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# Erythritol and xylitol differentially impact brain networks involved in appetite regulation in healthy volunteers

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**ABSTRACT**
**Background:** There is a growing consensus that sugar consumption should be reduced and the naturally occurring, low-calorie sweeteners xylitol and erythritol are gaining popularity as substitutes, but their effect on brain circuitry regulating appetite is unknown.
**Aim:** The study's objective was to examine the effects of the two sweeteners on cerebral blood flow (rCBF) and resting functional connectivity in brain networks involved in appetite regulation, and test whether these effects are related to gut hormone release.
**Methods:** The study was performed as a randomized, double-blind, placebo-controlled, cross-over trial. Twenty volunteers received intragastric (ig) loads of 50g xylitol, 75g erythritol, 75g glucose dissolved in 300mL tap water or 300mL tap water. Resting perfusion and blood oxygenation level-dependent data were acquired to assess rCBF and functional connectivity. Blood samples were collected for determination of CCK, PYY, insulin and glucose.
**Results:** We found: (i) xylitol, but not erythritol, increased rCBF in the hypothalamus, whereas glucose had the opposite effect; (ii) graph analysis of resting functional connectivity revealed a complex pattern of similarities and differences in brain network properties following xylitol, erythritol, and glucose; (iii) erythritol and xylitol induced a rise in CCK and PYY, (iv) erythritol had no and xylitol only minimal effects on glucose and insulin.
**Conclusion:** Xylitol and erythritol have a unique combination of properties: no calories, virtually no effect on glucose and insulin while promoting the release of gut hormones, and impacting appetite-regulating neurocircuitry consisting of both similarities and differences with glucose.

## Introduction

Excessive sugar consumption contributes to the worldwide rise in obesity and is a risk factor for cardiovascular disease and metabolic syndrome [1]. In response to this, the World Health Organization recommends reducing free sugars' daily intake to less than 10% of daily total energy [2]. In this context, sugar substitutes such as low-calorie artificial or natural sweeteners are gaining popularity. Erythritol and xylitol are carbohydrates naturally found in small amounts in various fruits and vegetables, which require little or no insulin for their metabolism [3,4]. This makes these sugar substitutes attractive for the dietary management of weight as well as glycemic control. Cholecystokinin (CCK) and peptide tyrosine tyrosine (PYY) are anorexigenic hormones released by enteroendocrine cells of the gut in response to food intake, thereby promoting satiation [5]. Administration of glucose stimulates GLP-1, PYY and CCK release, but after intake of artificial sweeteners such as sucralose or aspartame, gut hormone

release is not observed [6]. In contrast, we recently demonstrated that intragastric (ig) administration of the two natural sweeteners erythritol and xylitol leads to GLP-1 and CCK release similar to glucose [7]. Our findings were corroborated in another recent trial, where erythritol intake was also found to stimulate gut hormone release [8]. In brief, the unique combination of properties (low caloric content, low glycemic, yet stimulation of anorexigenic gut hormone release) renders them attractive as a sugar substitution for the target population with type 2 diabetes and obesity.

Functional neuroimaging techniques have allowed a deeper understanding of human gut-brain interactions. Studies combining functional magnetic resonance imaging (fMRI) with blood analyses have demonstrated a direct link between changes in plasma gut hormone concentrations and alterations in brain activity in regions that are part of the neural circuit of appetite [9–11] In addition, various neuroimaging methods have been used to quantify brain responses to oral as well as ig administration of glucose, and, to a lesser extent, of artificial sweeteners. Thus far, no trial has investigated the acute effects of xylitol and erythritol on brain function.

Therefore, this study's overall aim was to examine the effects of these two naturally occurring, low-calorie sweeteners compared to the caloric sweetener glucose and tap water on two measures of brain function, resting cerebral blood flow (rCBF) [12] and resting functional connectivity in a network of homeostatic and reward-related regions involved in appetite regulation, and to test whether these effects are related to the release of gut hormones. These measures are based on arterial spin labeling (ASL) and blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) sequences, respectively. They represent highly complementary measures of brain function, as the latter taps into temporal fluctuation in coordinated activity patterns *between* regions in a network, whereas the former measures neural activity *within* each region (or voxel), in our case of the same network. Hence, functional connectivity analyses of resting neuroimaging data are based on the concept of synchrony between the signal responses in spatially distinct brain regions. In addition to functional connectivity between specific regions, the functional properties of an entire brain network can be analyzed using a graph theoretical approach. Graph theory provides a theoretical framework in which the topology of complex networks can be examined, and can reveal important information about both the local and global organization of functional brain networks [13].

Given the lack of previous data on the impact of erythritol and xylitol on the brain and these circuits in particular, we refrained from formulating specific *a priori* hypotheses about the direction of the putative differences between the substances in these *a priori* hypothesized regions of interest. Therefore, this study should primarily be considered as exploratory and hypothesis-generating, even though we did have clear a priori regional hypotheses

## Material and methods

### Study subjects

The study was performed in accordance with the principles of the Helsinki Declaration of 1975 as revised in 2013. The protocol was approved by the State Ethical Committee of Basel (Ethikkommission Nordwest- und Zentralschweiz: EKNZ 2014-072; approval date: 02 April 2014) and registered at ClinicalTrials.gov (NCT02823249). Some deviations of the current study and the registered project at ClinicalTrials.gov require further explanations: First, the present data are part of a larger project, in addition to xylitol and erythritol, the effect of amino acids on various parameters (brain response, GI hormones, effect on subsequent liquid meal intake) are investigated. It was, however, not the purpose to compare the xylitol and erythritol with the amino acids. To avoid misunderstandings, the different parts of the larger project should have been registered separately. Second, we had planned to include GLP-1 and ghrelin measurements in the study as additional parameters. However, the material obtained during the MRI sessions was not enough and therefore, we had to limit our analyses and prioritized insulin/glucose and CCK. Each subject gave written informed consent and underwent a screening that included a medical interview and blood sampling (total blood cell count). Exclusion criteria were smoking, substance abuse, regular intake of medications, medical or psychiatric illness, any MRI contraindication (e.g. claustrophobia, non-removable metal devices), and any abnormalities detected upon laboratory screening. None of the subjects had a history of gastrointestinal disorders, food allergies or dietary restrictions. Subjects were instructed to abstain from alcohol, caffeine and strenuous exercise for 24 h and fast overnight for at least 10 h before each study visit. Twenty, healthy right-handed normoglycemic volunteers (10 male and 10 female) with a mean age of 27.7 years (range: 21–45 years) and mean BMI of 28.3 kg/m$^2$ (range: 20.0–38.9 kg/m$^2$) were included. This study's sample size was chosen based on practical considerations rather than statistical power estimation,

but power was maximized by the cross-over study design (see below). Moreover, the appropriate modeling of power for fMRI experiments is extremely challenging given the intrinsic noise, and spatiotemporal autocorrelation in the data. Furthermore, the issue of familywise error correction presents a particular challenge when estimating sample sizes to ensure sufficient power. Many of these same issues, in addition to others linked to the approach, are inherent in graph theoretical analysis. However, more importantly, the lack of prior data (regarding the effects of erythritol and xylitol) limited our ability to provide a realistic estimate of the likely effect size, a required input to any such analysis. Subject recruitment and follow-up visits were conducted over a period of 11 months (May 2014–April 2015).

## Study design

The study was performed as a randomized (counterbalanced), double-blind, placebo-controlled, cross-over trial. For an outline of a study, day see Figure 1. On the evening before each study day, subjects consumed a restricted simple carbohydrate standard dinner before 0800pm and fasted from 1200am (midnight) until the study visit, which started at 0800am on four separate occasions, at least 3 days apart. On arrival, a polyvinyl feeding tube was inserted into the stomach through an anaesthetized nostril. The rationale for intragastric administration of the test substances was to bypass orosensory cues to provide information on the isolated post-oral effects, which is crucial to increase our understanding of the role of the GI tract in the short-term control of appetite without confounding effects of cephalic and oral phases of ingestion, triggering hedonic responses and cognitions. An intravenous catheter was inserted into an antecubital vein for blood sample collection at specific time intervals (t = −15, −5, +15 and +60 min) for determination of plasma CCK, PYY, insulin and glucose concentrations. For details on blood sampling and laboratory analysis, see supplemental material (S1). For the baseline resting blood oxygenation level-dependent (rBOLD) and resting cerebral blood flow (rCBF) scans (starting at t = -15 min, duration 5 and 4 min, respectively), subjects were instructed to lie in dimmed light with their eyes open, to think of nothing in particular, and not to fall asleep. At t = 0 min, subjects received an ig load of one of the following test solutions, freshly prepared each morning, over 2 min: (i) 75 g glucose in 300 mL tap water (Haenseler AG, Herisau, Switzerland), (ii) 50 g xylitol in 300 mL tap water (Mithana GmbH, Zimmerwald, Switzerland), (iii) 75 g erythritol in 300 mL tap water (Mithana GmbH, Zimmerwald, Switzerland), (iv) 300 mL tap water (placebo). Concentrations were chosen based on the following considerations: 75 g of glucose as in a standard oral glucose tolerance test (with known effects on plasma glucose, insulin, gut hormones, and brain activity), 50 g of xylitol and 75 g of erythritol as the sweetness of the xylitol and erythritol concentrations correspond approximately to 75 g of glucose, resulting in equisweet loads. In order to maintain the blinding, different persons prepared and administered the treatment. At 6 and 21 min after administration, rBOLD (for 5 min) followed by rCBF (for 4 min) data were acquired, respectively. Five minutes of rBOLD is below the current standard of 8 to 10 min, which should be considered a limitation of our study. However, this was the time available to ensure that the subsequent sequences could be collected and that the entire protocol remained tolerable for the subjects, and such a duration was not uncommon at the time of data collection. Appetite-related sensations (hunger, prospective food consumption, satiety and fullness) were assessed by visual analog scales (VAS) after each blood sample collection [14]. VAS consisted of a horizontal, unstructured, 10-cm line with words anchored at each end, expressing the most positive and most negative rating (e.g. hunger: 0 cm: not at all hungry, 10 cm: as hungry as I have ever felt). During the study day, the volunteers were asked to report gastrointestinal symptoms such as nausea, bloating, and diarrhea.
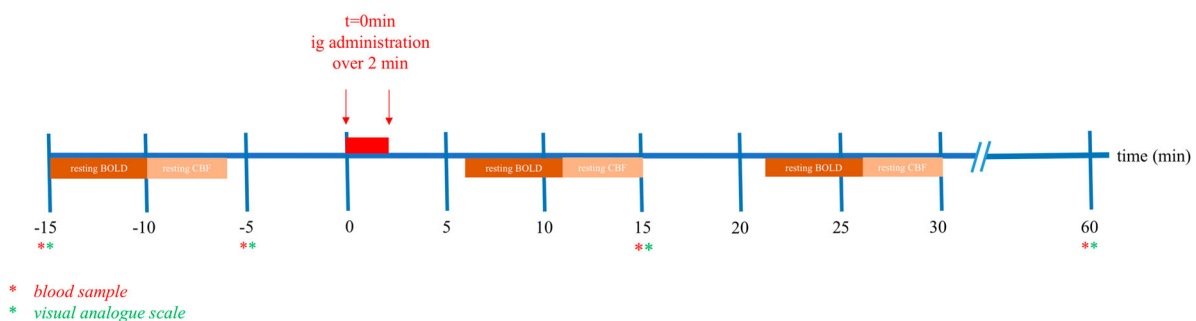


**Figure 1.** Outline of a study visit. BOLD, Blood Oxygen Level-Dependent; CBF, cerebral blood flow.

**Table 1.** Regions of interest (ROI) – taken as nodes of the network.

| | | atlas/study | name in atlas |
|---|---|---|---|
| Homeostatic | medulla (NTS) | Lassman et al. [15] | n/a |
| | hypothalamus | TD BA+ (Pickatlas) | hypothalamus |
| Reward | VTA | Murty et al. [16] | n/a |
| | caudate nucleus left + right | TD BA+ (Pickatlas) | caudate head + caudate body |
| | putamen left + right | TD BA+ (Pickatlas) | putamen |
| | nucleus accumbens left + right | IBASPM71 (Pickatlas) | nucleus accumbens |
| | pACC/vmPFC | Destrieux [17] | 6 [(p)ACC] |
| | medial OFC | Destrieux [17] | 31 (rectus) + 70 + 63 |
| | lateral OFC left + right | Destrieux [17] | 24 (orbital gyri) + 64 |
| | amygdala left + right | TD BA+ (Pickatlas) | amygdala |
| | anterior insula left + right | UCLA [18] | anterior insula |

All ROI were controlled and corrected for overlap. The ROIs were taken as nodes of the network. NTS, nucleus tractus solitarius; VTA, ventral tegmental area; pACC, accumbens; vmPFC, ventromedial prefrontal cortex; OFC, orbitofrontal cortex; n/a, not applicable.

### fMRI acquisition and pre-processing

For details on fMRI acquisition and pre-processing, see supplemental material (S2).

### fMRI analysis

For details on fMRI analysis, see supplemental material (S3), Figure 4A, and Table 1.

### Statistics

Data were analyzed in SAS 9.4 (SAS Institute, Cary, NC, USA) and shown as mean ± SEM unless otherwise stated. A two-tailed p-value < 0.05 was considered significant. To analyze the time course of the subjective and endocrine responses to the different infusions, marginal linear mixed model analyses were performed - one for each dependent variable. The optimal variance-covariance structure was chosen based on the observed variance-covariance matrix and the best fit indicated by the lowest value of Akaike's Information Criterion (AIC). If the assumption of normally distributed residuals was violated (based on a significant p-value of the Shapiro-Wilk test), box-cox transformations on the dependent variables were used to normalize the residual distribution. Observed untransformed values will be shown on graphs to facilitate interpretation and comparison with previous results. 'Time' [3 time points: immediately before (pre-infusion baseline, t0), and 15 and 60 min after infusion] and 'treatment' [erythritol (75 g), xylitol (50 g), glucose (75 g), and tap water (300 mL)] were included as within-subject categorical independent variables in the fixed effects model containing the two main effects and their interaction effect. The treatment-by-time interaction effect (testing the difference between the 4 treatments between the 3 time points) are the effects of interest. To follow up on the latter effect and test specific hypotheses on the difference in the change from pre-infusion baseline at each of the two post-infusion time points between the four treatments, planned contrast analyses were performed using paired Student's t-tests, with step-down Bonferroni (Holm) correction for multiple testing. Specifically, we compared the change from pre-infusion baseline at each of the two post-infusion timepoints between treatments. Further, we tested whether this change from pre-infusion baseline at each of the two post-infusion time points was significantly different from zero in each treatment separately by planned contrast analyses using one-sample Student's t-tests, with step-down Bonferroni (Holm) correction for multiple testing. Similar models and contrasts were used to analyze the brain data. Finally, to explore putative relationships between the differences in hormone response and brain response to the different sweet substances, we used Spearman's rank non-parametric correlations. Specifically, we used the hormone measurement at 15 min and the brain measurement at 21 min to calculate differences in change from baseline between treatments in case of a significant difference at both the hormone and brain level, and correlated the resulting values, with qFDR correction for multiple testing.

### Results

Twenty-three volunteers were recruited, but two did not meet the eligibility criteria, and one did not tolerate the nasogastric tube. This person's data was excluded from analysis and replaced by a new participant, giving a final total of 20 participants. For details, see the participant flow diagram (Figure 2). Administration of fifty grams of xylitol led to bloating and diarrhea in 40% of all subjects (8 out of 20), and 75 g of erythritol had the same side effects in 16.6% of all subjects (3 out of 20; xylitol vs. erythritol p = 0.16). Despite diarrhea (which usually stopped after 1–2 bowel movements), no study session had to be terminated prematurely. Data from 20 volunteers were obtained for analysis. For BOLD analysis, one scan for one subject had to be excluded because of excessive head motion (according to the criteria reported in supplement), and two scans of two subjects because of raw data quality problems. For arterial spin labeling (ASL) analysis, we
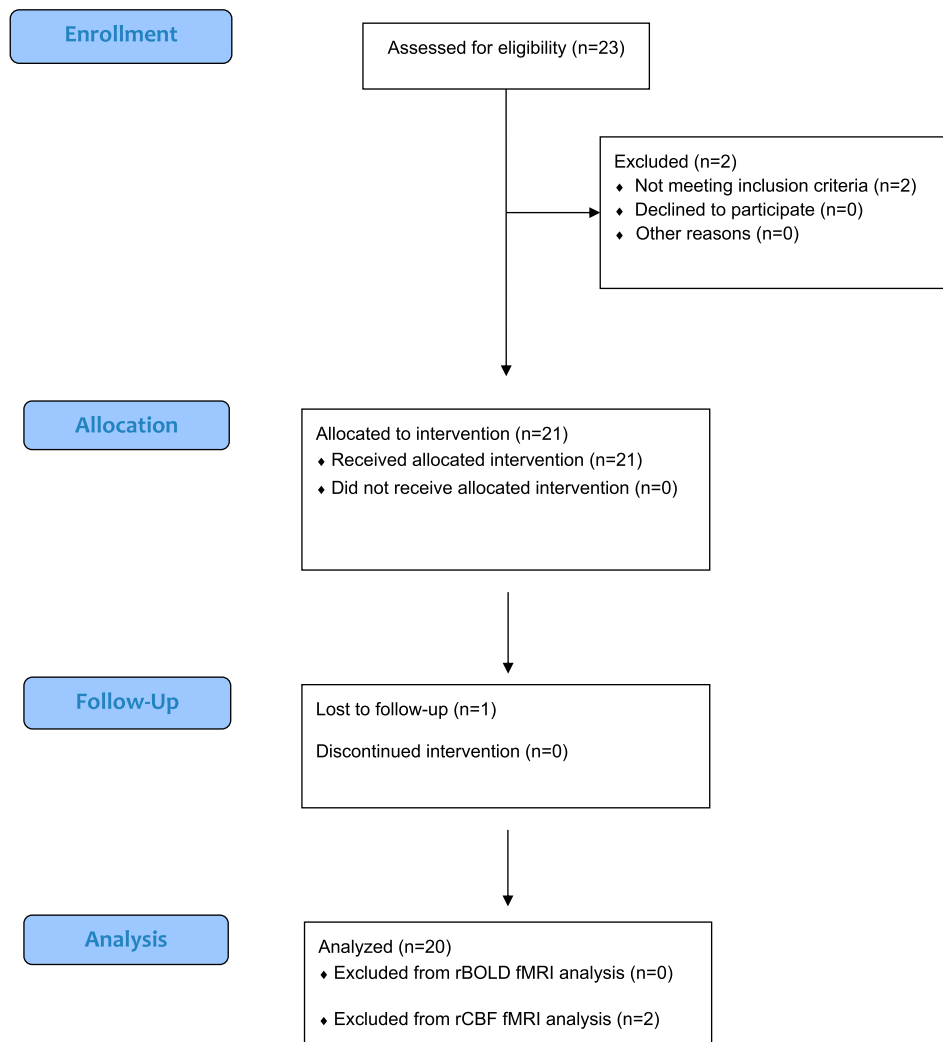
**Figure 2.** Participant flow diagram.

had to exclude two additional subjects because of data quality problems.

### Resting cerebral blood flow (rCBF) fMRI

The voxel-based analysis within the mask consisting of all ROIs did not reveal any significant treatment-by-time interaction effects, nor any main effects of treatment nor time. However, in the hypothalamus ROI analysis, a significant effect was found (local maximum MNI coordinates 6, −4, −12, voxel-level pFWE = 0.012, k = 9). To clarify this interaction effect, planned contrast analyses were performed on the average rCBF extracted from this cluster, revealing that it was driven by a difference after 11 min between glucose (a decrease from baseline) and xylitol (an increase from baseline) (pHolm = 0.005), and a trend for a similar difference between glucose and erythritol (a non-significant

increase from baseline) (pHolm = 0.072). No significant differences were found at 26 min. See Figure 3.
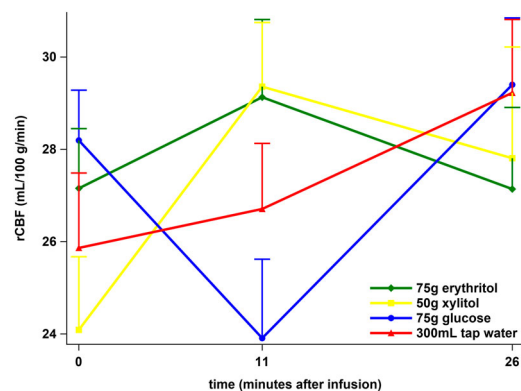


**Figure 3.** Resting cerebral blood flow (rCBF) fMRI. rCBF in response to 75 g erythritol, 50 g xylitol, and 75 g glucose dissolved in 300 mL tap water, respectively, and 300 mL tap water (placebo). Data are presented as mean ± SEM. N = 18.

## Resting Blood Oxygen Level-Dependent (BOLD) fMRI

For a summary of the graph theoretical results, see Figure 4(B).

### Node degree

*Definition and interpretation.* Important brain regions (hubs) often interact with many other regions, facilitate functional integration, and play a key role in network resilience to insult. Measures of node centrality variously assess importance of individual nodes on the above criteria. The degree of an individual node is equal to the number of links connected to that node, which in practice is also equal to the number of neighbors of the node. Individual values of the degree therefore reflect importance of nodes in the network. The degree has a straightforward neurobiological interpretation: nodes with a high degree are interacting, structurally or functionally, with many other nodes in the network [13].

*Global.* No significant effects were found at the global level, but planned contrast analyses revealed several effects at the nodal level.

*Anterior insula.* A stronger increase (from baseline) in node degree of the *left* anterior insula at 6 min after glucose administration compared to tap water (qFDR = 0.046) was found, as well as a more substantial decrease in the *right* anterior insula at 21 min after erythritol compared to tap water (qFDR = 0.035).

*Lateral orbitofrontal cortex (OFC).* A stronger decrease in the *left* lateral OFC was found at 6 min after xylitol compared to both erythritol (qFDR = 0.049) and tap water (qFDR = 0.024). In the *right* lateral OFC, a stronger increase was found at 6 min after glucose compared to both tap water and erythritol (qFDR = 0.0006 and 0.025, respectively), with statistical trends for a stronger increase at both 6 and 21 min after xylitol compared to tap water (qFDR = 0.069 and 0.074, respectively).

*Striatum.* A more robust increase was found in the *right caudate nucleus* at 21 min after erythritol compared to xylitol (qFDR = 0.022). In the *left putamen*, a difference was found at 6 min between glucose (decrease from baseline) compared to both tap water and xylitol (increase from baseline) (qFDR = 0.027 and 0.023, respectively). In the *right putamen*, a difference was found at both 6 and 21 min between xylitol (decrease from baseline) vs. tap water (an increase from baseline) (qFDR = 0.035 and 0.0078, respectively), as well as at

21 min between glucose (a decrease from baseline) vs. tap water (an increase from baseline) (qFDR = 0.030). In the *right nucleus accumbens*, a stronger increase (from baseline) was found at 6 min after xylitol compared to both erythritol (a decrease from baseline) and tap water (no change from baseline) (qFDR = 0.002 and 0.028, respectively).

### Clustering coefficient

*Definition and interpretation.* Functional segregation in the brain is the ability for specialized processing to occur within densely interconnected groups of brain regions. Measures of segregation primarily quantify the presence of such groups, known as clusters or modules, within the network. Simple measures of segregation are based on the number of triangles in the network, with a high number of triangles implying segregation. Locally, the fraction of triangles around an individual node is known as the *clustering coefficient* and is equivalent to the fraction of the node's neighbors that are also neighbors of each other. The mean clustering coefficient for the network hence reflects, on average, the prevalence of clustered connectivity around individual nodes [13].

*Global.* A more substantial decrease from baseline was found at 21 min after tap water compared to xylitol (qFDR = 0.025), with a similar statistical trend for glucose (qFDR = 0.076).

*Medial orbitofrontal cortex (OFC).* A stronger increase from baseline was found at 21 min after xylitol compared to both erythritol and tap water (both qFDR = 0.048).

### Characteristic path length

*Definition and interpretation.* Functional integration in the brain is the ability to rapidly combine specialized information from distributed brain regions. Measures of integration characterize this concept by estimating the ease with which brain regions communicate and are commonly based on the concept of a path. Paths are sequences of distinct nodes and links and in anatomical networks represent potential routes of information flow between pairs of brain regions. Lengths of paths consequently estimate the potential for functional integration between brain regions, with shorter paths implying stronger potential for integration. The average shortest path length between nodes in a network is known as the characteristic path length [13].

*Global.* A stronger decrease from baseline was found at 21 min after tap water compared to both xylitol and glucose (qFDR = 0.004 and 0.042, respectively).

*Lateral orbitofrontal cortex (OFC).* In the *left* lateral OFC, a difference was found at 6 min after xylitol (an increase from baseline) compared to both tap water and erythritol (both decrease from baseline) (qFDR = 0.015 and 0.028, respectively). In the *right* lateral OFC, a stronger decrease from baseline was found at 6 min after glucose compared to tap water (qFDR = 0.022).

*Striatum.* In the *left putamen*, a difference was found at 6 min after glucose (an increase from baseline) compared to both tap water and xylitol (both decrease from baseline) (qFDR = 0.015 and 0.028, respectively). In the *right putamen*, a difference was found at 6 min after xylitol (an increase from baseline) compared to tap water (a decrease from baseline) (qFDR = 0.010). Further, a difference was found at 21 min between tap water (a decrease from baseline) compared to both xylitol and glucose (an increase from baseline) (qFDR = 0.0016 and 0.012, respectively). In the *right nucleus accumbens*, differences were found at 6 min after xylitol (a decrease from baseline) compared to both erythritol and glucose (both increase from baseline) (qFDR = 0.021 and 0.039, respectively).

## Betweenness centrality

### Definition and interpretation.
Betweenness centrality is defined as the fraction of all shortest paths in the network that pass through a given node. Bridging nodes that connect disparate parts of the network often have a high betweenness centrality [13].

Given the substantial amount of zero-inflation (i.e. >30% of the distribution consisting of zeros), preventing normalization of the distribution by box-cox transformation, betweenness centrality was analyzed using hurdle models, each consisting of a mixed logistic regression part to model the probability of a zero versus a non-zero value, and a mixed linear regression part on the non-zero values, with correlated error terms.

*Global.* No significant effects were found at the global level, but several effects were found at the nodal level.

*Hypothalamus.* In the linear part of the hurdle model, a stronger increase from baseline was found at 21 min after erythritol compared to tap water (qFDR = 0.016), with similar statistical trends for xylitol and glucose compared to tap water (qFDR = 0.067 and 0.096, respectively).

*Striatum.* In the *right nucleus accumbens*, the linear part of the hurdle model showed a stronger decrease from baseline at 6 min after erythritol compared to tap water and xylitol (qFDR = 0.045 and 0.076, respectively).
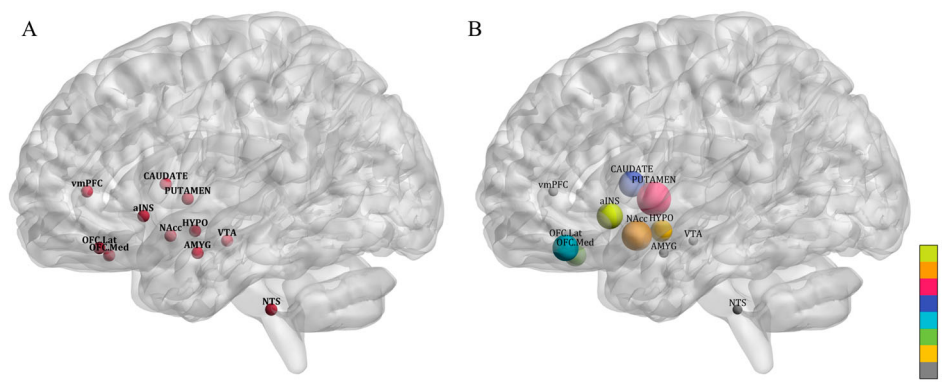


**Figure 4.** Overview of the nodes used in resting functional connectivity analysis and summary of the graph theoretical results. The nodes were visualized with the BrainNet Viewer (http://www.nitrc.org/projects/bnv/)37. NTS, nucleus of the solitary tract; VTA, ventral tegmental area; NAcc, nucleus accumbens; Hypo, hypothalamus; Amyg, amygdala; aINS, anterior insula; vmPFC, ventromedial prefrontal cortex; OFC.Lat, lateral orbitofrontal cortex; OFC.medial, medial orbitofrontal cortex. N = 20. Statistical tests: linear mixed model analyses followed by planned contrast analyses using paired Student's t-tests, with step-down Bonferroni (Holm) correction for multiple testing. (A) Overview of the nodes used in resting-state functional connectivity analyses. (B) Summary of the nodal graph theoretical results. The magnitude of the node indicates the number of nodal graph measures for which differences were found. Different colors/numbers indicate different patterns of results: 1 (grey): no effects. 2 (dark yellow): xylitol/erythritol/glucose >< water. 3 (green): xylitol >< erythritol/water. 4 (light blue): xylitol/glucose >< erythritol/water. 5 (dark blue): xylitol >< erythritol. 6 (red): left: xylitol >< glucose/erythritol/water; right: xylitol/glucose >< water. 7 (orange): xylitol >< erythritol/water/glucose. 8 (light yellow): glucose >< water >< erythritol. ><: different from.

### Hub score

Details on calculation of hub scores based on a combination of the abovementioned graph measures are provided in supplement.

The probability of being a hub was analyzed with generalized linear mixed models with logit link function for dichotomous outcomes (i.e. mixed logistic regression).

*Anterior insula.* In the *left* anterior insula, a difference in the probability of being a hub was found at 6 min after glucose (increased probability) compared to tap water (decreased probability) (qFDR = 0.009), and statistical trends were found for glucose compared to both erythritol and xylitol (both no significant change from baseline) (both qFDR = 0.089), as well as xylitol compared to tap water (qFDR = 0.089).

*Striatum.* In the *right caudate*, a lower probability of being a hub was found at 21 min after xylitol compared to erythritol (qFDR = 0.035). In the *left putamen*, a higher probability of being a hub was found at 6 min after glucose (an increase from baseline) compared to xylitol (a decrease from baseline), erythritol, and tap water (both no significant change from baseline) (qFDR = 0.0003, 0.009, and 0.044, respectively), and for erythritol compared to xylitol (qFDR = 0.034).

### Plasma CCK, PYY, insulin, and glucose concentrations

### Plasma CCK and PYY

The main effect of treatment was significant [CCK: F (3,56) = 42.6, p < 0.0001, and PYY: F(3,56) = 7.13, p = 0.0004], indicating a difference in CCK and PYY concentrations between the 4 treatments over all time points (including the pre-infusion baseline). Further, the treatment-by-time interaction effect was significant [CCK: F(6,109) = 22.75, p < 0.0001, and PYY: F (6,112) = 20.63, p < 0.0001], indicating that the difference between the 4 treatments changes significantly over time. Planned contrast analyses showed that at *15 min compared to pre-infusion baseline,* the increase in CCK was greater for erythritol, xylitol, and glucose vs. tap water (all three pHolm < 0.0001), with no differences between erythritol, xylitol, and glucose (all three pHolm = 1). The increase in PYY was greater for erythritol and glucose (significant pHolm = 0.038 and <0.0001, respectively) and xylitol (trend pHolm = 0.059) vs. tap water, with an attenuated increase after erythritol and xylitol vs. glucose (pHolm = 0.0019 and <0.0001, respectively), and no difference between erythritol and xylitol (pHolm = 0.67). At *60 min post-infusion compared to pre-infusion baseline*, the increase in CCK was again greater for erythritol, xylitol, and glucose vs. tap water (all three pHolm <= 0.0001), with a greater increase after xylitol vs. glucose (significant pHolm = 0.0004) and erythritol (trend pHolm = 0.093), and after erythritol vs. glucose (trend pHolm = 0.056) (Figure 5(A)), and the increase in PYY was again greater for erythritol, xylitol, and glucose vs. tap water (all three pHolm < 0.0001), with no differences between erythritol, xylitol and glucose (all three pHolm = 1) (Figure 5(B)).

### Plasma insulin and glucose

The main effect of treatment was significant [insulin: F (3,56) = 35.99, p < 0.0001, and glucose F(3,56) = 14.48, p < 0.0001], indicating a difference in insulin concentrations between the four treatments over all time points (including the pre-infusion baseline). Further, the treatment-by-time interaction effect was significant [insulin: F(6,109) = 31.56, p < 0.0001, and glucose: F (6,109) = 26.37, p < 0.0001], indicating that the difference between the four treatments changes significantly over time. Planned contrast analyses at *15 min compared to pre-infusion baseline* showed that the increase in insulin was greater for xylitol and glucose vs. erythritol and tap water (all pHolm < 0.0001, respectively), with an attenuated increase after xylitol vs. glucose (pHolm < 0.0001), and no difference between erythritol and tap water (pHolm = 0.49), both of which showed no increase. The increase in plasma glucose was greater for xylitol and glucose vs. erythritol and tap water (all pHolm < 0.001, respectively), with an attenuated increase after xylitol vs. glucose (pHolm < 0.0001), and no difference between erythritol and tap water (pHolm = 0.98), both of which showed no increase. At *60 min post-infusion compared to pre-infusion baseline*, the increase in insulin was again greater for xylitol and glucose vs. erythritol and tap water (all pHolm < 0.0001, respectively), with an attenuated increase after xylitol vs. glucose (pHolm < 0.0001), and no difference between erythritol and tap water (pHolm = 0.93), both of which showed no increase (Figure 5(C)). The increase in plasma glucose was greater for glucose vs. erythritol and tap water (significant pHolm = 0.0038 and 0.0299, respectively), and xylitol vs. erythritol (pHolm = 0.0023), with an attenuated increase after xylitol vs. glucose (trend pHolm = 0.056) and no difference between xylitol and tap water (pHolm = 0.30). Again no difference between erythritol and tap water (pHolm = 0.33) was found, both of which showed no increase (Figure 5(D)).
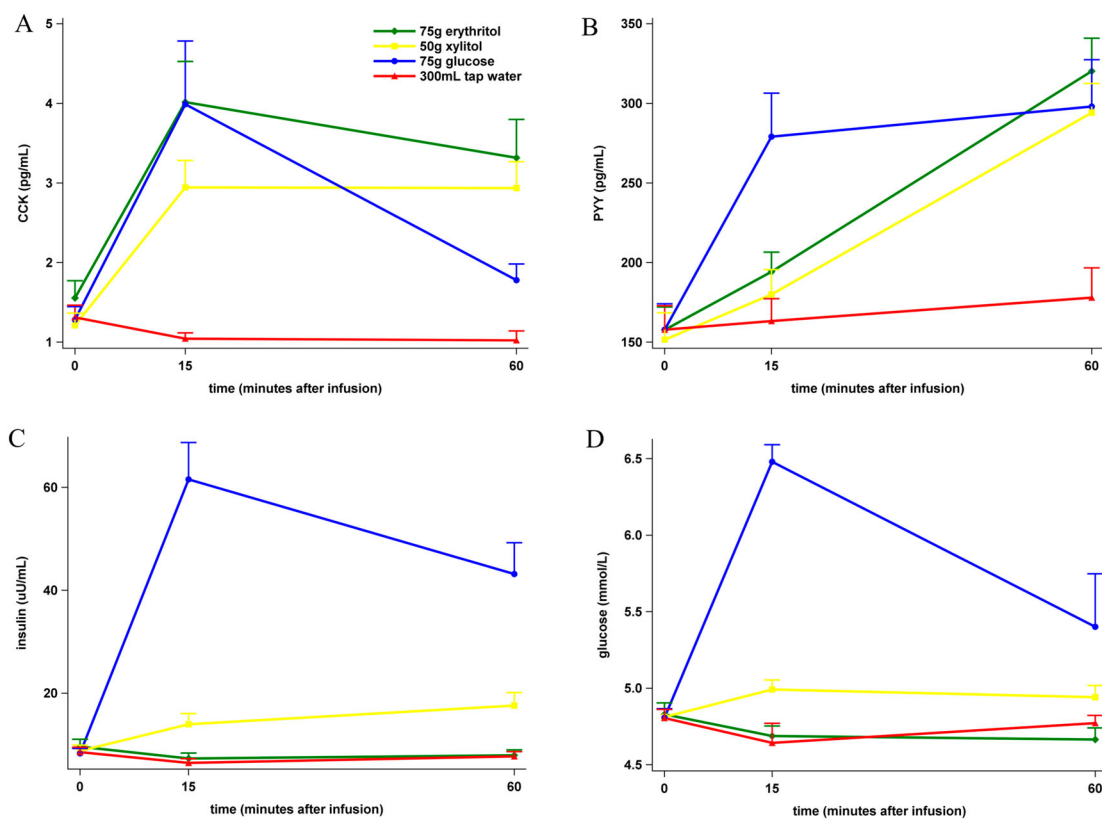
**Figure 5.** Plasma CCK, PYY, insulin, and glucose concentrations. Plasma CCK (A), PYY (B), insulin (C), and glucose (D) concentrations in response to ig 75 g erythritol, 50 g xylitol, and 75 g glucose dissolved in 300 mL tap water, respectively, and 300 mL tap water (placebo). Data are presented as mean ± SEM. N = 20.

### Hormone-brain correlations

For details on hormone-brain correlations, see supplemental material (S4). In brief, we did not find any significant correlations between changes in hormone levels and the respective changes in graph measures. However, when correlating the significant difference in hypothalamic resting cerebral blood flow change from baseline between glucose on the one hand and xylitol and erythritol on the other at 11 min. with the respective significant differences in glucose, insulin, and CCK levels at 15 min., we did find that most of these correlations were positive, indicating a stronger decrease in resting cerebral blood flow after glucose versus the other substances with a less strong increase in glucose and insulin levels.

### Appetite-related sensations

#### Hunger

The main effect of treatment was not significant [$F_{(3,55)} = 2.16$, $p = 0.10$]. The treatment-by-time interaction effect was significant [$F_{(6,109)} = 2.19$, $p = 0.0497$], indicating that the difference between the 4 treatments changes over time. Planned contrast analyses

showed that the decrease in hunger compared to pre-infusion baseline at 15 and 60 min did not differ between the treatments (Figure 6(A)).

### Prospective food consumption

The main effect of treatment was significant [$F_{(3,55)} = 5.99$, $p = 0.0013$], indicating a difference in prospective food consumption between the 4 treatments over all time points (including the pre-infusion baseline). The treatment-by-time interaction effect was not significant [$F_{(6,109)} = 1.19$, $p = 0.32$]. Planned contrast analyses showed that the decrease in prospective food consumption compared to pre-infusion baseline at 15 and 60 min did not differ between the treatments (Figure 6(B)).

### Satiety

The main effect of treatment was significant [$F_{(3,55)} = 3.11$, $p = 0.0338$], indicating a difference in satiety between the 4 treatments over all time points (including the pre-infusion baseline). The treatment-by-time interaction effect was not significant [$F_{(6,109)} = 0.97$, $p = 0.45$]. Planned contrast analyses showed that the increase in satiety compared to pre-infusion baseline
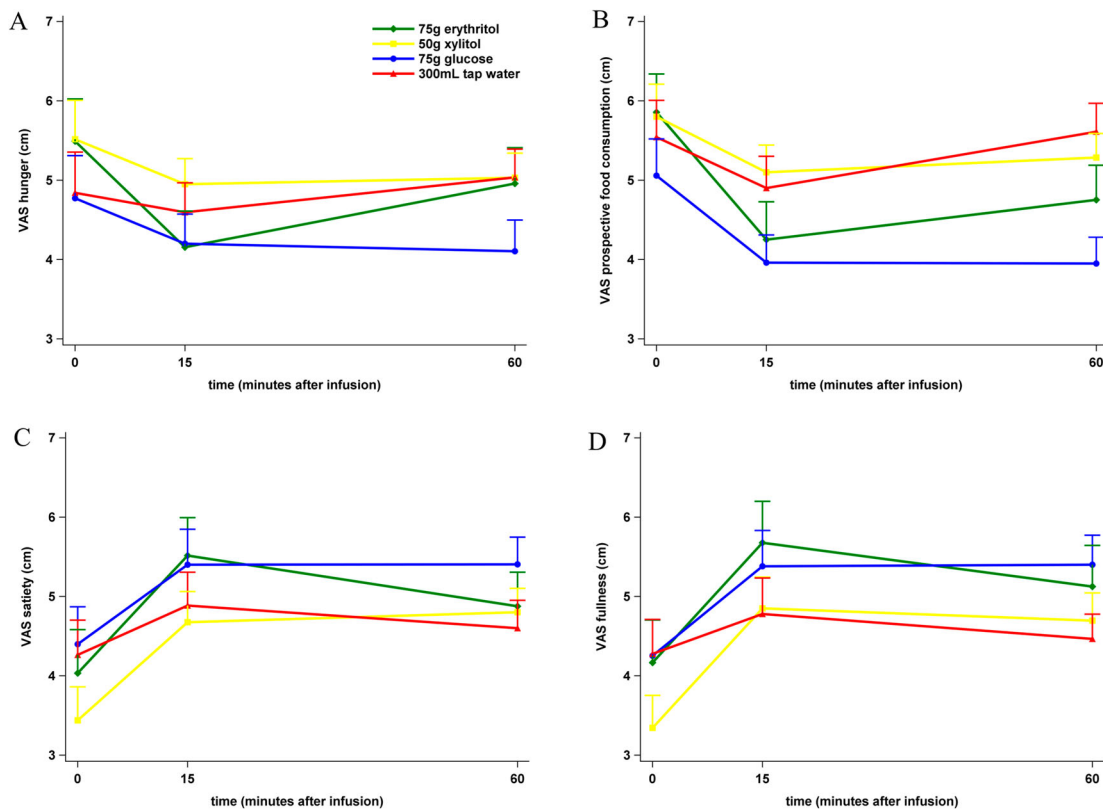
**Figure 6.** Appetite-related sensations. Hunger (A), prospective food consumption (B), satiety (C), and fullness (D) sensations in response to ig 75 g erythritol, 50 g xylitol, 75 g glucose dissolved in 300 mL tap water, respectively, and 300 mL tap water (placebo). Data are presented as mean ± SEM. N = 20.

at 15 and 60 min did not differ between the treatments (Figure 6(C)).

### Fullness

The main effect of treatment was not significant [F (3,55) = 2.57, p = 0.06], as was the treatment-by-time interaction effect [F(6,109) = 1.17, p = 0.33]. Planned contrast analyses showed that an increase in fullness compared to pre-infusion baseline at 15 and 60 min. did not differ between the treatments (Figure 6(D)).

### Discussion

The main findings can be summarized as follows: (i) xylitol, but not erythritol, increased cerebral blood flow and thus activity in the hypothalamus, whereas glucose had the opposite effect; (ii) graph analysis of resting functional connectivity revealed a complex pattern of similarities and differences in impact on global and nodal network properties between xylitol, erythritol, and glucose; (iii) both, erythritol and xylitol, induced a rise in CCK and PYY levels, albeit more slowly compared to glucose for the latter; (iv) erythritol had no

effect and xylitol only minimal effects on glucose and insulin.

The observed reduction in cerebral blood flow in response to glucose administration is consistent with prior fMRI studies in healthy volunteers, which observed a decrease in hypothalamic activity in response to oral or ig glucose loads [19–21]. In the present study, we show for the first time that the activity of the hypothalamus markedly differed following the ig administration of the low-calorie sweeteners xylitol and erythritol. While xylitol induced an increase in hypothalamic activity, erythritol did not affect the activity of the hypothalamus. Previous data by Page et al. suggest that the decrease in hypothalamic activity following the administration of glucose might be a central biomarker of satiety, whereas an increase in hypothalamic activity following the administration of fructose might rather be a sign of increased appetite [19]. While we found an increase in hypothalamic activity following the ig administration of xylitol, we did not find any effect on subjective feelings of hunger. Moreover, we showed an increase of the gut-derived satiation hormones GLP-1 and CCK after xylitol administration [7].

In addition to the three sweeteners' impact on hypothalamic cerebral blood flow, we investigated their effect on functional connectivity in a network of homeostatic and reward-related regions involved in appetite regulation.

Xylitol and erythritol had a differential impact on global and nodal network properties, including the ventral striatum, anterior insula, and OFC. The caloric sweetener glucose and xylitol affected global network properties similarly, both inducing an attenuated decrease in global clustering coefficient and characteristic path length when compared to tap water. These global changes may be at least partially driven by effects in the dorsal striatum, as the nodal findings in the right putamen mimic the results at the global level: similar to glucose, xylitol showed a decrease in node degree and an increase in characteristic path length compared to tap water, which implies less strong connectivity with the rest of the network after the ig administration of glucose and xylitol. In contrast, erythritol induced a stronger increase in node degree and a higher probability of being a hub in the caudate nucleus compared to xylitol, which implies stronger connectivity with the rest of the network after the ig administration of erythritol. As parts of the dorsal striatum, the putamen and caudate are implicated in reward processing in general and food reward in particular [22]. Taken together, these findings suggest a less central position of the dorsal striatum in the overall organization of the network after the ig administration of xylitol and, to a lesser extent, glucose. In contrast, the opposite applies to the ig administration of erythritol. Furthermore, in the OFC, xylitol induced a stronger increase in node degree and clustering coefficient than tap water and a stronger increase in clustering coefficient than erythritol, which implies a stronger connectivity with other nodes of the network after the ig administration of xylitol. The OFC is known to encode information related to the reward value of food, specifically representing its subjective pleasantness, and is involved in (food-related) decision-making [23,24]. Finally, erythritol induced a stronger decrease in node degree than tap water in the anterior insula, which implies diminished connectivity with other network nodes after the ig erythritol administration. The anterior insula processes information related to the taste of food and its hedonic valuation [25,26].

While xylitol and erythritol differentially impact the properties of key reward-related nodes in the network, similarities exist regarding their effects on the hypothalamus. Both erythritol and xylitol infusion induced a stronger increase in betweenness centrality compared to tap water. This effect was similar to the findings observed after the ig administration of the caloric sweetener glucose. The high betweenness centrality of a node implies a considerable influence within a network by virtue of the control over information passing between other nodes [13]. As a homeostatic gatekeeper, the hypothalamus plays a key role in sensing and responding to changes in circulating levels of hormones and nutrients, including glucose, and is known as the primary appetite regulatory brain area [27]. Our findings endorse that the hypothalamus has a considerable influence within the network after the ig administration of glucose. Moreover, we are able to show that xylitol and erythritol affect the hypothalamus in a similar manner.

Taken together, xylitol and erythritol differentially affect hypothalamic cerebral blood flow: while xylitol induced an increase in hypothalamic CBF, erythritol did not. In addition, while xylitol and erythritol differentially impact the properties of key reward-related nodes in the network, including the ventral striatum, anterior insula, and OFC, similarities exist regarding their impact on functional connectivity of the hypothalamus.

Studies combining fMRI with hormonal blood analyses have demonstrated a direct link between changes in plasma concentrations of gut hormones and alterations in brain activity in regions that are part of neural circuits of appetite control [9–11]. CCK and PYY are hormones released by enteroendocrine cells of the gut in response to food intake and promote satiation [5]. However, not all sweet-tasting substances, which stimulate sweet taste in the oral cavity, equally induce gut hormone secretion: artificial sweeteners such as sucralose or aspartame, for instance, do not stimulate gut hormone release. Furthermore, the caloric sweetener fructose only evokes a weak secretion of gut hormones [6]. To date, data on gut hormone secretion after xylitol and erythritol intake in humans is limited. In a recent trial, we found a stimulating effect of xylitol and erythritol on CCK and GLP-1 release [7]. We could reproduce our findings in terms of CCK release in the present study, and the stimulating effect of erythritol on PYY release is in line with a recent finding by Overduin et al. [8]. Both PYY [9,28] and CCK [15,20,29], when given as a peripheral infusion or after endogenous stimulated secretion, can affect brain activity in areas involved in the regulation of appetite, including homeostatic and reward-related brain regions. In the present study, no correlations were found between changes in hormone concentration and the respective changes in network properties or hypothalamic activity. However, the stronger decrease in resting cerebral blood flow in the hypothalamus after glucose versus xylitol and erythritol correlated with a reduced or blunted increase

in glucose and insulin levels. Our findings are in line with previous data by Little et al. [20], showing a correlation between glucose-induced decreases in hypothalamic activity and the increase in blood glucose and insulin levels. They have also shown that the reduction in hypothalamic BOLD signal was independent of the glucose-induced rise in CCK levels and suggested that changes in circulating glucose and insulin concentrations are likely to be key mediators of the central response to glucose. In contrast to glucose, xylitol exhibits a weak stimulating effect on blood glucose and insulin levels only; erythritol has no effect on glucose and insulin. Whether the observed central effects in response to xylitol and erythritol are mediated via a direct effect on the brain – rather than an indirect effect via hormones – is not possible to evaluate with the present study design.

Some limitations of our study require consideration: First, we studied the acute effects of relatively high doses of erythritol and xylitol in subjects who were not used to these substances. In future studies, the effects of lower doses, more common in everyday life, should be examined as well (e.g. 10 and 25 g). Second, we used an ig administration approach to stimulate gut hormones. It is known that a combination of gastric and intestinal nutrient stimuli elicits optimal satiation. However, the experimental design was too complex to include an additional intraduodenal infusion approach. Ig nutrient administration is more potent in stimulating gut hormones than comparable intraduodenal loads [30]. Third, the fMRI design (resting-state scans before and after infusion) only permits comparisons of resting-state connectivity and resting cerebral blood flow before versus after the ig administration, not a continuous recording of brain responses to the infusion over time. The latter should be studied using pharmacological MRI designs, as we and others did for other nutrients/ hormones in the past [15,20,31,32]. Fourth, the study should be considered exploratory and hypothesis-generating, for reasons outlined earlier. Fifth, five minutes of rBOLD is below the current standard of 8 to 10 min. However, this was the time available to ensure that the subsequent sequences could be collected and that the entire protocol remained tolerable for the subjects, and such a duration was not uncommon at the time of data collection. Finally, the spread of BMI across our cohort may introduce some variability to our data, however, the study was not focused on identifying BMI effects. It is important to note that all participants were glucose-tolerant and metabolically healthy, and future studies might want to include patients with diabetes, as they are an important target group for sugar substitutes.

In conclusion, different effects on the hypothalamus activity were observed for glucose on the one hand, and xylitol, and to a lesser extent, erythritol on the other. In addition, the impact of acute ig administration of the naturally occurring, low-calorie sweeteners erythritol and xylitol on functional connectivity properties of an appetite-regulation network consisting of homeostatic and reward-related brain regions is characterized by similarities as well as differences compared to glucose, with some additional differences between erythritol and xylitol as well. Both erythritol and xylitol - like glucose but unlike artificial sweeteners - lead to stimulation of gut hormone release (CCK and PYY), while there is no (erythritol) or only little (xylitol) effect on insulin release and glucose levels. Consequently, these two sugar substitutes have a unique combination of properties: no calories, virtually no effect on glucose and insulin combined with the induction of anorexigenic gut hormone release, and impact on appetite-regulating neurocircuitry consisting of both similarities and differences with glucose. Although these results require confirmation, ideally in a larger sample, we believe they warrant further consideration regarding these compounds' potential to constitute a rewarding and satiating alternative for glucose without its calories. This could contribute to the prevention and treatment of obesity and its complications such as type 2 diabetes.

## Disclosure statement

No potential conflict of interest was reported by the author (s). ACMG, CB, and BKW designed the research; ACMG and BKW conducted research; ACMG, JW, JFR, CLR, PD, OOD, and LVO analyzed data and performed statistical analysis; ACMG, LVO, and BKW wrote the manuscript. ACMG and BKW have primary responsibility for the final content. All authors read and approved the final manuscript.

## Ethics approval

The study was performed in accordance with the principles of the Helsinki Declaration of 1975 as revised in 2013. The protocol was approved by the State Ethical Committee of Basel (Ethikkommission Nordwest- und Zentralschweiz: EKNZ 2014-072; approval date: 02 April 2014) and registered at ClinicalTrials.gov (NCT02823249). Each subject gave written informed consent.

## Notes on contributors

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