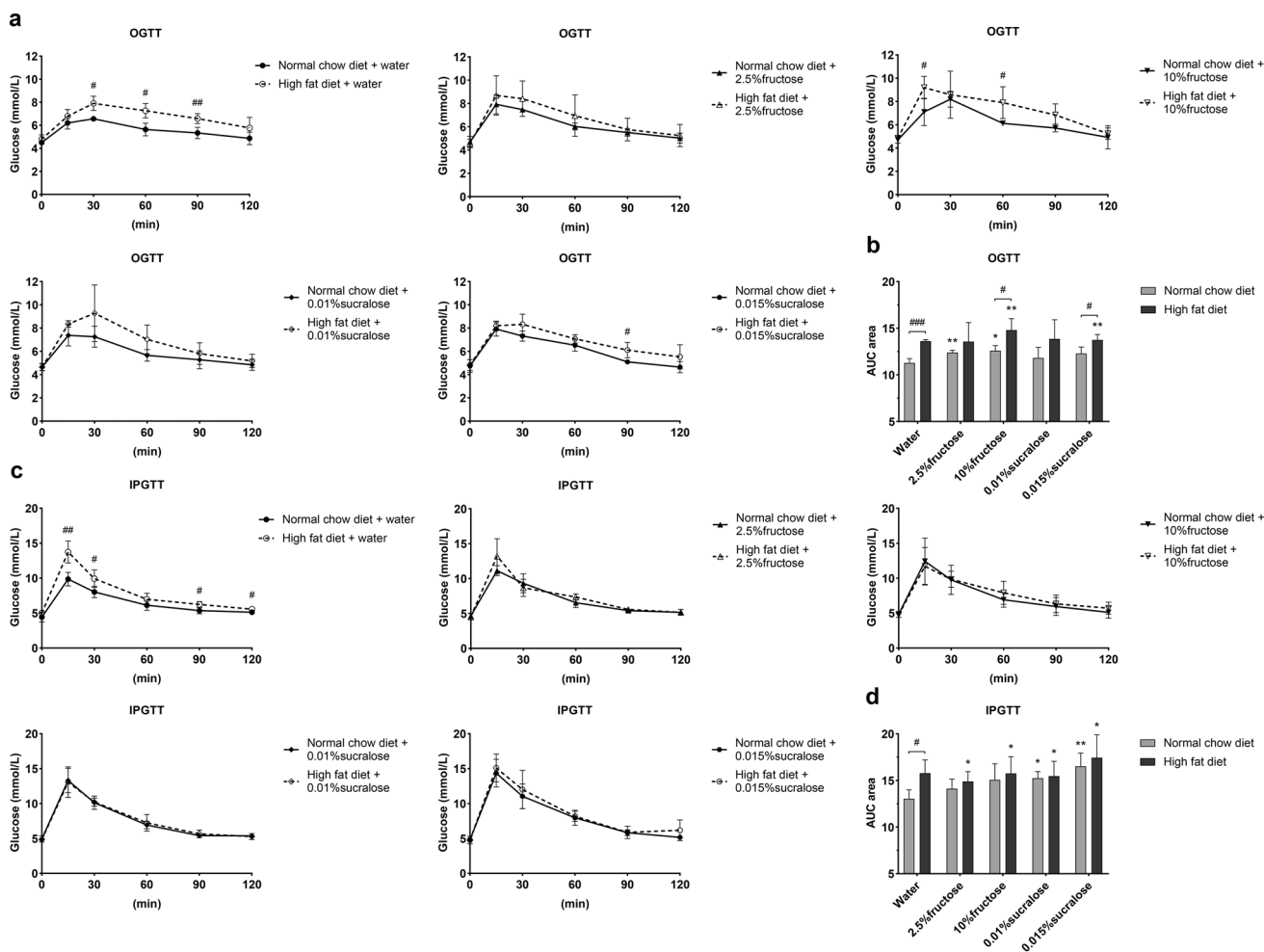


Fig. 3 Long-term supplementation of sweeteners and HFD impaired glucose tolerance. **a** Blood glucose levels at different time points during OGTT; **b** AUC in OGTT; **c** Blood glucose levels at different time points during IPGTT; **d** AUC in IPGTT. Data are presented as

mean \pm SD, $n = 5$ per group. Results are considered significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

Discussion

Our investigation started with exploring the effect of long-term consumption of sweeteners, in the presence or absence of HFD, on glucose homeostasis and obesity-related parameters. We found that persistent consumption of sweeteners, particularly 10% fructose and 0.015% sucralose, resulted in increased body weight, AUC_{OGTT} , AUC_{IPGTT} and higher blood glucose levels, which were further exacerbated in the presence of HFD. It is well recognized that the gastrointestinal tract is a major determinant of postprandial glycemic control, responsible for glucose transport and the release of diverse peptide hormones and neurotransmitters [33]. Our results demonstrated that rats fed NCD in combination with either fructose or sucralose showed an overall increased mRNA expression of T1R2 and T1R3 in the duodenum, jejunum and mainly in the ileum. However,

colonic expression of T1R2 and T1R3 was significantly up-regulated only in rats fed 0.01% sucralose or 0.015% sucralose among groups fed NCD. While a potential role of high fat diet in modulating intestinal STR expression has been indicated [25], in our study, the combination of HFD and sweetener had the most striking effect on the ileal STR expression. It is important to note that rats fed 0.01% sucralose or 0.015% sucralose plus HFD showed significantly up-regulated mRNA expression of T1R2 and T1R3 in all the four intestine segments. Evidence in rodents [34, 35], human cells and tissues [36, 37] strongly supports the functional role of STR, mainly expressed in gastrointestinal enteroendocrine cells, as an upstream sensor regulating GLP-1 secretion and the expression of glucose transporters, SGLT-1 and GLUT2 in the intestine. For example, GLP-1 secretion from the human L cell line NCI-H716 was promoted by sugars and sucralose but blocked

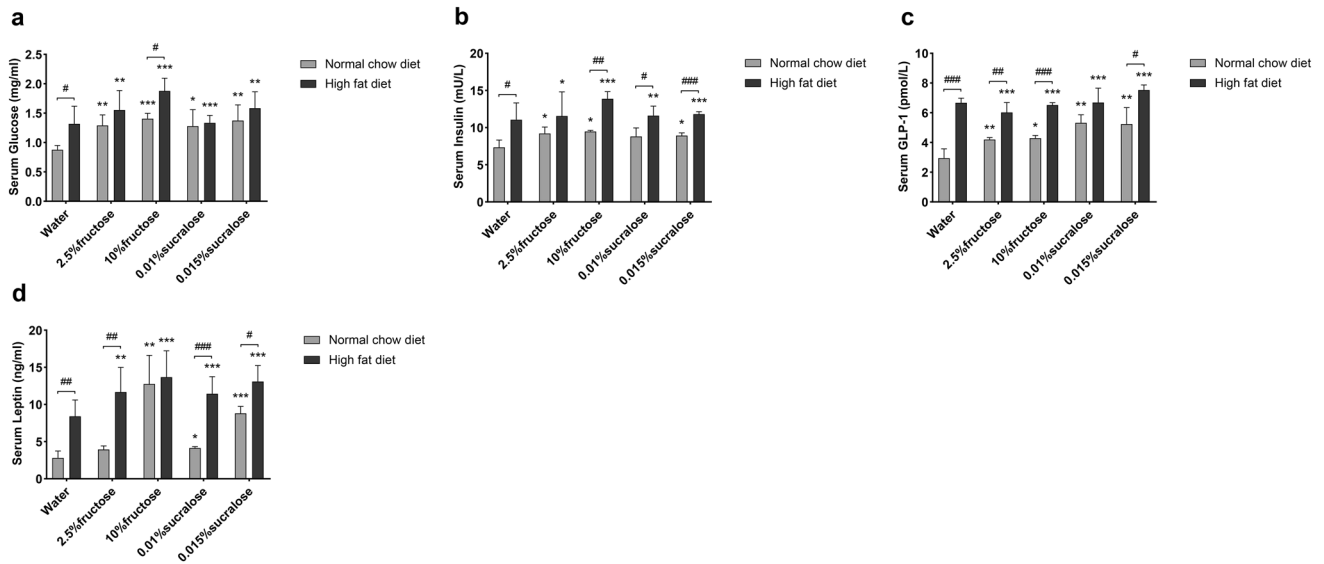


Fig. 4 Biochemical parameters. **a** Serum blood glucose levels; **b** Serum insulin levels; **c** Serum GLP-1 levels; **d** Serum leptin levels. Data are presented as mean ± SD, n = 5 per group. Results are

considered significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

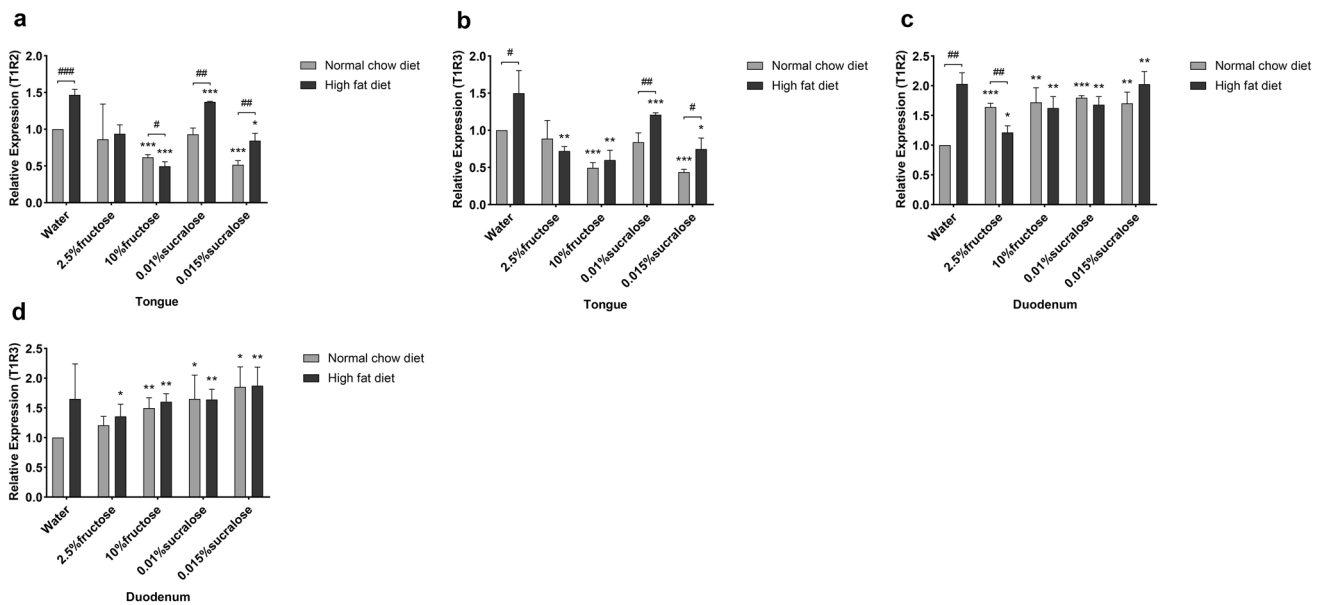


Fig. 5 STR expression in the tongue and duodenum. **a–b** T1R2 and T1R3 expression in the tongue; **c–d** T1R2 and T1R3 expression in the duodenum. Data are presented as mean ± SD, n = 5 per group. Results are considered significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$,

*** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

by the sweet receptor antagonist lactisole or siRNA for α -gustducin [38]. Besides, in knockout mice lacking T1R3 or α -gustducin, mRNA expression and protein abundance of SGLT-1 were not increased in response to dietary sugar and NNS supplementation, as occurred in wild-type mice [39]. Our study showed that intestinal STR dysregulation was accompanied by elevated fasting serum GLP-1 levels

and STR-dependent gains in mRNA expression of SGLT-1 and GLUT2 mainly in the ileum. We moreover noted that GLUT2 was stimulated to a higher extent than SGLT-1 by long-term consumption of fructose or sucralose whether in the presence or absence of HFD, suggesting an enhanced intestinal absorptive capacity especially when luminal glucose concentration is high after a meal, which further

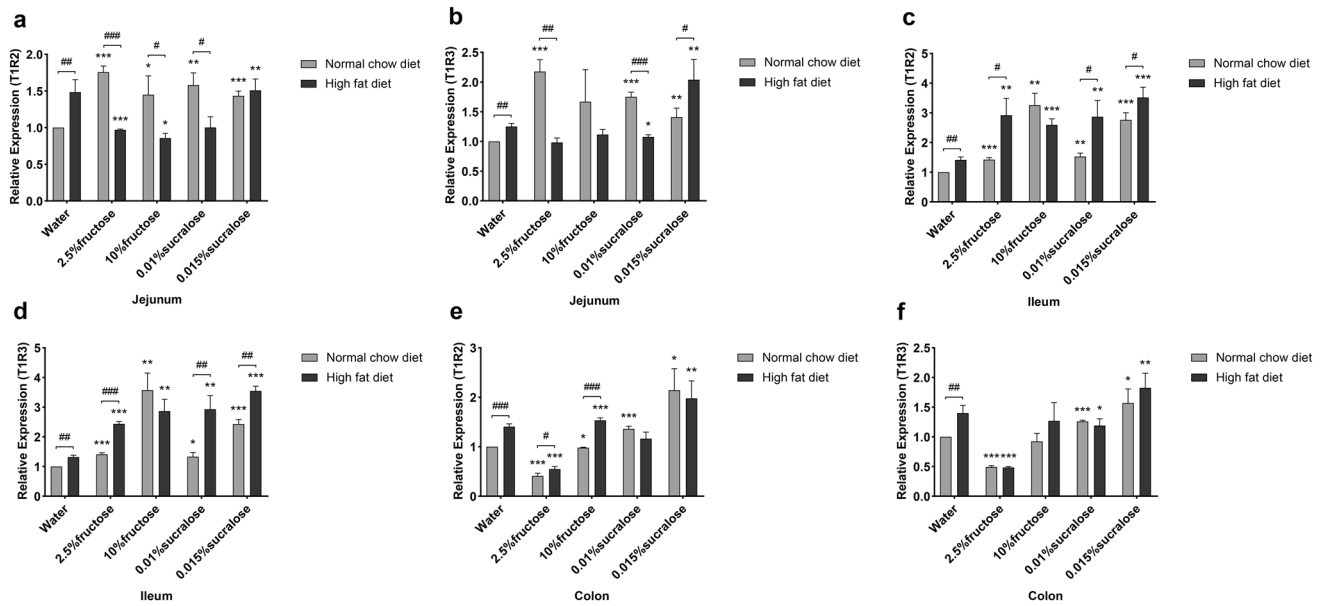


Fig. 6 STR expression in the jejunum, ileum and colon. **a–b** T1R2 and T1R3 expression in the jejunum; **c–d** T1R2 and T1R3 expression in the ileum; **e–f** T1R2 and T1R3 expression in the colon. Data are presented as mean \pm SD, $n = 5$ per group. Results are considered

significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

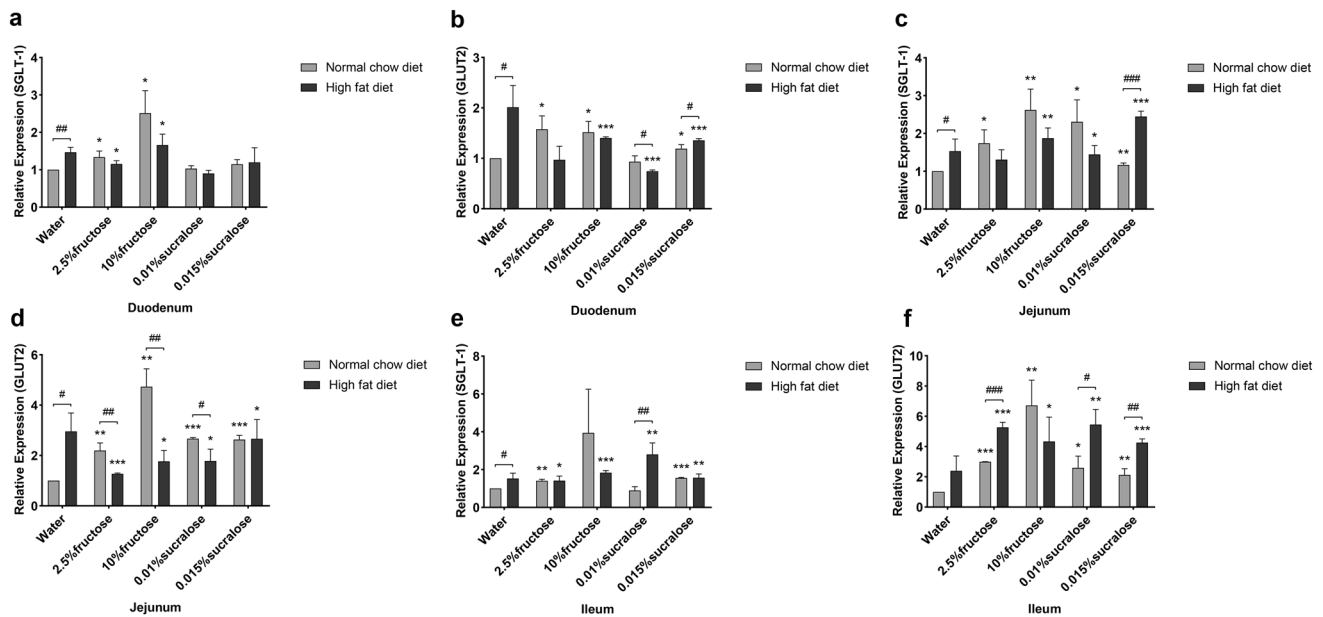


Fig. 7 Changes in expression of glucose transporters in the duodenum, jejunum and ileum. **a–b** SGLT-1 and GLUT2 expression in the duodenum; **c–d** SGLT-1 and GLUT2 expression in the jejunum; **e–f** SGLT-1 and GLUT2 expression in the ileum. Data are

presented as mean \pm SD, $n = 5$ per group. Results are considered significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

results in an increase in postprandial excursions [40], as shown in OGTT.

Evidence has been provided that different metabolic states could influence lingual and hypothalamic sweet

responses through modifying the expression of STRs, which further contributes to changes in feeding motivation and food intake [41, 42]. In our study, among groups fed NCD, rats fed 0.015% sucralose surprisingly ingested the

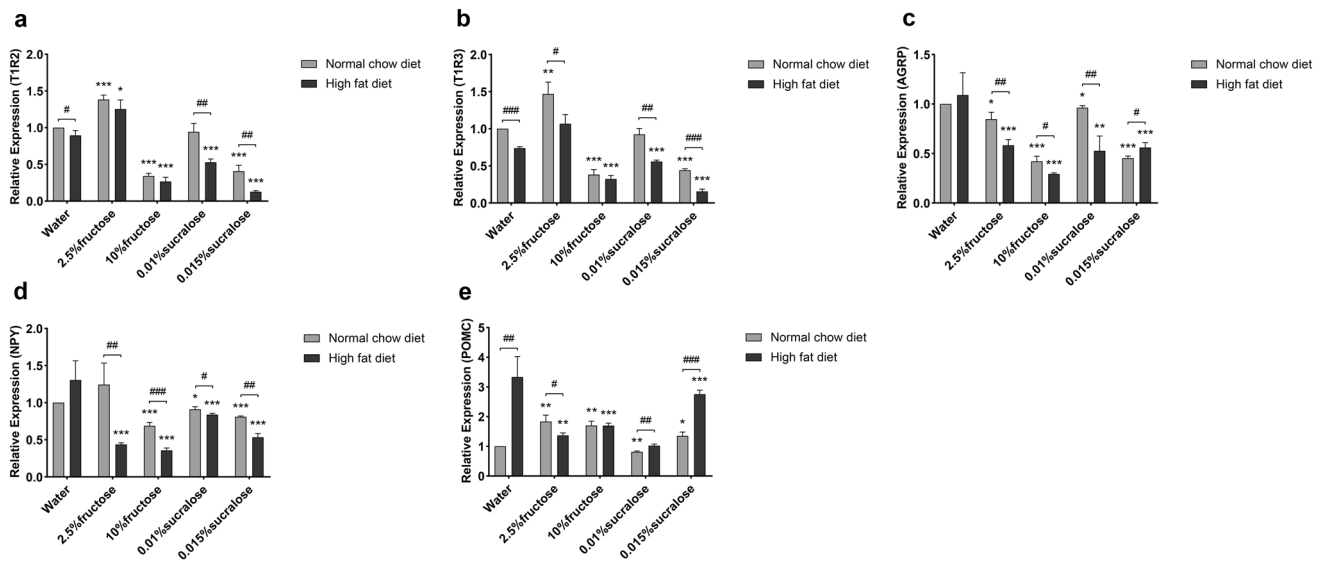


Fig. 8 Changes in expression of STR and appetite signals in the hypothalamus. **a–b** T1R2 and T1R3 expression in the hypothalamus; **c–e** AGRP, NPY and POMC expression in the hypothalamus. Data are presented as mean \pm SD, $n = 5$ per group. Results are considered

significant at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group (NCD + water); # $p < 0.05$, ### $p < 0.01$, ### $p < 0.001$ compared with the corresponding group fed NCD

most calories, even higher than those fed 10% fructose, while rats fed 2.5% fructose or 0.01% sucralose consumed equivalent energy to the control group (NCD + water), indicating that those fed 2.5% fructose compensated for the ingested liquid calories by reducing the ingestion of NCD. The presence of HFD increased energy intake in most of the groups, and rats fed 10% fructose consumed the most calories, followed by those fed 2.5% fructose or 0.015% sucralose. It has been previously revealed that impaired sweet taste sensitivity would promote obesity because a down-regulation in STRs could result in a different threshold for glucose and other sweet tastants both in the tongue and hypothalamus and increase food intake to compensate for the reduced sensitivity [41]. In line with these findings, we found that groups fed 10% fructose or 0.015% sucralose plus NCD showed significantly down-regulated STR expression both in the tongue and hypothalamus. Besides, our data indicated a potential role of HFD in modulating sweet taste perception in different nutrient-sensing tissues, since the presence of HFD exhibited opposite effects on lingual and hypothalamic STR expression in rats fed 0.01% sucralose, 0.015% sucralose or water.

Ingestive behavior is regulated by two major populations of neurons in the arcuate nucleus of hypothalamus: NPY and AgRP-coexpression neurons stimulate food intake; proopiomelanocortin-expression neurons decrease food intake [43]. In our study, in addition to significantly down-regulated STR expression, long term supplementation of 10% fructose or 0.015% sucralose resulted in an evident increase in proopiomelanocortin expression but a decrease

in AGRP/NPY expression whether in the presence of NCD or HFD, which is consistent with hypothalamic neuropeptide expression in animals exhibiting hyperphagia induced by palatable food [44, 45]. While the leptin-mediated inhibition of NPY and AgRP may partially explain the reduction of these orexigenic neuropeptides [45], our study also indicated a role for STR since activation of hypothalamic STR in the arcuate nucleus contributes to the increased firing of glucose-excitatory neurons, including proopiomelanocortin-expression neurons [15, 46], and decreased STR expression could therefore result in a high activation threshold of glucose-excitatory neurons and consequently weaken of the satiation effects of glucose. However, further studies will be needed to fully elucidate the relationship between the dysregulation of STR and alteration in ingestive behavior.

In conclusion, NNSs, typically considered to be metabolically inert, are actually not functionally inert and could result in disturbance in sweet taste perception in not only oral but also intestinal and central nervous tissues and further exert negative influence on global glycemic control, fuel utilization and energy homeostasis in a STR-dependent manner. It has to be pointed out that the difference between T1R2 and T1R3 expression may be due to the fact that T1R3 is also associated with umami sensing and could operate in a homomeric form [7]. We also investigate a new possibility that the combination of HFD may also influence sweet taste sensation and exacerbate the effects of different sweeteners. Alteration in protein levels and tissue-specific ablation of STRs will be further required to elucidate the specific

downstream signaling, by which STRs perform multiple functions and play a role in the development of obesity.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00394-023-03187-9>.

Author contributions The authors' responsibilities were as followed. YZ and HD designed research; YZ, LC, JG, YC, FL, XB conducted research; YZ and HD analyzed data; YZ wrote the paper; YZ and HD had primary responsibility for the final content. All authors read and approved the final version of the paper.

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Data availability The datasets used and analysed in this current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest Y. Zhang, L. Chen, J. Gao, Y. Cheng, F. Luo, X. Bai, H. Ding, no conflicts of interest.

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