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Non-caloric sweetener effects on brain appetite regulation in individuals across varying body weights

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Sucralose, a widely used non-caloric sweetener, provides sweet taste without calories. Some studies suggest that non-caloric sweeteners stimulate appetite, possibly owing to the delivery of a sweet taste without the post-ingestive metabolic signals that normally communicate with the hypothalamus to suppress hunger. In a randomized crossover trial (ClinicalTrials.gov identifier: NCT02945475), 75 young adults (healthy weight, overweight or with obesity) consumed a drink containing sucralose, sweetness-matched sucrose or water. We show that acute consumption of sucralose versus sucrose stimulates hypothalamic blood flow ($P < 0.018$) and greater hunger responses ($P < 0.001$). Sucralose versus water also increases hypothalamic blood flow ($P < 0.019$) but produces no difference in hunger ratings. Sucrose, but not sucralose, increases peripheral glucose levels, which are associated with reductions in medial hypothalamic blood flow ($P < 0.007$). Sucralose, compared to sucrose and water, results in increased functional connections between the hypothalamus and brain regions involved in motivation and somatosensory processing. These findings suggest that non-caloric sweeteners could affect key mechanisms in the hypothalamus responsible for appetite regulation.

Obesity rates have risen dramatically over the last three decades, posing a significant public health challenge[1]. A growing body of evidence links the rise in sugar-sweetened beverage consumption to weight gain and obesity[2–4]. To address this issue, non-caloric sweeteners are increasingly being consumed as a calorie-free alternative to satisfy the craving for sweetness[2]. Although non-caloric sweeteners are widely used, recent reviews have raised concerns about their potential adverse effects on cardiometabolic health[3,5–10], and their effects on body weight remain inconclusive. Although prospective cohort studies have associated non-caloric sweetener consumption with weight gain[4] and obesity[5], randomized controlled trials have reported neutral or beneficial effects on body weight[7,11–15]. Studies conducted in rodents suggest that non-caloric sweeteners stimulate hunger by interfering with the conventional neural responses to sweet taste and nutrient signalling that occur with caloric sugar[16]. Human studies using functional magnetic resonance imaging (fMRI) also indicate that the brain may respond differently

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to beverages containing non-caloric sweeteners than to those containing caloric sugar[11,17,18]. However, previous fMRI studies have often been constrained by small sample sizes comprising healthy-weight individuals[11,17–21]. Furthermore, prior studies have shown a lack of diversity in sex and race or ethnicity, primarily focusing on male and white participants, which limits their external validity[10–12,15,16,18].

In this study, we included a demographically diverse cohort to investigate how sucralose, a prevalent non-caloric sweetener[22], influences hypothalamic blood flow, plasma glucose, insulin and glucagon-like peptide 1 (GLP-1) across a range of body weights. This report extends our previous findings linking obesity with an increased brain response to food cues after sucralose consumption, contrasting with the response to sucrose[23]. Sucralose, chemically altered from sucrose by replacing hydroxyl groups with chlorine, offers sweetness without caloric absorption[24]. Drinks with sucralose were calibrated to match the sweetness of sucrose-containing drinks, allowing us to assess the differential effects of a sweet taste without nutrients (sucralose) compared to a sweet taste with nutrients (sucrose) on hypothalamic activity, glucose concentrations and hunger responses. We also compared sucralose with water to examine the specific effects of sweetness on hypothalamic activity and the corresponding physiological responses.

The hypothalamus has a crucial role in appetite and homeostatic metabolic regulation[25,26], and functional connectivity between the hypothalamus and other brain areas coordinates homeostatic energy balance[25,27,28]. Prior work has shown that ingestion of the simple sugar, glucose, exerts suppressive effects on hypothalamic activation (evidenced in MRI studies as reduced blood oxygen level-dependent (BOLD) signal or reduced cerebral blood flow (CBF))[26,29–35]. The glucose-linked reductions in hypothalamic activity are associated with reductions in hunger[33,34], whereas an increase in hypothalamic activity is associated with heightened hunger[36,37]. Based on prior findings, we reasoned that sucralose, compared to sucrose and water, would cause greater increases in hypothalamic blood flow and would alter functional connectivity between the hypothalamus and other brain regions. Additionally, we expected sucrose, but not sucralose, to raise blood glucose levels, inversely affecting hypothalamic blood flow. We expected to observe differences between the lateral and medial subfields of the hypothalamus, given their distinct functional roles. Finally, we projected that these hypothalamic responses would differ according to participants' weight status.

Results

Hypothalamic blood flow response: sucralose versus sucrose and water

This study included 75 adults (43 females), aged 18–35 years with healthy weight, overweight or obesity who participated in a randomized crossover trial (Table 1 and Fig. 1). Primary findings from pulsed arterial spin labelling (pASL) perfusion MRI analyses are presented for the sucralose, sucrose and water conditions (Fig. 2). There were no baseline differences in hypothalamic blood flow among the three drink sessions ($P = 0.40$). There was a significant effect of drink condition on the lateral hypothalamic blood flow response ($F_{2,363.88} = 5.05$, $P < 0.007$, R^2 model = 0.059). Specifically, the response in the lateral hypothalamic region of interest (ROI) was higher after consuming sucralose than after consuming sucrose (mean difference = 0.079 ± 0.028, $P < 0.018$) and sucralose compared to water (mean difference = 0.078 ± 0.028, $P < 0.019$), as shown in Fig. 3 and Supplementary Table 1. Although there was no interaction between drink and time on the hypothalamic response ($P = 0.70$), the hypothalamic response to drinks over time is shown in Fig. 3 and Supplementary Table 2 for completeness. No significant difference was observed in the medial hypothalamic ROI after sucralose compared to sucrose, although the response was greater after sucralose than after water (Supplementary Table 1). Additionally, differences in the Neudorfer ROI, defined by the high-resolution Neudorfer anatomical atlas of hypothalamic nuclei[38], were observed between sucralose and water (Supplementary Table 1). There were no differences in hypothalamic blood flow when comparing sucrose to water conditions across body mass index (BMI) groups (Supplementary Tables 1 and 3).

Table 1 | Participant characteristics ($n = 75$)

	Mean or freq (s.d. or freq %)	Range
Age (years)	23.33 (3.97)	18.15–34.51
BMI (kg m^{-2})	27.16 (5.17)	19.18–40.28
BMI group		
Obese	23 (30.7%)	
Overweight	24 (32%)	
Healthy weight	28 (37.3%)	
Sex		
Female	43 (57.33%)	
Male	32 (42.66%)	
Race and ethnicity		
Asian	23 (30.66%)	
Hispanic	19 (25.33%)	
Non-Hispanic black	12 (16%)	
Non-Hispanic white	21 (28%)	

Hypothalamic blood flow responses to drinks by weight status

In the lateral hypothalamic ROI, individuals with obesity had greater hypothalamic blood flow responses to sucralose versus water (β = 0.105 ± 0.052, $P = 0.042$) but not sucralose versus sucrose (β = 0.046 ± 0.051, $P = 0.37$). By contrast, individuals with healthy weight had greater hypothalamic responses to sucralose versus sucrose (β = 0.106 ± 0.048, $P = 0.027$) and no differences between sucralose versus water (β = 0.051 ± 0.047, $P = 0.279$). There were no differences in individuals with overweight to either sucralose versus sucrose (β = 0.081 ± 0.049, $P = 0.102$) or sucralose versus water (β = 0.081 ± 0.049, $P = 0.103$) (Fig. 4 and Supplementary Table 3). The Neudorfer and medial hypothalamic ROIs showed differential responses to sucralose relative to sucrose comparisons only among those with healthy weight (Supplementary Table 3).

Hypothalamic blood flow responses to drinks by sex

Post-hoc sex-stratified analyses in the lateral hypothalamus revealed differences in response between males and females. In the comparison between sucralose and sucrose, males showed no significant difference (β = 0.084, $P = 0.055$), whereas females exhibited a significant difference (β = 0.075, $P = 0.047$). For the sucralose versus water condition, males showed no significant difference (β = 0.052; $P = 0.22$), whereas females again demonstrated a significant difference (β = 0.098, $P = 0.0099$).

Hypothalamic seed-to-voxel connectivity

Functional connectivity analysis assessed hypothalamic connectivity with all other brain voxels after the ingestion of sucralose, sucrose or water. Statistical analysis of the connectivity from the bilateral hypothalamus to all other voxels in the brain resulted in different connectivity patterns after the ingestion of sucralose relative to sucrose and water. After ingestion of sucralose relative to sucrose, we observed increased connectivity between the left hypothalamus and anterior cingulate cortex (Fig. 5a). After ingestion of sucralose relative to water, we observed increased connectivity between the right hypothalamus

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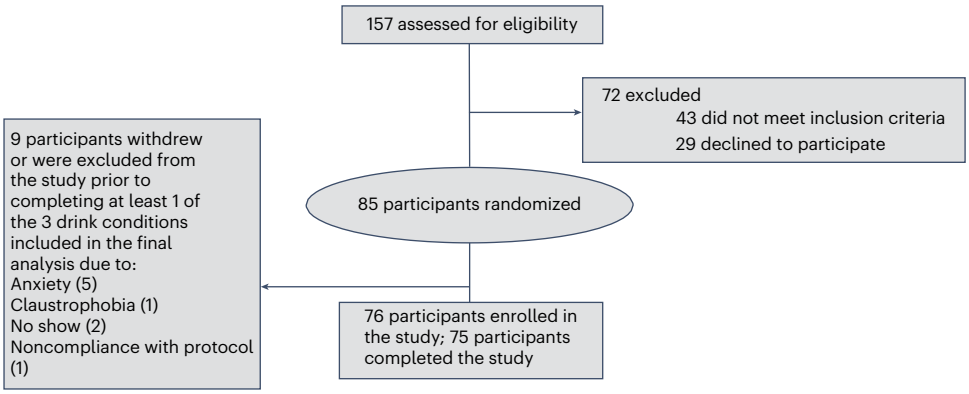

Fig. 1 | Participant enrollment flowchart for the Randomized Crossover Brain Response to Sugar II trial and final analysis. Of the 76 participants enrolled and who received at least one drink allocation, one participant did not receive any of the drinks (that is, sucralose, sucrose or water) included in this analysis because of dropout and was excluded from the analysis ($n = 75$).

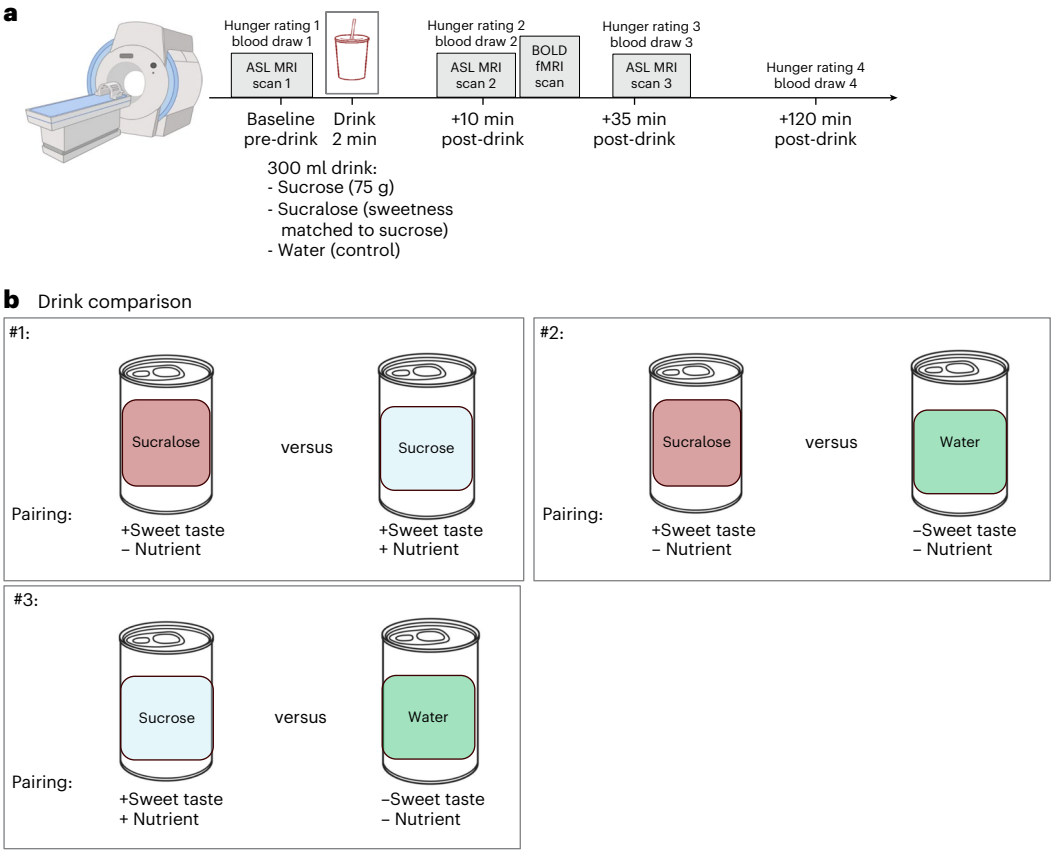

Fig. 2 | Schematic of study design. a, fMRI visit timeline. **b**, Drink comparisons for sucralose, sucrose and water for three different testing conditions. BOLD indicates blood oxygen level-dependent.

and left superior parietal lobule (Fig. 5b). After ingestion of sucrose relative to water, we observed increased connectivity between the right hypothalamus and precuneus cortex and decreased connection between right hypothalamus and occipital pole (Fig. 5c).

Hypothalamic seed-to-voxel connectivity by weight status

Compared to sucrose, sucralose ingestion in individuals with healthy weight increased connectivity between the left hypothalamus and parietal and frontal regions and decreased connectivity from the right hypothalamus to posterior cingulate gyrus, precuneus cortex and hippocampus. In those with overweight, changes were observed primarily in the right hypothalamus, with reduced connectivity to the postcentral gyrus, precentral gyrus and precuneus cortex. No significant changes were seen in individuals with obesity. Relative to water, sucralose increased connectivity from the hypothalamus to various cortical regions depending on weight status, with distinct patterns in healthy-weight, overweight and obesity groups. Similarly, sucrose ingestion relative to water induced unique connectivity changes across weight groups, notably involving the precentral gyrus, occipital cortex and frontal regions. Full data are detailed in Supplementary Table 4.

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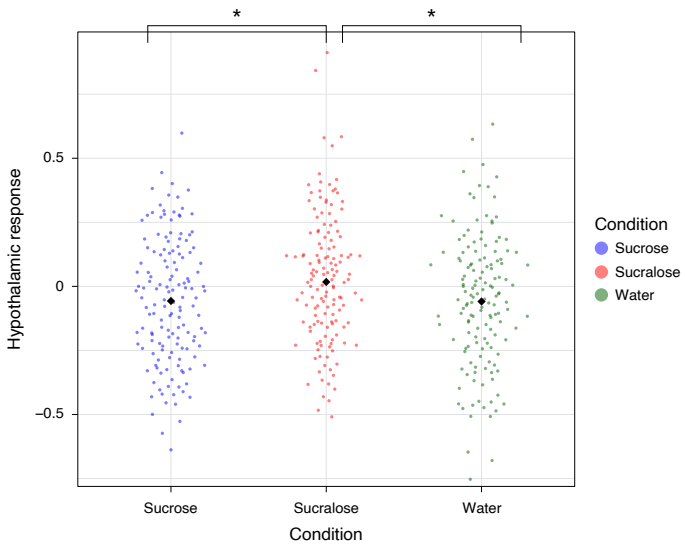

Fig. 3 | Differential hypothalamic response to drink comparisons. Significant increases in hypothalamic blood flow were observed after sucralose versus sucrose ($P = 0.018$) and sucralose versus water ($P = 0.019$). Statistical analyses were performed using linear mixed-effects models with condition, BMI group, age, sex and race/ethnicity as fixed effects and subject as a random intercept. Comparisons were adjusted for multiple comparisons using a Bonferroni correction. Data showing lateral hypothalamic ROI ($n = 75$).

Circulating glucose, insulin and GLP-1 responses

Results for the effects of sucrose, sucralose and water on changes in peripheral glucose, insulin and GLP-1 levels have been previously reported[23]. There were no differences in baseline systemic levels of glucose ($P = 0.97$), insulin ($P = 0.67$) or GLP-1 ($P = 0.67$) between the sucralose, sucrose and water conditions, as previously reported[23]. There was a significant effect of drink on the peripheral glucose response ($F_{2,552.65} = 139.36$, $P < 0.00001$, R^2 model = 0.34), peripheral insulin response ($F_{2,540.57} = 320.78$, $P < 0.00001$, R^2 model = 0.52) and peripheral GLP-1 response ($F_{2,551.08} = 68.65$, $P < 0.00001$, R^2 model = 0.21). Post-hoc analysis showed a marked increase in peripheral glucose ($P < 0.0003$), insulin ($P < 0.0001$) and GLP-1 ($P < 0.0002$) following sucralose compared to sucrose intake, but no differences were observed in peripheral glucose ($P = 0.99$), insulin ($P = 0.96$) or GLP-1 ($P = 0.48$) levels when comparing sucralose to water (Supplementary Table 5). There was an interaction between time and drink on peripheral glucose ($P < 0.00002$), insulin ($P < 0.00009$) and GLP-1 ($P < 0.00002$) levels (Fig. 6 and Supplementary Table 2). No interactions were observed between weight status and drink for the area under the curve of plasma glucose ($P = 0.92$), insulin ($P = 0.07$) or GLP-1 ($P = 0.44$); similarly, no sex-by-drink interactions were found for area under the curve of plasma glucose ($P = 0.32$), insulin ($P = 0.08$) or GLP-1 ($P = 0.71$), as previously reported[23].

Hunger responses

There were no differences in baseline self-reported hunger ratings among the drink sessions ($P = 0.678$); however, there was a significant effect of drink condition on changes in hunger ($F_{2,580.21} = 10.79$, $P < 0.00002$, R^2 model = 0.153). Post-hoc analysis revealed a significant increase in hunger after sucralose compared to sucrose (mean difference = 0.575 ± 0.16, $P < 0.001$) but no differences after sucralose versus water (mean difference = −0.090 ± 0.16, $P = 0.99$) (Supplementary Table 6 and Fig. 6). Although there was no interaction between time and drink on hunger ratings ($P = 0.38$), drink comparisons by time on hunger are shown in Fig. 6 and Supplementary Table 2 for informational purposes.

Circulating glucose, insulin, GLP-1 and hypothalamic blood flow links

There was a significant relationship between changes in circulating glucose levels and blood flow in the medial hypothalamus 35 min after consuming sucrose ($\beta = -0.005 \pm 0.002$, $P < 0.007$) (see Fig. 7a and Supplementary Table 7). However, no significant association was found between changes in circulating glucose and hypothalamic blood flow after consuming sucralose ($P = 0.19$; Fig. 7a). In an exploratory analysis stratified by weight status, negative associations were observed between increments in peripheral glucose and the medial hypothalamic response to sucrose in individuals with healthy weight and those with overweight but not in individuals with obesity (see Supplementary Table 8). We found no significant associations between GLP-1 levels and medial hypothalamic response at either 10-min or 35-min post sucrose ingestion ($\beta = 0.001$, $P = 0.67$; $\beta = 0.002$, $P = 0.78$, respectively). Similarly, no significant associations were observed between insulin levels and medial hypothalamic response to sucrose at either time point ($\beta = 0.002$, $P = 0.104$; $\beta = 0.004$; $P = 0.79$, respectively).

Hypothalamic blood flow and hunger links

Decreases in medial hypothalamic blood flow observed within 10 min after consuming sucrose were associated with reduced hunger post ingestion (Supplementary Table 9 and Fig. 7b). No such associations were found following sucralose ingestion (Supplementary Table 9 and Fig. 7b).

Peripheral insulin sensitivity and hypothalamic blood flow responses

Recent reports have highlighted the importance of insulin sensitivity in the hypothalamic regulation of appetite and eating behaviour[39,40]. Therefore, we conducted post-hoc analyses to explore the association between peripheral insulin sensitivity and the differential hypothalamic blood flow response to sucralose versus sucrose and water. We used

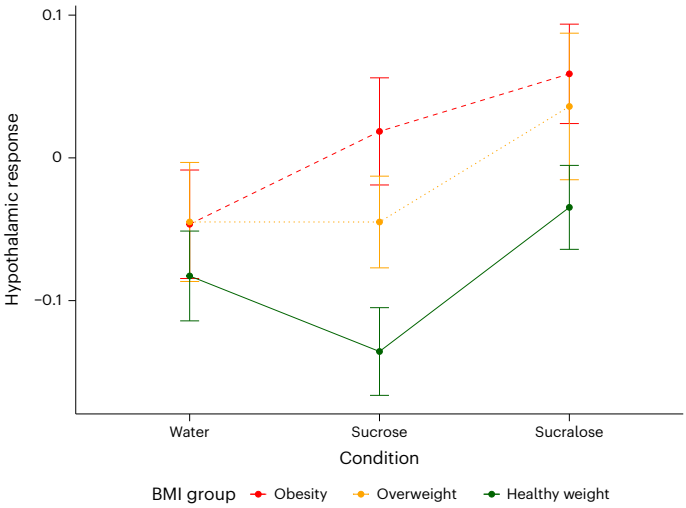

Fig. 4 | Difference in hypothalamic response to drinks by weight status. Mean change in lateral hypothalamic blood flow after water, sucrose or sucralose ingestion stratified by weight status ($n = 75$). Individuals with healthy weight had greater hypothalamic blood flow after sucralose versus sucrose ($P = 0.027$) but not sucralose versus water ($P = 0.279$). Individuals with obesity had greater hypothalamic activation after sucralose versus water ($P = 0.042$) but not sucralose versus sucrose ($P = 0.370$). Individuals with overweight had no differences in hypothalamic response to either drink comparison. Statistical analyses were performed using linear mixed-effects models with condition, BMI group, age, sex and race/ethnicity as fixed effects and subject as a random intercept. Data are presented as mean ± s.e.m., and statistical models are reported in Supplementary Table 3.

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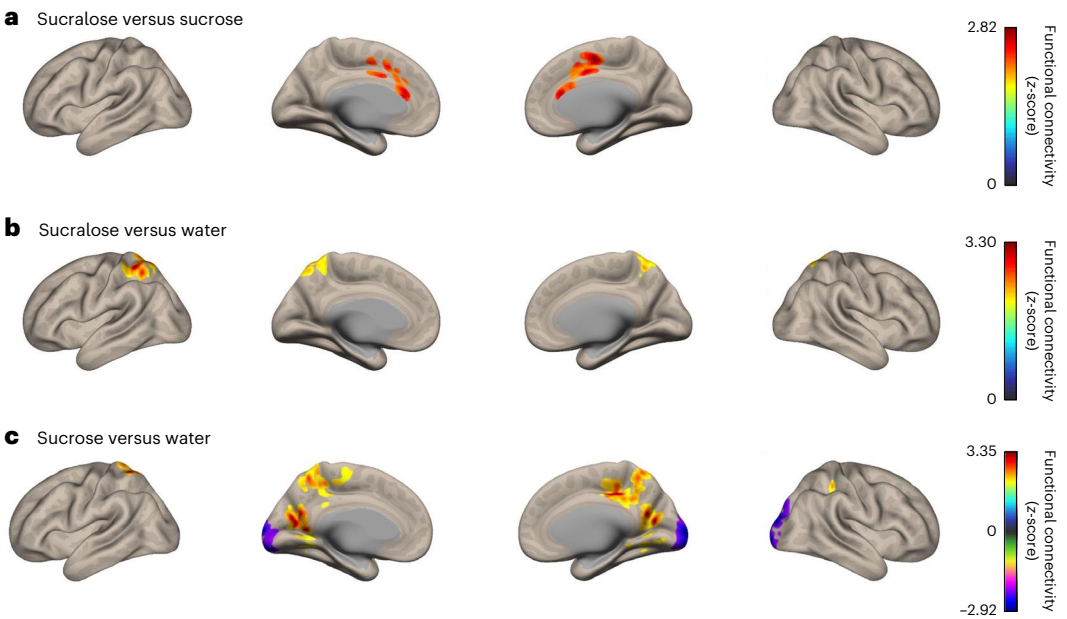

Fig. 5 | Differential functional connectivity from hypothalamus seed region after sucralose ingestion relative to sucrose and water. Seed-to-voxel analysis comparing functional connectivity between the hypothalamus (seed region) and other brain regions after sucralose ingestion relative to sucrose or water, adjusting for age, sex, BMI, and race/ethnicity. **a**, Sucralose compared to sucrose resulted in increased connectivity between the left hypothalamus and anterior cingulate cortex, **b**, Sucralose compared to water resulted in increased connectivity between the right hypothalamus and left superior parietal lobule. **c**, Sucrose compared to water resulted in increased connectivity between the right hypothalamus and precuneus cortex and decreased connectivity between the right hypothalamus and the occipital pole. Group-level analyses were performed using a weighted general linear model, which evaluated voxel-level hypotheses and accounted for random effects across subjects and sample covariance estimation across multiple measurements. Significance was considered at $P < 0.05$ with correction for multiple comparisons using the false discovery rate ($q < 0.05$). Hot colours in red, orange and yellow indicate a positive *z*-score, suggesting greater connectivity after sucralose relative to the comparison drink. Neudorfer hypothalamic ROI was used as the seed region. Five participants had excessive motion during the BOLD acquisition and were excluded from the functional connectivity analysis ($n = 70$).

the whole-body insulin sensitivity index (ISI) as a continuous variable and stratified participants into tertiles of insulin sensitivity (0.797, 4.11 and 7.414) to examine these associations. In the sucralose versus sucrose comparison, we observed a difference in lateral hypothalamic response in the lowest ISI tertile ($\beta = 0.095$, $P = 0.029$) and the middle ISI tertile ($\beta = 0.0863$, $P = 0.005$) but no significant difference in the highest ISI tertile ($\beta = 0.078$, $P = 0.08$), adjusting for age, sex, BMI and race/ethnicity (Supplementary Table 10). For the sucralose versus water comparison, we observed a difference in the hypothalamic response in the lowest ISI tertile ($\beta = 0.103$, $P = 0.015$) and the middle ISI tertile ($\beta = 0.085$, $P = 0.005$) but no significant difference in the highest ISI tertile ($\beta = 0.078$, $P = 0.12$), adjusting for age, sex, BMI and race/ethnicity (Supplementary Table 10).

Discussion

In this randomized crossover trial involving healthy young adults of varying weights, we show that drinks sweetened with sucralose, a non-caloric sweetener, increased hypothalamic blood flow—a purported MRI marker of hunger[33,34,36,37]—compared to caloric sugar (sucrose) and water. Sucrose, compared to sucralose, had a hunger-dampening effect while also raising peripheral glucose levels, which corresponded to reduced medial hypothalamic blood flow. These results support the notion, initially observed in rodents[16,41], that non-caloric sweeteners may alter appetite by interfering with the conventional neural responses to sweet taste and nutrient signalling observed with caloric sugar. Sucralose triggered a stronger hypothalamic response than water, suggesting that sweet taste alone can modulate hypothalamic activity, potentially owing to the presence of sweet taste receptors (T1R2 and T1R3) on glucose-sensing neurons in the hypothalamus[42–44].

Discrepancies in the literature about the effects of non-caloric sweeteners on body weight[12–15,45] may arise from various factors, including the specific type of non-caloric sweeteners used, the characteristics of the study participants, the non-caloric intake amount and the duration of consumption. Our study focused on acute responses to sucralose compared to sucrose and water under controlled conditions to better understand the neural and hormonal mechanisms involved. Previous fMRI studies have shown a greater reduction in hypothalamic activity following the acute consumption of glucose-containing drinks than to drinks containing non-caloric sweetener[11,17] and no difference in the hypothalamic response to sucralose compared to water[17]. However, these studies were limited to a small number of lean male participants, leaving a significant gap in understanding how weight status and sex may influence hypothalamic responses to caloric versus non-caloric sweeteners. Understanding this relationship may have important implications for the use of non-caloric sweeteners in weight management strategies.

To address these limitations, in addition to examining differences in hypothalamic responses to sucralose compared to sucrose and water among the whole cohort, we also stratified the results by weight status to test our hypothesis that different weight-status groups would exhibit distinct responses to caloric versus non-caloric sweeteners. Stratified analyses revealed distinct differences in the hypothalamic responses to sucralose compared to sucrose and water across weight-status groups. Among the individuals with healthy weight, sucralose produced greater activation of the hypothalamus than sucrose. Individuals with obesity showed a greater response in the lateral hypothalamic response to sucralose relative to water, whereas in the healthy-weight group, these differences were present in the medial hypothalamus. These findings align with rodent studies suggesting a role of sweet taste in hypothalamus signalling[42–44]. Although we did not observe significant

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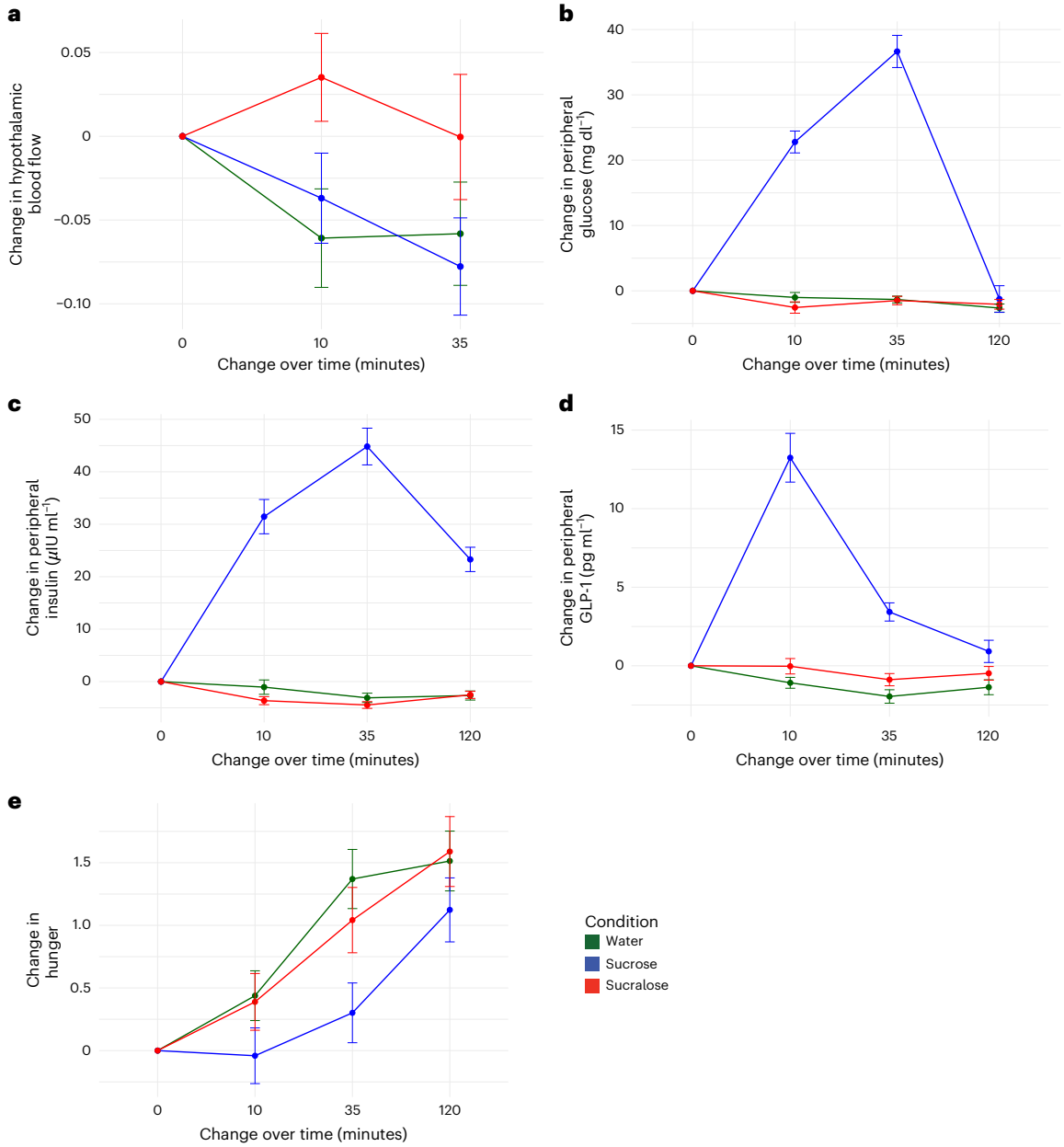

Fig. 6 | Visual display of changes in hypothalamic, peripheral glucose, insulin, GLP-1 and hunger responses over time. Change in **a**, hypothalamic response (lateral hypothalamus) ($n = 75$), **b**, peripheral glucose ($n = 72$), **c**, peripheral insulin ($n = 72$), **d**, peripheral GLP-1 ($n = 72$) and **e**, self-reported hunger ratings ($n = 75$) in response to each drink condition: water, sucrose and sucralose. Data are presented as mean values ± s.e.m. and are reported in Supplementary Table 2.

differences between sucralose and sucrose or water in the overweight group, the response patterns in the hypothalamus fell between those of the healthy-weight and obese groups.

Sex-stratified analyses indicated that females exhibited more pronounced hypothalamic responses to sucralose than to sucrose and water. This finding aligns with previous studies showing that females generally have greater brain responses to food cues compared to males[46,47]. Additionally, our previous report[23] demonstrated that females have greater cortical brain responses to food cues after the ingestion of sucralose compared to sucrose.

Our findings revealed that oral intake of sucrose led to increased peripheral glucose levels and reduced hypothalamic activity, whereas sucralose had no such effect. This supports existing evidence suggesting that metabolic signals like glucose are tightly connected to changes in hypothalamic activity[29,30,33,36,48]. Importantly, the relationship between peripheral glucose fluctuations and hypothalamic activity was less evident in individuals with obesity, further reinforcing the idea that obesity may disrupt glucose signalling within the hypothalamus. Although we observed associations between circulating glucose levels and hypothalamic response to sucrose, there were no associations between circulating GLP-1 or insulin levels and hypothalamic responses to sucrose. This could suggest that the hypothalamic blood flow response is more sensitive to the changes in peripheral glucose levels than to glucose-induced changes in insulin or GLP-1 levels. Nonetheless, it is well known that GLP-1 and insulin have important roles in the central feedback regulation of appetite and glucose homeostasis. Studies in rodent models have shown that GLP-1 influences hypothalamic and brainstem pathways involved in appetite regulation and glucose sensing[49,50]. Similarly, insulin signalling in the brain affects energy homeostasis and glucose metabolism[51,52]. These mechanisms may contribute to feedback regulation, even if no direct linear correlation with hypothalamic blood flow was observed in our study.

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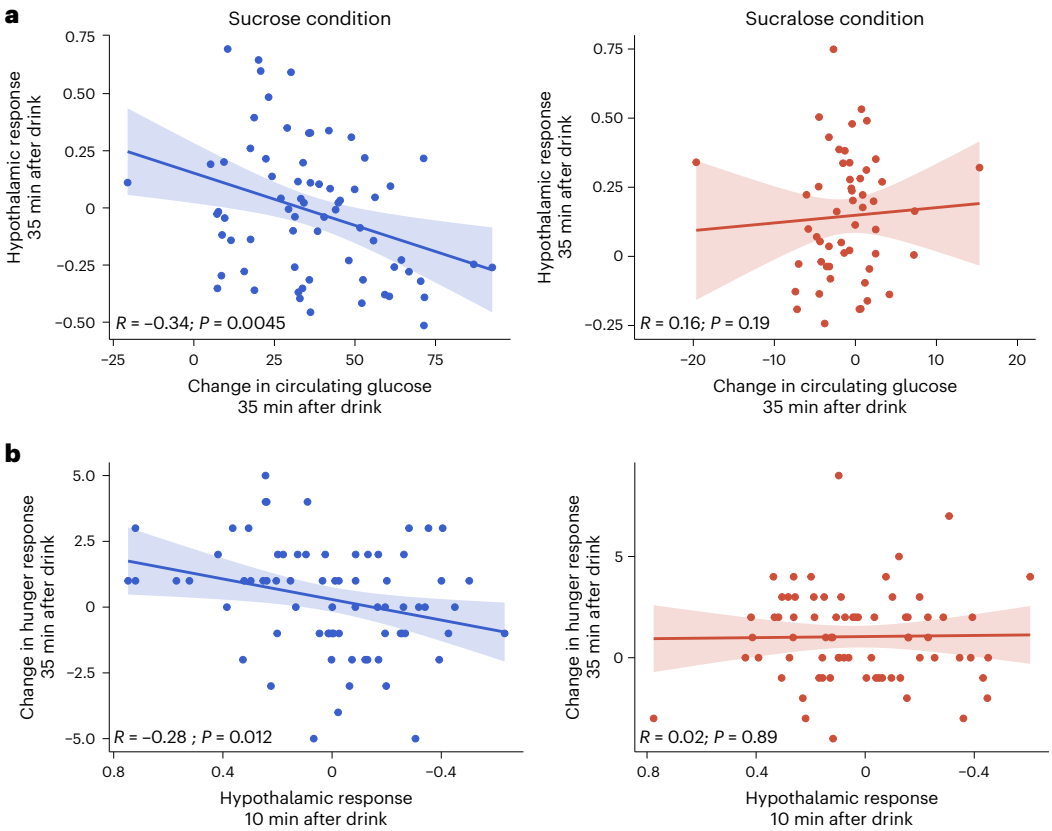

Fig. 7 | Associations between peripheral glucose, hunger and changes in medial hypothalamic blood flow. Figures show scatterplots with Pearson correlations for visual purposes. Linear regression models were used in the statistical analysis and data are reported in Supplementary Tables 7 and 9. Data are presented as mean values ± s.e.m.

Our results also suggest that insulin resistance (that is, lower insulin sensitivity), independent of BMI, is associated with heightened hypothalamic responses to sucralose compared to sucrose and water. Although exploratory, these findings point to a potential role of insulin resistance in modulating the hypothalamic response to non-caloric sweeteners. These findings underscore the need for further research into neural and metabolic effects of non-caloric sweeteners, particularly in populations with insulin resistance, diabetes and other metabolic disorders that may alter brain pathways related to appetite regulation and energy balance.

Although we did not observe a significant time × drink interaction on hypothalamic response, the time course of the hypothalamic responses showed dynamic changes over time. The greatest response to sucrose occurred 35 min post ingestion, whereas the largest response to sucralose occurred earlier. These differences in timing align with the distinct metabolic and sensory responses elicited by the two sweeteners.

We anticipated that sucralose would increase hunger more than water; however, we speculate that the absence of a significant difference may be because of the complex interplay between subjective hunger and the mismatch between the expectation of caloric intake and the actual lack of caloric content. Sucralose does not suppress hunger, and it may lead to reduced satisfaction and potentially increased cravings over time, although we did not directly test this concept in our study, and further research is needed to determine longer term effects of sucralose on hunger and cravings.

By including two distinct subfields of the hypothalamus—the lateral hypothalamus and the medial hypothalamus, which comprises the ventromedial hypothalamic nucleus (VMH) and the arcuate nucleus—we aimed to explore subfield-specific roles. Previous research in rats showed that bilateral lesions in the VMH led to hyperphagia, while lesions in the lateral hypothalamic area resulted in hypophagia[53]. These foundational studies identified the VMH as the 'satiety centre', which suppresses food intake, and the lateral hypothalamic area as the 'hunger centre', which stimulates eating. Our findings revealed that sucralose, compared to sucrose or water, induced the most pronounced differences in the lateral hypothalamic subfield. However, the response patterns in the medial hypothalamic subfield and the hypothalamus ROI defined by the Neudorfer high-resolution atlas were similar. Importantly, associations between changes in peripheral glucose levels post sucrose ingestion were notable in the medial hypothalamic subfield but not in the lateral subfield. Furthermore, reductions in blood flow in the medial hypothalamus correlated with decreased hunger. These findings align with prior studies demonstrating that glucose ingestion reduced the fMRI signal in the hypothalamic area corresponding to the VMH, correlating with lower fasting plasma glucose and insulin levels, highlighting the significant role of the medial hypothalamus in nutrient sensing[29].

Finally, our functional connectivity analysis revealed that acute consumption of sucralose, as opposed to sucrose, significantly increased coupling between the hypothalamus and the anterior cingulate cortex—an area of the brain that has a crucial role in attention, motivation and reward processing[54]. Additionally, compared to water, sucralose intake led to greater connectivity between the right hypothalamus and the left superior parietal lobule, a region integral to somatosensory integration[55]. These results suggest that sucralose may enhance the functional connection between brain regions, coordinating appetite with reward and motivation and potentially influencing food-seeking behaviour. Furthermore, connectivity differences varied by weight status. Among individuals

with healthy weight, sucralose versus sucrose intake increased connectivity between the left hypothalamus and the supramarginal gyrus, angular gyrus, postcentral gyrus and superior frontal gyrus, while reducing connectivity from the right hypothalamus to regions involved in memory and introspective processing. This pattern was more pronounced in the healthy-weight group than in the overweight and obese groups, who exhibited fewer or no significant connectivity changes after sucralose relative to sucrose consumption. Additionally, distinct connectivity patterns emerged in the sucralose versus water comparison across weight groups, suggesting that neural sensitivity to sweeteners may differ based on body weight. These findings highlight how weight status may shape neural responses to non-caloric sweeteners, with potential implications for appetite regulation and eating behaviour.

Limitations and future research considerations

The primary focus of this study was to investigate the isolated effects of sucralose; however, considering that beverages are frequently consumed with meals containing carbohydrates[56] and proteins, further investigation into whether hypothalamic responses are differentially modulated by the ingestion of sucralose within a mixed-meal context would be valuable, particularly given prior evidence showing altered brain responses when sucralose and carbohydrates are co-consumed[56]. Additionally, given that the unique chemical structure of each type of non-caloric sweetener may elicit varying physiological responses[14], future studies should examine whether altered hypothalamic and metabolic responses are also observed with other types of non-caloric sweeteners.

We investigated both the medial and lateral hypothalamic subfields to determine whether the varying effects of sucralose, compared to sucrose and water, could be attributed to specific areas within the hypothalamus. However, owing to the limitations in spatial resolution, we were unable to precisely attribute these effects to individual neurons within these hypothalamic subfields.

Although previous research has shown higher habitual non-caloric sweetener consumption among individuals with obesity and in females[57], our data did not reflect this trend. This discrepancy could be caused by common limitations in self-reported dietary data as well as the inability of nutrient databases like the Nutrition Data System for Research to capture all sources of non-caloric sweeteners, which could have made it difficult to detect subtle differences between weight groups. Additionally, our study included young, healthy adults who were weight-stable and not actively engaged in weight-loss efforts, which may partly explain the lack of higher non-caloric intake among women and individuals with obesity, as non-caloric sweeteners are often used in weight-loss efforts that were not being pursued by our participants[58].

Although our study matched the sucralose and sucrose drinks for sweetness, assessing sweet perception and sweet liking would have provided further insights[13], particularly regarding how subjective perceptions of sweetness might influence hypothalamic responses to non-caloric and caloric sweeteners[59]. Although this study is one of the largest that we know of to examine acute brain and metabolic responses to caloric and non-caloric sweeteners, the limited sample size in our stratified analyses highlights the need for further research into how taste and metabolic factors interact across BMI categories and metabolic states. Finally, our study on acute neurophysiological responses to non-caloric sweeteners provided a controlled environment to identify specific neural and hormonal mechanisms. This work sets the stage for subsequent research on repeated exposures and chronic consumption. Given that rodent studies have shown that chronic consumption of non-nutritive sweeteners can alter central appetite signalling mechanisms[60], more research addressing the long-term effects of non-caloric sweeteners is warranted.

Conclusion

Our findings indicate that the non-caloric sweetener sucralose can affect key mechanisms in the hypothalamus responsible for appetite regulation. Individual characteristics, such as sex, adiposity and insulin resistance, appear to affect how the hypothalamus interprets sweet taste and metabolic signals related to appetite control. This may be particularly relevant for non-caloric sweeteners like sucralose, which create a mismatch between the expectation of caloric intake and the absence of actual energy. Our findings suggest that this mismatch leads to different hypothalamic activation patterns compared to caloric sweeteners, which may ultimately influence appetite regulation and metabolic responses over time. Considering the prevalent consumption of non-caloric sweeteners, it is vital to conduct comprehensive studies to clarify their long-term health ramifications.

Methods

Study overview

Data are from the Brain Response to Sugar study, an investigation of neuroendocrine responses to high-reward foods (ClinicalTrials.gov identifier: NCT02945475)[23]. Data presented are the primary results of the pASL MRI analyses from the sucralose, sucrose and water conditions from the randomized crossover trial (Fig. 1). Glucose was included in the larger trial to test differences in equicaloric sugars on outcomes[23]. Participants provided written informed consent compliant with the University of Southern California Institutional Review Board (IRB no. H-09-00395). This study followed Consolidated Standards of Reporting Trials (CONSORT) guidelines (trial protocol can be found in the online digital repository[61]).

The study included an initial screening visit and MRI visits performed at the Dornsife Cognitive Neuroimaging Center of the University of Southern California at approximately 08:00 h after a 12 h overnight fast. During the screening visit, height was measured to the nearest 0.1 cm using a stadiometer and weight was measured to the nearest 0.1 kg using a calibrated digital scale. BMI was calculated as weight (in kg) divided by height (in m^2). Sex was self-reported by participants based on their sex assigned at birth. Patient compensation was up to $370 in a gift card for participating in all screening and MRI study visits. Enrollment occurred between July 2016 and March 2020.

The three MRI visits were performed in blinded, random order (using function randperm, a computer-generated randomization procedure in MATLAB) on separate days between 2 days and 2 months apart with ingestion of 300 ml drinks containing either sucrose (75 g), sucralose (individually sweetness-matched to sucrose, as previously described[23]) or a water control to test their effects on changes in hypothalamic blood flow, circulating glucose levels and ratings of hunger (Fig. 2). Participants and experimenters were blind to the drink provided during the study sessions. Hypothalamic blood flow (measured by pASL perfusion MRI), hunger ratings and glycemic, insulin and GLP-1 responses were measured fasting, +10 min and +35 min after drink ingestion. Hunger ratings and glycemic responses were additionally measured 120 min after drink ingestion. Drinks were flavoured with 0.25 tsp (1.07 g) of non-sweetened cherry flavouring (Kraft Foods Kool-Aid unsweetened cherry drink mix) to improve palatability. Females underwent MRI visits during the follicular phase of the menstrual cycle to reduce variability in hunger[62,63]. Whole-body ISI was estimated using the 2 h oral glucose tolerance test[64]. Habitual non-caloric sweetener intake was assessed with repeated 24 h dietary recalls (an average of five per participant) using the Nutrition Data System for Research (v.2018). Daily intake of acesulfame potassium, sucralose, saccharin and aspartame was quantified in mg. Each participant's average intake across all recalls was calculated to reflect their typical non-caloric sweetener use. Participants were classified as non-caloric sweetener users if their average daily intake exceeded zero and non-users if it was equal to zero.

Of the 76 participants enrolled who received at least one drink allocation (that is, sucralose, sucrose or water), one participant dropped out and was excluded from the analysis (Fig. 1). Participants ultimately included in this analysis were 75 adults (43 female) ages 18–35 years with healthy weight, overweight or obesity (Table 1). Sample sizes were originally estimated at 120 participants to detect a minimum effect of 0.31 s.d. of the difference in sweeteners on activation within brain ROIs, controlling for the false discovery rate among brain regions, assuming a paired two-sided *t*-test, $\alpha = 0.05$ and 80% power. The study was halted on 13 March 2020 because of the COVID-19 pandemic, with a recruited sample of 76 participants. We calculated that with this sample, we would have 80% power to detect a minimum effect size of 0.40 s.d.[23]. Participants were right-handed, nonsmokers, weight-stable for at least 3 months before the study visits, nondieters, not taking medication (except oral contraceptives) and with no history of diabetes, eating disorders, illicit drug use or other medical diagnoses. There were no significant differences in non-caloric sweetener use between weight groups whether measured as a continuous variable (healthy weight, 14.36 ± 29.77 mg day^{-1}; overweight, 30.08 ± 70.15 mg day^{-1}; obesity, 20.11 ± 47.80 mg day^{-1}; $P = 0.537$) or as a categorical variable (percentage of non-caloric sweetener users by group: healthy weight, 35.7%; overweight, 48.0%; obesity, 39.1%; $P = 0.649$). There were also no sex differences in non-caloric sweetener intake (females, 16.76 ± 37.24 mg day^{-1}; males, 27.15 ± 65.05 mg day^{-1}; $P = 0.38$; female users, 39.5%; male users, 42.4%; $P = 0.98$).

The prespecified primary outcome was relative changes in hypothalamus blood flow in response to acute sucralose versus sucrose and water among the whole cohort and stratified by weight status (healthy weight, overweight, obese). Secondary outcomes included (1) associations between changes in circulating glucose, insulin and GLP-1 levels, hypothalamic blood flow and ratings of hunger in response to sucralose and sucrose; and (2) functional connectivity analysis to investigate brain regions with MR signal responses that were correlated with the hypothalamic response after sucralose relative to sucrose and water. Post-hoc exploratory outcomes included sex-stratified hypothalamic blood flow responses, weight-status-stratified functional connectivity and associations between insulin sensitivity and hypothalamic blood flow responses to sucralose versus sucrose and water.

MRI acquisition parameters. Participants were scanned at the USC Dana and David Dornsife Neuroimaging Center. Data were collected using a 3T Siemens MAGNETOM Prismafit MRI System with a 32-channel head coil. A high-resolution 3D magnetization prepared rapid gradient echo (MPRAGE) sequence (TR = 1,950 ms; TE = 2.26 ms; bandwidth = 200 Hz per pixel; flip angle = 9°; slice thickness = 1 mm; field of view (FOV) = 224 mm × 256 mm; matrix = 224 × 256) was used to acquire structural images for multi-subject registration.

pASL was used to quantify CBF changes in response to acute consumption of the different drinks. pASL provides a measure of CBF by magnetically tagging arterial blood directly before it enters the brain and measuring the amount of tagged blood to reach specific brain areas[65,66]. To acquire CBF maps, pASL images were obtained with a PICORE-Q2TIPS (proximal inversion with control for off-resonance effects—quantitative imaging of perfusion by using a single subtraction) sequence by using a frequency offset corrected inversion pulse and echo planar imaging readout for acquisition[67]. The pASL acquisition parameters used in this study were: FOV = 192 mm; matrix = 64 × 64; bandwidth = 2,232 Hz per pixel; slice thickness = 5 mm; in-plane resolution = 3 × 3 mm^2; interslice spacing = 0 mm; TR = 4,000 ms; flip angle = 90; bolus duration (TI1) = 0.7 s, inversion time (TI2) = 1.8 s; number of label/control pairs = 60. The first control volume of the pASL sequence was used as a calibration image for CBF quantification. The temporal stability of pASL complements the longer curve of glucose responses that we measured with the accompanying blood draws during the study sessions. BOLD-fMRI was acquired with a multi-band interleaved gradient echo planar imaging sequence. A total of 88 1.5 mm-thick slices covering the whole brain were acquired using the following parameters: TR = 1,000 ms; TE = 43.20 ms; bandwidth = 2,055 Hz per pixel; flip angle = 52°; multi-band factor = 8; FOV = 128 mm × 112 mm; matrix = 128 × 112; and number of volumes = 376.

ASL analysis. We used the Bayesian Inference for Arterial Spin Labelling (BASIL) toolbox, part of the Oxford FMRIB Software Library (FSL), MATLAB (2017a) and Python (3.7.3) software to determine mean CBF across the entire brain as well as regional CBF in the hypothalamus and hypothalamic subfields. Following motion correction of the whole image sequence, CBF quantification was performed based on a single compartment model and voxel-wise calibration. The hypothalamic response to drink was measured as the ratio of hypothalamic CBF to whole brain CBF after the drink (averaged across post-drink time points). This post-drink value was then subtracted from the corresponding pre-drink value, which was calculated in the same manner (hypothalamic CBF divided by whole brain CBF before the drink). This approach corrects for baseline CBF differences and normalizes hypothalamic blood flow relative to global changes in CBF. To reduce confounding effects owing to limited spatial resolution, we adjusted for partial volume effects. Computer codes for data analysis are published in https://osf.io/tuw93.

Hypothalamus ROIs

We examined the hypothalamic response to different drink conditions by focusing on two distinct hypothalamic subfields: the lateral hypothalamic and the medial hypothalamic. This approach was driven by the well-recognized functional differences between these two areas of the hypothalamus[25]. The lateral and medial hypothalamic subfield ROI masks were anatomically defined according to prior work[68] and previously used to examine hypothalamic appetite regulation[69] (Extended Data Fig. 1). We included an additional exploratory subfield based on the high-resolution Neudorfer anatomical atlas of hypothalamic nuclei related to energy regulation to enable direct comparison with future studies that may use similar methodologies[38]. The Neudorfer hypothalamus ROI includes all hypothalamic nuclei related to energy regulation: lateral hypothalamus, ventromedial nucleus, dorsomedial hypothalamic nucleus, arcuate nucleus and paraventricular nucleus. A single mask was created and normalized into MNI152 space. The Neudorfer ROI was used in bilateral functional connectivity analyses, using the comprehensive mask encompassing hypothalamic nuclei involved in energy regulation (Extended Data Fig. 1).

Functional connectivity analysis

BOLD-fMRI data were collected during a visual food-cue task, in which food and non-food images were presented to participants in random order as previously described[23]. To explore the primary effects of different drinks on hypothalamic activity and its functional connections, we performed an analysis across the whole fMRI time series including both food and non-food stimuli. This approach allowed us to identify differences in hypothalamic functional connections after the consumption of sucralose relative to sucrose and water. A similar methodology was previously applied to investigate how increments in peripheral glucose affect brain connectivity during visual food and non-food tasks[70]. Data were processed using Conn Toolbox (v.21.a; https://www.nitrc.org/projects/conn)[71,72] and Statistical Parametric Mapping (SPM v.12.7; https://www.fil.ion.ucl.ac.uk/spm)[73]. Preprocessing included motion alignment, regression of physiological noise fluctuations and bandpass filtering between 0.008 Hz and 0.09 Hz. Noise-corrected fMRI images were then co-registered to anatomical T1 weighted images, normalized into MNI standard space. We then performed seed-to-voxel analyses starting from a seed in the hypothalamus as described above. Results were used in second-level group analyses comparing functional connectivity between drink conditions using a full factorial model.

This model incorporated drink condition as a between-subjects factor and included four covariates, correcting for age, sex, BMI and race/ethnicity. Group-level analyses were performed using a weighted general linear model[74], which evaluated voxel-level hypotheses and accounted for random effects across subjects and sample covariance estimation across multiple measurements. Statistical inferences for clusters were based on Gaussian random field theory[74,75], with significance determined using a voxel-level threshold of $P < 0.001$ and a false discovery rate corrected cluster size threshold of $P < 0.05$ (ref. 76). See Supplementary File 1 for more details of methods.

Glucose and hormone assays

Plasma glucose was measured enzymatically using glucose oxidase (YSI 2300 STAT PLUS Enzymatic Electrode-YSI analyzer, Yellow Springs Instruments). Plasma insulin and GLP-$1_{(7-36)}$ (active) were measured by Luminex multiplex technology (Millipore). Blood samples for GLP-1 analysis were collected in tubes containing a DPP-IV inhibitor to prevent degradation of GLP-1. Plasma was promptly separated and stored at −80 °C. All samples were assayed in duplicate.

Hunger ratings

Visual analogue scales were used to assess feelings of hunger on a scale from 1 to 10, in which 1 was 'not at all' and 10 was 'very much'. Hunger was assessed at baseline, +10 min, +35 min and +120 min after drink consumption. Prior studies have demonstrated good reproducibility and validity of these VAS scores for assessing subjective sensations of hunger[77].

Statistical analysis

Descriptive statistics were used to characterize frequency for categorical variables and mean (s.d.) and median (interquartile range) for continuous variables and to check distributional properties. Linear mixed-effects models were used for comparisons between sucralose and sucrose, aiming to assess the impact of sweet taste without calories (sucralose) versus sweet taste with calories (sucrose), and sucralose versus water, to investigate the effects of a sweetened drink without calories (sucralose) versus a non-sweet drink without calories (water). A linear contrast with a significance threshold of $P < 0.05$ was used to compare changes from before to after ingestion between the sucralose versus sucrose and sucralose versus water conditions. Residual diagnostics confirmed that model assumptions, including normality of residuals and homoscedasticity, were met, indicating that the data satisfied the assumptions of the statistical tests used. Given that there was no significant interaction between drink condition and time on hypothalamic response, drink condition was collapsed across both time points. Glycemic, insulin and GLP-1 measures and hunger ratings were collapsed across three time points. We also examined the main effect of BMI group on the hypothalamic response across all drinks, tested for interactions between BMI group and drink comparisons and stratified drink comparisons by weight-status group and by sex. Linear mixed-effects models assumed random intercept for each subject. General linear regression models were used to assess the association of changes in circulating glucose, insulin or GLP-1 (independent variable) with hypothalamic response (dependent variable) after drink ingestion and the association between hypothalamic responses (independent variable) with hunger ratings (dependent variable) after drink ingestion. Linear regression analyses were also conducted to explore associations between insulin sensitivity and the differential hypothalamic response to sucralose versus sucrose and water. Models were adjusted for age, sex, BMI and race/ethnicity. To evaluate differences in habitual non-caloric sweetener use by weight groups and sex, we performed ANOVA or an independent two-sample *t*-test for continuous variables (average intake in mg) and chi-squared tests for categorical variables (percentage of users versus non-users). Post-hoc comparisons of drink contrasts and time points were adjusted for multiple comparisons when necessary, using a Bonferroni correction, with significance levels set at $\alpha = 0.05$. Model fits were examined using the r2beta function from the r2glmm package. All statistical analyses were performed using Rstudio (version 2023.06.1).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author (K.A.P.) on reasonable request, and all brain imaging data are available in the Open Science Framework repository (https://osf.io/tuw93). Access to individual-level data is restricted owing to ethical and legal concerns. However, data may be shared for scientific collaborations upon request, contingent on the execution of appropriate data-sharing agreements. All requests will undergo review and approval by investigators, and will be in compliance with relevant local and national regulations and data-sharing policies. To request access, please contact the corresponding author. An initial response to requests will be provided within four weeks. Source data are provided with this paper.

Code availability

Computer codes used for data analyses are published in the following Open Science Framework repository: https://osf.io/tuw93/.

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Author contributions

K.A.P. conceived and designed the study. All authors were involved with the acquisition, analysis or interpretation of data. S.P.C. and K.A.P. drafted the manuscript; S.P.C., K.A.P., S.K., R.V., K.J., J.R.M., A.H.X. and A.G.Y. critically reviewed the manuscript for important intellectual content. S.P.C. performed statistical analysis. S.P.C., H.L. and B.A. visualized the project. H.L., A.G.Y., B.A., K.J. and R.V. provided administrative, technical or material support. K.A.P. obtained funding and supervised the research.

Competing interests

The authors declare no competing interests.

Additional information

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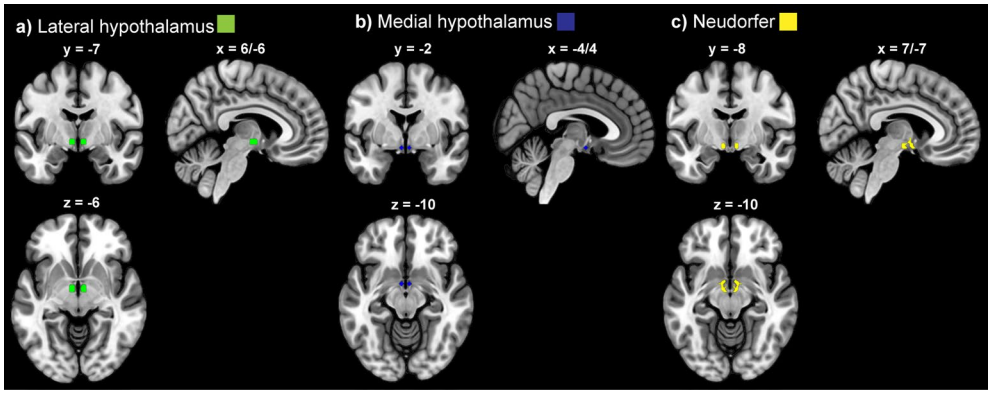

Extended Data Fig. 1 | Visual Display of Hypothalamic ROI. Hypothalamic Region of interest (ROIs) and corresponding coordinates. (**a**) Lateral hypothalamus (green), (**b**) Medial hypothalamus (blue), (**c**) Neudorfer (yellow). The images are displayed in neurological convention.

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Software and code

Policy information about availability of computer code

Data collection	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	pASL and fMRI data were preprocessed and first and second level analyses were run using: FSL (6.0.1), Matlab (2017a), Python (3.7.3). fMRI seed to voxel functional connectivity analysis was run suing CONN Toolbox (v21.a) and SPM (v12.7). Statistical analyses were performed using Rstudio (version 2023.06.1), Model fits were examined using the r2beta function from the r2glmm package. Computer codes for data analysis are published in: https://osf.io/tuw93/.

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Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We included female and male subjects. Sex was self-reported by participants based on their sex assigned at birth, which reflects the categorization recorded at the time of birth. Gender identity was not explicitly assessed in this study.Sex characteristics shown in detail in Table 1. See Results: Hypothalamic Responses to Sucralose vs Sucrose and Water Stratified by Sex (page 18) for exploratory sex based analysis results. We did not collect any gender data.
Reporting on race, ethnicity, or other socially relevant groupings	We included self reported race/ethnicity data.
Population characteristics	Population characteristics shown in detail in Table 1.
Recruitment	Participants in the greater Los Angeles area were recruited by flyers and advertisements and social media outlets. Recruiting participants through flyers, advertisements, and social media outlets might favor the recruitment of individuals who are more motivated, health-conscious, or have a greater interest in research studies related to appetite regulation and metabolic outcomes. To mitigate this bias, we actively sought to recruit a diverse participant pool, ensuring representation across race/ethnicity and gender (including both females and males). We believe that our use of standardized protocols, a repeated within-subject design, and fasting conditions helps maintain the internal validity of our findings. However, we acknowledge that this recruitment strategy may impact the generalizability of the results, which should be considered when interpreting our study outcomes.
Ethics oversight	This randomized, controlled, crossover trial (NCT02945475) followed Consolidated Standards of Reporting Trials (CONSORT) guidelines (trial protocol can be found in online digital repository: https://osf.io/tuw93/). Participants provided written informed consent that was compliant with the University of Southern California Institutional Review Board (IRB #H-09-00395).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Life sciences study design

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Sample size	Of the 76 participants enrolled, who received at least 1 drink allocation, 1 participant did not receive any of the drinks (i.e., sucralose, sucrose, or water) included in this analysis because of dropout, and was excluded from this analysis (Figure 2). Participants included in this analysis were 75 adults (43 female) ages 18 to 35 years with healthy weight, overweight, or obesity (Table 1). Sample sizes were originally estimated a sample size of 120 participants to detect a minimum effect of 0.31 SD of the difference in sweeteners on activation within brain ROIs, controlling for the false discovery rate among brain regions, assuming a paired 2-sided t test, $\alpha = .05$, and 80% power. The study was halted on March 13, 2020 because of the COVID-19 pandemic, with a recruited sample of 76 participants. We calculated that, with this sample, we would have 80% power to detect a minimum effect size of 0.40 SD
Data exclusions	Of the 157 participants assessed for eligibility, 72 were excluded prior to randomization, with 43 not meeting inclusion criteria and 29

Data exclusions	declining to participate. Among the 85 participants randomized, 9 were excluded from the final analysis due to anxiety (5 participants), claustrophobia (1 participant), no-shows for study visits (2 participants), and noncompliance with the protocol (1 participant). Of the 76 participants enrolled, one participant dropped out before completing any of the required drink conditions (sucralose, sucrose, or water) and was excluded from the final analysis, resulting in a final sample size of 75 participants. All exclusions were based on pre-established criteria outlined in the study protocol.
Replication	We employed a repeated within-subject design to ensure consistency in our findings, with all study visits conducted in the morning after an overnight fast to reduce variability. The MRI acquisitions were selected based on protocols previously shown to be reliable for assessing appetite regulation (Yunker et al, 2021). Metabolic assays were conducted in duplicate to help ensure consistency and reliability. Minor variations observed were within expected ranges, and our results align with previously established findings using these protocols (Page et al, JAMA 2013; Page et al, Journal of Clinical Investigation, 2011; Jastrebroff et al, Diabetes Care DOI:10.2337/db15-1216)
Randomization	The drink order given during the fMRI sessions will be randomized using a computer-generated sequence.
Blinding	Participants and investigators are blinded to study drink condition.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

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Clinical trial registration	NCT02945475
Study protocol	The trial protocol can befound in an online digital repository: https://osf.io/tuw93/.
Data collection	Study enrollment occurred between July 2016 and March 2020 in Los Angeles, California. Data collection occurred at Dana & David Dornsife Cognitive Neuroscience Imaging Center and Diabetes and Obesity Research Institute at the Keck School of Medicine, University of Southern California.
Outcomes	The primary outcome was the relative changes in hypothalamic blood flow in response to acute ingestion of sucralose, sucrose, and water. This was measured using pulsed arterial spin labeling (pASL) MRI, focusing on lateral, medial, and Neudorfer hypothalamic subfields. Secondary outcomes included changes in peripheral glucose, insulin, and GLP-1 levels, subjective hunger ratings, and functional connectivity between the hypothalamus and other brain regions. Peripheral biomarkers were measured from blood samples collected at fasting and post-ingestion time points (+10, +35, and +120 minutes) and quantified using biochemical assays. Hunger ratings were assessed via standardized self-report scale. Functional connectivity was evaluated through BOLD fMRI seed-to-voxel analysis.

Plants

Seed stocks — *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

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Magnetic resonance imaging

Experimental design

Design type: perfusion (pASL). functional connectivity was conducted using resting state data.

Design specifications: The study included three blinded MRI sessions conducted in random order, each session involving 300 mL drink ingestion (sucralose, sucrose, or water). MRI measurements were collected at fasting, +10, and +35 minutes after drink ingestion.

Behavioral performance measures: Hunger ratings were measured at fasting, +10, +35, and +120 minutes post-drink ingestion. Circulating glucose, GLP-1, and insulin levels were also measured at corresponding time points to assess physiological responses to drink conditions.

Acquisition

Imaging type(s): Functional (BOLD fMRI) and perfusion (pASL).

Field strength: 3 Tesla (3T Siemens MAGNETOM Prismafit MRI System).

Sequence & imaging parameters: Structural imaging: High-resolution 3D magnetization-prepared rapid gradient echo (MPRAGE): TR=1950 ms, TE=2.26 ms, flip angle=9°, slice thickness=1 mm, FOV=224 mm × 256 mm, matrix=224 × 256.
Perfusion imaging (pASL): FOV=192 mm, matrix=64 × 64, TR=4000 ms, TE unspecified, flip angle=90°, slice thickness=5 mm, interslice spacing=0 mm.
BOLD-fMRI: TR=1000 ms, TE=43.2 ms, flip angle=52°, slice thickness=1.5 mm, FOV=128 mm × 112 mm, matrix=128 × 112.

Area of acquisition: Whole-brain acquisition (Hypothalamus-specific subfield analysis were conducted).

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software: BASIL toolbox (part of FSL 6.0.1) for CBF analysis; Conn Toolbox v21.a and SPM v12.7 for functional connectivity analysis. Preprocessing included motion correction, regression of physiological noise, bandpass filtering (0.008–0.09 Hz), co-registration with T1-weighted images, and automated pipeline execution for functional connectivity and ASL analyses.

Normalization: Data were normalized to MNI152 space using FSL and CONN Toolbox pipelines.

Normalization template: MNI152.

Noise and artifact removal: Motion correction, physiological noise regression, and partial volume effect adjustments were conducted to reduce artifacts and improve CBF measurement accuracy. Automated artifact removal scripts in Python (3.7.3) were employed for ASL data preprocessing.

Volume censoring: Volumes with framewise displacement exceeding the 75th percentile plus 1.5 times the interquartile range were excluded as outliers.

Statistical modeling & inference

Model type and settings: Linear mixed-effects models were employed for repeated measures data to compare drink conditions (sucralose vs sucrose and sucralose vs water) and their effects on the independent variable of interest. Random intercepts were modeled for each subject. Models were adjusted for age, sex, BMI, and race/ethnicity. Random intercepts were modeled for each subject.

Effect(s) tested: Voxel-wise analysis was conducted, evaluating individual voxel-level hypotheses for regional CBF and connectivity patterns.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s): The analysis focused on hypothalamic subfields (lateral and medial hypothalamus) and the Neudorfer hypothalamus mask. These ROIs were defined using the Baroncini atlas and the high-resolution Neudorfer atlas, normalized into MNI152 space.

Statistic type for inference (See Eklund et al. 2016): Voxel-wise analysis was conducted, evaluating individual voxel-level hypotheses for regional CBF and functional connectivity patterns.

Correction: Statistical significance was corrected for multiple comparisons using a false discovery rate (FDR) correction with an additional cluster-size threshold based on Gaussian Random Field theory. Bonferroni corrections were applied for specific drink condition contrasts.

Models & analysis

n/a	Involved in the study
☐	☒ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity: Seed-to-voxel connectivity measures were modeled using a weighted GLM with covariates (age, sex, BMI, and race/ethnicity). Significance was determined using Gaussian Random Field theory with FDR-corrected p-values (voxel-level $p < 0.001$, cluster-level $p < 0.05$)).