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Original research

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

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ABSTRACT

Objective Animal studies suggest that prebiotic, plant-derived nutrients could improve homoeostatic 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 assayed potential microbial and metabolic markers.

Design 59 overweight young adults (19 females, 18–42 years, body mass index 25–30 kg/m^2) underwent functional task MRI before and after 14 days of supplementary intake of 30 g/day of inulin (prebiotics) and equicaloric 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 *Bifidobacteriaceae*, 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.

INTRODUCTION

Plant-based diets, recognised as a major effector of planetary health,[1] are more beneficial for cardiovascular and brain health compared with conventional Western diets.[2 3] Plant-based food and related prebiotic nutrients are less dense in calories and have been claimed to modulate brain function[4]

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 underly 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.

including feeding[5] and psychological functioning[6] 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[7] to modulate hypothalamic signalling.[8] First experimental studies showed that oral intake of the SCFA butyrate or of the butyrate-producing bacteria *Akkermansia* spp lowered body weight (in humans[9]) and restored obesity-induced functional brain changes (in mice[10]). Moreover, 1 week of colonic SCFA delivery modulated hypothalamic-pituitary-adrenal axis-dependent stress-induced cortisol response in a study including 66 healthy men,[11] and intake of autologous faeces-derived microbiota from a
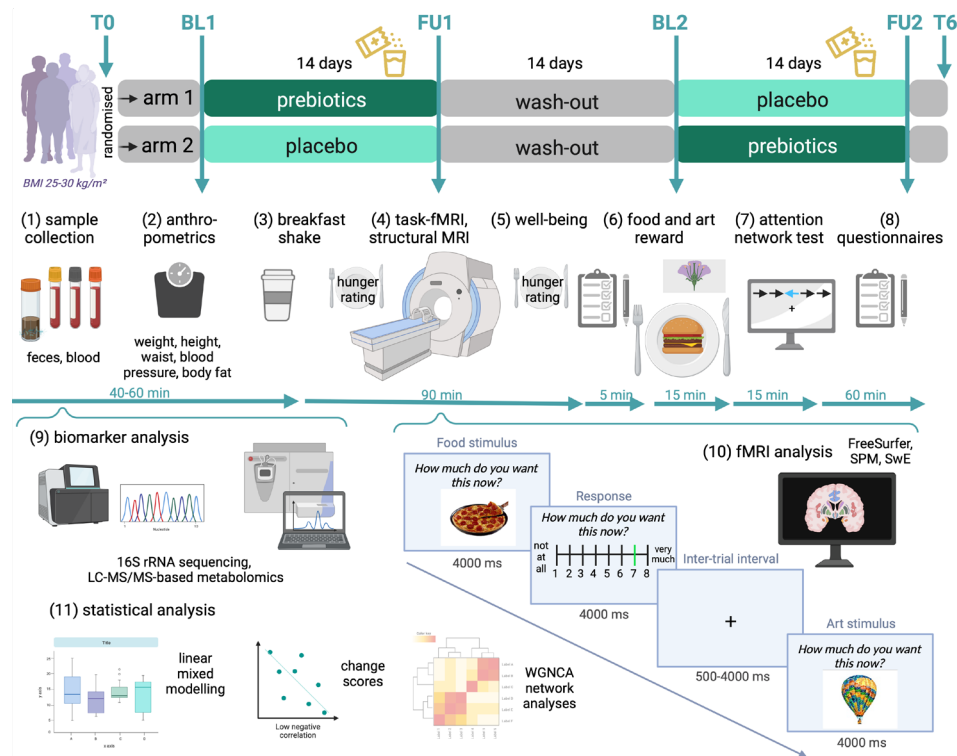
Figure 1 Study design. Within-subject cross-over dietary intervention design with two study arms and up to six measurement timepoints (upper panel, T0: screening; BL1/2: baseline 1/2, FU1/2: follow-up 1/2, T6: additional follow-up). Participants were randomly assigned to receive first prebiotics and 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.

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 ghrelin[14 15] (both n<50). In another randomised clinical trial (RCT) in >100 patients with obesity, inulin compared with placebo induced greater weight loss[16] 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 *Parabacterioides*, 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 axis[25] 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 and assessed food wanting using fMRI before and after 14 days of daily 30 g supplementary intake of inulin (prebiotic fibre) and equicaloric maltodextrin (placebo), respectively. Suggested microbial and metabolic mediators of potential effects were measured using faeces and serum proxies collected at all four timepoints. The study and analyses were preregistered at ClinicalTrials.gov/NCT03829189 and osf.io/ynkxw.

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
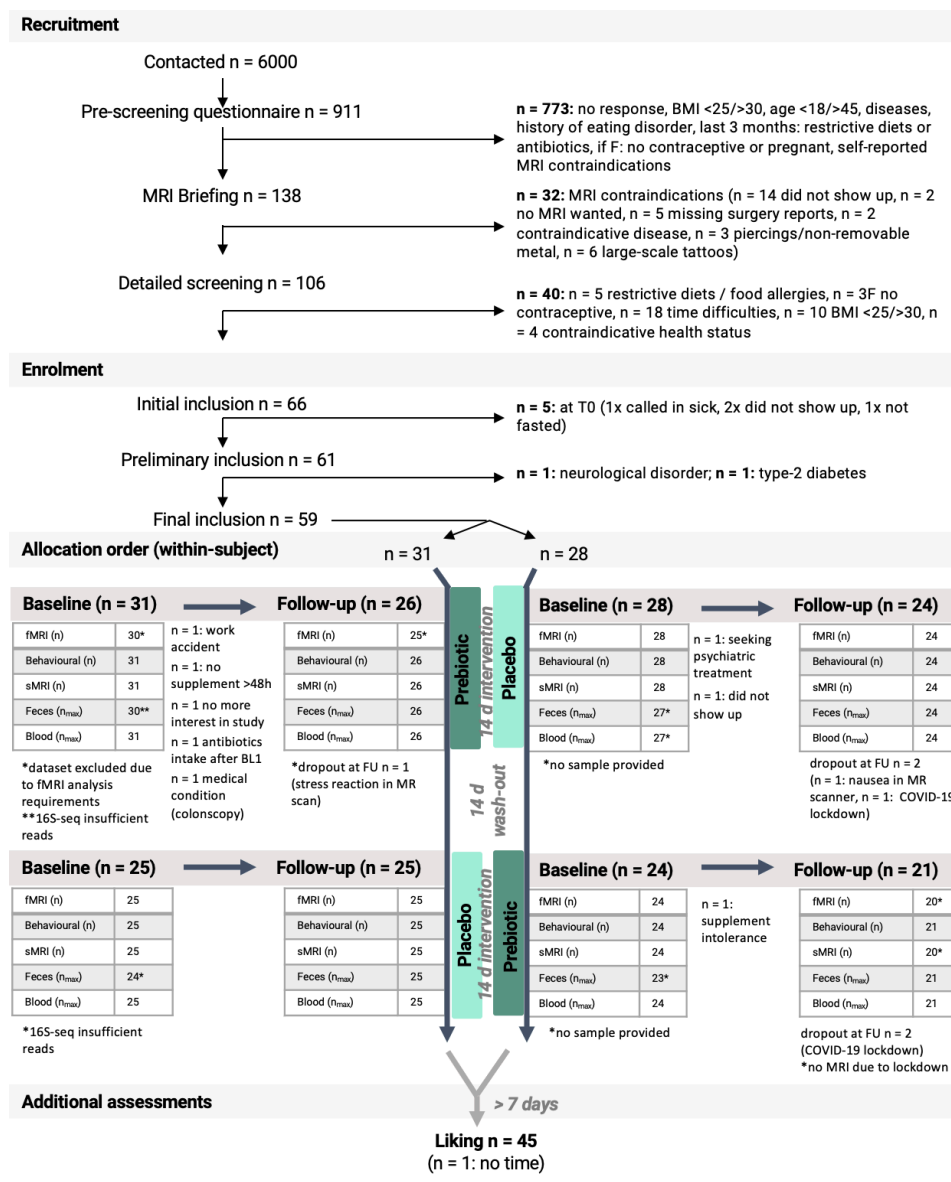
Figure 2 Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Participants underwent a randomised controlled dietary intervention trial in a within-subject cross-over design. BMI, body mass index; fMRI, functional MRI.

Synergy1, BENEO, Mannheim, Germany) per day compared with calorie-matched placebo consisting of 16 g maltodextrin (63 kcal, 0 g 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 fMRI (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 Prismafit 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 interstimulus 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 fMRIPprep V.1.2.5.[26] 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_fMRI 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, TNFalpha) 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 2×250 bp 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.[27] 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 'hypothalmus' 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 χ^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.
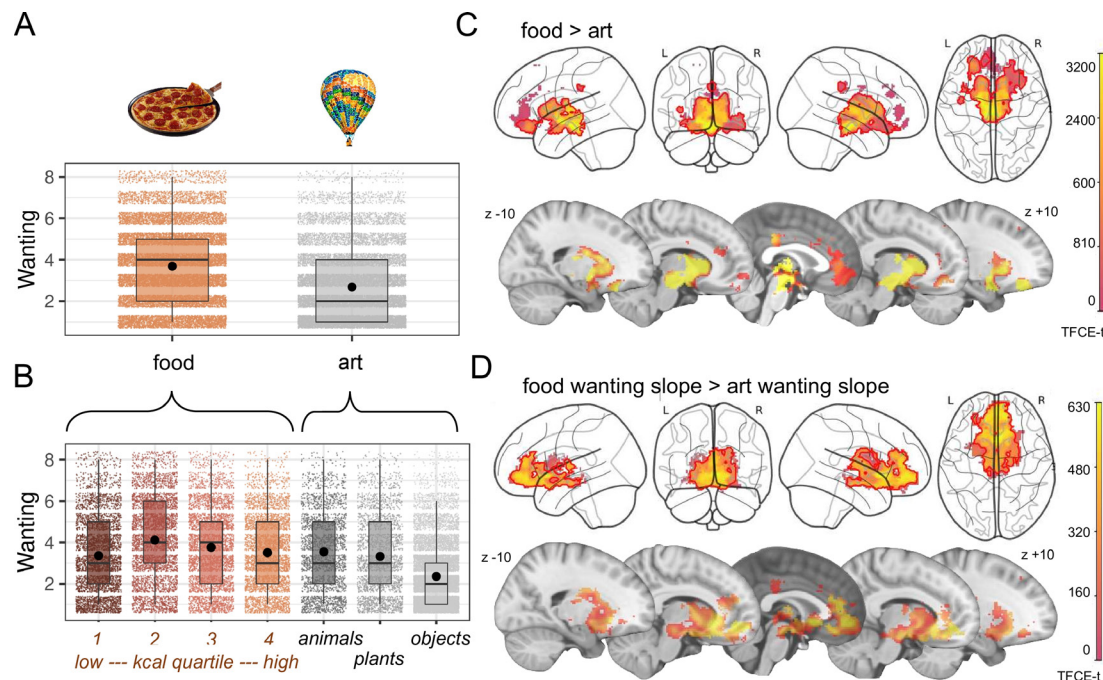
Figure 3 Behavioural (A, B) and neural response (C, D) to food and art stimuli in overweight adults during decision-making. Participants responded to food with higher wanting scores compared with art (n_{obs}=32 111, n_{subj}=59) (A), showing highest mean values for moderately high caloric stimuli, and lowest mean values for art objects (n_{obs}=32 111, n_{subj}=59) (B). Food compared with art valuation elicited stronger brain activation particularly in subcortical areas of the reward network (n_{subj}=57) (C), while additional parametric modulation with wanting scores indicated a stronger brain activation in ventromedial prefrontal cortex and orbitofrontal cortex when comparing food versus art (n_{subj}=57) (D). Statistics were done with linear mixed effect modelling, up to 4 time points per participant×120 stimuli on wanting scores (main analysis) (A), (exploratory analysis) (B) and on voxel-wise blood-oxygen-level-dependent signal using the sandwich estimator toolbox with threshold-free cluster enhancement (TFCE) family wise-error correction (FWE) of multiple comparisons (C,D),(main analyses) (C,D). Colour bars depict parametric TFCE statistic (TFCE-t >50 for visualisation purposes) with wild-boot strapped p_{FWE}<0.05 marked in red outline.

de/gut_brain_study/food-wanting/fmri-analysis.[29] Statistical MRI maps are available at https://identifiers.org/neurovault.collection:14111.

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	
		n=59
Gender (n)	Women	19
	Men	40
Age (years)	Mean (SD)	28.3 (6.55)
	Median (min, max)	28 (19.0, 45.0)
BMI (kg/m²)	Mean (SD)	27.3 (1.51)
	Median (min, max)	27.0 (25 30)
SES index (score)	Mean (SD)	14.5 (2.98)
	Median (min, max)	14.4 (5.10, 19.2)
Habitual dietary fibre (g/day)	Mean (SD)	16.3 (6.31)
	Median (min, max)	15.4 (1.54, 30.5)
Blood HbA1c (%)	Mean (SD)	5.31 (0.20)
	Median (min, max)	5.30 (4.6, 5.8)
	Missing	2 (3.4%)

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}=32 111, 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, including ventral tegmental area (VTA), hypothalamus, nucleus accumbens (NAc), basal ganglia and ventromedial thalamus, as well as anterior insula, amygdala, cingulate, vmPFC and distinct parts of the orbitofrontal cortex (OFC) (*H_neural_1*, n=57, design A, p_{FWE}<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}=32 111/16,071, n_{subj}=59, b_{all} < |0.07|, t_{all} < |1.0|; p_{all}>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 (approximatively −0.3 points on the Likert scale, figure 4A;
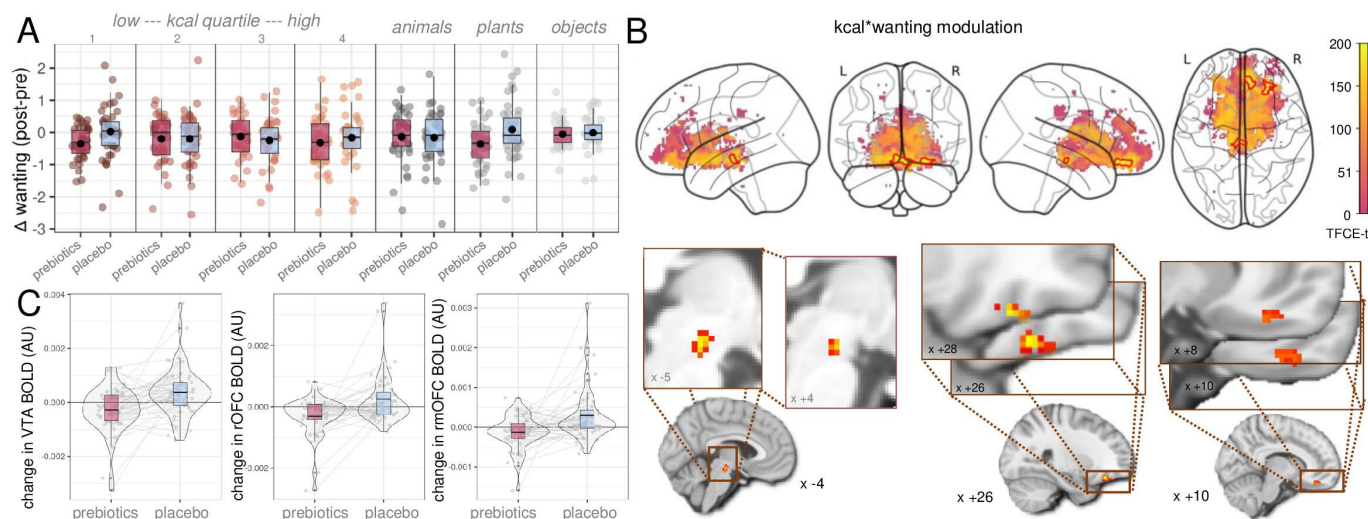
Figure 4 Effects of prebiotic intervention on food decision-making. After the intervention, participants decreased wanting scores for food from caloric quartiles 1 and 4 as well as animals (exploratory analysis, n_{subj}=59, (A). At the neural level, brain activation decreased in the ventral tegmental area (VTA) and in two clusters in the orbitofrontal cortex (OFC) towards high-caloric, wanted food stimuli (main analysis, n_{subj}=57, (B, C). Statistics according to linear mixed effects modelling, up to 4 time points per participant×120 stimuli on wanting scores and on voxel-wise blood-oxygen-level-dependent signal using the sandwich estimator toolbox with threshold-free cluster enhancement (TFCE) family wise-error correction (FWE) of multiple comparisons. Colour bars depict parametric TFCE statistic with wild-boot strapped p_{FWE}<0.05 marked in red outline (upper right panel) and as enlargement (lower right panel).

n_{obs}=32 111, n_{subj}=59, group×time×subcategory: all p<0.01; online supplemental file_fMRI-Results).

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 ($p_{FWE-corr}$=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_{obs}=200, n_{subj}=57, all p<0.001 figure 6B–D online supplemental file_microbiome_table 1). Beta diversity on Amplicon Sequencing Variant was significantly different after prebiotic intervention (NMDS, prebiotics: p_{adj}=0.001; figure 6E), and there were abundance changes in families of *Actinobacteria* and *Firmicutes* (all p_{adj}<0.02, figure 6F). Zooming at the genera level, prebiotics induced significant shifts in various abundances, including profound increases in

Table 2 Localisation of significant changes in brain activation to visual food and art stimuli during functional MRI, after prebiotic compared with placebo intervention (main analysis)

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

NAc, nucleus accumbens; OFC, orbitofrontal cortex; TFCE, threshold-free cluster enhancement; unc, uncorrected; VTA, ventral tegmental area.
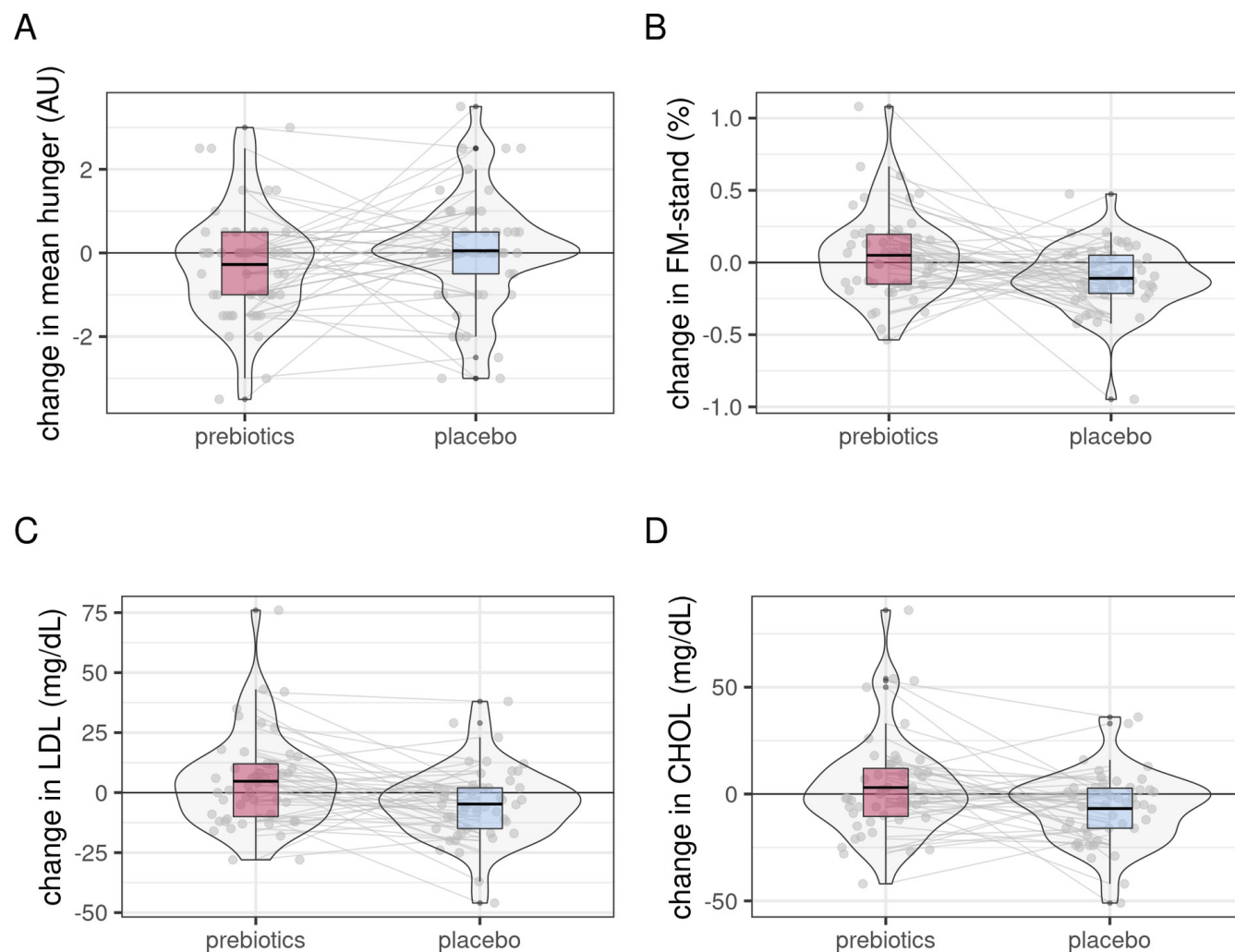
Figure 5 Changes in secondary eating behaviour-related outcomes after prebiotic (red) compared with placebo condition (light blue). Hunger ratings during fMRI significantly decreased after prebiotics (A), while gender-standardised body fat mass (FM-stand, B), serum lipid markers low-density lipoprotein (LDL, C) and cholesterol (CHOL, D) significantly decreased after placebo (linear mixed effects modelling, all p<0.05, exploratory analyses). FMRI, functional magnetic resonance imaging.

Bifidobacteria (table 3, online supplemental file_microbiome table 2).

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, $n_{obs} \geq 122$, $n_{subj} \geq 40$, $p_{all} > 0.39$, table 4).

Next, we explored changes induced by microbial shifts on the metagenomic level according to KEGG[28] analysis. Changes in KEGG orthologue relative abundance were significantly different after prebiotics (figure 7A, NMDS: prebiotics p_{adj}=0.001, placebo p_{adj}=0.99, posthoc-pairwise permutational multivariate analysis of variance (PERMANOVA): F=11.46, p_{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 pathays related to carbohydrate, protein and fat metabolism,
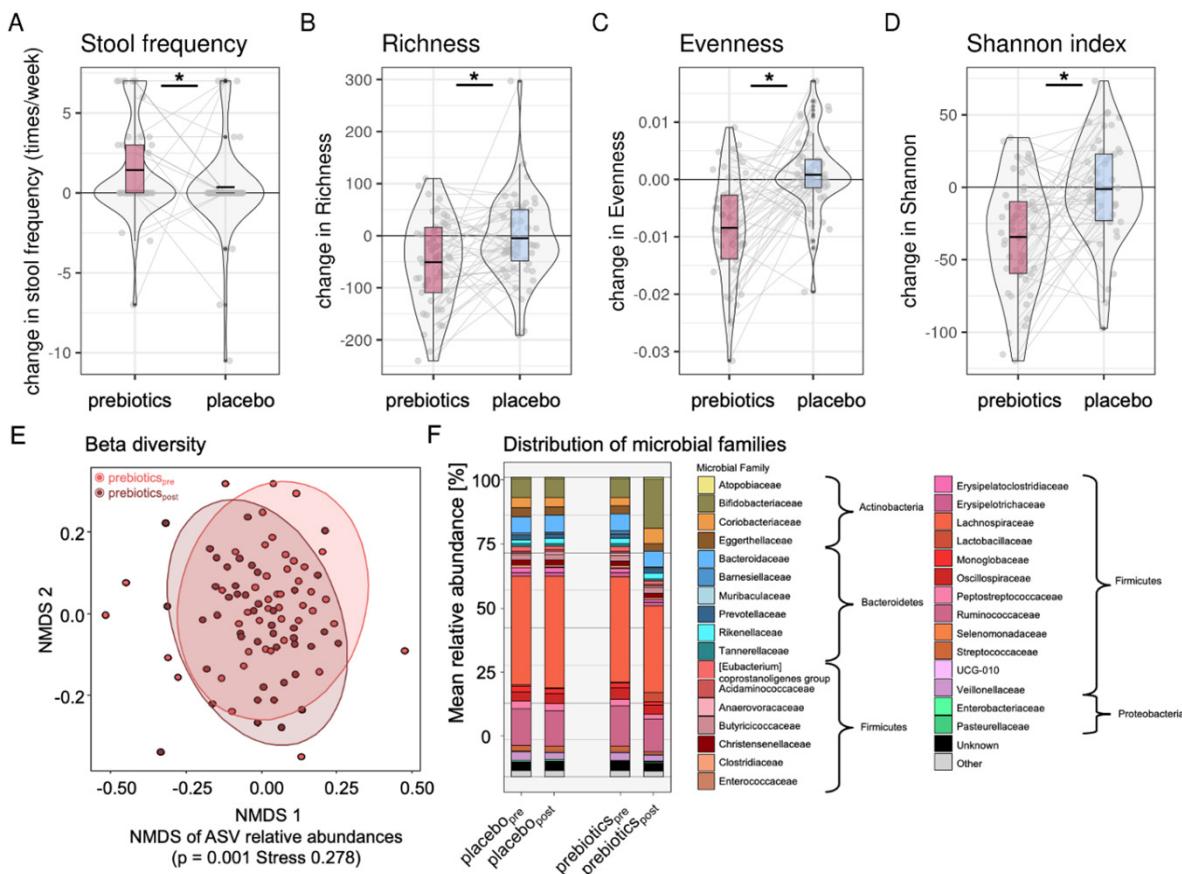
Figure 6 Microbiota-related shifts after 2-week prebiotic intervention (exploratory analyses). Increases in stool frequency (A) and decreases in (B) microbiota richness, (C) evenness, (D) Shannon index, (E) beta diversity changes compared by dissimilarity gradients according to group and timepoint after prebiotics (pink) compared with placebo (blue), and (F) shifts in microbial family distribution. Asterisks in (A–D) indicating significant ANOVA results for null-full model comparisons (p<0.05). ANOVA, analysis of variance; ASV, amplicon sequencing variant; NMDS, non-metric multidimensional scaling.

plant degradation or cell repair ($p_{adj}<0.05$, online supplemental file_microbiome 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 $r_{all}>0.32$, $p_{all}<0.05$). 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 significant decreases in pathways involved in flavonoid and stilbenoid biosynthesis, two-component signal transduction, biofilm formation, amino sugar and nucleotide sugar metabolism, citrate cycle ($r_{all}>0.37$, $p_{all}<0.05$), and with significant increases in ATP-binding cassette transporters (ABC, $r=-0.39$, $p<0.05$, exemplary figure 7D,E). Decreases in rOFC activation after prebiotics correlated with significant decreases in aromatic hydrocarbon degradation ($r=0.32$, $p<0.05$). Decreases in rmOFC activation after prebiotics correlated with significant increases in oxidative phosphorylation ($r=-0.31$, $p<0.05$). For details, see online_supplemental_file_microbiome figure 2a and 2b.

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, over-weight 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.[30] Another randomised trial reported that 4 weeks of probiotic

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

	Interaction effect **time (follow-up)×intervention (prebiotic)**		ANOVA null model comparison	
Increased abundance	**b**	**t**	**P value**	**p$_{adj}$**
Anaerostipes	0.73	3.01	0.003	0.017
Bifidobacterium	9.82	10.42	<0.001	<0.001
Collinsella	2.66	4.96	<0.001	<0.001
Holdemanella	0.37	3.13	0.002	0.011
Lachnospiraceae FCS020 group	0.21	3.31	0.001	0.006
Lacticaseibacillus	0.10	2.05	<0.001	0.002
Lactiplantibacillus	0.03	2.82	<0.001	<0.001
Lactobacillus*	2.08	2.65	0.008	0.045
Ligilactobacillus	0.28	2.67	0.008	0.045
Limosilactobacillus	0.28	5.10	<0.001	<0.001
Decreased abundance				
Desulfovibrio	0.20	3.41	0.001	0.006
Eggerthella	0.33	3.46	0.001	0.006
Eubacterium brachy group	0.11	3.18	0.002	0.011
Eubacterium eligens group	0.21	2.76	0.006	0.033
Roseburia	1.10	3.86	<0.001	0.001
Ruminococcus gauvreauii group	0.69	3.86	<0.001	0.001
Shuttleworthia	0.08	2.78	0.006	0.033
Subdoligranulum	1.30	2.82	0.005	0.028

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 n_{obs} =204 in n_{subj}=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.

supplementary powder containing *Bifidobacteria* and *Lactobacillae* 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] neuroimaging studies indicate neural responses within VTA and OFC to underly dopamine-related 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.[16 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 4 Concentrations of faeces and serum SCFA levels

	Total (µmol/g)						Butyrate (µmol/g)						Acetate (µmol/g)						Propionate (µmol/g)					
	n	Pre, mean±SD	n	Post, mean±SD	Change (%)	P value	n	Pre, mean±SD	n	Post, mean±SD	Change (%)	P value	n	Pre, mean±SD	n	Post, mean±SD	Change (%)	P value	n	Pre, mean±SD	n	Post, mean±SD	Change (%)	P value
Faeces																								
Prebiotics	42	26.4±10.9	40	24.6±8.5	−6.8	0.39	42	18.3±8.7	41	16.8±8.0	−8.2	0.70	41	7.9±2.7	42	8.1±2.4	2.5	0.78	42	7.1±2.4	41	6.2±3.2	−12.7	0.60
Placebo	42	23.9±8.8	42	23.2±12.2	−2.9		42	16.2±6.9	42	15.5±9.4	−4.3	42	7.4±2.4	42	7.4±3.2	0.0		42	7.1±3.1	42	6.6±3.4	−7.0	
Serum																								
Prebiotics	27	5.8±1.3	27	5.5±1.5	−5.2	0.65	36	0.5±0.3	34	0.5±0.4	0.0	0.59	30	3.1±3.2	32	3.8±3.0	22.6	0.88	28	0.5±0.5	24	0.6±0.6	20.0	0.84
Placebo	29	5.5±1.4	25	5.3±1.5	−3.6		34	0.5±0.4	37	0.4±0.3	−20.0	32	2.4±2.3	28	3.1±3.2	29.2		27	0.4±0.4	28	0.4±0.4	0.0	

P, according to ANOVA null-full model p value, total=sum of butyrate, acetate and proprionate.
ANOVA, analysis of variance; SCFA, short chain fatty acid.

related to the ABC transporters (ko02010). It has been shown that *Lactobacillus* use dietary fibre (e.g., inulin) via ABC transporters to produce acetate,[45] which can be further degraded to butyrate.[46] Multiple of the upregulated microbiota genera after prebiotics have been classified in previous studies to produce SCFA, eg, *Anaerostipes*, *Bifidobacterium* and *Holdemanella*.[47] 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).[48] Others observed SCFA increases, for example, in type 2 diabetes mellitus (n=25,[49]), or even decreases in faecal SCFA (n=30).[42] 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.[50] A recent human study further reported spikes in liver enzymes, cytokines and cholesterol in some participants after 30 g/day inulin,[51] underlining the possibility that the dosage of inulin in the current study might have exceeded optimal levels. For serum SCFA, others did find short-term increases in SCFA after inulin,[52] and the postprandial increase in SCFA correlated with decreases in serum ghrelin.[53] Prebiotics and SCFA also stimulate the expression of PYY and GLP-1 in the gut,[54] that may contribute to changes in central reward-related food responses. In humans, PYY injections induced changes in BOLD-related fMRI signalling in the hypothalamus, VTA and OFC.[55 56] We found that decreases in fasting PYY correlated with decreases in brain activation in the VTA and OFC clusters after intervention, pointing to a similar mechanisms. 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
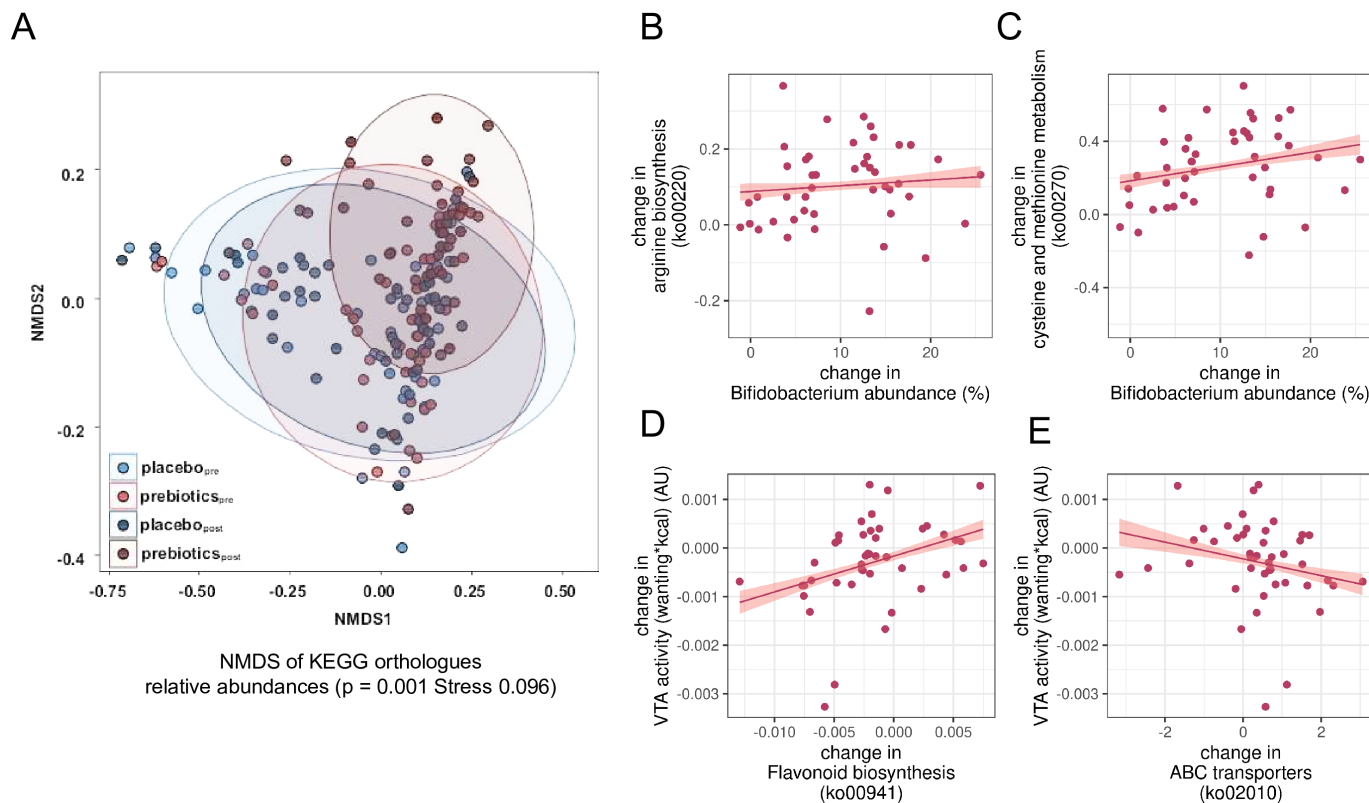
Figure 7 Predicted functional shifts and their correlations with changes in microbiota genera and in reward-related brain activation after prebiotic intervention (exploratory analyses). (A) Dissimilarity of functional composition of microbiome preprebiotic to versus postprebiotic intervention based on NMDS stress test (p=0.001) and principal component analysis of relative abundance of predicted KEGG orthologues statistics, calculated by PERMANOVA (p_{adj}=0.002). (B) Change scores of *Bifidobacterium* abundance and arginine biosynthesis (ko00220), (C) *Bifidobacterium* abundance and cysteine and methionine metbolism (ko00270), (D) flavonoid biosynthesis (ko00941) and changes in reward-related brain response, (E) stilbenoid, gingerol biosynthesis (ko00945) and reward-related brain response. (B–E), all r>0.32, all p<0.05 according to Spearman's correlation, line gives regression fit with 95% CI. KEGG, Kyoto Encyclopaedia of Genes and Genomes; NMDS, non-metric multidimensional scaling; PERMANOVA, permutational multivariate analysis of variance; VTA, ventral tegmental area.

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 cross-over design, however, interindividual differences at baseline determining microbiota responses could be kept to a minimum. Also, participants belonged to a Western, Educated, Industrialised, 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,[57] 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.

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