Original research

Prebiotic diet changes neural correlates of food decision-making in overweight adults: a randomised controlled within-subject cross-over trial

Evelyn Medawar, Frauke Beyer, Ronja Thieleking, Sven-Bastiaan Haange, Ulrike Rolle-Kampczyk, Madlen Reinicke, Rima Chakaroun, Martin von Bergen, Michael Stumvoll, Arno Villringer, A Veronica Witte

ABSTRACT
Objective Animal studies suggest that prebiotic, plant-derived nutrients could improve homeostatic and hedonic brain functions through improvements in microbiome–gut–brain communication. However, little is known if these results are applicable to humans. Therefore, we tested the effects of high-dosed prebiotic fibre on reward-related food decision-making in a randomised controlled within-subject cross-over study and assessed potential microbial and metabolic markers.

Design 59 overweight young adults (19 females, 18–42 years, body mass index 25–30 kg/m²) underwent functional task MRI before and after 14 days of supplementary intake of 30 g/day of inulin (prebiotics) and equi-caloric placebo, respectively. Short chain fatty acids (SCFA), gastrointestinal hormones, glucose/lipid and inflammatory markers were assayed in fasting blood. Gut microbiota and SCFA were measured in stool.

Results Compared with placebo, participants showed decreased brain activation towards high-caloric wanted food stimuli in the ventral tegmental area and right orbitofrontal cortex after prebiotics (preregistered, family wise error-corrected p <0.05). While fasting blood levels remained largely unchanged, 16S-rRNA sequencing showed significant shifts in the microbiome towards increased occurrence of, among others, SCFA-producing Biildobacteriaceae, and changes in >60 predicted functional signalling pathways after prebiotic intake. Changes in brain activation correlated with changes in Actinobacteria microbial abundance and associated activity previously linked with SCFA production, such as ABC transporter metabolism.

Conclusions In this proof-of-concept study, a prebiotic intervention attenuated reward-related brain activation during food decision-making, paralleled by shifts in gut microbiota.

Trial registration number NCT03829189.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ Targeting high-caloric food craving and unhealthy eating behaviour is crucial for prevention and treatment of the worldwide obesity pandemic. The gut microbiome has been implicated in feeding behaviour through modifying gut-brain crosstalk, for example, short chain fatty acid production.

WHAT THIS STUDY ADDS
⇒ We here present causal evidence for effects of supplementary prebiotics on reward-related food decision making in a group of 59 well-characterised overweight adults. Leveraging advanced neuroimaging, next-generation sequencing and multiomics, our results suggest functional microbial changes that underlie these effects.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ Our findings strengthen the hypothesis that dietary prebiotics cause a reduction of reward-related brain activation in response to high-caloric food stimuli. A better understanding of underlying microbiome–gut–brain mechanisms could help to develop novel strategies towards fostering healthier eating behaviour in humans.

INTRODUCTION
Plant-based diets, recognised as a major effector of planetary health, are more beneficial for cardiovascular and brain health compared with conventional Western diets. Plant-based food and related prebiotic nutrients are less dense in calories and have been claimed to modulate brain function including feeding and psychological functioning via the microbiota–gut–brain axis, however, direct experimental evidence is still limited.

Microbiota-derived metabolites of plant-based dietary fibre such as short-chain fatty acids (SCFA), can cross the blood–brain barrier to modulate hypothalamic signalling. First experimental studies showed that oral intake of the SCFA butyrate or of the butyrate-producing bacteria Akkermansia spp lowered body weight (in humans) and restored obesity-induced functional brain changes (in mice). Moreover, 1 week of colonic SCFA delivery modulated hypothalamic-pituitary-adrenal axis-dependent stress-induced cortisol response in a study including 66 healthy men, and intake of autologous faeces-derived microbiota from a
Gut microbiota dietary weight-loss period enhanced weight loss maintenance in humans.12 Earlier trials in humans showed that supplementary intake of prebiotic fibre such as inulin-type fructans reduced subjective hunger and improved gut hormonal-driven appetite regulation through changes in postprandial glucagon-like peptide (GLP)-1, neuropeptide y (PYY)13 (n=10) and ghrelin14 15 (both n<50). In another randomised clinical trial (RCT) in >100 patients with obesity, inulin compared with placebo induced greater weight loss16 and exploratory results indicated mood improvements in a microbiota-based subgroup with elevated relative Coprococcus abundance at baseline.17 Own results from two cross-sectional analyses indicated that habitual overall dietary fibre intake links to specific microbiota genera including Parabacteriodes, which in turn explained variance in eating behaviour in adults with overweight and treatment success after bariatric surgery.18 However, neuroimaging evidence of how prebiotic diets and diet-related microbial changes affect the brain with regard to eating behaviour remains to be shown. At the brain level, food decision-making is thought to rely on a complex interplay of homoeostatic and hedonic signalling, orchestrated by a variety of subcortical and cortical networks involving the brainstem and hypothalamus, striatum and prefrontal cortex areas.19 The neurobiological underpinnings of (unhealthy) eating behaviour and their neuroimaging correlates, however, have not been fully understood. Functional MRI (fMRI) studies indicated that presentation of highly palatable food cues leads to a stronger brain response in reward areas than equicaloric, non-palatable food cues.20 In parallel, disinhibition and unhealthy food craving, sometimes controversially described as food addiction,21 have been linked with subtle structural differences in the reward network,22 23 and with differential brain activation in the ventromedial prefrontal cortex (vmPFC) in response to high-caloric food stimuli.24 Whether these effects can be mitigated by prebiotic dietary targeting the gut–brain axis25 is yet unknown.

We here aimed to test the hypothesis that a high-dosed prebiotic fibre intervention can alter the gut microbiome and thereby neural activation patterns of food reward in a population at risk for weight gain and insulin resistance. To this end, we conducted an RCT in overall healthy adults in a randomised within-subject cross-over design, participants were randomly assigned to receive first prebiotics and second placebo (arm 1), or vice versa (arm 2), for 14 days each, separated by a 14-day wash-out period. Following the same timeline, at BL1, FU1, BL2 and FU2, participants provided stool samples and underwent fasting blood draw (1), anthropometric measurements (2), received a standard breakfast shake (3) and MRI assessments (4), followed by brief surveys (5), food remuneration (6) and further tests and questionnaires (7–8). Steps (9–11) indicate data processing and statistical analysis. Screens give fMRI wanting task paradigm scheme and timing. BL, baseline; FU, follow-up; fMRI, functional magnetic resonance imaging (MRI); LC-MS/MS, liquid chromatography–mass spectrometry; SPM, statistical parametric mapping. SwE, sandwich estimator, WGNCA, weighted graph network correlational analysis. Created with BioRender.com.

Figure 1  
Study design. Within-subject cross-over dietary intervention design with two study arms and up to six measurement timepoints (upper panel, T0: screening; BL1/2: baseline 1/2, FU1/2: follow-up 1/2, T6: additional follow-up). Participants were randomly assigned to receive first prebiotics and second placebo (arm 1), or vice versa (arm 2), for 14 days each, separated by a 14-day wash-out period. Following the same timeline, at BL1, FU1, BL2 and FU2, participants provided stool samples and underwent fasting blood draw (1), anthropometric measurements (2), received a standard breakfast shake (3) and MRI assessments (4), followed by brief surveys (5), food remuneration (6) and further tests and questionnaires (7–8). Steps (9–11) indicate data processing and statistical analysis. Screens give fMRI wanting task paradigm scheme and timing. BL, baseline; FU, follow-up; fMRI, functional magnetic resonance imaging (MRI); LC-MS/MS, liquid chromatography–mass spectrometry; SPM, statistical parametric mapping. SwE, sandwich estimator, WGNCA, weighted graph network correlational analysis. Created with BioRender.com.

**METHODS**

**Study design**

In this within-subject cross-over design, participants underwent screening and, if eligible, received both verum and placebo in a randomised order (two arms) for 14 days each, separated by a wash-out period of at least 2 weeks (figure 1). Verum (prebiotic fibre) consisted of 30 g inulin (63 kcal, 26.7 g fibre, Orafti Beneo...
Synergy1, BENE0, Mannheim, Germany) per day compared with calorie-matched placebo consisting of 16g maltodextrin (63 kcal, 0g fibre), each provided as two sachets per day.

Data acquisition took place between 2019 and 2022 with some breaks due to lockdown regulations during the SARS-CoV-2 pandemic. All participants were invited to baseline and follow-up visits for each condition, resulting in four study visits with faeces and fasting blood sample collection, fMRI and questionnaires. Briefly, after fasting blood draw and anthropometrics (~45 min), participants received a neutral drink covering 10% of their individual daily energy requirement. Right after, the MRI assessment followed (~2 hours), which was then followed by further computer-based assessments (~1.5 hours) (see online supplemental file_general for further details).

Participants
Volunteers of all gender were recruited via online and local advertisements and the institute's local database. Inclusion criteria were a body mass index of 25–30 kg/m², no MRI contraindications, aged 18–45 years, women: intake of oral contraceptives. Exclusion criteria were: neurological or psychiatric disease; intake of medication acting on the central nervous system; diabetes mellitus type 2; severe untreated internal disease including the gastrointestinal tract, lung, heart, vasculature, liver and kidneys; eating disorder or unconventional eating habits; women: pregnancy, breastfeeding as well as daily consumption of >50 g alcohol, >10 cigarettes, or >6 cups of coffee. Out of 106 initially recruited volunteers with screening assessment, 59 participants (19 women, 40 men) took part in the study, with 45 completing all 4 measurement visits (figure 2). For power analysis and sample size rationale, see online supplemental file_general.

Registration and blinding
Participants received a small reimbursement of €9–€10/hour for testing days and additionally €30 for study completion. The study was registered at https://clinicaltrials.gov/ct2/show/NCT03829189 and https://osf.io/f6qz5 (14 January 2019).
prior to recruitment and data acquisition. Additionally, details on IMRI (pre)processing were uploaded before the start of data analysis https://osf.io/ynkxw (11 May 2021). Participants and staff members were blinded regarding the study intervention/placebo allocation. Sachets were labelled with either A or B through a random assignment performed by author AVW, who was not involved in data collection, prior to the study. Allocation to the A-B or B-A study arm was determined following a randomised order generated using the R software’s ‘sample()’ function by author RT. Authors EM and RT enrolled participants and assigned them to the intervention arm accordingly.

**Patient and public involvement**

The authors acknowledge a missed opportunity of not following a tailored approach to involve patients or the public in the design of the study. We invited and collected comments and assessments from all participants throughout the study to inform the design of upcoming research studies.

**MRI**

MRI was performed on a 3T Siemens Prisma-fit scanner with a 32-channel head coil. FMRI was done in an event-related design assessing wanting of food and art, respectively. Participants were presented with four sets of images across four sessions (randomised order). Each stimulus was shown for 4000 ms with the question ‘How much do you want this now?’, followed by a 4000 ms response period, followed by 500–4000 ms inter-stimulus interval with a 500 ms jitter until the next stimulus was presented (figure 1). Wanting ratings were done on a 8-point Likert scale with 1 labelled as ‘not at all’ and 8 as ‘absolutely’. Participants were informed about receiving a reward right after the scanning session outside the scanner, for food and art, respectively, based on their highest ratings in that session. The reward was given as a dish to eat right away and as a carton-based art print to take home with.

Preprocessing was done using fMRIPrep V.1.2.5. As preregistered, first-level contrasts of interest were global difference between food and art viewing, food compared with art wanting slope, and wanting modulation (design A), food wanting by caloric or fibre density (design B) and considering liking ratings as modulator (design C). See online supplemental file IMRI for further details.

**Additional behavioural assessments**

Dietary habits, lifestyle factors including gastrointestinal quality of life, sleep, physical activity, mental well-being and mood were assessed at each timepoint. Additionally, we assessed potential traits associated with food decision-making at baseline, that is, on personality, eating behaviour, anxiety and well-being, as well as on art knowledge (see online supplemental file behav for details).

**Blood and faeces markers**

To assess serum SCFA, gut hormones (ghrelin, GLP-1, PYY), markers of glucose/lipid metabolism (glucose, insulin, glycated haemoglobin A1c, high and low density lipoprotein, triglycerides), inflammatory markers (high sensitive C reactive protein, interleukin-6, TNFalp) and other markers (trimethylamine-n-oxid and amino acids), blood was obtained in fasting state (12.5±2.2 hours fasted) at the same time per participant for each session. Stool samples were taken within 1–2 days before the testing day to assess faecal SCFA and microbial markers.

**Microbial analysis**

For 16S-rRNA gene profiling, DNA was extracted and V3–V4 variable regions of the 16S-rRNA genes were amplified by PCR and a library was constructed, followed by paired-end 2×250bp Illumina sequencing. Raw sequencing data analysis was done on the inhouse Galaxy server using a pipeline implemented with the DADA2 R-package processed data in fastq format. For further details, see online supplemental file microbiome.

**Statistical analysis**

On a behavioural level, we hypothesised that participant’s wanting ratings scored higher for food compared with art (H_behav_1), and that wanting would change after prebiotic intervention (H_behav_2), dependent on caloric density of the food item (H_behav_3). Linear mixed models were performed in R (version>3.6) using lmer(), for a model-of-interest and a null model for each effect of interest. Model residuals were tested for normal distribution using the R package performance() with the command check_normality(x, effects='random'), see online supplemental file behav for details.

On a neural level, we hypothesised that food evaluation elicits different regional brain activation compared with art evaluation (H_neural_1), and that this differential brain response changes after prebiotic intervention (H_neural_2). Inference tests were performed using a homoeostatic and reward-related region-of-interest brain mask on first-level contrasts (designs A–C) and second-level factors time (baseline, follow-up), group (prebiotics/placebo), and time×group interactions, using the Sandwich Estimator (SwE V.2.2.2, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Swe, implemented in SPM V.12.7486 run in MATLAB V.>9.0) and R (V.>3.6). All main analyses were run in a homoeostatic regulation and reward-related region-of-interest brain mask defined by a combination of two meta-analyses of available previous independent studies at neurosynth.org using the keywords ‘hypothalamus’ and ‘reward’, respectively, integrating functional brain responses of 922 and 98 studies, respectively (created in April 2021; figure 3 in online supplemental file fMRI). Significant results were reported according to threshold-free cluster enhancement methods with alpha <0.05 and family wise error (FWE) correction for multiple comparisons. For details, see fMRI preregistration and online supplemental file fMRI.

Further exploratory analyses were done with the aim to generate hypotheses on potential mechanisms between changes in the microbiome/metabolism and changes in brain activation. First, intervention effects versus placebo were explored in anthropometrics and blood and faeces markers according to mixed effects inference with (restricted) maximum likelihood fitting and \( \chi^2 \) test for comparison. Microbiome composition and predicted functional pathways based on Kyoto Encyclopaedia of Genes and Genomes (KEGG 28) were analysed using Stress test on non-metric multidimensional scaling (NMDS) prior to individual genera/pathway testing with linear mixed effects modelling. Second, bivariate correlation analyses were done on the difference (delta) post versus pre after prebiotic treatment, in those outcomes that showed a significant group×time interaction effect only. Significance threshold for exploratory analyses was set at \( p<0.05 \), follow-up microbiome analyses were corrected for multiple comparisons using false discovery rate.

**Data and code availability**

Data are available at https://doi.org/10.17605/OSF.IO/FC4 and code is available at https://gitlab.gwdg.de/gut_brain_study/food-wanting/task-fmri-behavior-analysis and https://gitlab.gwdg.de.
Gut microbiota

RESULTS
A total of 59 well-characterised overweight/obese adults were included in main analyses (19 women, 40 men, mean age 28 years±6.2 SD, body mass index (BMI) 27.3 kg/m²±1.4 SD, socioeconomic status 14.2±3.2; table 1, online supplemental file_general-table1).

Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Women, 19; Men, 40</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean (SD), 28.3 (6.55)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean (SD), 27.3 (1.51)</td>
</tr>
<tr>
<td>SES index (score)</td>
<td>Mean (SD), 14.5 (2.98)</td>
</tr>
<tr>
<td>Habitual dietary fibre (g/day)</td>
<td>Mean (SD), 16.3 (8.31)</td>
</tr>
<tr>
<td>Blood HbA1c (%)</td>
<td>Mean (SD), 5.31 (0.20)</td>
</tr>
</tbody>
</table>

BMI, body mass index; HbA1c, glycated haemoglobin A1c; Max, maximum; Min, minimum; SD, standard deviation; SES, socioeconomic status.

Neurobehavioural correlates of reward-related decision-making
Overall, wanting and liking ratings in the fMRI preference task were higher for food than for art stimuli (H_behav_1; n_obs=32111, n_subj=59, b=1.03, t=7.78, 8, p<0.001, figure 3A,B, online supplemental file_behav table 2). Food evaluation activated large parts of the reward network (H_neural_1, n=57, design A, pFWE<0.05; figure 3C). Similarly, higher wanting ratings for food compared with art elicited higher brain activation ubiquitously across these brain areas, yet particularly in the vmPFC and OFC (design A, p_FWE<0.05; figure 3D).

Effect of prebiotics on food decision-making
At the behavioural level, individuals’ overall wanting scores were not different after the 2-week prebiotic intervention regarding food versus art and when accounting for calories or fibre, contrary to our hypothesis (H_behav_2+3; n_obs=32111, n_subj=59, b<0.07, t<1.0, p<0.32, online supplemental file_behav tables 7a, 10 and 11). Exploratory analysis showed however that prebiotics compared with placebo led to significantly lower overall wanting scores (online supplemental file_behav table 7b). That is, when looking at stimulus subcategory, participants reported decreases in wanting for very low and very high caloric content as well as for plants after prebiotics (approximately −0.3 points on the Likert scale, figure 4A;
According to fMRI (H_neural_2), we did not observe changes in regional brain response after prebiotics in food compared with art viewing, food compared with art wanting slope, or wanting modulation (design A). However, brain activation towards wanted, high-caloric food (design B) decreased after prebiotics compared with placebo in three clusters, in the VTA (pFWE-corr=0.042), in the right OFC (rOFC, p FWE-corr<0.05) and in the right medial OFC (rmOFC, p FWE-corr<0.05) (n=57, figure 4B,C, table 2). In addition, art liking compared with food liking increased in a small cluster in the right NAc after prebiotics compared with placebo (design C, table 2). See online supplemental file_fMRI-Results for secondary and sensitivity results.

In addition, after prebiotics, participants reported less subjective hunger during the fMRI task, compared with placebo (exploratory analyses, ß=−0.39, p<0.001; figure 5A, online supplemental file_behav tables 18 and 19).

While both intervention and placebo supplements contained the same amounts of calories and participants reported equally high compliance in taking the daily supplements, we observed in exploratory analysis decreases in body fat after placebo (time-point×intervention, b=0.16, p=0.005; figure 5B). In addition, lipid markers were significantly lower after placebo intake compared with prebiotics, as well as alanin-aminotransferase (b_all>0.09, t_all>2.4, p_all<0.013; examples figure 5C,D). BMI, waist-to-hip ratio, and blood pressure did not change significantly, which was also true for fasting ghrelin, GLP-1 and PYY, glucose, insulin, amino acids, as well as inflammatory markers (see online supplemental file_general tables 2–5).

In exploratory bivariate correlation analysis on change scores after the prebiotic intervention, mean bold activation in the three outlined VTA and OFC clusters decreased in correlation with decreases in fasting PYY (Spearman’s r_all>0.32, p_all<0.05).

Changes in gut microbiota and parameters

The prebiotic intervention led to increases in stool frequency (b=1.2, t=2.1, p=0.04, figure 6A). Through 16S-rRNA analysis, we detected significantly decreased richness, evenness and alpha diversity after prebiotics compared with placebo (n_all=200, n_subj=57, all p<0.001 figure 6B–Donline supplemental file_microbiome_table 1). Beta diversity on Amplicon Sequencing Variant was significantly different after prebiotic intervention (NMDS, prebiotics: padj=0.001; figure 6E), and there were abundance changes in families of Actinobacteria and Firmicutes (all p_all<0.02, figure 6F).

Zooming at the genera level, prebiotics induced significant shifts in various abundances, including profound increases in

---

**Table 2**

<table>
<thead>
<tr>
<th>Prebiotic compared with placebo</th>
<th>TFCE P (FW-corr)</th>
<th>TFCE cluster size</th>
<th>Peak Z</th>
<th>Peak (unc)</th>
<th>X (mm)</th>
<th>Y (mm)</th>
<th>Z (mm)</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parametric modulation, food wanting×kcal; decreases in activation</td>
<td>0.038</td>
<td>51</td>
<td>3.595</td>
<td>0.002</td>
<td>26</td>
<td>32</td>
<td>16</td>
<td>Right OFC</td>
</tr>
<tr>
<td></td>
<td>0.042</td>
<td>43</td>
<td>3.388</td>
<td>0.001</td>
<td>4</td>
<td>20</td>
<td>14</td>
<td>VTA</td>
</tr>
<tr>
<td></td>
<td>0.043</td>
<td>41</td>
<td>3.075</td>
<td>0.003</td>
<td>10</td>
<td>36</td>
<td>20</td>
<td>Right (medial) OFC</td>
</tr>
<tr>
<td>Art liking×food liking slope; increases in activation</td>
<td>0.039</td>
<td>3</td>
<td>3.827</td>
<td>0.001</td>
<td>8</td>
<td>16</td>
<td>6</td>
<td>Right NAc</td>
</tr>
</tbody>
</table>

NAC, nucleus accumbens; OFC, orbitofrontal cortex; TFCE, threshold-free cluster enhancement; unc, uncorrected; VTA, ventral tegmental area.
Changes in microbiota genera link to changes in neurobehavioural outcomes

We further explored whether the observed changes in microbial genera predicted intervention-induced changes in neurobehaviour. According to these exploratory analyses, a less severe decrease in *Subdoligranulum* correlated with intervention-induced decreases in VTA brain activation towards wanted, high caloric food stimuli after prebiotic intervention (r=−0.38, p=0.01). Note that bacterial abundance was measured in percentage, thus a relative decrease in *Subdoligranulum* does not necessarily display absolute decrease after prebiotics. Additionally, increases in *Lactiplantibacillus* (lactic acid producing bacteria) were significantly related to increases in rmOFC activation (r=0.40, p=0.008), however abundance of this bacterium did not change in all participants.

Complementary weighted network analyses in a subgroup of available participant data from all four timepoints (n=35) did not provide compelling evidence that clusters of microbial taxa related to neurobehavioural outcomes (exploratory analyses, online supplemental file_microbiome).

SCFA and microbial functional capacity prediction

We could not detect changes in SCFA acetate, butyrate and propionate after intervention, neither in fasting serum nor in faecal concentrations (exploratory analyses, n_{obs}≥122, n_{subj}≥40, p_{all}>0.39, table 4).

Next, we explored changes induced by microbial shifts on the metagenomic level according to KEGG analysis. Changes in KEGG orthologue relative abundance were significantly different after prebiotics (figure 7A, NMDs: prebiotics \( P_{\text{adj}}=0.001 \), placebo \( P_{\text{adj}}=0.99 \), posthoc-pairwise permutational multivariate analysis of variance (PERMANOVA): F=11.46, \( P_{\text{adj}}=0.002 \), online supplemental file_microbiome table 3). The KEGG orthologues were annotated to 158 pathways out of which about 44%, that is, 69 were significantly altered in relative abundance after prebiotic intervention compared with placebo, including pathways related to carbohydrate, protein and fat metabolism.
plant degradation or cell repair (padj<0.05, online supplemental file table 4).

More specifically, exploratory analyses indicated that increases in relative abundance of Bifidobacteria correlated significantly with increases in metabolic pathways related to taurine, seleno-compounds, nicotinate and amino acids, and with decreases related to porphyrin metabolism, steroid degradation, (unsaturated) fatty acid biosynthesis, and DNA repair functions (exemplary figure 7B,C; Spearman’s r>0.32, p<0.05). In addition, increases in Lactobacillus and decreases in Gordonibacter correlated with increases in pyruvate metabolism pathway, a precursor of SCFA (note that not all participants changed in Lactobacillus and Gordonibacter abundance, though). Further exploratory analyses indicated that decreases in VTA brain activation after prebiotic intervention correlated with intervention-induced changes in relative abundance and predicted metabolic pathways correlated with changes in VTA brain activation. While fasting gut hormones, inflammatory markers and SCFA in blood and faeces remained unchanged, we observed that prebiotics-induced decreases in brain activation in reward areas related to decreases in fasting PYY.

**DISCUSSION**

In this proof-of-concept study, we tested the effects of a prebiotic intervention on food decision-making in a randomised within-subject cross-over design including 59 well-characterised, overweight adults. In preregistered analyses, we found that 14 days of high-dose dietary prebiotics, compared with placebo, led to decreases in bold-related brain activation towards high caloric, wanted food in the VTA and right OFC measured using 3T fMRI. In parallel, prebiotics led to significant shifts in relative abundance of the gut microbiota, including increases in SCFA-producers such as Bifidobacteria and Collinsella. Exploratory analyses indicated intervention-induced changes in relative abundance and predicted metabolic pathways correlated with changes in VTA brain activation. While fasting gut hormones, inflammatory markers and SCFA in blood and faeces remained unchanged, we observed that prebiotics-induced decreases in brain activation in reward areas related to decreases in fasting PYY.

**Changes in functional brain activation**

Only few studies with moderate sample size have addressed whether manipulating the microbiome can alter brain functions. A parallel trial in 34 females indicated that 4 weeks of fermented milk consumption (including Bifidobacteria) induced resting-state functional connectivity changes in the midbrain. Another randomised trial reported that 4 weeks of probiotic...
supplementary powder containing *Bifidobacteria* and *Lactobacillus* resulted in changes in microbial genera abundance that correlated with improvements of emotional attention and memory, paralleled by differences in related brain activation.31

Our findings now present prebiotics-induced changes in brain activation with potential implications for food craving and decision-making: While the neuronal processes underlying human eating behaviour are far from fully understood,32 neuro-imaging studies indicate neural responses within VTA and OFC related to reward anticipation and subjective value attribution of food, respectively, linking stronger BOLD-related activation to higher reward values and decision-making.33

Indeed, midbrain and medial OFC activation during fMRI in response to milkshake taste predicted the amount of milkshake intake after the scan.34

Consistently, drivers of reward considering food (such as caloric content) modulate subjective value particularly in the OFC,35 and the right OFC has been specifically implicated in food-related motivation.36 Notably, decreases in brain activation towards high caloric food cues such as ice-cream in the OFC has for example been shown using fMRI when participants were instructed to consider health aspects or long-term consequences of consumption, compared with ‘naive’ viewing.37 The intervention-induced decreases in VTA and rOFC in the current study might thus indicate a diminished anticipation of reward, and a smaller subjective value attribution to high-caloric wanted foods after prebiotic treatment, potentially translating in a subtle reduction of the desire for high-caloric food. At the behavioural level, we could not confirm a general reduction in food wanting ratings, yet exploratory analysis indicated less wanting of very high and very low caloric food, as well as certain art objects, and less hunger after prebiotics. We also observed a marginal increase in body fat after prebiotics which was not statistically significant when comparing pre versus post, but in the interaction model, that is, when taking into account a marginal decrease after placebo (discussed below). This anthropometric data might speak against a significant translation of the observed changes in brain activation to healthier eating behaviour, however two weeks may be too short to generate robust trends in body composition and studies incorporating longer durations are needed.

### Microbiota-related mechanisms

The gut microbiome has only recently been shown to be relevant for host nutritional foraging in rats, for example, through changing circulating amino acids and bacterial tryptophan.38

Another faecal transplantation rat study indicated that microbiota from obese donors resulted in changes in food preference and expression of dopaminergic markers in the striatum.39

In humans, a single-group study in 26 females suggested that increased consumption of vegetables rich in inulin-type fructans over two weeks increased *Bifidobacteria* and decreased the desire to eat sweet, salty, and fatty food.40 In the current study, we similarly observed changes in multiple bacterial genera abundances after prebiotics compared with placebo, mainly increases in Actinobacteria phylum (eg, *Bifidobacteria*) and Firmicutes phylum (eg, *Lactobacillus*). This suggests a marked increase in fiber-degrading, SCFA producing bacteria that are present in the gut, which is in line with previous human trials.41–44

Functional capacity prediction analyses further yielded a multitude of different pathways that were selectively changed after prebiotic intervention, among them pathways involved in SCFA production capable to modify systemic SCFA signalling. For example, one of the most strongly upregulated pathways

### Table 3  Significant shifts in microbiota relative abundances on the genera level after prebiotic intervention, according to 16S-rRNA sequencing and linear mixed effects modelling after FDR-correction for multiple comparisons

<table>
<thead>
<tr>
<th>Increased abundance</th>
<th>Interaction effect time (follow-up)×intervention (prebiotic)</th>
<th>ANOVA null model comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>t</td>
</tr>
<tr>
<td>Anaerostipes</td>
<td>0.73</td>
<td>3.01</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>9.82</td>
<td>10.42</td>
</tr>
<tr>
<td>Collinsella</td>
<td>2.66</td>
<td>4.96</td>
</tr>
<tr>
<td>Holdemanella</td>
<td>0.37</td>
<td>3.13</td>
</tr>
<tr>
<td>Lachnospiraceae FCS020 group</td>
<td>0.21</td>
<td>3.31</td>
</tr>
<tr>
<td>Lactcaseibacillus</td>
<td>0.10</td>
<td>2.05</td>
</tr>
<tr>
<td>Lactiplantibacillus</td>
<td>0.03</td>
<td>2.82</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>2.08</td>
<td>2.65</td>
</tr>
<tr>
<td>Ligilactobacillus</td>
<td>0.28</td>
<td>2.67</td>
</tr>
<tr>
<td>Limosilactobacillus</td>
<td>0.28</td>
<td>5.10</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desulfovibrio</td>
<td>0.20</td>
<td>3.41</td>
</tr>
<tr>
<td>Eggerthella</td>
<td>0.33</td>
<td>3.46</td>
</tr>
<tr>
<td>Eubacterium brachy group</td>
<td>0.11</td>
<td>3.18</td>
</tr>
<tr>
<td>Eubacterium eligens group</td>
<td>0.21</td>
<td>2.76</td>
</tr>
<tr>
<td>Roseburia</td>
<td>1.10</td>
<td>3.86</td>
</tr>
<tr>
<td>Ruminococcus gauvreauii group</td>
<td>0.69</td>
<td>3.86</td>
</tr>
<tr>
<td>Shuttleworthia</td>
<td>0.08</td>
<td>2.78</td>
</tr>
<tr>
<td>Subdoligranulum</td>
<td>1.30</td>
<td>2.82</td>
</tr>
</tbody>
</table>

Linear mixed effects modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with p<0.05): with the Formula: bacterial_genus_of_interest−time point×intervention+time point+intervention+(1+(intervention+time point)|subject). All models run on nobs =204 in nsubj=58 and listed in alphabetical order of genera of interest.

*Statistics refer to models without random slopes due to non-convergence. ANOVA, analysis of variance; FDR, false-discovery rate.
related to the ABC transporters (ko02010). It has been shown that *Lactobacillus* use dietary fibre (e.g., inulin) via ABC transporters to produce acetate, which can be further degraded to butyrate. Multiple of the upregulated microbiota genera after prebiotics have been classified in previous studies to produce SCFAs, e.g., *Anaerostipes, Bifidobacterium* and *Holdemania*.

Moreover, pointing to a dose–effect relationship, a less severe decrease in relative *Subdoligranulum* abundance (also SCFA producers), as well as the increases in prebiotics-induced upregulation of ABC transporters, correlated with significant decreases in prebiotics-induced VTA brain activation in the current study. This may suggest a potential mechanistic route of higher SCFA production leading to lessened reward anticipation, however, these considerations need to be taken with caution due to the lack of direct evidence.

In contrast to our *a priori* hypothesis, we did not observe changes in faecal or fasting blood levels of acetate, butyrate or propionate, suggesting that, in principle, changes in brain activity may have been driven by other indirect factors. Similar to our trial, previous small scale studies could not show increases in faecal SCFA after inulin, for example, in healthy young adults (subgroup, n=49). Others observed SCFA increases, for example, in type 2 diabetes mellitus (n=25), or even decreases in faecal SCFA (n=30). These conflicting results might be explained by unknown complexity of local and systemic microbial effects, and/or by pre-existing differences such as microbiota patterns at baseline (note higher relative *Firmicutes* in our overweight/obese group compared with obesity studies), or differences in stool frequency, weight and fluidity (note significant changes in Bristol stool scale after prebiotics in the current study). The latter opens the possibility that changes in, for example, gut motility (specifically anticipatory contractions on seeing food stimuli in the scanner) may underlie the observed changes in brain responses.

Body fat and lipid markers slightly improved after placebo condition and worsened after prebiotics in the current study. While we did not observe changes in lifestyle habits according to questionnaires, beneficial effects of for example increased energy expenditure in the placebo phase cannot be ruled out. Also, inulin, particularly at high doses, might challenge liver cholesterol metabolism, as postulated in mice under certain conditions. A recent human study further reported spikes in liver enzymes, cytokines and cholesterol in some participants after 30 g/day inulin, underlining the possibility that the dosage of inulin in the current study might have exceeded optimal levels. For serum SCFAs, others did find short-term increases in SCFAs after inulin, and the postprandial increase in SCFA correlated with decreases in serum ghrelin. Prebiotics and SCFA also stimulate the expression of PYY and GLP-1 in the gut, and decreases in fasting PYY correlated with decreases in brain activation in the VTA and OFC clusters after intervention, pointing to a similar mechanism. However, a (postprandial) increase in serum SCFA or gut hormones due to prebiotics in our sample might have been masked after overnight fasting.

**Limitations**

Our study should be discussed in light of several limitations. First, 14 days of intervention can be considered too short to induce long-lasting effects on neuronal processes involved in eating behaviour. Also, secondary analyses did not replicate the

**Table 4**

<table>
<thead>
<tr>
<th>Total (μmol/g)</th>
<th>Butyrate (μmol/g)</th>
<th>Acetate (μmol/g)</th>
<th>Propionate (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre</strong></td>
<td><strong>Post</strong></td>
<td><strong>Pre</strong></td>
<td><strong>Post</strong></td>
</tr>
<tr>
<td>n</td>
<td>value</td>
<td>mean±SD</td>
<td>n</td>
</tr>
<tr>
<td>Feces</td>
<td>Probiotics</td>
<td>42</td>
<td>2.0±10.9</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>42</td>
<td>2.3±9.8</td>
</tr>
<tr>
<td>Serum</td>
<td>Probiotics</td>
<td>37</td>
<td>5.8±1.3</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>39</td>
<td>5.6±1.4</td>
</tr>
</tbody>
</table>

*ANOVA analysis of variance, SCFA: short chain fatty acid.*

For serum SCFAs, others did find short-term increases in SCFAs after inulin, and the postprandial increase in SCFA correlated with decreases in serum ghrelin. Prebiotics and SCFA also stimulate the expression of PYY and GLP-1 in the gut, and decreases in fasting PYY correlated with decreases in brain activation in the VTA and OFC clusters after intervention, pointing to a similar mechanism. However, a (postprandial) increase in serum SCFA or gut hormones due to prebiotics in our sample might have been masked after overnight fasting.

**Limitations**

Our study should be discussed in light of several limitations. First, 14 days of intervention can be considered too short to induce long-lasting effects on neuronal processes involved in eating behaviour. Also, secondary analyses did not replicate the
exact same activation clusters at the whole brain level or when further constraining fMRI analyses to very small peak areas of the reward network. By following recommendations to fully preregister the applied brain mask and statistical thresholding in addition to further preprocessing steps, we, however, aimed to ensure confidence in the robustness of the observed effects. Exploratory analyses need to be interpreted with caution due to their non-confirmative nature. In addition, KEGG analyses need to be considered indirect only and microbiome samples were not time-locked to MRI sessions. Due to the within-subject crossover design, however, interindividual differences at baseline determining microbiota responses could be kept to a minimum. Also, participants belonged to a Western, Educated, Industrially developed, Rich and Democratic society and we did not recruit representative shares of female and diverse gender, limiting generalisability of results difficult.

CONCLUSIONS

According to preregistered RCT analysis of advanced 3T-fMRI, this proof-of-concept study suggests that a high-dosed microbiome-changing prebiotic intervention decreases brain responses to high-caloric food cues during decision-making within 2 weeks in overweight adults. Based on 16S-rRNA combined with functional pathway prediction and metabolomics, exploratory findings offer the possibility of a mechanistic link between prebiotic dietary intake, related changes in SCFA production, gut motility or PYY and reduced reward-related brain activation during food-decision making. While the current data does not allow us to conclude that the prebiotic treatment-induced changes in brain responses were beneficial for behavioural control, neural response in reward-related areas during fMRI have previously shown to predict behaviour change, underlining implications for the treatment of unhealthy eating behaviours or overnutrition using microbiome-changing interventions. Future studies are needed to explore whether such treatments could open avenues for less invasive approaches to obesity.

Author affiliations

1Department of Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany
2Berlin School of Mind and Brain, Humboldt-Universität zu Berlin, Berlin, Germany
3Charité Universitätmedizin Berlin, Berlin, Germany
4Cognitive Neurology, University of Leipzig Medical Center, Leipzig, Germany
5Department of Molecular Systems Biology, Helmholtz-Centre for Environmental Research - UFZ, Leipzig, Germany
6Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig Medical Center, Leipzig, Germany
7Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden
8Medical Department III Endocrinology Nephrology Rheumatology, University of Leipzig Medical Center, Leipzig, Germany

Twitter Evelyn Medawar @EvelynMedawar and A Veronica Witte @witte1veronica
Acknowledgements We thank all the individuals who took part in the study. For participant support, we thank Maria Dreyer, Ramona Menger, Bettina Joehst and Susan Prejawa and for technical support at the MRI we thank all MTAs and specifically Nicole Pampus, Sylvie Neubert, Mandy Jochemey, Anke Kummer and Domenica Klank, and Torsten Schlumm. For fMRI analysis support we thank Hannah Sophie Heinrichs. For lab support we thank Laura Hezel, Charlotte Wiegank, Lina Eisenberg, Emmy Töws and Anna-Luise Wehle. For all other data collection, we highly appreciate the support of all our interns and student assistants Leonie Disch, Lukas Recker, Emira Shehabi, Niklas Hübke, Lynn Moseskü, Larissa de Biasi, Hannah Stock, Lennard Schneidewind, Christian Schneider and Anne-Kathrin Brecht. We thank Lorenz Lemcke and Anna Bujanow for medical assistance. For SCFA analysis, Michael Stumvoll http://orcid.org/0000-0001-6225-8240.

References


Gut first published as 10.1136/gutjnl-2023-330365 on 4 October 2023. Downloaded from http://gut.bmj.com/ on November 5, 2023 by guest. Protected by copyright.
38 Trevelise BK, Kohl KD. The gut microbiome influences host diet selection behavior. Proc Natl Acad Sci U S A 2022;119:e2117537119.