

# The SWEET spot for weight maintenance

Sarah H. Schmitz & Louis J. Aronne

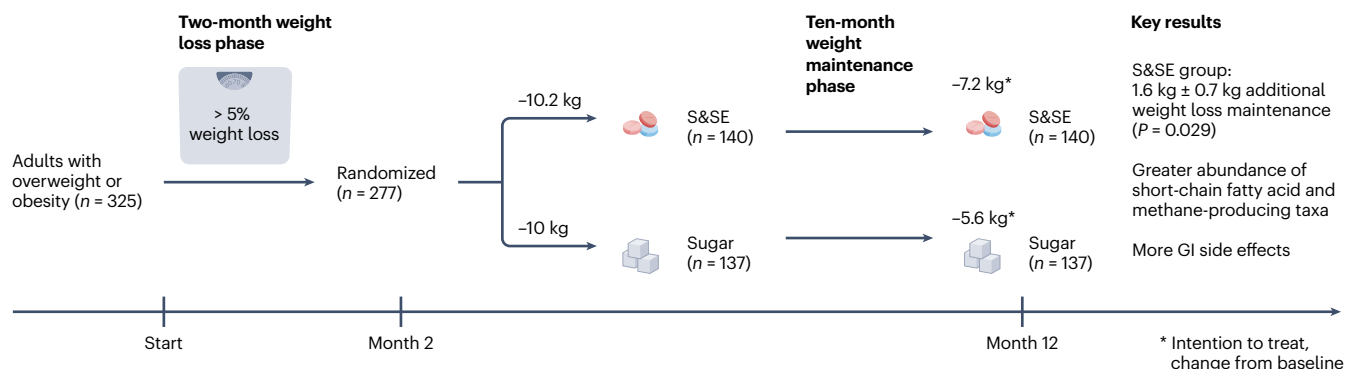
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**A 12-month multicentre randomized clinical trial finds that replacing added sugar in foods and beverages with sweeteners and sweetness enhancers supports modest weight loss maintenance and alters gut microbiota composition, with no safety concerns identified.**

Sustaining weight loss is notoriously difficult, as persistent hormonal adaptations drive increased hunger and promote weight regain<sup>1</sup>, reversing many of the cardiometabolic benefits achieved through weight loss<sup>2</sup>. Replacing added sugars with calorie-free sweeteners and sweetness enhancers has been proposed as a means to reduce caloric intake without sacrificing dietary palatability. Both retrospective and prospective studies have suggested that such substitutions can aid in weight loss maintenance<sup>3,4</sup>; however, questions remain regarding the long-term effect of sweeteners and sweetness enhancers on body weight, metabolic health and the gut microbiome. Although the World Health Organization advises limiting free sugar intake to less than 10% of total energy, and ideally below 5% (ref. 5), adherence to these targets is low<sup>6</sup>. Although sweeteners and sweetness enhancers could help to bridge this gap, observational studies have produced conflicting results related to body weight and metabolic outcomes, potentially due to reverse causality and residual confounding<sup>7</sup>, and most interventional studies in humans have been short in duration and limited to beverages<sup>8</sup>. Mechanistic work has raised the possibility that sweeteners and sweetness enhancers could alter the gut microbiota in ways that affect glucose tolerance and appetite regulation<sup>9</sup>.

In this issue of *Nature Metabolism*, Blaak, Raben and colleagues<sup>10</sup> report the results of their 1-year multicentre randomized trial, SWEET, which compared the effect of sweeteners and sweetness enhancers on weight loss maintenance in both adults and children with overweight or obesity. Although the study was affected by the COVID-19 pandemic, the authors were able to complete a comprehensive study assessing the effect of sweeteners and sweetness enhancers on weight loss maintenance, cardiometabolic health and gut microbial composition. The study was conducted in Denmark, Greece, the Netherlands and Spain, enrolling 341 adults and 38 children with overweight or obesity. The subsequent discussion will focus on adults, as the paediatric cohort was not powered for statistical analysis and findings in this group were considered exploratory. All participants completed a 2-month weight loss phase that consisted of a low-energy diet and needed to achieve at least 5% weight loss to qualify for the maintenance phase. Participants then entered the 10-month weight maintenance phase during which they were randomized to one of two ad libitum healthy diets consisting of less than 10% energy from added sugar. The sweeteners and sweetness enhancers group were allowed food and drinks with sweeteners and sweetness enhancers, aiming to replace sugar-containing items unit-for-unit. The sugar group was told to avoid sweeteners and sweetness enhancers and to limit added sugar to less than 10% of energy intake. The two primary endpoints were change in body weight and change in gut microbiota composition at 1 year. Secondary endpoints included changes in metabolic markers, occurrence of adverse events and gastrointestinal side effects (Fig. 1).

At the end of the 2-month weight loss phase, participants in both groups had lost an average of 10 kg, corresponding to more than 10% weight loss. Over the subsequent 10-month weight maintenance period, weight was gradually regained, but both groups retained more than half of their initial loss. Notably, in the intention-to-treat



**Fig. 1 | Sweeteners and sweetness enhancers support modest weight loss maintenance and alter gut microbiota composition.** Participants completed a 2-month weight loss phase before being randomized to either a sweeteners and sweetness enhancers (S&SE) group, who replaced sugar in their diet with sweeteners and sweetness enhancers, or a sugar group, who avoided consuming sweeteners and sweetness enhancers, during a 10-month weight maintenance

phase. Both groups limited their intake of sweeteners and sweetness enhancers or sugar to less than 10% of total energy. The primary endpoints were change in body weight and gut microbiota composition after 1 year, and the secondary endpoints included changes in metabolic markers, occurrence of adverse events and gastrointestinal (GI) side effects.

analysis, those consuming sweeteners and sweetness enhancers sustained an additional  $1.6 \pm 0.7$  kg of weight loss compared with the sugar group ( $P = 0.029$ ). Among individuals who completed the trial, the difference was similar (1.7 kg) but no longer statistically significant, a finding probably influenced by the higher-than-expected dropout rate (40% versus the planned 30%). Participants in the sweeteners and sweetness enhancers group reduced their sugar intake twice as much as the sugar group, and the highest dietary compliance was associated with the largest weight difference (3.7 kg). This aligns with previous research indicating that the degree of adherence, rather than the specific diet composition, is the strongest predictor of weight loss outcomes<sup>11</sup>. At 6 months, the sweeteners and sweetness enhancers group had significantly greater reductions in body mass index, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol; however, these differences were no longer evident at 12 months, possibly related to weight regain. Notably, no adverse effects of sweeteners and sweetness enhancers on cardiometabolic markers were observed.

In a subset of 137 adults who completed the trial, gut microbiota analysis revealed that intake of sweeteners and sweetness enhancers shifted community composition towards a greater abundance of short-chain fatty acid and methane-producing taxa. Baseline microbiota profiles predicted, with reasonable accuracy, which participants in the sweeteners and sweetness enhancers group would maintain or improve their HbA1c levels and avoid weight regain – an observation consistent with the principles of precision nutrition, in which individual microbiome features inform dietary recommendations. Gastrointestinal adverse events, including abdominal pain or cramps, loose stools and excess intestinal gas, were more frequently reported in the sweeteners and sweetness enhancers group than in the sugar group, but were generally mild and rarely led to discontinuation.

The SWEET trial stands out in literature dominated by short-term beverage trials. Its inclusion of a wide variety of commercially available foods and drinks containing sweeteners and sweetness enhancers reflects real-world patterns of consumption, and the multicentre European design enhances generalizability across different cultural and dietary contexts. The run-in phase ensured participants started the intervention in a state of recent, clinically significant weight loss, a time when weight maintenance strategies are most needed. That said, the effect on weight maintenance was modest, and the trial was not designed to detect differences in long-term morbidity or mortality. The attenuation of cardiometabolic differences by 12 months suggests that even with the use of sweeteners and sweetness enhancers, weight loss maintenance remains challenging. The microbiome findings are intriguing but remain correlative; whether the observed taxa

contribute causally to body weight regulation or glucose metabolism remains to be determined.

The results of this trial strengthen the case for sweeteners and sweetness enhancers as part of a sugar-reduction toolkit in weight maintenance, provided they are being used to replace sugar in the diet. Future studies could adopt cross-over designs to reduce between-person variability, include metabolomic and functional microbiome assays to probe mechanisms, and stratify by baseline microbiota to test personalized dietary prescriptions. Long-term follow-up would help to further elucidate the effect of sweeteners and sweetness enhancers on the incidence of type 2 diabetes and cardiovascular disease.

The SWEET trial offers reassurance that replacing sugar with a variety of sweeteners and sweetness enhancers in both foods and drinks can support weight maintenance and does not seem to harm cardiometabolic health over a year. The findings also hint at a role for the gut microbiome in determining who will benefit most, a reminder that even in the long-running sweetener debate, the answer is never one-size-fits-all.

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## Competing interests

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# Effect of sweeteners and sweetness enhancers on weight management and gut microbiota composition in individuals with overweight or obesity: the SWEET study

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Consumption of sweeteners and sweetness enhancers (S&SEs) is a popular strategy to reduce sugar intake, but the role of S&SEs in body weight regulation and gut microbiota composition remains debated. Here, we show that S&SEs in a healthy diet support weight loss maintenance and beneficial gut microbiota shifts in adults with overweight or obesity. In this multi-centre, randomized, controlled trial, we included 341 adults and 38 children with overweight or obesity. Adults followed a 2-month low-energy diet for  $\geq 5\%$  weight loss, followed by a 10-month healthy ad libitum diet with  $<10\%$  energy from sugars. One group replaced sugar-rich products with S&SE products (S&SEs group), while the other did not (sugar group). Primary outcomes included changes in body weight and gut microbiota composition at 1 year. Secondary outcomes included changes in cardiometabolic parameters. The S&SEs group, compared to the sugar group, maintained greater weight loss at 1 year ( $1.6 \pm 0.7$  kg,  $P = 0.029$ ) and exhibited distinct gut microbiota shifts, with increased short-chain fatty acid and methane-producing taxa ( $q \leq 0.05$ ). No significant differences were observed in cardiometabolic markers or in children. Overall, our findings indicate that prolonged consumption of S&SEs in a healthy diet is a safe strategy for obesity management. ClinicalTrials.gov identifier: [NCT04226911](https://clinicaltrials.gov/ct2/show/study/NCT04226911).

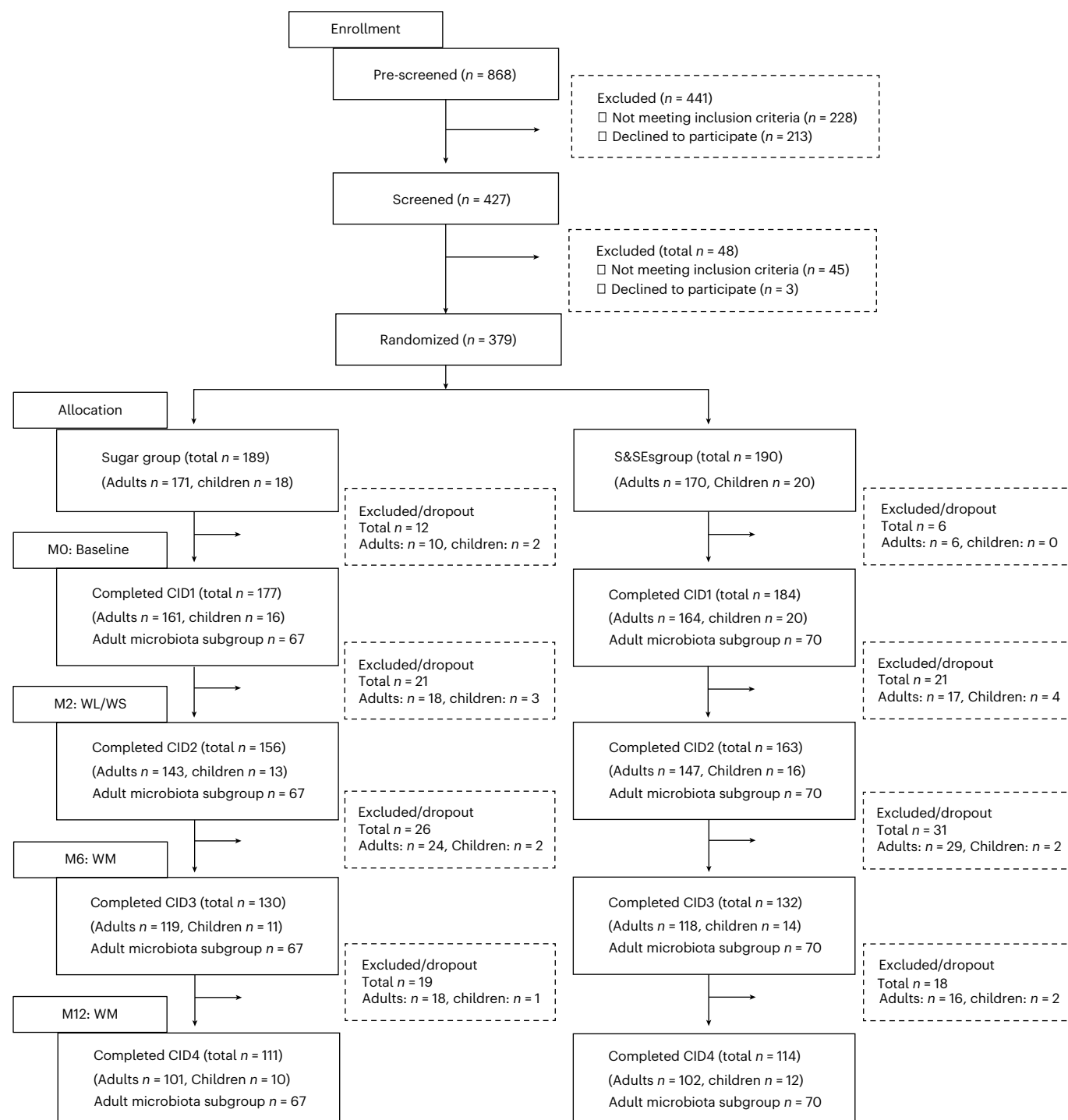
The prevalence of overweight and obesity has increased globally, raising the risk of non-communicable diseases such as type 2 diabetes (T2D) and cardiovascular disease (CVD)<sup>1,2</sup>. A shift towards a Westernized diet (high in saturated fat and added sugar, low in dietary fibre) has been proposed to be a key contributor to the development of obesity-related cardiometabolic complications<sup>3</sup>. Added sugar, in particular, increases dietary energy density, which may lead to greater energy intake and the development of obesity.

In 2015, the World Health Organization (WHO) strongly recommended that free sugar intake should be less than 10% of total energy intake (E%) and preferably even less than 5 E% as a conditional recommendation<sup>4</sup>. The latter is still not being fulfilled by large parts of

the global population, including Denmark, Greece, Spain and the Netherlands<sup>5–9</sup>. One common strategy to reduce sugar intake is replacing it with S&SEs. Consequently, the worldwide consumption of foods and beverages containing S&SEs has substantially increased over recent years<sup>10</sup>.

Although S&SEs are generally considered safe, their long-term effects on cardiometabolic health remain debated. Cohort studies have raised concerns about potential risks, prompting the WHO to issue a conditional recommendation against using non-sugar sweeteners for weight control or reducing the risk of non-communicable diseases<sup>4,11,12</sup>. Nevertheless, observational evidence is not in line with data from short-term studies<sup>13,14</sup>, and the limited number of long-term

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**Fig. 1 | Study participant flow chart.** Flow chart of participant enrollment, allocation, and follow-up in the study. A total of 868 individuals were pre-screened, with 379 randomized into two groups: the sugar group ( $n = 189$ ) and the S&SEs group ( $n = 190$ ). The figure shows exclusions, dropouts, and the number

of adults and children completing each study visit (CID1–CID4) at baseline (M0), weight loss (WL)/weight stability (WS) (M2), mid-weight maintenance (WM) (M6), and after weight maintenance (M12), including the adult microbiota subgroup.

randomized controlled trials (RCTs) have shown neutral or beneficial effects, including modest weight loss and no negative impact on T2D or CVD risk markers<sup>13,14</sup>. Like the WHO, the overall review conclusions seem to depend on which evidence the authors choose to cite and emphasize<sup>15</sup>.

Another emerging concern is the potential impact of S&SEs on gut microbiota composition. Some studies suggest that S&SEs may alter the gut microbiota, potentially affecting metabolic health<sup>13</sup>. A previous

study<sup>14</sup> demonstrated a link between saccharin-induced alterations in the gut microbiota and glucose intolerance in mice. In addition, in a small post hoc human trial, they showed that supplementation of saccharin ( $5 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) for 1 week increased glycaemic response, which was associated with microbiota alterations in a small group of study participants clustered as ‘responders’, while no response was found in the other participants (‘non-responders’)<sup>14</sup>. The poor glycaemic response in the ‘responders’ was replicated in mice upon faecal

transplantation. Interestingly, the microbiota composition of responders was distinct before saccharin exposure, suggesting individual variability in response to S&SEs and the potential for the gut microbiome to predict susceptibility. The same group later exhibited person-specific gut microbiome shifts related to altered glycaemic responses following 2 weeks of S&SE supplementation at doses lower than the acceptable daily intake compared to sachet-contained vehicle glucose or no supplement<sup>16</sup>. However, other studies<sup>14</sup> and a crossover RCT found no effect of saccharin, sucralose or aspartame on gut microbiota or glucose regulation after 2 weeks in healthy individuals<sup>14,15</sup>. A recent cohort study linked sugar-sweetened beverage intake to gut microbiota changes and metabolites associated with diabetes risk<sup>17</sup>. These conflicting results highlight the controversy in the current evidence. Controlled, long-term studies are needed to directly assess the impact of replacing sugar with S&SEs on microbiota and metabolic outcomes.

Although the inclusion of S&SEs in sugar-reduced diets may assist in sustaining weight loss by improving palatability and adherence, the long-term effects of S&SE intake on the gut microbiota and their potential influence on cardiometabolic health and safety remain to be elucidated. Therefore, the aim of this RCT in the SWEET project ('Sweeteners and sweetness enhancers: prolonged effects on health, obesity and safety') was to assess the effect of combined and prolonged use of S&SEs (in both foods and drinks)—as part of a healthy sugar-reduced ad libitum diet—on weight loss maintenance, cardiometabolic risk factors and gut microbiota composition in adults with overweight or obesity. In children with overweight or obesity, the trial focused on weight control and cardiometabolic outcomes<sup>18</sup>.

We reasoned that the inclusion of S&SEs in foods and drinks would increase the palatability of the diet and thereby compliance with the recommendations for a healthy sugar-reduced diet, resulting in improved control of body weight and related risk factors, with no effect on gut microbiota or other safety concerns associated with their long-term use compared with a diet excluding S&SEs. The primary outcomes were 1-year changes in body weight and gut microbiota composition in adults. Secondary outcomes included 1-year changes in risk factors for T2D and CVD, body mass index (BMI)-for-age z-score in children, intrahepatic lipid (IHL) content, the occurrence of (serious) adverse events (AEs), gastrointestinal symptoms and use of concomitant medication in adults with overweight or obesity.

## Results

In total, 341 adults and 38 children were included, and 203 adults and 22 children (60% and 58%) completed the 1-year trial. The number of participants in each intervention group is shown in Fig. 1. The most common causes for dropout were either personal reasons or unknown. All explanations for dropout and exclusion can be found in Extended Data Table 1. This trial was conducted during the COVID-19 pandemic; therefore, we encountered obstacles such as disrupted participant follow-up, increased dropout rates and logistical challenges related to travel restrictions and safety protocols. For the analysis of gut microbiota composition, a subgroup of 137 adults was included (Copenhagen,  $n = 26$ ; Pamplona,  $n = 26$ ; Harokopio,  $n = 25$ ; Maastricht,  $n = 60$ ).

### Participant characteristics

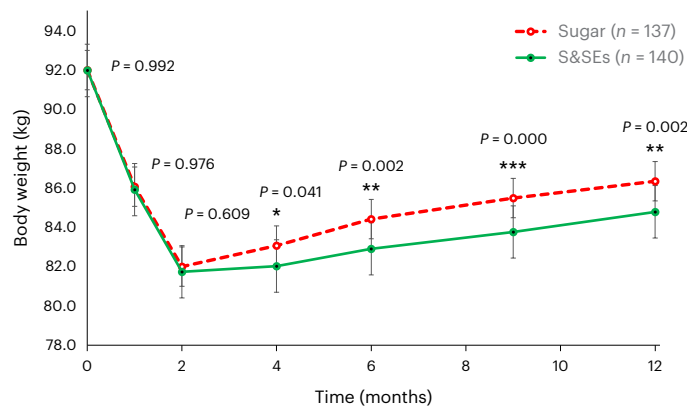
The baseline visit (month 0, M0) was completed by 95% of the included adults (median (Q1–Q3) age, 47 years (40–50 years), 71% female; Table 1). The majority (95% of the adults and 92% of the children) had citizenship in the country in which the trial was conducted, and 3.4% of the adults and 14% of the children reported that they belonged to a minority group (self-reported). The 277 adults who completed the weight loss period lost  $10.1 \pm 3.6$  kg (mean  $\pm$  s.d.) with no difference between the groups (Extended Data Table 2). The participant characteristics after the weight loss period (M2) are shown in Table 1.

**Table 1 | Baseline characteristics of adult participants who completed the first CID ( $n=325$ ) and of those who successfully completed the weight loss period ( $n=277$ )**

|                                            | Baseline (M0)                     | After weight loss (M2)           |                                 |                   |
|--------------------------------------------|-----------------------------------|----------------------------------|---------------------------------|-------------------|
|                                            | All participants ( $n=325$ )      | Sugar group ( $n=137$ )          | S&SEs group ( $n=140$ )         | Pvalue            |
| <b>Anthropometric</b>                      |                                   |                                  |                                 |                   |
| Body weight (kg)                           | 91.0<br>(79.6–103.4)              | 81.5<br>(69.0–93.0)              | 79.3<br>(71.2–88.9)             | 0.66              |
| BMI ( $\text{kg m}^{-2}$ )                 | 31.7 (28.7–35.2)                  | 28.4<br>(25.3–31.1)              | 27.6<br>(25.6–30.0)             | 0.16 <sup>a</sup> |
| Fat mass (kg)                              | 37.1 (30.9–44.0)                  | 30.3<br>(24.4–36.8)              | 30.2<br>(24.8–36.4)             | 0.97              |
| Fat mass (%)                               | 43.0 (37.5–47.8)                  | 39.4<br>(33.3–43.8) <sup>b</sup> | 38.9<br>(33.1–44.5)             | 0.80              |
| Fat-free mass (kg)                         | 51.0 (45.4–60.8)                  | 48.9<br>(43.3–58.7)              | 48.2<br>(42.6–58.5)             | 0.95              |
| VAT (g)                                    | 1,031<br>(574–1,680) <sup>c</sup> | 630<br>(368–1,158) <sup>d</sup>  | 635<br>(358–1,027) <sup>e</sup> | 0.36              |
| WC (cm)                                    | 101.3<br>(92.3–111.0)             | 92.0<br>(82.5–100.0)             | 90.0<br>(84.0–100.0)            | 0.52              |
| HC (cm)                                    | 113.0<br>(107.0–121.5)            | 105.5<br>(100.0–113.0)           | 107.0<br>(100.5–113.0)          | 0.99              |
| <b>Blood pressure and heart rate</b>       |                                   |                                  |                                 |                   |
| Systolic (mmHg)                            | 119 (109–128)                     | 110 (104–119)                    | 110 (101–118)                   | 0.42              |
| Diastolic (mmHg)                           | 80 (73–86)                        | 74 (68–80)                       | 72 (68–80)                      | 0.45              |
| Heart rate (beats per min)                 | 70 (63–79)                        | 68 (62–75)                       | 69 (60–75) <sup>f</sup>         | 0.90              |
| <b>Lipid profile</b>                       |                                   |                                  |                                 |                   |
| Total cholesterol ( $\text{mmol l}^{-1}$ ) | 5.03<br>(4.53–5.64) <sup>g</sup>  | 4.22<br>(3.65–4.84)              | 4.13<br>(3.51–4.50)             | 0.09              |
| HDL-C ( $\text{mmol l}^{-1}$ )             | 1.32 (1.19–1.55) <sup>g</sup>     | 1.16<br>(1.03–1.32)              | 1.16<br>(1.05–1.34)             | 0.25              |
| LDL-C ( $\text{mmol l}^{-1}$ )             | 2.77 (2.35–3.21) <sup>h</sup>     | 2.22<br>(1.91–2.64)              | 2.09<br>(1.83–2.48)             | 0.05              |
| Non-HDL-C ( $\text{mmol l}^{-1}$ )         | 3.67 (3.16–4.22) <sup>g</sup>     | 3.03<br>(2.59–3.10)              | 2.82<br>(2.46–3.29)             | 0.03              |
| Triglycerides ( $\text{mmol l}^{-1}$ )     | 1.02 (0.76–1.53) <sup>h</sup>     | 0.89<br>(0.71–1.13)              | 0.83<br>(0.64–1.06)             | 0.12 <sup>a</sup> |
| <b>Glucose metabolism</b>                  |                                   |                                  |                                 |                   |
| Glucose ( $\text{mmol l}^{-1}$ )           | 5.33<br>(5.06–5.77) <sup>a</sup>  | 5.16<br>(4.83–5.44)              | 5.11<br>(4.77–5.55)             | 0.62              |
| Insulin ( $\text{pmol l}^{-1}$ )           | 48.3<br>(34.8–68.9) <sup>a</sup>  | 34.2<br>(24.6–48.0)              | 30.9<br>(22.7–45.9)             | 0.29 <sup>a</sup> |
| HbA1c (%)                                  | 5.3 (5.1–5.5) <sup>a</sup>        | 5.2 (4.9–5.4) <sup>b</sup>       | 5.2 (5.0–5.4)                   | 0.97              |

All values are unadjusted medians (Q1–Q3). Data are given for baseline (M0, before weight loss) and after weight loss (M2, before initiation of the WM period), with exclusion of those who did not achieve  $\geq 5\%$  weight loss according to the intervention during the WM period. Pvalues represent the analysis of group differences at M2. Differences between groups at M2 were analysed by ANCOVA adjusted for age, sex, baseline body weight and site. <sup>a</sup>Values were log transformed before analysis; <sup>b</sup> $n=136$ ; <sup>c</sup> $n=239$ ; <sup>d</sup> $n=96$ ; <sup>e</sup> $n=101$ ; <sup>f</sup> $n=139$ ; <sup>g</sup> $n=322$ ; <sup>h</sup> $n=321$ . HC, hip circumference; VAT, visceral adipose tissue; WC, waist circumference.

For children, 95% of the included participants completed the baseline visit (median (Q1–Q3) age 10 years (9–11 years), 61% girls; Supplementary Table 1). The 28 children who completed M2 and had a parent who achieved  $\geq 5\%$  weight loss tended to lose weight ( $-0.5 \pm 0.14$  kg,  $P = 0.07$ ) and increase height ( $1.2 \pm 0.8$  cm,  $P < 0.0001$ ), resulting in a  $0.19 \pm 0.18$  reduction in BMI-for-age z-score ( $P < 0.0001$ ). The changes in body weight (mean  $\pm$  s.d., sugar group,  $-0.6 \pm 1.6$  kg versus S&SEs group,  $-0.4 \pm 1.2$  kg), height (sugar group,  $1.4 \pm 0.8$  cm



**Fig. 2 | Body weight unadjusted means from M0 to M12 for the 277 adult participants who completed the weight loss period (M0–M2) with a weight loss  $\geq 5\%$  of initial body weight.** For the 74 adult participants who dropped out after successful weight loss, missing data are imputed by the last observation carried forward. Body weight was measured in the fasting state except at months 0.5, 1, 4 and 9. Statistical differences between groups were assessed using ANCOVA linear mixed models. Interaction time  $\times$  group:  $P < 0.0001$ . Post hoc analyses: groups differ \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Error bars, s.e.m.

versus S&SEs group,  $1.0 \pm 0.7$  cm) and BMI-for-age z-score (sugar group,  $-0.22 \pm 0.20$  versus S&SEs group,  $-0.17 \pm 0.14$ ) did not differ between the groups ( $P = 0.65$ ,  $P = 0.31$  and  $P = 0.66$ , respectively) (Supplementary Table 1). Waist and hip circumferences were also reduced (mean  $\pm$  s.d.,  $1.2 \pm 2.5$  cm,  $P = 0.02$  and  $-1.3 \pm 3.4$  cm,  $P = 0.047$ , respectively) with no differences between groups ( $P > 0.05$ ). No other changes were observed at M2 (Supplementary Table 1).

### Body weight

The adults' body weight over time (in the intention-to-treat (ITT) population) is shown in Fig. 2. The 1-year change in adult body weight (M12 – M0) was  $-6.4 \pm 6.5$  kg (mean  $\pm$  s.d.) for the whole ITT population. A similar lowering of body weight ( $-6.3 \pm 7.2$  kg) was observed for completers (Extended Data Fig. 1b). For the ITT population (last observation carried forward), the S&SEs group maintained a  $1.6 \pm 0.7$  kg larger weight loss (mean  $\pm$  s.e.m.,  $P = 0.03$ ) than the sugar group (Table 2). For completers, the difference was similar but not significant ( $1.7 \pm 1.0$  kg,  $P = 0.08$ ) (Table 2). An interaction between time and intervention group was observed ( $P = 0.0002$ ), with the sugar group weighing more than the S&SEs group at M4 ( $1.0 \pm 0.5$  kg,  $P = 0.04$ ), M6 ( $1.6 \pm 0.5$  kg,  $P = 0.002$ ), M9 ( $2.1 \pm 0.6$  kg,  $P = 0.0002$ ) and M12 ( $1.7 \pm 0.5$  kg,  $P = 0.002$ ). Imputation of missing body weight values for the ITT population based on the same model showed that the S&SEs group maintained a  $1.8 \pm 0.7$  kg larger weight loss (mean  $\pm$  s.e.m.) ( $P = 0.01$ ) than the sugar group (Table 2).

Per-protocol analyses were carried out using the compliance scores (Table 2 and Extended Data Table 3). Results for participants scoring at least two points resulted in similar weight differences between groups as the ITT and completers analyses. However, by increased dietary compliance, the weight difference between groups increased (participants scoring three or four points). In both cases, the S&SEs group had the largest weight loss maintenance (Table 2).

### Gut microbiota composition

The clinical characteristics of the subgroup analysed for gut microbiota ( $n = 137$ ) were comparable to those of the total adult population ( $n = 341$ ) (Supplementary Table 2). Consistent with the findings in all adults of the total study population, the gut microbiota subgroup showed significantly lower body weight regain in the S&SEs group compared to the sugar group over 1 year ( $3.4 \pm 0.7$  kg versus  $5.6 \pm 0.8$  kg,  $P = 0.016$ ) (Supplementary Table 2).

**Table 2 | The 1-year change in body weight of adults and differences between groups in different populations (ITT, completers and per protocol)**

|                                 | 1-year change in body weight (kg) |                        | Difference between groups (kg) | Pvalue |
|---------------------------------|-----------------------------------|------------------------|--------------------------------|--------|
|                                 | Sugar group                       | S&SE group             |                                |        |
| ITT population (LOCF)           | $-5.6 \pm 5.7$ (n=137)            | $-7.2 \pm 7.0$ (n=140) | $1.6 \pm 0.7$                  | 0.029  |
| ITT population (repeated model) | $-5.2 \pm 4.9$ (n=137)            | $-6.9 \pm 6.9$ (n=140) | $1.8 \pm 0.7$                  | 0.011  |
| Completers                      | $-5.4 \pm 6.3$ (n=101)            | $-7.1 \pm 8.0$ (n=102) | $1.7 \pm 1.0$                  | 0.082  |
| Per protocol $\geq 2$ points    | $-5.1 \pm 5.5$ (n=78)             | $-6.6 \pm 6.6$ (n=80)  | $1.6 \pm 0.9$                  | 0.085  |
| Per protocol $\geq 3$ points    | $-5.2 \pm 5.8$ (n=58)             | $-7.4 \pm 7.1$ (n=68)  | $2.2 \pm 1.1$                  | 0.059  |
| Per protocol 4 points           | $-4.0 \pm 4.7$ (n=39)             | $-7.7 \pm 7.0$ (n=36)  | $3.7 \pm 1.4$                  | 0.009  |

The 1-year change for each group is presented as unadjusted mean  $\pm$  s.d., and the difference between groups is the adjusted mean  $\pm$  s.e.m. Pvalues represent the analysis of group differences with 1-year change as the outcome. Difference between groups analysed by ANCOVA adjusted for age, sex, baseline weight and intervention site unless otherwise specified. LOCF, last observation carried forward.

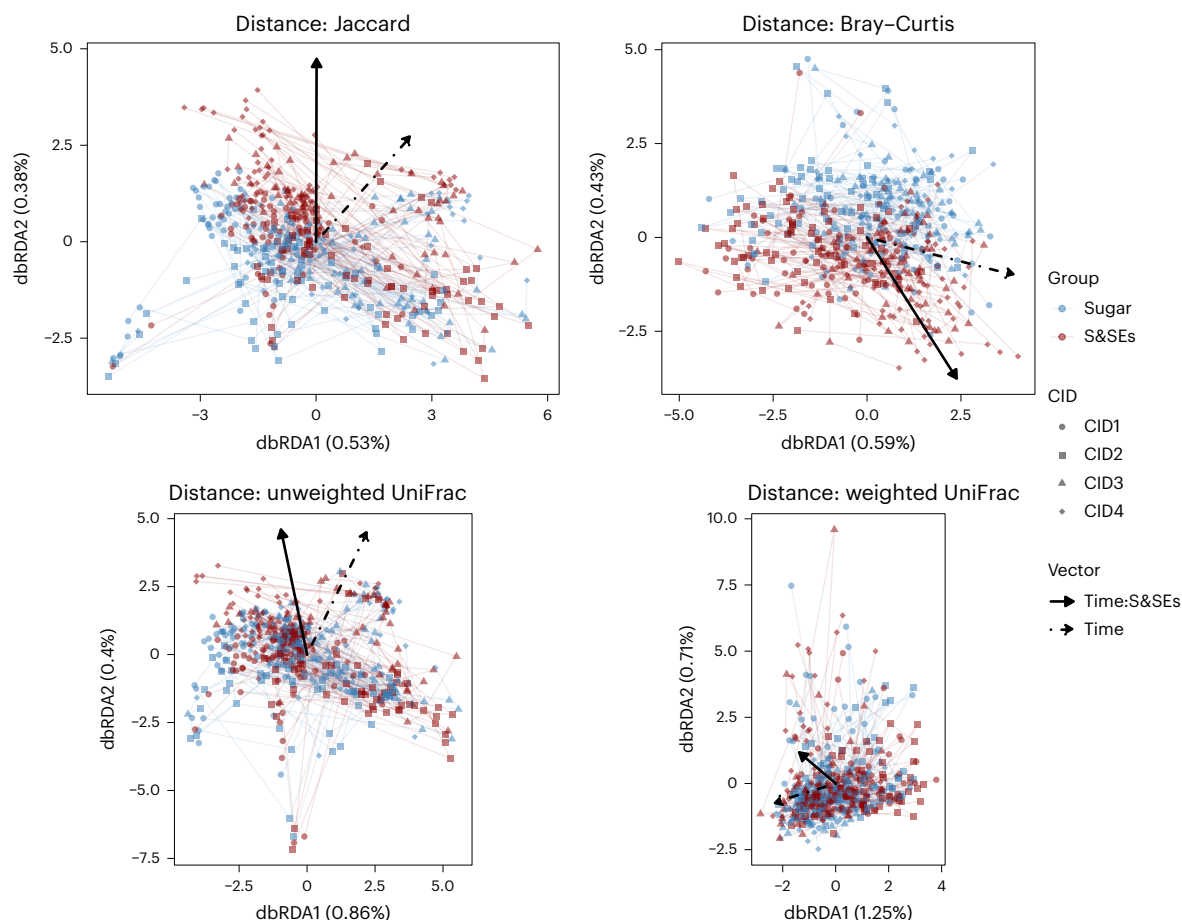
The trends in alpha diversity change over time was not different between groups (Supplementary Fig. 1). We observed a significant interaction between overall microbiota composition change in time and intervention group (PERMANOVA, time  $\times$  group interaction,  $P < 0.005$ ) (Fig. 3). A total of 46 taxa exhibited differential trends in relative abundance over time between the groups (Extended Data Fig. 2).

The distinct shift in microbial communities between groups over time revealed increased overall abundance of multiple short-chain fatty acid (SCFA)-producing genera in the S&SEs group, including *Megasphaera*, *Megamonas*, *Dialister*, *Catenibacterium*, *Eubacterium eligens*, *Lachnospiraceae* ND3007, *Prevotella*, *Alloprevotella*, *Porphyromonas*, *Butyrivibrio*, *Oscillospira*, *Eubacterium siraeum* and *CAG:56* (Fig. 4a–j,m,o–p and Extended Data Fig. 2). In line with this finding, higher abundances of SCFA-producing families were observed in the S&SEs group for *Veillonellaceae*, *Prevotellaceae* and *Porphyromonadaceae* (Supplementary Fig. 2a–c). Additionally, higher abundances for other SCFA-producing families were found in the S&SEs group compared to the sugar group (*Peptococcaceae*, *Actinomycetaceae*, *Peptostreptococcales*, *Tissierellales* and *Clostridia vadinBB60*; Supplementary Fig. 2d–g). Only three genera—*Saccharimonadales*, *Candidatus Competibacter* and *Clostridium sensu stricto 1*—exhibited lower abundance in the S&SEs group compared to the sugar group (Fig. 4k,l,n), corresponding to the lower abundance of *Saccharimonadales* and *Competibacteraceae* at the family level (Supplementary Fig. 2h,i).

Additionally, among the most strongly affected genera with a higher abundance in the S&SEs group was *Methanobrevibacter*, a methane ( $\text{CH}_4$ )-producing genus (Fig. 4q). This pattern was also observed for *Methanosarcinaceae* at the family level (Supplementary Fig. 2j). Several taxa, such as *Megasphaera*, *Catenibacterium* and *Methanobrevibacter*, were different between groups at baseline. However, the outcomes remained the same, and the observed pattern aligns closely with taxa that showed no differences at the baseline.

### Prediction of pathways based on 16S rRNA taxonomy

Pathway analyses executed by PICRUSt2 MetaCyc pathways showed that in the S&SE group, methanogenesis (METHANOGENESIS-PWY, coenzyme F<sub>430</sub> biosynthesis (PWY-5196), methyl-coenzyme M oxidation to  $\text{CO}_2$  (PWY-5209), superpathway of methanogenesis (PWY-6830),



**Fig. 3 | Microbial community composition.** Ordination plot of dbRDA using different dissimilarity metrics (Jaccard,  $P = 0.001$ ;  $F = 2.427$ ;  $R^2 = 0.005$ ; Bray-Curtis,  $P = 0.001$ ;  $F = 1.948$ ;  $R^2 = 0.004$ ; unweighted UniFrac,  $P = 0.001$ ;  $F = 3.967$ ;  $R^2 = 0.008$ ; and weighted UniFrac,  $P = 0.001$ ;  $F = 3.923$ ;  $R^2 = 0.008$ ). The first two constraint axes are plotted, and the amount of variation captured by an axis is displayed in parentheses after the axis name. Individual samples are represented

by points; the shape corresponds to the collection time point (CID) and the colour corresponds to the intervention group; samples from the same individual but different CIDs are connected by a line. Black arrows show the direction and relative size of the variables' influence on ordination. The significance of the interaction between time and group was tested with PERMANOVA, and results for each distance are indicated.

tetrahydromethanopterin biosynthesis (PWY-6148)) was upregulated, reflecting enhanced  $\text{CH}_4$ -producing potential (Extended Data Fig. 3 and Supplementary Table 3). In line with the increased abundance of SCFA-producing taxa, SCFA fermentation pathways are potentially upregulated (notably acetate, P341-PWY, PWY-5517, 5532, 6344, 6328, 6185, 5392). Additionally, aromatic compound degradation (PWY-5430, PWY-6185) and L-arabinose degradation (PWY-5517) also show upregulation, suggesting higher breakdown of complex plant-derived compounds and carbohydrates. Finally, compounds related to aromatic amino acids (chorismate), vitamin biosynthesis and cofactor production (PWY-6160, PWY-6165, PWY-5507) are upregulated. Downregulated pathways in the S&SE group highlight a reduction in photosynthesis (chlorophyll biosynthesis), polyunsaturated fatty acid synthesis (for example, linoleate biosynthesis, PWY-5995) and phospholipid remodelling (PWY-7409).

Analysis of the microbiota patterns in responders and non-responders was done by random forest analysis. This analysis showed that change in glycated haemoglobin A1c (HbA1c) and body weight regain after weight loss in the S&SEs group but not in the sugar group could be predicted with a degree of confidence (Extended Data Fig. 4). With reasonable accuracy, baseline microbiota composition (M0) classified individuals that did not change or decreased HbA1c during weight maintenance (WM) (responders) versus those that showed an increase in HbA1c (area under the curve (AUC), 0.76; Fig. 5). Furthermore, microbial composition after weight loss (M2) gave

a weaker but reasonable prediction of the extent of body weight regain (AUC, 0.69), indicating the presence of detectable differences in microbiota composition between responders and non-responders. Notably, microbiota composition after weight loss (M2) performed considerably worse in predicting change in HbA1c (AUC, 0.62) than at baseline. Taxa significantly contributing to the classification of HbA1c with microbial composition at baseline included *Petrotoga*, *SH\_PL14*, *Acetobacter*, *CAG:56*, *Faecalibacterium* and *Desulfotomaculales*. At M2, the key taxa were *Rothia*, *TMX7* and *Flavonifractor* (Fig. 5). For body weight classification with microbial composition at M2, important taxa included *Turicibacter*, *Family\_XIII\_AD3011*, *Eisenbergiella* and *Hungatella*.

In the total RCT ( $n = 341$ ), Bristol stool scale (BSS) values were collected on all clinical investigation days (CIDs). No differences in BSS scores were observed between groups at the different time points. Both the S&SEs and sugar groups experienced more constipation or harder lumps after weight loss (M2), based on the decrease in BSS score ( $P < 0.001$  and  $P = 0.001$ , respectively) with a shift towards types one and two (Supplementary Fig. 3a). During the WM period, BSS scores increased in the S&SEs group (M2 versus M12,  $P = 0.014$ ), with the most substantial difference observed between M2 (after weight loss) and M6 ( $P = 0.023$ ) with a shift towards more normal stool types (types three and four) (Supplementary Fig. 3a,b). For the sugar group, no differences in BSS scores were observed during the WM period (Supplementary Fig. 3a–c). Overall, changes over time as well as differences between groups were minor.

**Intervention diets and compliance.** For both adults and children, total sugars constituted 15 E% at baseline (M0) (Supplementary Table 4). Large variability was observed for food groups at M0, and intake was not normally distributed, especially for S&SE-containing foods. Data on added sugar intake was only available from the Danish nutritional software ( $n = 52$ ) and showed that adults in the S&SEs group reduced E% of added sugar by  $3.4 \pm 1.2$  percentage points more than the sugar group ( $P = 0.05$ ). For all adults, total sugar intake was reduced by  $12.0 \pm 5.5$  g day<sup>-1</sup> ( $P = 0.03$ ) ( $2.4 \pm 0.9$  percentage points,  $P = 0.01$ ) more in the S&SEs group than the sugar group. No other differences were observed for adults or children (Supplementary Table 4).

Both adults and children reduced their total intake of sugar-rich products by  $142 \pm 240$  g day<sup>-1</sup> (mean  $\pm$  s.d.) ( $P < 0.0001$ ) and  $163 \pm 187$  g day<sup>-1</sup> ( $P = 0.008$ ), respectively. For adults, the S&SEs group had a  $107 \pm 31$  g day<sup>-1</sup> larger reduction in sugar-rich products than the sugar group ( $P = 0.0007$ ), whereas no differences between groups were observed for children ( $P = 0.36$ ). For S&SE-containing products, adults reduced the total amount of S&SE products in the sugar group and increased it in the S&SE group (difference of  $229 \pm 36$  g day<sup>-1</sup>,  $P < 0.0001$ ). For children, the difference of  $237 \pm 110$  g day<sup>-1</sup> between groups tended to differ but was not significant ( $P = 0.07$ ) (Supplementary Table 4). For adults, the differences between groups were mainly explained by consumption of beverages, milk, sugar, honey or jam and candy (Supplementary Table 5). Data for other products can be found in Supplementary Table 5.

In the subgroup for microbiota analyses, data on energy intake, S&SE intake, added sugar intake and urinary S&SE excretion patterns reflected the total study population (Supplementary Table 6).

Change in urinary nitrogen excretion (reflecting protein intake) at M6 and M12 was similar in both groups. Likewise, there were no differences in excretion of glucose, sucrose and fructose. Biomarkers of S&SE intake, except Steviol acyl glucuronide, increased in the S&SE group and decreased in the sugar group ( $P < 0.001$  at M12). The 1-year change in excretion was generally larger than the change at M6 (Extended Data Table 4).

In addition, no differences between groups in a subgroup at Maastricht were found in physical activity, including sedentary time, moderate or vigorous physical activity and step count (all  $P \geq 0.1$ ) (Supplementary Table 7).

## Secondary and explorative outcomes

**Cardiometabolic health.** For adult completers, the S&SEs group had a larger reduction in BMI, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and non-HDL-cholesterol (non-HDL-C) at M6 than the sugar group (Table 3). At M12, however, only hip circumference was significantly more reduced in the S&SEs group ( $1.8 \pm 0.8$  cm) than the sugar group ( $P = 0.04$ ), whereas total cholesterol, BMI and waist circumference tended to differ ( $P \leq 0.1$ ). Otherwise, no differences were observed (Table 3). Similarly, no differences in risk markers for T2D and CVD were found between groups in the subgroup for the gut microbiota analysis (Supplementary Table 2). Moreover, the 1-year change in IHL content during the WM period, determined in a subgroup at Maastricht ( $n = 27$ ),

remained similar between the S&SEs and sugar groups ( $1.0 \pm 0.8\%$  versus  $0.4 \pm 0.2\%$ , respectively;  $P = 0.213$ ) (Table 3).

**Children's BMI-for-age z-score.** Between M0 and M12, the BMI-for-age z-score of the children decreased by  $0.30 \pm 0.39$  ( $P = 0.001$ ) (Supplementary Fig. 4), with no differences between groups (sugar group,  $-0.25 \pm 0.38$  versus S&SEs group,  $-0.34 \pm 0.41$ ,  $P = 0.48$ ) (Supplementary Table 8). There were also no differences in other outcomes (all  $P \geq 0.18$ ) (Supplementary Table 8).

**Adverse events and concomitant medication use.** Nine serious AEs were reported during the WM period, of which five were in the sugar group and four were in the S&SEs group (Extended Data Table 5). The serious AEs in the sugar group included a surgical procedure unrelated to the intervention, a shoulder lesion caused by an accident, postoperative ileus unrelated to the intervention and angina pectoris, in which the participants recovered without further consequences. Furthermore, one participant in the sugar group was diagnosed with hypothyroidism. In the S&SEs group, the serious AEs included laparoscopic cholecystectomy, diverticulitis and pulmonary embolism, in which the participants recovered without further consequences. Additionally, one participant in the S&SEs group reported a serious AE, involving an illness of which no specific details were disclosed and was not presumed to be serious.

During the WM period, the number of reported AEs was higher in the S&SEs group than in the sugar group, of which the S&SEs group was found to be a predictor ( $P = 0.047$ ) (Extended Data Table 5). In addition, the number of AEs related to gastrointestinal symptoms was higher in the S&SEs group than in the sugar group ( $P = 0.026$ ) (Extended Data Table 5). More specifically, the S&SEs group reported more abdominal pain or cramps ( $P = 0.012$ ), loose stools ( $P = 0.014$ ) and excess intestinal gas ( $P = 0.002$ ).

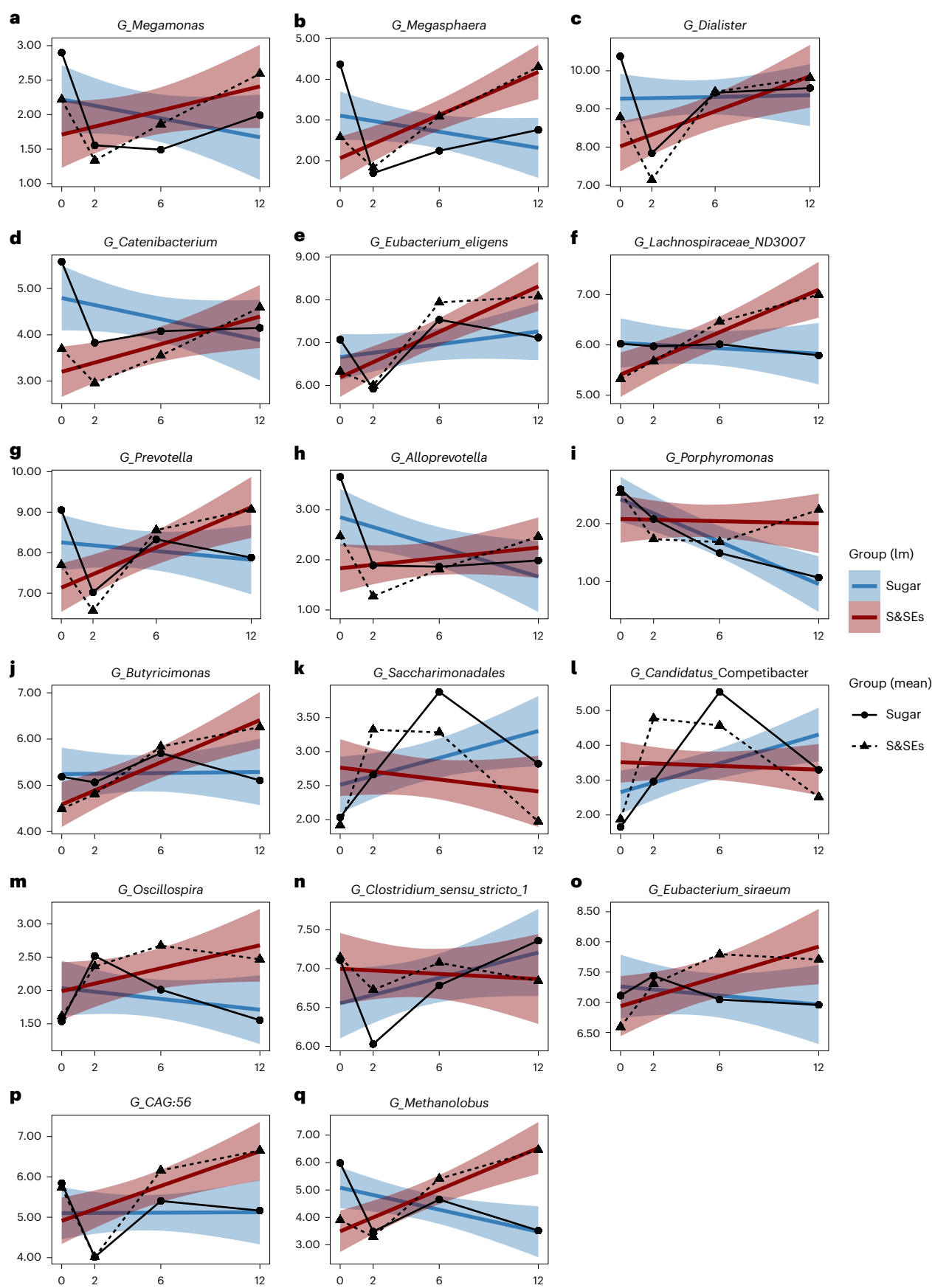
For concomitant medications, the use of S&SEs did not result in a differential use of total concomitant medication ( $P = 0.775$ ) (Supplementary Table 9). Only for hormonal agents was the amount of reported concomitant medication lower in the S&SEs group, and the S&SEs group was found to be a predictor ( $P = 0.035$ ). However, when analysing the type of hormonal agents separately, no differences were found between groups in reported glucocorticoids, sex hormones or thyroid drugs ( $P = 0.649$ ,  $P = 0.491$  and  $P = 1.000$ , respectively).

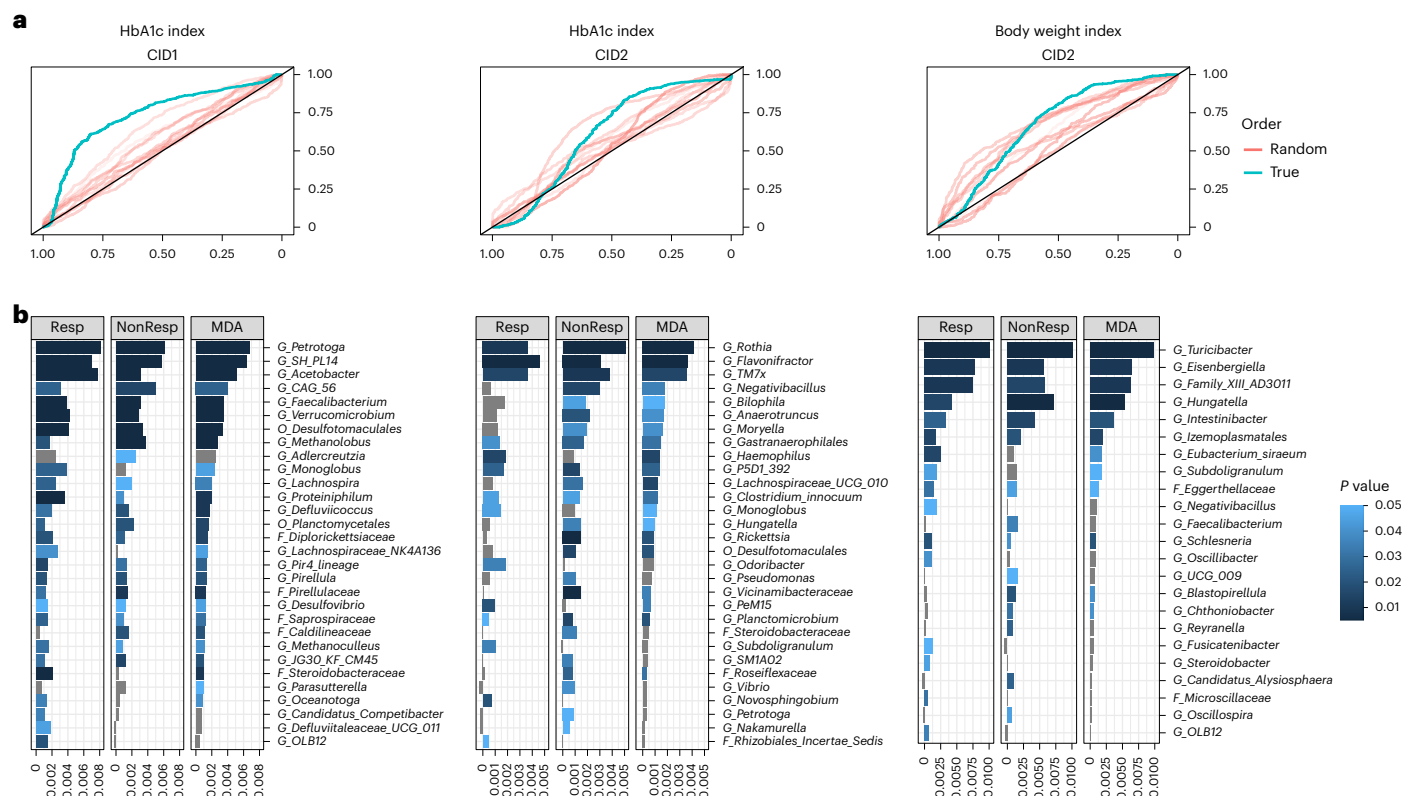
## Discussion

A slightly better weight loss maintenance at 1 year was observed in adults consuming S&SE products compared with those who did not (sugar group), both in the context of healthy diets low in added sugars. The improved weight loss maintenance was accompanied by altered gut microbiota composition, with a higher abundance of SCFA-producing and CH<sub>4</sub>-producing bacterial taxa in the S&SEs group. Furthermore, the S&SE diet led to significantly larger decreases in BMI, total cholesterol, LDL-C, HDL-C and non-HDL-C at 6 months and in hip circumference after 1 year. To our knowledge, this is the first study to demonstrate long-term beneficial health impacts of S&SE intake in adults with overweight or obesity.

**Fig. 4 | SCFA-producing genera.** Spaghetti plots depicting change in abundance over time for genera involved in SCFA production. **a**, *Megamonas* ( $\beta = 0.56$ , s.e. = 0.22,  $P = 0.0531$ ). **b**, *Megasphaera* ( $\beta = 1.27$ , s.e. = 0.25,  $P < 0.0001$ ). **c**, *Dialister* ( $\beta = 0.52$ , s.e. = 0.20,  $P = 0.0458$ ). **d**, *Catenibacterium* ( $\beta = 0.68$ , s.e. = 0.21,  $P = 0.0110$ ). **e**, *Eubacterium eligens* ( $\beta = 0.52$ , s.e. = 0.19,  $P = 0.0458$ ). **f**, *Lachnospiraceae* ND3007 ( $\beta = 0.58$ , s.e. = 0.16,  $P = 0.0047$ ). **g**, *Prevotella* ( $\beta = 0.90$ , s.e. = 0.20,  $P = 0.0002$ ). **h**, *Alloprevotella* ( $\beta = 0.78$ , s.e. = 0.26,  $P = 0.0210$ ). **i**, *Porphyromonas* ( $\beta = 0.75$ , s.e. = 0.24,  $P = 0.0183$ ). **j**, *Butyrivibrio* ( $\beta = 0.69$ , s.e. = 0.20,  $P = 0.0094$ ). **k**, *Saccharimonadales* ( $\beta = -0.59$ , s.e. = 0.22,  $P = 0.0463$ ). **l**, *Candidatus Competibacter* ( $\beta = -0.86$ , s.e. = 0.26,  $P = 0.0103$ ). **m**, *Oscillospira* ( $\beta = 0.45$ , s.e. = 0.18,  $P = 0.0623$ ). **n**, *Clostridium sensu stricto* 1

( $\beta = -0.42$ , s.e. = 0.18,  $P = 0.0752$ ). **o**, *Eubacterium siraeum* ( $\beta = 0.43$ , s.e. = 0.18,  $P = 0.0737$ ). **p**, *CAG:56* ( $\beta = 0.51$ , s.e. = 0.21,  $P = 0.0591$ ). **q**, *Methanobrevibacter* ( $\beta = 1.61$ , s.e. = 0.31,  $P < 0.0001$ ). The y axis shows CSS normalized and log-transformed read counts (abundance), and the x axis indicates time (month). Coloured by intervention groups, straight lines indicate the fit of a simple linear regression with corresponding 95% confidence intervals. The lines signify the mean of each group, with dashed lines and squares denoting the S&SEs group and solid lines and dots for the sugar group. Statistical importance of differences in trends between groups was tested with linear mixed-effect models as implemented in LinDa; outcomes are indicated above.  $P$  values adjusted using false discovery rate.





**Fig. 5 | Results of random forest classification of responders and non-responders. a**, Receiver operating characteristic (ROC) curves for random forest models in which prediction was considerably better than chance. Facet labels indicate the index used to define responders or non-responders and the CID from which samples were used to build the model. ROC curve colours indicate the nature of the response variable used: green for the original definition of responders or non-responders, and red for a randomly assigned definition. **b**, Genera that significantly contributed to the classifications shown in **a**. If a genus was not taxonomically assigned, the lowest assigned taxonomic level was

used as the name, preceded by a capital letter indicating the taxonomic rank. The x axis shows each taxon's numeric contribution to classification, and the y axis lists the corresponding taxa. Facet labels indicate the classification group (Resp, responders; NonResp, non-responders) for which contribution was measured, or the mean decrease in accuracy (MDA) for the model overall. Bar colour reflects significance (*P* value); grey bars indicate no significant contribution. *P* values were estimated with a permutation test as implemented in the rfPermute package with no adjustment for multiple testing.

Both groups maintained a large weight loss after 1 year, with the S&SEs group achieving a 1.6 kg greater weight loss. Participants reduced sugar intake twice as much in the S&SEs group, suggesting that replacing sugar with S&SEs supports WM better. The combined scoring system also demonstrated that the highest level of dietary compliance resulted in the largest weight difference (3.8 kg), suggesting that more consistent adherence could further amplify the observed differences after 1 year, making the results more clinically relevant. Our trial demonstrated almost twice as large a difference in body weight compared to the systematic review and meta-analysis (SRMA) of the WHO, in which an average weight loss of 0.71 kg with S&SEs was reported. The WHO SRMA, however, included studies with different durations and control groups, and the main effect was driven by interventions comparing S&SEs with added sugar as the control, whereas the present trial used a healthy sugar-reduced diet as the control. Consistently, most clinical studies or meta-analyses reported no effects or even beneficial effects of S&SEs on body weight control<sup>19,20</sup>. Notably, the recommendations of the WHO SRMA were conditional, meaning that the assessment panel was less confident regarding their judgement and that the link between S&SEs and disease outcomes might be confounded by baseline characteristics of participants and complicated patterns of S&SEs use. Therefore, reverse causality and the influence of other lifestyle factors are probably in the population-based studies upon which the recommendations were mainly based<sup>21</sup>. To support this idea, a recent substitution analysis of prospective cohort studies found that intake of S&SE beverages was associated with a reduction in body weight,

incidence of obesity, coronary heart disease and all-cause mortality compared with sugar-sweetened beverages and was no different than water when the influence of reverse causality and residual confounding was mitigated<sup>22</sup>.

As previously mentioned, different conclusions in prior studies and reviews could arise from differences in the choice of comparator, such as a control contributing with (for example, sugar) or without energy (for example, water)<sup>23,24</sup>. Recently, the Diabetes and Nutrition Study Group (DSNG) of the European Association for the Study of Diabetes (EASD) addressed the limitations of previous SRMAs<sup>25</sup>. In their SRMA, they found that S&SE-sweetened beverages reduced body weight and cardiometabolic risk factors<sup>24</sup> and were associated with reductions in both risk of obesity and CVD outcomes<sup>22</sup> when replacing sugar-sweetened beverages. This analysis and the critiques on joint analyses of energy and non-energy comparators led to the second WHO-commissioned SRMA, which included the intended replacement of energy-containing sugars with low and no-energy sweeteners. The present trial aimed to meet the previous critiques both in terms of trial duration (1 year) and choice of comparator<sup>26–28</sup>.

In the present trial, significant reductions in some CVD risk markers were observed at M6 in the S&SEs group compared with the sugar group, but these did not last until M12. Fading compliance and/or a decreased number of participants in the last part of the trial might explain this difference. Still, urinary excretion of S&SEs was significantly higher in the S&SEs group versus the sugar group at M12, so the lack of significant differences is probably a result of the lower number

**Table 3 | Changes in anthropometric markers, blood pressure, lipid profile, glucose metabolism and subjective appetite for adult completers at 6 months (*n*=237) and 1 year (*n*=203)**

|                                           | Sugar group                |                           | S&SEs group               |                           | Pvalue<br>(6 months) | Pvalue<br>(1 year) |
|-------------------------------------------|----------------------------|---------------------------|---------------------------|---------------------------|----------------------|--------------------|
|                                           | 6-month changes<br>(n=119) | 1-year changes<br>(n=101) | 6-month change<br>(n=118) | 1-year changes<br>(n=102) |                      |                    |
| Anthropometric                            |                            |                           |                           |                           |                      |                    |
| Body weight (kg)                          | -7.5±5.3                   | -5.4±6.3                  | -9.1±6.0                  | -7.1±8.0                  | 0.02                 | 0.08               |
| BMI (kg m <sup>-2</sup> )                 | -2.6±1.8                   | -1.9±2.2                  | -3.1±2.0                  | -2.4±2.7                  | 0.02                 | 0.09               |
| Fat mass (kg)                             | -                          | -4.2±5.0 <sup>a</sup>     | -                         | -5.3±6.3 <sup>b</sup>     | -                    | 0.14               |
| Fat mass (%)                              | -                          | -2.5±3.2 <sup>a</sup>     | -                         | -3.1±4.0 <sup>3</sup>     | -                    | 0.29               |
| Fat-free mass (kg)                        | -                          | -1.1±2.3 <sup>a</sup>     | -                         | -1.4±2.3 <sup>b</sup>     | -                    | 0.43               |
| VAT (g)                                   | -                          | -216±332 <sup>c</sup>     | -                         | -218±312 <sup>d</sup>     | -                    | 0.87               |
| WC (cm)                                   | -7.4±5.8                   | -5.4±6.6                  | -8.5±6.3                  | -7.0±7.7                  | 0.16                 | 0.10               |
| HC (cm)                                   | -5.8±5.9                   | -3.4±5.1                  | -6.8±5.8                  | -5.1±6.9                  | 0.17                 | 0.04               |
| Cardiometabolic health                    |                            |                           |                           |                           |                      |                    |
| Systolic blood pressure (mmHg)            | -3±11 <sup>e</sup>         | -5±11 <sup>b</sup>        | -4±13                     | -3±10                     | 0.58                 | 0.24               |
| Diastolic blood pressure (mmHg)           | -3±7 <sup>e</sup>          | -3±8 <sup>b</sup>         | -4±9                      | -2±7                      | 0.37                 | 0.46               |
| Heart rate (beats per min)                | -1±9 <sup>e</sup>          | -2±8 <sup>b</sup>         | -3±9                      | -4±8                      | 0.07                 | 0.19               |
| Total cholesterol (mmol l <sup>-1</sup> ) | -0.02±0.56 <sup>f</sup>    | -0.05±0.54 <sup>g</sup>   | -0.31±0.68 <sup>h</sup>   | -0.21±0.73 <sup>g</sup>   | <0.01                | 0.06               |
| HDL-C (mmol l <sup>-1</sup> )             | 0.08±0.15 <sup>f</sup>     | 0.01±0.15 <sup>g</sup>    | 0.03±0.18 <sup>h</sup>    | -0.02±0.19 <sup>g</sup>   | 0.02                 | 0.19               |
| LDL-C (mmol l <sup>-1</sup> )             | -0.11±0.39 <sup>f</sup>    | -0.18±0.49 <sup>g</sup>   | -0.27±0.49 <sup>h</sup>   | -0.25±0.58 <sup>g</sup>   | 0.02                 | 0.36               |
| Non-HDL-C (mmol l <sup>-1</sup> )         | -0.10±0.53 <sup>f</sup>    | -0.06±0.48 <sup>g</sup>   | -0.33±0.66 <sup>h</sup>   | -0.19±0.67 <sup>g</sup>   | <0.01                | 0.10               |
| Triglycerides (mmol l <sup>-1</sup> )     | -0.13±0.50 <sup>f</sup>    | -0.12±0.46 <sup>g</sup>   | -0.18±0.55 <sup>h</sup>   | -0.16±0.48 <sup>g</sup>   | 0.65                 | 0.57               |
| Glucose (mmol l <sup>-1</sup> )           | -0.17±0.48 <sup>f</sup>    | -0.21±0.44 <sup>g</sup>   | -0.13±0.49 <sup>h</sup>   | -0.10±0.50 <sup>g</sup>   | 0.67                 | 0.14               |
| Insulin (pmol l <sup>-1</sup> )           | -14.2±49.6 <sup>f</sup>    | -12.2±33.0 <sup>g</sup>   | -15.4±34.0 <sup>h</sup>   | -14.9±24.8 <sup>g</sup>   | 0.81                 | 0.47               |
| HbA1c (%)                                 | -0.1±0.2                   | -0.0±0.2 <sup>i</sup>     | -0.1±0.2 <sup>h</sup>     | -0.0±0.2 <sup>g</sup>     | 0.88                 | 0.98               |
| IHL content (%) <sup>j</sup>              | -                          | -2.0±3.6 <sup>k</sup>     | -                         | -2.6±3.9 <sup>l</sup>     | -                    | 0.21               |
| Subjective appetite sensations            |                            |                           |                           |                           |                      |                    |
| Desire for savoury foods (%)              | -3±26 <sup>h</sup>         | -4±27 <sup>m</sup>        | -6±27 <sup>n</sup>        | -1±23 <sup>o</sup>        | 0.39                 | 0.46               |
| Desire for sweet foods (%)                | -8±29 <sup>h</sup>         | -5±30                     | -14±26 <sup>n</sup>       | -10±24                    | 0.12                 | 0.27               |
| Hunger (%)                                | -1±22 <sup>h</sup>         | -0±27                     | -4±26 <sup>n</sup>        | -2±26                     | 0.39                 | 0.55               |
| Satiety (%)                               | -4±23 <sup>h</sup>         | -1±26                     | -3±27 <sup>n</sup>        | -1±23                     | 0.80                 | 0.93               |
| Fullness (%)                              | -2±24 <sup>h</sup>         | -1±24                     | -7±24 <sup>n</sup>        | -4±24                     | 0.07                 | 0.32               |

All 6-month and 1-year changes are unadjusted mean±s.d. P values represent the analysis of group difference with change as the outcome. Differences between groups were analysed by ANCOVA adjusted for age, sex, baseline weight and intervention site unless otherwise specified. <sup>a</sup>*n*=99; <sup>b</sup>*n*=100; <sup>c</sup>*n*=79; <sup>d</sup>*n*=75; <sup>e</sup>*n*=118; <sup>f</sup>*n*=116; <sup>g</sup>*n*=98; <sup>h</sup>*n*=117; <sup>i</sup>*n*=96; <sup>j</sup>subset of participant in Maastricht only, adjusted for body weight (changes) and baseline differences; <sup>k</sup>*n*=12; <sup>l</sup>*n*=15; <sup>m</sup>*n*=97; <sup>n</sup>*n*=114; <sup>o</sup>*n*=95.

of participants. We observed no effects of the S&SE diet on risk markers for T2D and CVD, including IHL content, upon 10 months of S&SE intake. Therefore, our findings from our 1-year intervention study are consistent with those from the WHO SRMA on short-term RCTs<sup>29</sup>, and together, these data do not support the notion of potential undesirable health effects with long-term use of S&SEs<sup>4</sup>.

Previous short-term RCTs (1–12 weeks) have indicated AEs of S&SEs on glycaemic response, which was driven by alterations in gut microbial composition and functionality; however, the data are not consistent<sup>14,16,30</sup>. In the current trial, distinct shifts in gut microbiota composition were observed in a subgroup of the S&SEs group, characterized by a higher abundance of taxa associated with SCFA and CH<sub>4</sub> production. Additionally, pathway analysis inferred by PICRUSt2 MetaCyc pathways confirmed an increase in methanogenesis, and

potentially in fermentation and SCFA production, among other pathways. SCFAs are known to promote beneficial health effects, such as increased energy expenditure through enhanced lipid oxidation and improved satiety by modulating gut–brain signalling through incretins<sup>31</sup>. Therefore, SCFAs may prevent and/or counteract obesity and associated cardiometabolic risk factors. Additionally, microbial composition at baseline and/or after initial weight loss can classify with reasonable accuracy variation in body weight regain as well as changes in HbA1c. This is in line with previous studies indicating that there may be responders and non-responders to S&SEs intervention driven by their microbiota composition<sup>14,16</sup>. Overall, our trial indicates a shift towards saccharolytic fermentation (SCFA-producing taxa) in the S&SEs group, which may have contributed to the positive effects on body weight maintenance.

Interestingly, the higher abundance in *Methanobrevibacter*, known for its ability to generate  $\text{CH}_4$  as a metabolic byproduct, in the S&SEs group was accompanied by more gastrointestinal symptoms. Increased levels of  $\text{CH}_4$  may contribute to gastrointestinal symptoms by inhibiting gastrointestinal motility, potentially causing slow-transit constipation and related issues like abdominal pain<sup>32,33</sup>. Additionally, some S&SEs, such as sugar alcohols (for example, sorbitol, xylitol and mannitol), are incompletely absorbed in the small intestine, reaching the colon where they act as osmotic laxatives<sup>13,34,35</sup>. The osmotic effects, however, vary between different S&SEs, influencing the type and severity of symptoms<sup>36</sup>. Whether the experienced gastrointestinal symptoms translate into a substantial clinical burden remains to be determined. Additionally, the amount of reported concomitant medication use was not affected by long-term S&SE intake.

A strength of the present RCT is the investigation of the long-term (10 months) effects of S&SEs compared to no S&SEs on weight loss maintenance in the context of an ad libitum, low-sugar, healthy diet, whereas most previous studies have investigated single S&SEs<sup>37</sup> and only a few included both foods and drinks<sup>38,39</sup>. Furthermore, longer-term studies ( $\geq 6$  months) are scarce. In addition, the effect of 10 months of S&SE intake on the human gut microbiome was investigated for the first time in a real-life, controlled setting. Another important aspect of this study is the examination of the long-term effects of S&SEs on IHL content, the occurrence of (serious) AEs and gastrointestinal symptoms and the use of concomitant medication. Moreover, this multi-centre RCT included participants from northern, central, southern and south-eastern Europe, providing a comprehensive representation across diverse geographic locations in Europe. In addition, this study reflects realistic consumption patterns and dosages of various S&SEs in locally available products. Finally, the inclusion of urinary biomarkers to assess dietary compliance further strengthens the results and supports the dietary intake data. Although participants were recruited from diverse European regions with varying dietary habits, the use of standardized dietary guidance and objective compliance measures (that is, urinary biomarkers) increases confidence that cultural differences had limited impact on adherence and outcomes.

However, this study had limitations. The dropout rate was higher than expected (40% versus 30%), which resulted in a lower number of completers than required for 90% power. However, with 203 completers, the power for a 1-year change in weight loss was 86%, which can be deemed satisfactory. Furthermore, results on energy intake should be interpreted cautiously owing to underreporting, with baseline intake about 25% lower than the estimated energy need, as previously observed<sup>40</sup>. The same caveat applies to the children's results, which should be interpreted with caution because of the limited sample size and suboptimal compliance. Moreover, the absence of direct measurements of SCFAs may have limited our ability to fully interpret the metabolic implications of the observed microbial shifts. Further detailed analysis of the functionality of the microbiota by metagenomic analysis or analysis of microbial metabolites, in addition to the current taxonomic classification and prediction of functionality, as done by PICRUSt2 MetaCyc pathways, would have provided more mechanistic insights into the link between changes in gut microbiota compositions and changes in clinical parameters. Future research is warranted to investigate the effects of long-term intake of S&SEs on human gut microbial functionality, allowing for a more comprehensive assessment of long-term physiological effects in humans.

In conclusion, the present RCT showed that compared to adults consuming drinks and foods without S&SEs, those who included S&SEs in a healthy, ad libitum, sugar-reduced diet exhibited improved 1-year weight loss maintenance and gut microbiota composition (in terms of a higher abundance of SCFA-producing and  $\text{CH}_4$ -producing bacterial taxa) without affecting cardiometabolic health markers. Moreover, based on initial microbial composition or composition directly after weight loss, we can classify with reasonable accuracy the extent of body

weight regain or change in HbA1c, suggesting a significant contribution of the altered microbiota composition to weight loss maintenance. The personalized microbiota composition-related effect on HbA1c and body weight during WM warrants further mechanistic deepening to better understand the clinical implications.

## Methods

### Ethics statement

This trial has been registered at ClinicalTrials.gov (NCT04226911), was approved by national ethical committees (protocols and amendments) and was conducted in accordance with the Declaration of Helsinki. The study was monitored for Good Clinical Practice compliance by the European Clinical Research Infrastructure Network, as described previously<sup>18</sup>. All participants provided written informed consent.

### Trial design

The SWEET trial was a two-armed parallel group RCT conducted at four intervention sites: Athens (Harokopio University of Athens, Greece), Copenhagen (University of Copenhagen, Denmark), Maastricht (Maastricht University, the Netherlands) and Pamplona (University of Navarra, Spain), effectively covering northern, central, southern and south-eastern Europe.

The 1-year RCT consisted of an initial 2-month weight loss period followed by a 10-month, randomized, two-armed parallel WM period. The full 1-year trial on which the majority of outcomes are reported (months M0–M12) comprises the combined periods and is thus termed WM. For adults, the goal was first to achieve a weight loss of  $\geq 5\%$  of the initial weight and second, to maintain their new body weight. For children, the first goal was to achieve weight stability and second, to maintain their BMI-for-age z-score. CIDs were carried out at baseline (M0), after weight loss and weight stability (M2) and twice during WM (M6 and M12).

### Participants

In total, 341 adults and 38 children were included in the trial (Fig. 1). The analysis of gut microbiota composition was done in a subgroup of 137 adult completers. Participants were enrolled between June 2020 and October 2021. The recruitment procedure and all inclusion and exclusion criteria are described elsewhere<sup>18</sup>. In brief, 18–65-year-old men and women (self-reported sex) with  $\text{BMI} \geq 25 \text{ kg m}^{-2}$  and 6–12-year-old boys and girls (self-reported sex) with a BMI-for-age of  $>85\text{th}$  percentile were included. Children were included in a family setting with at least one recruited parent. Participants were required to have a regular consumption of sugar-containing or sugar-sweetened products. Adult participants were excluded at screening if, for example, they had been surgically treated for obesity, were taking medication affecting body weight, had been diagnosed with diabetes, had a fasting glucose  $>7.0 \text{ mmol l}^{-1}$  or had systolic blood pressure of  $>160 \text{ mmHg}$  and/or diastolic blood pressure of  $>100 \text{ mmHg}$ . Children were excluded if, for example, they performed  $>10 \text{ h}$  of intensive physical training per week, had self-reported eating disorders, were diagnosed with diabetes or used medication that affected their body weight. All adults received low-energy diet products free of charge. Participants in Copenhagen, Pamplona and Athens did not receive reimbursement for their participation; travel expenses and financial compensation were provided for participants in Maastricht.

### Intervention

The trial lasted 1 year for each participant. The first CID (M0) was on 24 August 2020, and the last participant's final visit (M12) was on 6 October 2022. During the initial 2-month period, adults—regardless of randomization—received the low-energy diet (Cambridge Weight Plan). For children, weight stability should be achieved by following the dietary recommendations of the American Academy of Pediatrics on the prevention, assessment and treatment of overweight and obesity<sup>18</sup>.

All participants were randomly allocated to one of two diet groups in a 1:1 ratio by a site-specific, computerized randomization list created by a person in Copenhagen not involved in the RCT. Stratification was done by sex, age (<40 or ≥40 years) and BMI (<30 or ≥30 kg m<sup>-2</sup>) in blocks of four. Each household, including children, was randomized to the same intervention group determined by the oldest member of the household. If there was more than one eligible child per household, it was still the randomization group of the oldest adult participant that determined the child allocation.

Although randomization was done after inclusion (at screening), it was not revealed to the participants before completion of the 2-month weight loss or weight stability period. The two ad libitum intervention diets were a healthy diet with <10 E% added sugar, allowing foods and drinks with all types of S&SE products commercially available (S&SEs group), and a healthy diet with <10 E% added sugar, not allowing S&SE products (sugar group). The maximum allowed sugar intake was calculated individually at M2 and recalculated at M6 and then converted to a simple trial-specific unit system (one unit = 10 g sugar). The participants received their maximum unit intake and lists with sugar-rich products, including the unit content. Lists were divided into different categories; for example, drinks, breakfast or desserts. The lists also provided a corresponding product with S&SEs (similar in weight or volume; details described elsewhere<sup>18</sup>). For the S&SEs group, the aim was to replace as many sugar-containing products as possible with S&SEs products, whereas S&SEs products were not allowed in the sugar group. S&SEs included high-potency sweeteners (for example, aspartame, acesulfame-K, saccharin, thaumatin, neotame, stevia glycosides), polyols (for example, erythritol, sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol), slowly digestible carbohydrates (for example, sucromalt, isomaltulose) and sweet fibres or oligosaccharides (inulin-type oligosaccharides). Owing to the characteristics of the trial, blinding was not possible, but all efforts to blind trial staff taking measurements and doing statistical analyses were made. During the intervention period, participants were supervised by dietitians at least every third month. The goal for adults was to maintain weight loss, and for children, the goal was to maintain BMI-for-age z-score. Further reduction in body weight or BMI-for-age z-score was allowed if the participant was compliant with the intervention.

### Data collection and outcomes

Data were collected according to common standard operating procedures, which were used in all intervention sites. Most data were collected at the CIDs after ≥10 h of overnight fast. All data were stored in a central data hub in Copenhagen, from which pseudo-anonymized data can be requested until 2032 through a data-sharing contract. As of 2032, fully anonymized data can be transferred.

**Primary outcomes.** *Body weight.* Body weight was measured to the nearest 0.1 kg on a digital scale, with participants wearing underwear or light clothes. Fasting body weight was measured at screening and CIDs, but fasting was not required at other visits.

*Gut microbiota composition.* Participants were selected based on whether they completed the full intervention and whether faecal spot samples were available at all time points. The participants were stratified based on age, sex and centre. Faecal spot samples were collected and immediately frozen (−20 °C) at home by the participants before all CIDs. At CIDs, the faecal spot samples were stored at −80 °C at the intervention sites. Barcoded amplicons from the V3–V4 region of 16S rRNA genes (341 F, 5′-CCTACGGGNGGCWGCAG-3′; 785 R, 5′-GACTACHVGGGTATCTAATCC-3′) were generated using the Illumina two-step PCR protocol, and sequencing on an Illumina MiSeq with the paired-end (2×) 300 bp protocol (Nextera XT index kit). After each PCR step, the products were purified (QIAquick PCR Purification Kit), the size of the PCR products was checked on a fragment analyzer (Advanced

Analytical), and concentration was quantified by fluorometric analysis (Qubit dsDNA HS Assay Kit). For genomic DNA isolation, QIAamp Fast DNA Stool Mini Kits (Qiagen) were used. All outcomes were determined at M0, M2, M6 and M12. Stool consistency was assessed using BSS for each sample<sup>41</sup>.

**Secondary outcomes.** Secondary outcomes included changes in anthropometry and body composition, risk factors for T2D and CVD, IHL content, the occurrence of (serious) AEs, gastrointestinal symptoms and use of concomitant medication in adults with overweight or obesity.

*Anthropometry and body composition.* The methods for measuring anthropometry (BMI, waist and hip circumference) and body composition (fat percentage, fat mass, fat-free mass and computed visceral adipose tissue (CoreScan software; Encore v.17.0) using Dual-energy X-ray absorptiometry) have been extensively described elsewhere<sup>18</sup>.

*Risk factors for T2D and CVD.* Risk factors for T2D include elevated levels of glucose, insulin and HbA1c; for CVD, risk factors include high cholesterol, elevated triglycerides and high blood pressure. During CIDs, fasting venous blood samples were drawn. Whole blood samples were collected in EDTA tubes for HbA1c analysis and immediately stored locally at −80 °C. Blood samples were collected in serum separator tubes for analyses of lipids (triglycerides, total cholesterol, LDL-C and HDL-C) and insulin. Whole blood samples with fluoride were collected for glucose analysis, with EDTA for CCK, GLP-1 and EDTA plus aprotinin for ghrelin analysis. All blood samples were centrifuged at 1,500g for 10 min at 4 °C. Supernatant (serum or plasma) samples were aliquoted and stored locally at −80 °C until shipment to the central lab at Bioatriki (Athens). Glucose concentration was measured by the enzymatic Hexokinase/G-6-PD method (Alinity c Glucose Reagent Kit 07P55, Abbott) using an Alinity c analyser (a clinical chemistry analyser from Abbott). A conversion factor for glucose, from mg dl<sup>-1</sup> to mmol l<sup>-1</sup>, was applied by multiplying by 0.0555. Insulin concentration was measured by a chemiluminescent microparticle immunoassay (Alinity i Insulin Reagent kit, Abbott) using an Alinity i analyser (immunoassay analyser from Abbott). A conversion factor for insulin, from μIU ml<sup>-1</sup> to pmol l<sup>-1</sup>, was applied by multiplying by 6. HbA1c was measured by an enzymatic assay (Alinity c Haemoglobin A1c Reagent kit, Abbott) using an Alinity c analyser, clinical chemistry (Abbott). One participant had results below the lower detection limit during two CIDs. These latter results were reported as 5.0%. Total cholesterol was measured by an enzymatic assay (Alinity c Cholesterol Reagent kit (Abbott)), and LDL-C and HDL-C were measured by liquid (Alinity c Direct LDL Reagent kit) and accelerator (Alinity c Ultra HDL Reagent kit) selective detergent methods, respectively (both Abbott). All cholesterol concentrations were analysed using an Alinity c analyser. A conversion factor for cholesterol (total, LDL and HDL), from mg dl<sup>-1</sup> to mmol l<sup>-1</sup>, was applied by multiplying by 0.02586. Triglycerides were measured by a glycerol phosphate oxidase method (Alinity c Triglyceride Reagent kit, Abbott) using an Alinity c analyser. A conversion factor for triglycerides, from mg dl<sup>-1</sup> to mmol l<sup>-1</sup>, was applied by multiplying by 0.01129. CCK was measured by a competitive enzyme immunoassay method (RayBio Human CCK Enzyme Immunoassay kit, RayBiotech) using a BioTek Quant analyser (BioTek Instruments). Two participants had results above the range of quantification (4,000 pg ml<sup>-1</sup>) during two CIDs, and their CCK results were reported as 4,000 pg ml<sup>-1</sup>.

*IHL content.* IHL content was analysed in a subgroup (*n* = 27) of adults at Maastricht (S&SEs group, *n* = 15; sugar group, *n* = 12). Proton-magnetic resonance spectroscopy (<sup>1</sup>H-MRS) was performed using a 3T MR system (Achieva 3T-X Philips Healthcare) at M0, M2 and M12. A 32-channel sense cardiac/torso coil (Philips Healthcare) was used, and a 30 × 30 × 30 mm voxel was placed in the lower hepatic lobe. The STEAM (repetition time,

4,500 ms; echo time, 20 ms; number of signal averages, 128) sequence was used, as described previously<sup>42,43</sup>. VAPOR water suppression was applied, and an additional water reference scan was obtained (number of signal averages, 16). A custom-written MATLAB script (MATLAB 2014b, The MathWorks) was used to post-process the spectra. Phasing, frequency alignment and eddy current correction were all performed on spectra before signal averaging. CH<sub>2</sub> resonance, as a percentage of the sum of CH<sub>2</sub> + H<sub>2</sub>O resonances (CH<sub>2</sub>/(CH<sub>2</sub> + water)), was used as a parameter of IHL content.

**Changes in (serious) AEs and concomitant medication use.** Any experienced (serious) AEs or changes in medication use were registered during the CIDs. During the WM period, the participants were asked, regardless of intervention, directly about AEs potentially related to the consumption of S&SEs, including gastrointestinal symptoms and headache.

**Other outcomes.** Subjective appetite sensations over the last 7 days were reported in a questionnaire delivery platform on-site or at home within  $\pm 7$  days of each CID. The participants answered five questions: (1) how strong was your desire to eat savoury foods; (2) how strong was your desire to eat sweet foods; (3) how satiated have you felt; (4) how hungry have you felt; and (5) how full have you felt? All questions were developed in English and translated into the local language at each site. A visual analogue scale with extremes anchored at each end (0, not at all; 100, extremely) was used. Depending on the device that was used, lines were not necessarily 10 cm long, but the rating was presented as a percentage; for example, a mark at 4 cm on an 8 cm line would correspond to 50%.

**Compliance.** To assess compliance, participants completed 4-day weighed records of all foods and drinks (three weekdays and one weekend day) at M0 and M12. Only records of a minimum of 3 days were deemed valid and used for further analysis. Daily average intake of energy and macronutrients was calculated by national dietary software at the four intervention sites. In Copenhagen, all information from food records was manually entered into the software programme DankostPro<sup>44</sup>. This software is based on the official Danish national food composition database (v.4) developed by the National Food Institute at the Technical University of Denmark<sup>45</sup>. In Maastricht, food intake data were analysed by the Eetmeter food diary and analysis tool (Voedingscentrum). In Harokopio, food intake data were analysed with Nutritionist V diet analysis software (v.2.1, 1999; First Databank), extensively amended to include traditional Greek foods and recipes, as described in the Food Composition Tables and Composition of Greek Cooked Food and Dishes<sup>46</sup>. Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains. In Pamplona, food records were manually entered in the online application Nutrium (Nutrium.com), which is based on two food databases (BEDCA & CESNID from Spain and the US Department of Agriculture).

Furthermore, intake (g) of products with sugar and S&SEs and the corresponding units were estimated. Products of interest are described elsewhere<sup>18</sup>. As an objective measure, 24 h urine samples were collected at M0, M6 and M12. The urine samples were weighed and volumes registered (Maastricht, Harokopio and Pamplona) or calculated from the urinary density (Copenhagen). If urinary volume was not registered, urinary weight was divided by 1.0165 g ml<sup>-1</sup> (M0,  $n = 7$ ; M6,  $n = 0$ ; M12,  $n = 2$ ). Urinary biomarkers of S&SEs (acesulfame-K, saccharin, sucralose, cyclamate and steviol glucuronide) as well as glucose, fructose and sucrose were analysed by ultra-pressure liquid chromatography coupled to tandem mass spectrometry<sup>47</sup>. Preparation and analyses followed procedures described elsewhere<sup>47</sup> and were conducted at Wageningen University. After correction for dilution, urinary concentrations (ng ml<sup>-1</sup>) were multiplied by 24 h urine volume and converted to daily excretions (mg day<sup>-1</sup>). Urinary urea concentration was analysed locally

and converted to daily urinary nitrogen excretion (g day<sup>-1</sup>) by multiplying urea excretion (g day<sup>-1</sup>) by 0.4664. At Copenhagen and Maastricht, urea was measured by an enzymatic UV test (colourimetry) (ABX Pentra Urea CP, Horiba ABX) using an ABX Pentra 400. At Harokopio, urea was measured by an enzymatic colourimetric (Urease) Alinity c Urea Nitrogen Reagent kit (Abbott Laboratories). At Pamplona, urea was measured by an enzymatic kinetic test (COBAS 8000, Roche Diagnostics).

For the per-protocol population, participants' compliance was estimated using points (minimum zero points and maximum four points) in relation to four criteria: intake of sugar units and S&SE units and urinary excretion of S&SEs at M6 and at M12. In three out of the four criteria, 75% of the group with the highest (>Q1) or lowest (<Q3) value—depending on the outcome assessed—received one point, and the remaining 25% received no points. Extended Table 6 shows Q1, median and Q3 for the four compliance criteria for each group, including the cut-point for receiving compliance points.

Physical activity was measured in a subgroup at Maastricht for seven consecutive days at M0, M6 and M12 using a triaxial accelerometer (activPAL 3TM micro, PAL Technologies). The activPAL was attached to the anterior thigh of the participants and measured posture allocation, step count and 24 h physical activity, distinguishing between sleeping time, low-to-moderate physical activity (time spent standing or walking <100 steps per min), moderate-to-vigorous physical activity (time spent walking  $\geq 100$  steps per min or cycling) and sedentary time (time spent sitting or lying down).

### Sample size

Sample size calculation was based on body weight results from a previous trial<sup>48</sup>. It was estimated that a mean difference of 1.5 kg, with a s.d. of  $\pm 3.5$  kg, 90% power and a two-sided alpha level of 0.05, would require 231 completers. With an estimated dropout of 30%, a minimum of 330 adult participants should be included (approximately 25% per intervention site). The sample size for the 1-year change in gut microbiota required a minimum of 100 participants ( $n = 50$  per intervention group) and was based on a calculation taking into account -10% change in 20 of the 50 most abundant operational taxonomic units with an alpha of <0.05%. According to this calculation, a total of 40 participants would be enough to detect compositional changes. Furthermore, considering previous work<sup>49</sup> in which  $n = 75$  was used to determine differences in beta-diversity, the estimated number of  $n = 100$  participants in our statistical power calculation would indeed be sufficient. No power calculation was performed for the children; hence, the analyses of BMI z-scores were exploratory only.

### Statistical analysis

Statistical analyses were conducted in R (v.4.3.1). Baseline characteristics before (M0) and after weight loss or weight stability (M2) are presented as medians (Q1–Q3), and changes at M2 and M12 are presented as unadjusted mean  $\pm$  s.d. Differences between groups are presented as adjusted mean  $\pm$  s.e.m. Given that randomization was completed at M0 (that is, 2 months before the 10-month intervention period was initiated), differences in the participant characteristics between groups after weight loss or weight stability (M2) and in changes during the 2-month period (M2–M0) were analysed by analysis of covariance (ANCOVA) adjusted for sex, age, baseline body weight and site. Sex was included as a covariate, given that we know from previous studies that different outcomes can vary between men and women<sup>50</sup>. The trial was not powered to analyse data from men and women separately.

Change in body weight was calculated as the difference between M0 and M12 (M12 – M0). For the primary ITT analysis, the population was defined as all randomized participants who obtained the required  $\geq 5\%$  weight loss at M2. For the ITT analysis, missing data (that is, body weight at M12) were imputed as the last observation carried forward. The analysis of differences between the groups was conducted using an ANCOVA linear mixed model, with individual change in body weight

as the response, and intervention group, baseline body weight, age, sex and site as fixed effects. Additionally, a complete-case analysis (all dropouts omitted) and a per-protocol analysis (only compliant participants defined by the point scoring system) were analysed with the same ANCOVA model. Finally, adult body weight was analysed with the inclusion of all visits (time points) as a linear mixed-effect model with repeated measurements. This analysis had body weight as the response variable and included the fixed effects time-intervention interaction, age, sex and baseline body weight. Site and participants were included as random effects. If there was a significant interaction, post hoc tests were conducted with the R extension package 'emmeans' to calculate the estimated marginal mean and s.e.m. at each time point for comparison of the groups. This model was also used to impute missing body weight values for post hoc ITT analyses. For secondary outcomes on continuous data, the main analysis compared the 6-month and 12-month mean changes between the treatment groups by use of the ANCOVA linear mixed model defined above, without imputation of missing values (that is, complete-case analyses). All models were graphically checked by residual plots and quantile–quantile plots to assess model assumptions, mainly the normality assumption, and when relevant, transformed (for example, by logarithm). Furthermore, Poisson regression analysis was used to analyse the predictive effect of group intervention on reported AEs and concomitant medications.

**Microbiota analysis.** The complete microbiota data processing and analysis pipeline is available at [https://github.com/AlexanderUm/SWEET\\_microbiome](https://github.com/AlexanderUm/SWEET_microbiome). Running the pipeline with the corresponding data will reproduce all of the presented microbiota analysis results and figures. In short, raw reads were preprocessed with the CASAVA pipeline (v.1.8.3) and the Quantitative Insights Into Microbial Ecology 2 (QIIME2; v.2023.9.1) platform. Demultiplexed reads were de-noised into amplicon sequence variants (ASVs) with the DADA2 plug-in<sup>51</sup> and were taxonomically annotated with the Naive Bayes classifier trained on the SILVA (v.138) database<sup>52</sup>. A phylogenetic tree was constructed using the FastTree algorithm and MAFFT alignment. PICRUSt2 (ref. 53) was used to infer metabolic pathways using default settings, and the unstratified MetaCyc pathways abundance table was used for further pathways analysis. Phylogenetic tree, taxonomic, pathways abundance and ASV tables were imported in the R statistical and programming environment (v.4.3.2)<sup>54</sup> using the qiime2R package<sup>55</sup>. For data transformation and visualization, the R packages tidyverse<sup>56</sup>, ggvegan<sup>57</sup>, cowplot<sup>58</sup>, broom<sup>59</sup>, ComplexHeatmap<sup>60</sup>, circlize<sup>61</sup> and RColorBrewer<sup>62</sup> were used. Before further analysis, ASVs with fewer than 50 reads across all samples and those taxonomically assigned to mitochondria or chloroplasts or to kingdoms other than *Bacteria* or *Archaea*, as well as those not assigned to any phylum, were removed from the dataset. An appropriate normalization of ASV counts, as specified below, was applied in correspondence with the performed analysis or visualization.

Microbial alpha diversity metrics (Chao1, observed species, Shannon and Simpson indexes) were calculated, and a linear mixed-effects regression analysis (LMM) was performed as implemented in the MicrobiomeStat<sup>63</sup> R package for all indexes except observed species. For observed species, a generalized linear mixed-effect model (GLMM) for a Poisson distribution (log link function) was performed using the lme4 package<sup>64</sup>. Alpha diversity metrics were calculated using the unfiltered ASV table rarefied at even depth (40k). The LMM and GLMM used intervention group (main effect), time of sampling (M0, M2, M6 and M12) (time variable), intervention centre (adjustment variable) as fixed effects and subject ID as the random effect.

Differences in overall microbial composition between intervention groups were assessed using distance-based redundancy analysis (dbRDA) followed by an ANOVA-like permutation test (PERMANOVA) as implemented in the vegan<sup>65</sup> R package. The following model was used for dbRDA: DistanceMatrix ~ Time × Intervention-Group + Condition(Country). The PERMANOVA was performed with

999 permutations and by model terms. Dissimilarity distances (Jaccard, Bray–Curtis, unweighted and weighted UniFrac) for dbRDA were calculated as implemented in the phyloseq<sup>66</sup> R package from the ASV count table with total-sum scaling and log<sub>2</sub>-transformed count.

Differences in individual taxa and metabolic pathway abundance trends over time between intervention groups were tested using the LinDA method as implemented in the MicrobiomeStat R package. Differential abundance was assessed at the genus and family taxonomic levels; in addition, inferred MetaCyc pathways and default count normalization implemented in LinDA was used. Before differential abundance analysis, features with prevalence less than 50% were removed from the dataset. The *P* values calculated for the LMM were adjusted for multiple testing with false discovery rate correction, and *q* values less than or equal to 0.1 were considered significant. The LMM model used for differential abundance analysis was identical to the model used for analysis of alpha diversity trends.

Responders and non-responders were identified based on body weight regain (WM index = (weight at CID4 – weight at CID2)/(weight at CID1 – weight at CID2)) or changes in HbA1c and fasting glucose during the WM period (CID4 – CID2)<sup>67</sup> (Extended Data Fig. 1a). Microbial composition at each CID was then used to predict the response with a random forest. For classification, genera with a minimum prevalence of 50% in any treatment group were normalized using total-sum-scaling and then log<sub>2</sub>-transformed. The random forest model was built as implemented in the randomForest package<sup>68</sup> and validated using the caret<sup>69</sup> package. The random forest models were built with 1,999 trees, and the number of variables per split (mtry) was set to the default. Classification accuracy was assessed with 25 times repeated fivefold cross-validation as implemented in the caret package, and AUC was estimated. As additional validation, the response variable was permuted, and the accuracy of the corresponding random forest model was estimated. This process was repeated ten times, and the results were compared with the original model. The significance of the feature's contribution to the classification model was evaluated using the permutation approach (199 permutations) as implemented in the rfPermute<sup>70</sup> package. The pROC<sup>70</sup> package was used to build ROC curves.

## Reporting summary

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

## Data availability

Any additional information required to reanalyse the data reported in this paper is available from the lead contacts upon request. Upon approval of a synopsis with a research idea (within 4 weeks of submission), individual de-identified data will be shared. In principle, all reasonable requests will be approved.

Raw fastq files of 16S rRNA gene amplicon sequences are available from NCBI BioProject under accession number [PRJNA1276528](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1276528). Source data are provided with this paper.

## Code availability

Full microbiome analysis is available at [https://github.com/AlexanderUm/SWEET\\_microbiome](https://github.com/AlexanderUm/SWEET_microbiome).

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## Author contributions

M.D.P. and L.K. co-authored and edited the manuscript. M.D.P., J.J.A.J.B., S.S.H.A., S.N.-C. and K.R. performed the measurements and collected the data. A.U. analysed and generated the gut microbiome data and results. M.D.P. and L.K. analysed and generated all other data and results. J.J.A.J.B. and M.M.S. conducted faecal sample preparations before analysis and monitored the microbiome analysis. M.D.P., L.K., J.J.A.J.B., M.M.S., A.U., T.C.A.M., G.H.G., A.R. and E.E.B. evaluated the results. The SWEET EU-project was initiated by J.C.G.H., A.R. and J.H. The protocol for the SWEET intervention trial was written by L.K., A.R. and Y.M., with contributions from E.E.B., G.H.G. and J.A.M. A.R., Y.M., E.E.B. and J.A.M. are principal investigators at the four intervention sites, where M.D.P., J.J.A.J.B., M.M.S., A.U., L.K., S.S.H.A., S.N.-C., K.R., T.C.A. and G.H.G. are co-investigators. E.J.M.F., T.L., H.M., G.F. and C.E.H. were responsible for specific methods, platforms or analyses, and M.d.A. is responsible for monitoring of the trial. A.R. and E.E.B. had the primary responsibility for the final content. All authors reviewed the manuscript and approved the final version.

## Competing interests

A.R. has received honoraria from Nestlé, Unilever and the International Sweeteners Association and is currently employed by Novo Nordisk. J.C.G.H. and J.H. have received project funds from the American Beverage Association. T.L. works for a company (NetUnion) that has no conflict of interest in the trial outcome. C.E.H.’s research centre provides consultancy to, and has received travel funds to present research results from, organizations supported by food and drink companies. The remaining authors declare no competing interests.

## Additional information

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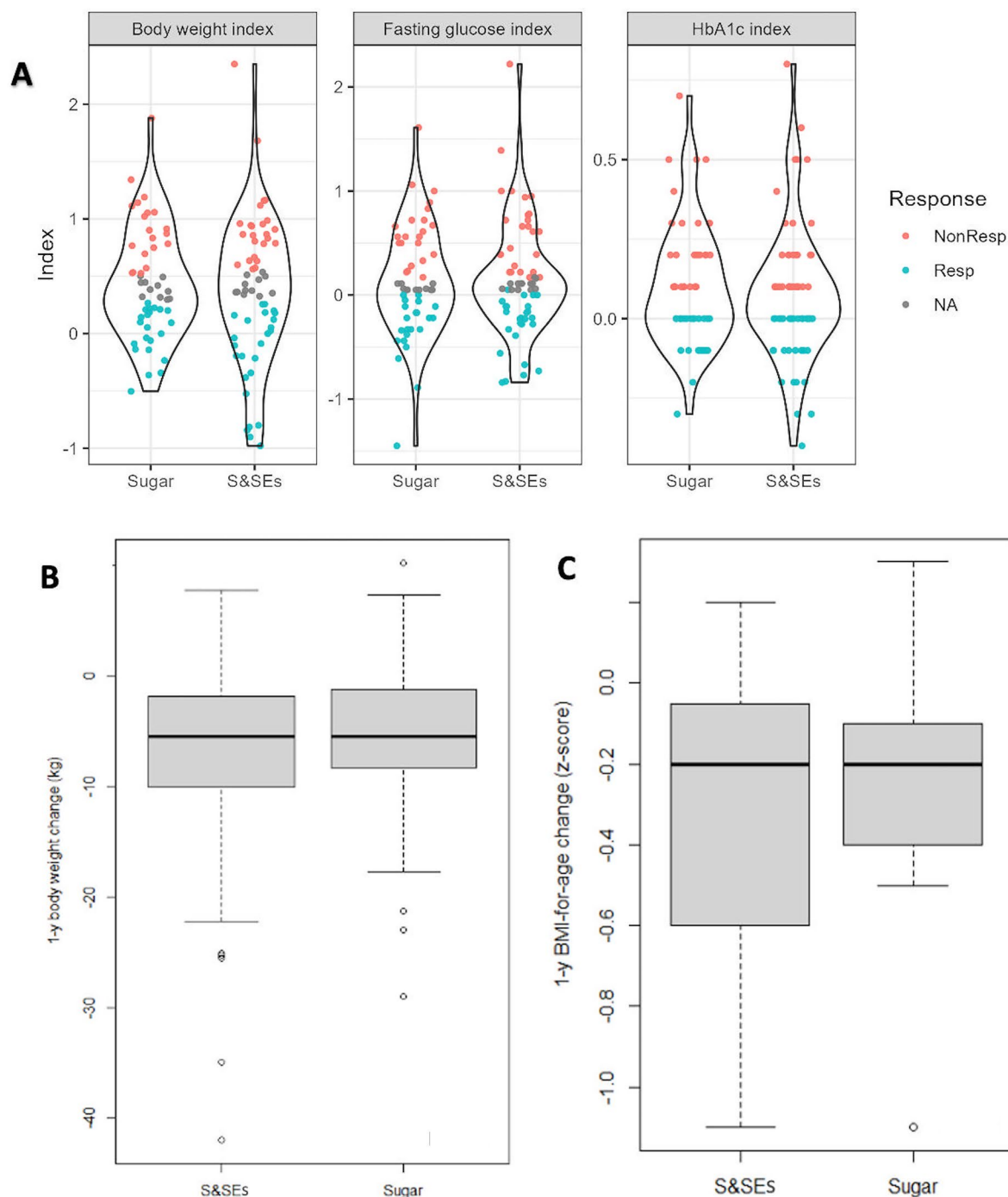
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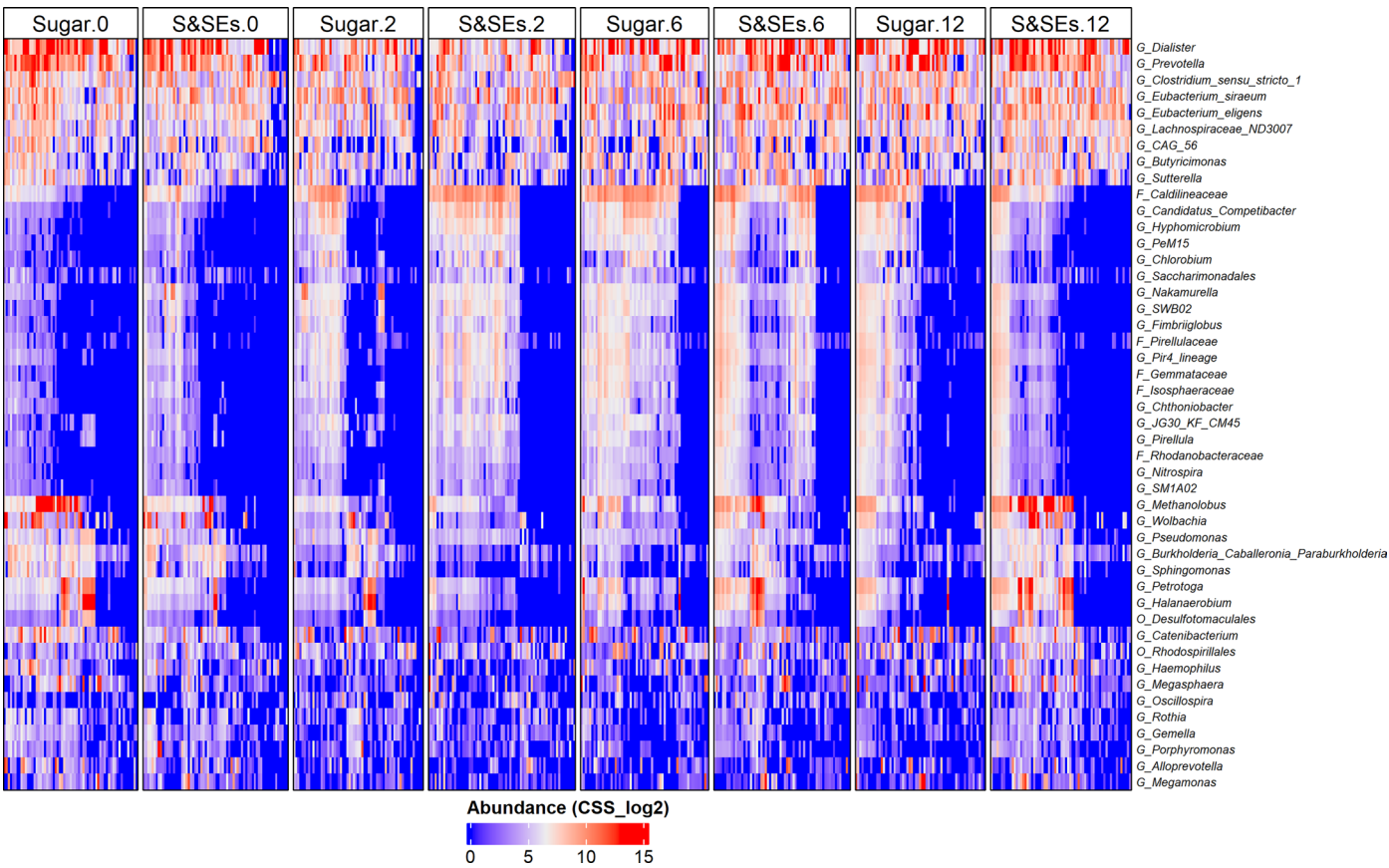
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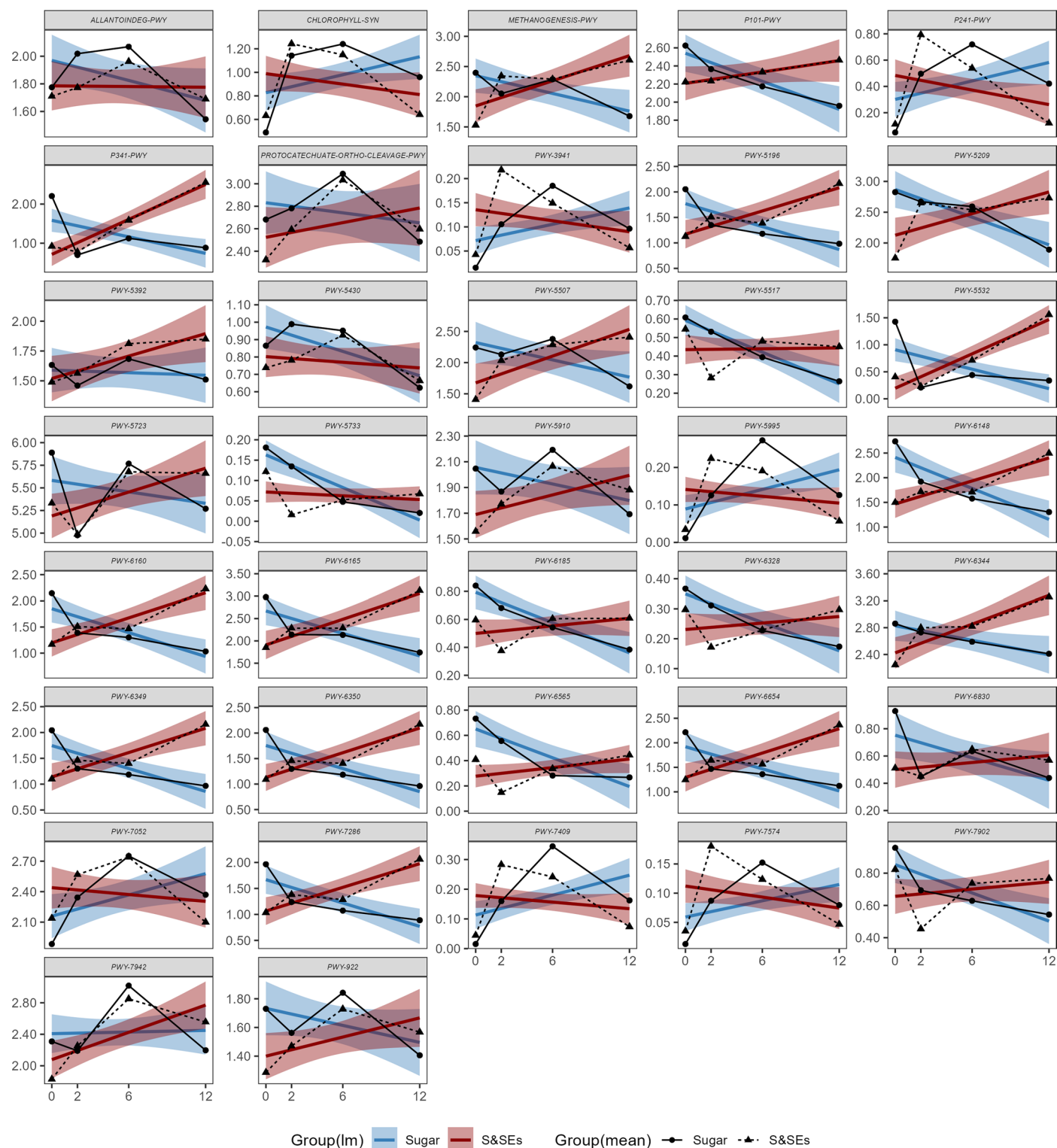


**Extended Data Fig. 1 | Classification of responders and body weight outcomes in adults and children across intervention groups. a**, Violin plots of body weight regain, fasting glucose, and HbA1c indices during the weight maintenance phase (CID4 – CID2), with participants in the Sugar and S&SEs groups classified as responders or non-responders. Body weight regain was reflected in a weight maintenance index = (weight at CID4 – weight at CID2)/(weight at CID1 – weight at CID2)). For body weight regain (left panel) and fasting glucose (middle panel), participants were split into five tertiles within both S&SEs and Sugar

group. For HbA1c, participants with a shift above zero were considered non-responders; the rest were considered responders (right panel). The colour of the dots corresponds to the placement of a participant into the response (Resp), non-response (NonResp) group, or exclusion from the further analysis (NA). **b**, Boxplot of 1-y change in body weight (kg) of the completers in each of the intervention groups in adults. **c**, Boxplot of BMI-for-age (z-score) of completers in each intervention group in children. IB; completers  $n = 203$ . Abbreviations: S&SEs, sweeteners and sweetness enhancers.

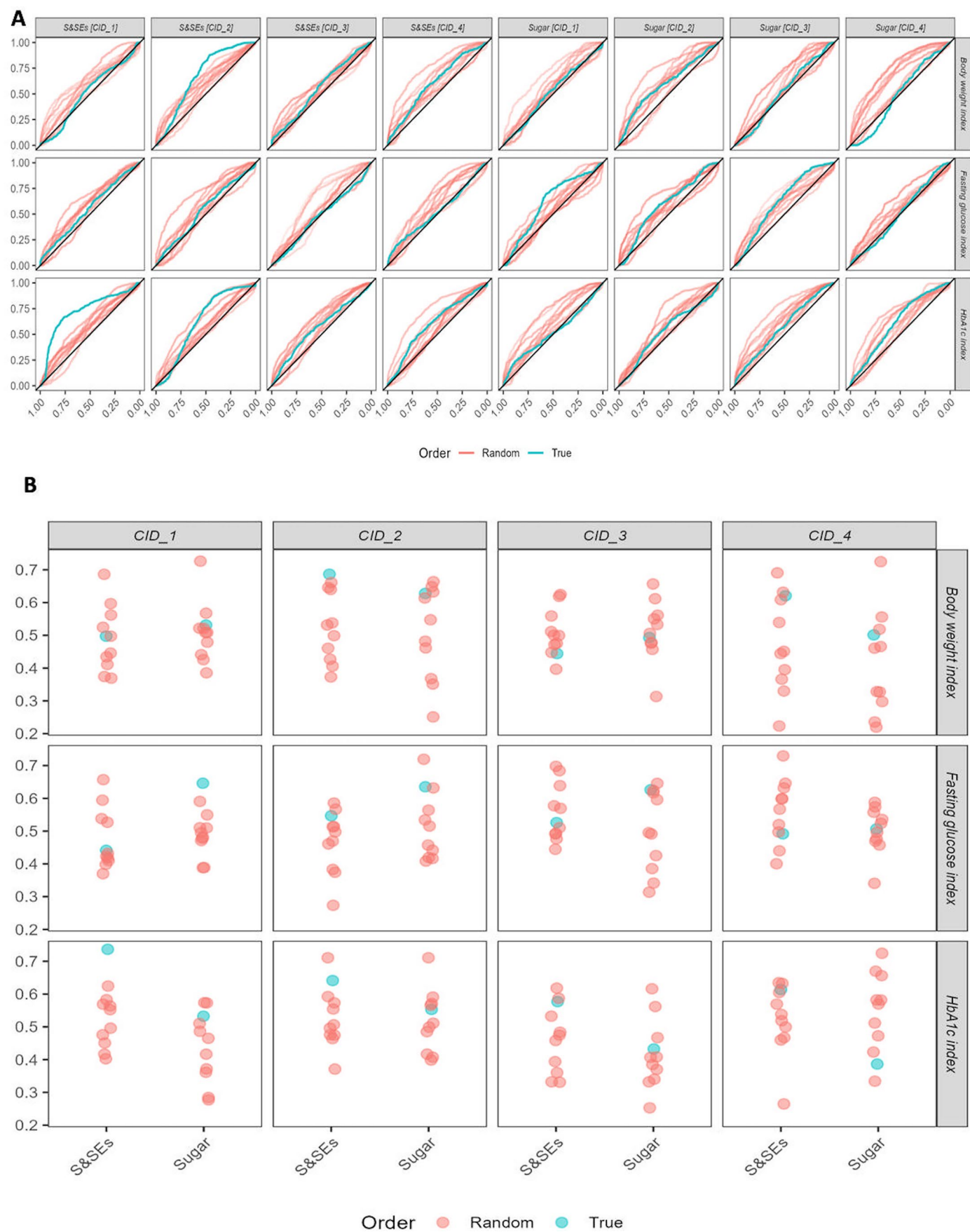


**Extended Data Fig. 2 | Microbial genera with differential abundance trends between intervention groups over time.** Heat map of microbial genera (rows) CSS normalized and log2 transformed abundance per sample with significant different abundance trends over time between the intervention groups.



**Extended Data Fig. 3 | Spaghetti plots of pathway analysis based on inferred by PICRUSt2 MetaCyc pathways.** Colored by intervention groups straight lines indicate the fit of a simple linear regression with corresponding 95% confidence intervals: red lines for the S&SEs group and blue lines for the Sugar group. The black lines signify the mean of each group, with dashed lines and squares

denoting the S&SEs group and solid lines and dots for the Sugar group. Statistical importance of differences in trends between groups was tested with linear mixed effect models as implemented in LinDa and outcomes are indicated above. P-values adjusted using False Discovery Rate.



Extended Data Fig. 4 | See next page for caption.

**Extended Data Fig. 4 | Random Forest classification of responders vs non-responders.** (A) Receiver operating characteristic (ROC) curves based on Random Forest classification of responders and non-responders, (B) Dot plots of the Area under the curve (AUC) based on Random Forest classification of responders and non-responders Responder and non-responders were defined by changes in HbA1c, fasting glucose, or weight maintenance index (as indicated

in grid row names), (A) Treatment group and CID are indicated in the grid column names (B) CID is indicated in the grid columns names and treatment group in the x-axis labels The colour of ROC curves or dots corresponds to the order of the response variable used to build the prediction model: green - original definition of the responders/non-responders; red - definition of the responders/non-responders is random.

Extended Data Table 1 | Reasons for dropping out or being excluded during the trial

|                                                 |                                    | Total | Adults |       | Children |       |
|-------------------------------------------------|------------------------------------|-------|--------|-------|----------|-------|
|                                                 |                                    |       | Sugar  | S&SEs | Sugar    | S&SEs |
| Pre-screened (n=868)                            |                                    |       |        |       |          |       |
| Dropout /exclusion                              | Total                              | 441   | -      | -     | -        | -     |
|                                                 | Not meeting inclusion criteria (n) | 228   | -      | -     | -        | -     |
|                                                 | Declined to participate (n)        | 213   | -      | -     | -        | -     |
| Screened (n=427)                                |                                    |       |        |       |          |       |
| Dropout /exclusion                              | Total                              | 18    | -      | -     | -        | -     |
|                                                 | Not meeting inclusion criteria (n) | 45    | -      | -     | -        | -     |
|                                                 | Declined to participate (n)        | 3     | -      | -     | -        | -     |
| Randomized (n=379)                              |                                    |       |        |       |          |       |
| Dropout/exclusion                               | Total (n)                          | 18    | 10     | 6     | 2        | 0     |
|                                                 | Personal reason (n)                | 9     | 5      | 2     | 2        | 0     |
|                                                 | Excluded (n)                       | 2     | 1      | 1     | 0        | 0     |
|                                                 | Illness (n)                        | 1     | 1      | 0     | 0        | 0     |
|                                                 | Non-compliance (n)                 | 1     | 0      | 1     | 0        | 0     |
|                                                 | Unknown (n)                        | 1     | 1      | 0     | 0        | 0     |
|                                                 | Other (n)                          | 4     | 2      | 2     | 0        | 0     |
| Completed M0: Baseline (n=361)                  |                                    |       |        |       |          |       |
| Dropout/exclusion                               | Total (n)                          | 42    | 18     | 17    | 3        | 4     |
|                                                 | Adult termination (n)              | 6     | -      | -     | 3        | 3     |
|                                                 | Personal reason (n)                | 13    | 8      | 4     | 0        | 1     |
|                                                 | Excluded (n)                       | 5     | 4      | 1     | 0        | 0     |
|                                                 | Illness (n)                        | 3     | 2      | 1     | 0        | 0     |
|                                                 | Non-compliance (n)                 | 1     | 1      | 0     | 0        | 0     |
|                                                 | Unknown (n)                        | 8     | 0      | 8     | 0        | 0     |
|                                                 | Other (n)                          | 6     | 3      | 3     | 0        | 0     |
| Completed M2: WL/WS (n=319)                     |                                    |       |        |       |          |       |
| Dropout/exclusion                               | Total (n)                          | 57    | 24     | 29    | 2        | 2     |
|                                                 | Adult termination (n)              | 2     | -      | -     | 1        | 1     |
|                                                 | Personal reason (n)                | 23    | 11     | 10    | 1        | 1     |
|                                                 | Illness (n)                        | 4     | 1      | 3     | 0        | 0     |
|                                                 | Non-compliance (n)                 | 3     | 3      | 0     | 0        | 0     |
|                                                 | SAE (n)                            | 1     | 0      | 1     | 0        | 0     |
|                                                 | Unknown (n)                        | 7     | 2      | 5     | 0        | 0     |
|                                                 | Others (n)                         | 17    | 7      | 10    | 0        | 0     |
| Completed M6: WM (n=262)                        |                                    |       |        |       |          |       |
| Dropout/exclusion                               | Total (n)                          | 37    | 18     | 16    | 1        | 2     |
|                                                 | Adult termination (n)              | 1     | -      | -     | 0        | 1     |
|                                                 | Personal reason (n)                | 17    | 8      | 7     | 1        | 1     |
|                                                 | Excluded (n)                       | 1     | 1      | 0     | 0        | 0     |
|                                                 | Non-compliance (n)                 | 1     | 0      | 1     | 0        | 0     |
|                                                 | Unknown (n)                        | 10    | 6      | 4     | 0        | 0     |
|                                                 | Others (n)                         | 7     | 3      | 4     | 0        | 0     |
| Completed M12: WM (n=225)                       |                                    |       |        |       |          |       |
| Total dropout/exclusion after randomization (n) |                                    | 154   | 70     | 68    | 8        | 8     |

Abbreviation: M, month; SAE, serious adverse event; S&SEs, sweeteners and sweetness enhancers; WL, weight loss; WM, weight maintenance; WS, weight stability.

**Extended Data Table 2 | Change during the 2-month weight loss period in adults for those who lost a minimum of 5% body weight (n=277)<sup>1</sup>**

|                           | WL change<br>(M2-M0)                         | WL change (M2-M0)        |                           |                |
|---------------------------|----------------------------------------------|--------------------------|---------------------------|----------------|
|                           | All<br>successful<br>participants<br>(n=277) | Sugar group<br>(n=137)   | S&SEs<br>group<br>(n=140) | P <sup>2</sup> |
| <b>Anthropometric</b>     |                                              |                          |                           |                |
| Body weight (kg)          | -10.1±3.6                                    | -10.0±3.6                | -10.2±3.6                 | 0.66           |
| BMI (kg/m <sup>2</sup> )  | -3.5±1.1                                     | -3.5±1.1                 | -3.5±1.1                  | 0.92           |
| Fat mass (kg)             | -6.8±2.7                                     | -6.8±2.8                 | -6.8±2.7                  | 0.70           |
| Fat mass (%)              | -3.6±2.4 <sup>3</sup>                        | -3.6±2.4 <sup>4</sup>    | -3.5±2.4                  | 0.29           |
| Fat free mass (kg)        | -2.8±2.3                                     | -2.7±2.4                 | -2.9±2.1                  | 0.44           |
| VAT (g)                   | -410±375 <sup>5</sup>                        | -449±395 <sup>6</sup>    | -373±352 <sup>7</sup>     | 0.02           |
| WC (cm)                   | -9.4±4.8                                     | -9.3±4.9                 | -9.6±4.8                  | 0.79           |
| HC (cm)                   | -6.9±3.9                                     | -6.8±4.0                 | -6.9±3.7                  | 0.96           |
| <b>Blood pressure</b>     |                                              |                          |                           |                |
| Systolic (mmHg)           | -8±11                                        | -7±11                    | -8±11                     | 0.49           |
| Diastolic (mmHg)          | -6±8                                         | -6±8                     | -6±8                      | 0.58           |
| Pulse (beats/min)         | -3±9 <sup>3</sup>                            | -2±9 <sup>4</sup>        | -3±9                      | 0.37           |
| <b>Lipid profile</b>      |                                              |                          |                           |                |
| Total-CHOL (mmol/L)       | -0.96±0.74 <sup>8</sup>                      | -0.87±0.67 <sup>9</sup>  | -1.04±0.78                | 0.07           |
| HDL-CHOL (mmol/L)         | -0.18±0.21 <sup>8</sup>                      | -0.18±0.21 <sup>9</sup>  | -0.19±0.20                | 0.83           |
| LDL-CHOL (mmol/L)         | -0.62±0.52 <sup>10</sup>                     | -0.57±0.50 <sup>11</sup> | -0.66±0.54                | 0.18           |
| Non-HDL-CHOL (mmol/L)     | -0.77±0.70 <sup>8</sup>                      | -0.69±0.63 <sup>9</sup>  | -0.85±0.75                | 0.06           |
| Triglycerides (mmol/L)    | -0.26±0.59 <sup>10</sup>                     | -0.23±0.60 <sup>11</sup> | -0.30±0.57                | 0.39           |
| <b>Glucose metabolism</b> |                                              |                          |                           |                |
| Glucose (mmol/L)          | -0.26±0.47 <sup>8</sup>                      | -0.29±0.48 <sup>9</sup>  | -0.22±0.45                | 0.30           |
| Insulin (pmol/L)          | -23.3±50.3 <sup>8</sup>                      | -23.7±62.0 <sup>9</sup>  | -23.0±35.6                | 0.97           |
| HbA1c (%)                 | -0.1±0.2 <sup>10</sup>                       | -0.2±0.2 <sup>11</sup>   | -0.1±0.2                  | 0.10           |

LEGEND: <sup>1</sup>All values are unadjusted means±SD. Data are given for those participants who achieved a minimum of 5% weight loss as a collected group and according to the intervention during the following weight maintenance period. <sup>2</sup>p-value for analysis of group difference with WL change (M2-M0) as response variable. Difference between groups was analysed by ANCOVA adjusted for age, sex, baseline body weight and site. <sup>3</sup>n=276, <sup>4</sup>n=136, <sup>5</sup>n=196, <sup>6</sup>n=95, <sup>7</sup>n=101, <sup>8</sup>n=275, <sup>9</sup>n=135, <sup>10</sup>n=274, <sup>11</sup>n=134. Abbreviations: BMI, body mass index; CHOL, cholesterol; HbA1c, glycated hemoglobin A1c; HDL, high density lipoprotein; HC, hip circumference; LDL, low density lipoprotein, S&SEs, sweeteners and sweetness enhancers; VAT, visceral adipose tissue; WC, waist circumference; WL, weight loss.

**Extended Data Table 3 | Compliance evaluation where the population with either highest (>Q3) or lowest (<Q1) received points**

|                                             |        | Sugar group  |                   | S&SEs group  |                 |
|---------------------------------------------|--------|--------------|-------------------|--------------|-----------------|
|                                             |        | Within group | Points given if   | Within group | Points given if |
| <b>Sugar intake units/d</b>                 | Q1     | <b>0.9</b>   | >0.9              | (0.0)        |                 |
|                                             | Median | 1.9          |                   | (0.6)        |                 |
|                                             | Q3     | <b>3.3</b>   |                   | (1.7)        | <3.3            |
| <b>S&amp;SEs intake units/d</b>             | Q1     | (0.0)        |                   | <b>0.7</b>   | >0.7            |
|                                             | Median | (0.0)        |                   | 3.1          |                 |
|                                             | Q3     | (0.0)        | <6.2 <sup>1</sup> | <b>6.2</b>   |                 |
| <b>M6 urinary S&amp;SEs excretion mg/d</b>  | Q1     | 0.5          |                   | <b>5.4</b>   | >5.4            |
|                                             | Median | 3.3          |                   | 15.0         |                 |
|                                             | Q3     | <b>10.0</b>  | <10.0             | 29.5         |                 |
| <b>M12 urinary S&amp;SEs excretion mg/d</b> | Q1     | 0.8          |                   | <b>6.6</b>   | >6.6            |
|                                             | Median | 2.9          |                   | 16.2         |                 |
|                                             | Q3     | <b>7.5</b>   | <7.5              | 32.3         |                 |

The maximum points are 4 and more points correspond to higher compliance. Dietary intake of units was measured at month 12. Urine was samples at month 6 and 12. The limit is the Q3 in the S&SEs group. The limit is the Q3 in the Sugar group. Abbreviation: M, month; S&SEs, sweeteners and sweetness enhancers.

**Extended Data Table 4 | Baseline, 6-month and 1-year changes in urinary excretion of sugars, S&SEs and nitrogen for adults<sup>1</sup>**

|                                       | Sugar group                       |                              |                              | S&SE group                        |                              |                             | P <sup>2</sup><br>6M | P <sup>2</sup><br>1-y |
|---------------------------------------|-----------------------------------|------------------------------|------------------------------|-----------------------------------|------------------------------|-----------------------------|----------------------|-----------------------|
|                                       | Baseline<br>(n=161)               | 6M<br>change<br>(n=119)      | 1-year<br>change<br>(n=101)  | Baseline<br>(n=164)               | 6M<br>change<br>(n=118)      | 1-year<br>change<br>(n=102) |                      |                       |
| Sugar excretion                       |                                   |                              |                              |                                   |                              |                             |                      |                       |
| Glucose<br>(mg/d)                     | 70.2 <sup>3</sup><br>(48.0:103.2) | -38.9 <sup>4</sup><br>±258.3 | -78.0 <sup>5</sup><br>±549.2 | 71.5 <sup>6</sup><br>(50.3:102.0) | -23.9 <sup>7</sup><br>±69.0  | -19.0 <sup>8</sup><br>±61.0 | 0.91                 | 0.89                  |
| Sucrose<br>(mg/d)                     | 4.9 <sup>3</sup><br>(2.2:8.3)     | 2.8 <sup>4</sup><br>±51.7    | 4.0 <sup>5</sup><br>±62.6    | 4.6 <sup>6</sup><br>(2.5:11.7)    | -5.0 <sup>7</sup><br>±15.6   | -3.9 <sup>8</sup><br>±17.7  | 0.12                 | 0.16                  |
| Fructose<br>(mg/d)                    | 42.7 <sup>3</sup><br>(24.4:82.5)  | -24.8 <sup>4</sup><br>±73.6  | -31.7 <sup>5</sup><br>±89.9  | 44.9 <sup>6</sup><br>(25.7:89.1)  | -17.2 <sup>7</sup><br>±63.9  | -17.6 <sup>8</sup><br>±74.9 | 0.94                 | 0.50                  |
| Total sucrose+<br>fructose (mg/d)     | 47.7 <sup>3</sup><br>(29.6:90.3)  | -22.0 <sup>4</sup><br>±97.2  | -27.7 <sup>5</sup><br>±117.7 | 52.0 <sup>6</sup><br>(31.8:97.0)  | -22.2 <sup>7</sup><br>±70.1  | -21.4 <sup>z</sup><br>±82.1 | 0.65                 | 0.90                  |
| S&SEs excretion                       |                                   |                              |                              |                                   |                              |                             |                      |                       |
| Cyclamate<br>(mg/d)                   | 0.05 <sup>3</sup><br>(0.01:2.98)  | -1.51 <sup>4</sup><br>±6.89  | -2.09 <sup>5</sup><br>±7.02  | 0.10 <sup>6</sup><br>(0.02:4.70)  | -0.15 <sup>7</sup><br>±11.80 | 1.81 <sup>8</sup><br>±12.16 | 0.005                | <0.001                |
| Sucralose<br>(mg/d)                   | 0.00 <sup>3</sup><br>(0.00:0.03)  | -0.01 <sup>4</sup><br>±0.16  | -0.01 <sup>5</sup><br>±0.15  | 0.00 <sup>6</sup><br>(0.00:0.05)  | 0.05 <sup>7</sup><br>±0.39   | 0.15 <sup>8</sup><br>±0.86  | 0.16                 | <0.001                |
| Acesulfame<br>(mg/d)                  | 0.6 <sup>3</sup><br>(0.0:7.3)     | -2.1 <sup>4</sup><br>±10.2   | -3.0 <sup>5</sup><br>±10.2   | 0.9 <sup>6</sup><br>(0.0:5.8)     | 2.4 <sup>7</sup><br>±21.9    | 5.1 <sup>8</sup><br>±26.0   | 0.03                 | <0.001                |
| Saccharine<br>(mg/d)                  | 0.1 <sup>3</sup><br>(0.0:0.4)     | -0.5 <sup>4</sup><br>±2.7    | -0.3 <sup>5</sup><br>±2.9    | 0.1 <sup>6</sup><br>(0.0:0.5)     | 0.9 <sup>7</sup><br>±4.6     | 2.0 <sup>8</sup><br>±5.0    | <0.001               | <0.0001               |
| Steviol acyl<br>glucuronide<br>(mg/d) | 0.4 <sup>3</sup><br>(0.1:1.3)     | 1.6 <sup>4</sup><br>±14.5    | 1.0 <sup>5</sup><br>±7.5     | 0.4 <sup>6</sup><br>(0.2:1.8)     | 1.1 <sup>7</sup><br>±8.0     | -0.6 <sup>8</sup><br>±7.9   | 0.18                 | 0.68                  |
| Total S&SEs<br>(mg/d)                 | 5.4 <sup>3</sup><br>(0.8:15.3)    | -2.5 <sup>4</sup><br>±20.3   | -4.3 <sup>5</sup><br>±17.4   | 5.5 <sup>6</sup><br>(1.2:21.8)    | 4.3 <sup>7</sup><br>±31.2    | 8.4 <sup>8</sup><br>±38.5   | <0.001               | <0.001                |
| Total S&SEs ex.<br>Saccharin (mg/d)   | 5.0 <sup>3</sup><br>(0.7:13.9)    | -2.0 <sup>4</sup><br>±19.7   | -4.0 <sup>5</sup><br>±17.0   | 5.2 <sup>6</sup><br>(0.8:18.2)    | 3.4 <sup>7</sup><br>±31.1    | 6.4 <sup>8</sup><br>±37.2   | <0.001               | <0.001                |
| Nitrogen excretion                    |                                   |                              |                              |                                   |                              |                             |                      |                       |
| Nitrogen<br>(g/d)                     | 10.3 <sup>9</sup><br>(6.8:13.0)   | 2.5 <sup>10</sup><br>±6.9    | -0.2 <sup>11</sup><br>±5.1   | 10.4 <sup>12</sup><br>(7.9:13.4)  | 1.6 <sup>7</sup><br>±5.7     | -0.3 <sup>8</sup><br>±5.0   | 0.42                 | 0.85                  |

<sup>1</sup>Baseline excretion reported as median (Q1:Q3), and changes as unadjusted means±SD. <sup>2</sup>p-value for analysis of group difference with change as the outcomes. Except for nitrogen, all other outcomes were right skewed and therefore log-transformed before calculation of change. Difference between groups was analyzed by ANCOVA adjusted for age, sex, baseline body weight and intervention site unless otherwise specified. <sup>3</sup>n=158, <sup>4</sup>n=98, <sup>5</sup>n=81, <sup>6</sup>n=161, <sup>7</sup>n=87, <sup>8</sup>n=83, <sup>9</sup>n=148, <sup>10</sup>n=94, <sup>11</sup>n=88, <sup>12</sup>n=152 Abbreviation: ex, excluding; S&SEs, sweeteners and sweetness enhancers.

**Extended Data Table 5 | Adverse events reported during the 10-month WM phase in the S&SEs group and the Sugar groups**

|                                                | S&SE group<br>(n=170) |      | Sugar group<br>(n=171) |      | P-<br>value |
|------------------------------------------------|-----------------------|------|------------------------|------|-------------|
|                                                | n                     | %    | n                      | %    |             |
| S&SEs possibly related AEs                     |                       |      |                        |      |             |
| Gastrointestinal symptoms                      | 186                   | 62.6 | 118                    | 55.1 | 0.026*      |
| Abdominal pain or cramps                       | 18                    | 6.1  | 5                      | 2.3  | 0.012*      |
| Opened bowels more frequently than usual       | 12                    | 4.0  | 15                     | 7.0  | 0.582       |
| Opened bowels less frequently than usual       | 9                     | 3.0  | 6                      | 2.8  | 0.419       |
| Constipation                                   | 12                    | 4.0  | 7                      | 3.3  | 0.249       |
| Loose stools                                   | 17                    | 5.7  | 5                      | 2.3  | 0.014*      |
| Semi-loose stools                              | 18                    | 6.1  | 11                     | 5.1  | 0.195       |
| Bloating                                       | 17                    | 5.7  | 11                     | 5.1  | 0.256       |
| Belching                                       | 4                     | 1.3  | 6                      | 2.8  | 0.536       |
| Excess gas                                     | 37                    | 12.5 | 14                     | 6.5  | 0.002*      |
| Nausea                                         | 5                     | 1.7  | 6                      | 2.8  | 0.775       |
| Stomach acid/ reflux                           | 10                    | 3.4  | 11                     | 5.1  | 0.850       |
| Mucus in stool                                 | 3                     | 1.0  | 1                      | 0.5  | 0.334       |
| Stool retention sensation                      | 6                     | 2.0  | 5                      | 2.3  | 0.762       |
| Relieved feeling after opening bowels          | 13                    | 4.4  | 12                     | 5.6  | 0.826       |
| Other                                          | 5                     | 1.7  | 3                      | 1.4  | 0.469       |
| Headache                                       | 18                    | 6.1  | 18                     | 8.4  | 0.971       |
| Other AEs                                      |                       |      |                        |      |             |
| Psychiatric disorder                           | 7                     | 2.5  | 7                      | 3.4  | 0.984       |
| Nervous system disorder                        | 3                     | 1.1  | 5                      | 1.4  | 0.582       |
| Infections and infestations                    | 48                    | 16.2 | 41                     | 19.2 | 0.529       |
| Musculoskeletal and connective tissue disorder | 6                     | 2.0  | 8                      | 3.7  | 0.649       |
| Injury, poisoning and procedural complications | 15                    | 5.1  | 10                     | 4.7  | 0.342       |
| Respiratory disorder                           | 2                     | 0.7  | 2                      | 0.5  | 0.992       |
| Skin and subcutaneous tissue disorders         | 3                     | 1.1  | 2                      | 1.0  | 0.645       |
| Neoplasms benign (incl cysts and polyps)       | 5                     | 1.7  | 0                      | 0.0  | 0.053       |
| Reproductive system and breast disorders       | 3                     | 1.0  | 1                      | 0.5  | 0.334       |
| Other                                          | 1                     | 0.4  | 2                      | 1.0  | 0.576       |
| Total AEs (n)                                  | 297                   |      | 214                    |      | 0.047*      |

% is calculated by the amount of a certain AE divided by the total amount of AEs reported in the group. S&SEs, sweeteners and sweetness enhancers; AEs, adverse events. P-value was assessed via poisson regression analysis. \* denote significance ( $P < 0.05$ ).

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- ☐ ☒ For null hypothesis testing, the test statistic (e.g.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☐ ☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's  $d$ , Pearson's  $r$ ), indicating how they were calculated

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### Software and code

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Data collection All clinical protocols are available and the clinical trial has been registered at a clinical trial register (NCT04226911)

Data analysis Code for microbial analysis will be made available via Github.com: [https://github.com/AlexanderUm/SWEET\\_microbiome](https://github.com/AlexanderUm/SWEET_microbiome).

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Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request. 16S rRNA gene amplicon sequences were submitted to European Nucleotide archive PRJNA1276528

## Research involving human participants, their data, or biological material

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|                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reporting on sex and gender                                        | Sex of individuals was based on self report                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Reporting on race, ethnicity, or other socially relevant groupings | No reporting is included on that                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Population characteristics                                         | Individual characteristics' are reported in table 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Recruitment                                                        | In total, 341 adults and 38 children were included in the trial (Figure 1). The analysis of gut microbiota composition was done in a subgroup of 137 adult completers. Participants were enrolled between June 2020 and October 2021, and all gave written informed consent. The recruitment procedure and all in- and exclusion criteria are described elsewhere (doi: 10.1136/bmjopen-2022-061075). In brief, 18-65 year old men and women (self-reported sex) with BMI $\geq 25$ kg/m <sup>2</sup> and 6-12 year old boys and girls (self-reported sex) with a BMI-for-age $> 85$ th percentile were included. Children were included in a family setting with at least 1 recruited parent. |
| Ethics oversight                                                   | The trial was registered at clinicaltrials.gov NCT04226911, approved by national ethical committees (protocols and amendments) and conducted in accordance with the Declaration of Helsinki.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

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

All studies must disclose on these points even when the disclosure is negative.

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample size     | Sample size calculation was based on body weight results from a previous trial (25). It was estimated that a mean difference of 1.5 kg, SD of $\pm 3.5$ kg, 90% power, 2-sided $\alpha$ of 0.05, would require 231 completers. With an estimated dropout of 30%, a minimum of 330 adult participants should be included (approximately 25% per intervention site). The sample size for the 1-year change in gut microbiota, required a minimum of 100 participants (n=50 per intervention group) and was based on a calculation taking into account ~10% change in 20 of the 50 most abundant operational taxonomic units (OTU) with an alpha of $< 0.05$ percent. According to this calculation a total of 40 participants would be enough to detect compositional changes. Furthermore, considering the work by Sievenpiper (27), where n=75 was used to determine differences in beta-diversity, the estimated number of n=100 participants in our statistical power calculation would indeed be sufficient. No power calculation was performed for the children, hence the analyses of BMI z-scores were exploratory only. |
| Data exclusions | The number of participants in each intervention group is shown in Figure 1. Most common reasons for drop out were either personal or unknown. All reasons for dropout and exclusion can be found in Supplemental Table 2. Furthermore, this trial was conducted during the COVID-19 pandemic, encountering obstacles such as disrupted participant follow-up, increased dropout rates, and logistical challenges related to travel restrictions and safety protocols. For the analysis of gut microbiota composition a subgroup of 137 adults were included (Copenhagen: n=26, Pamplona: n=26, Harokopio: n=25, Maastricht: n=60).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Replication     | This is an extensive human study, so replication requires a whole new extensive study,                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Randomization   | All participants were randomly allocated to 1 of 2 diet groups in a 1:1 ratio by a site-specific, computerized randomisation list created by a person in Copenhagen not involved in the RCT. Stratification was done by sex, age ( $< 40$ or $\geq 40$ years) and BMI ( $< 30$ or $\geq 30$ kg/m <sup>2</sup> ) in blocks of 4. Each household, including children, was randomised to the same intervention group determined by the oldest member of the household. If there was more than one eligible child per household, it was still the randomisation group of the oldest adult participant that determined the child allocation.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Blinding        | Due to the characteristics of the trial, blinding was not possible, but all efforts to blind trial staff taking measurements and doing statistical analyses were done.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

## 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.

## Materials &amp; experimental systems

|                                     |                                                        |
|-------------------------------------|--------------------------------------------------------|
| n/a                                 | Involved in the study                                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Clinical data      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                        |

## Methods

|                                     |                                                 |
|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Clinical trial registration | NCT04226911                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Study protocol              | Study protocol is available at clinical trial register, the METC protocol from Maastricht University has been uploaded                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Data collection             | Data were collected according to common standard operation procedures (SOPs) to be used in all intervention sites. Most data were collected at the CIDs after ≥10 hours overnight fast. All data were stored in a central data-hub at Copenhagen from where pseudo-anonymised data can be requested until 2032 via a data sharing contract. As of 2032, fully anonymised data can be transferred.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Outcomes                    | <p>Primary outcomes</p> <p>Body weight. Body weight was measured to the nearest 0.1 kg on a digital scale with participants wearing underwear/light clothes. Fasting body weight was measured at screening and CIDs, but fasting was not required at other visits.</p> <p>Gut microbiota composition. Participants were selected based on whether they completed the full intervention and whether fecal spot samples were available at all timepoints. The participants were stratified based on age, sex, and center. Secondary outcomes included changes in anthropometry and body composition, risk factors for T2D and CVD, IHL content, the occurrence of (serious) AEs, gastrointestinal symptoms, and use of concomitant medication in adults with overweight or obesity. Other outcomes were subjective appetite sensations and compliance. The measurement of all outcomes is described in the article.</p> |

## 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.                                                                                                                                                                                                                                                                                          |
| Novel plant genotypes | Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied. |
| Authentication        | Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.                                                                                                                                                                                                                                       |