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.

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.

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] 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

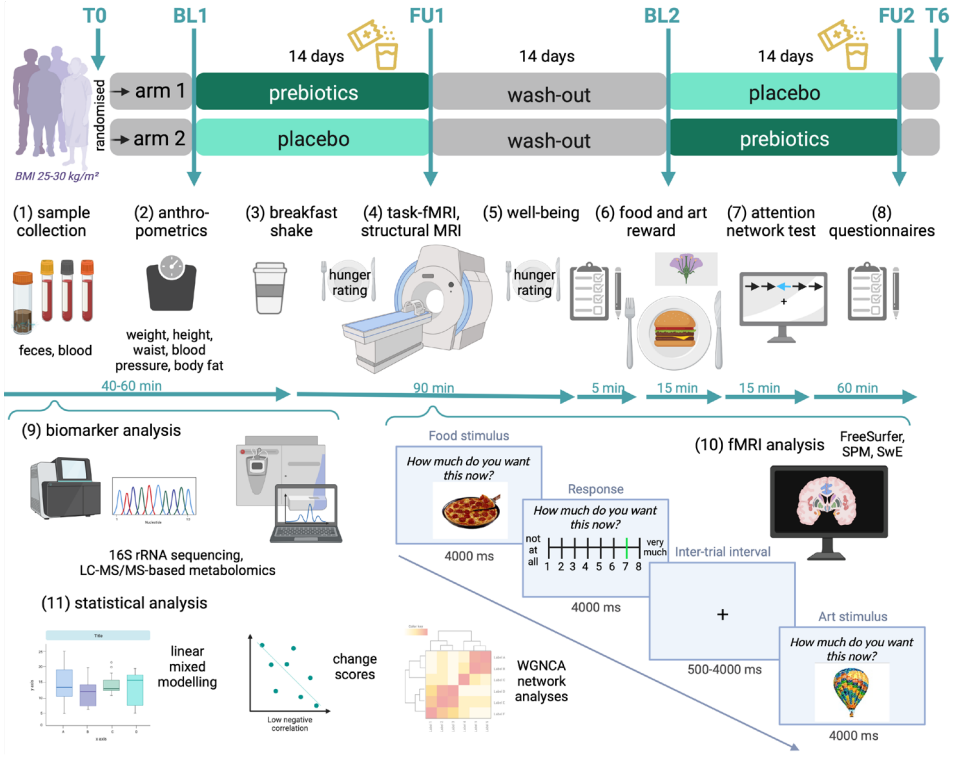

Figure 1 Study design. Within-subject cross-over dietary intervention design with two study arms and up to six measurement timepoints (upper panel, T0: screening; BL1/2: baseline 1/2, FU1/2: follow-up 1/2, T6: additional follow-up). Participants were randomly assigned to receive first prebiotics and 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 *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 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

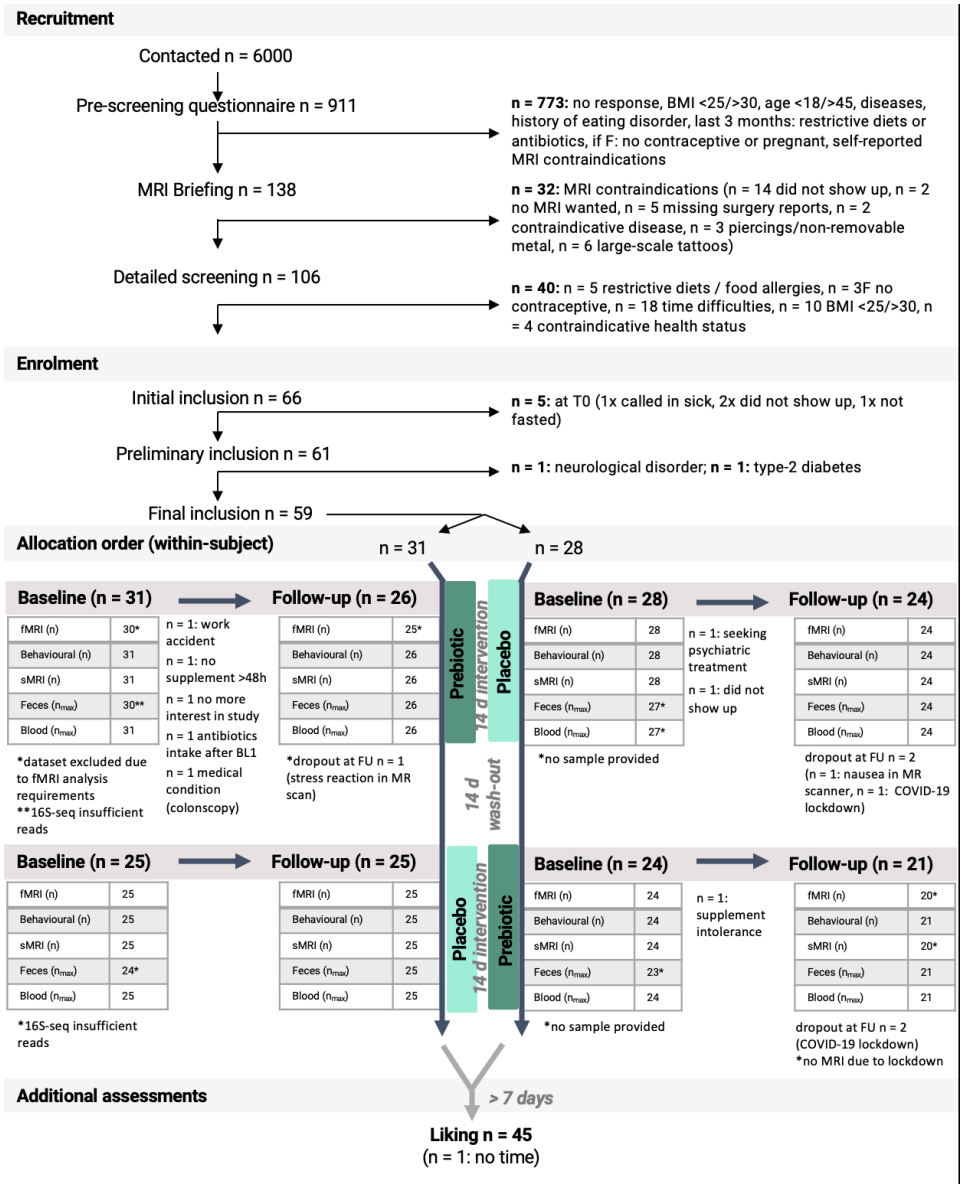

Figure 2 Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Participants underwent a randomised controlled dietary intervention trial in a within-subject cross-over design. BMI, body mass index; fMRI, functional MRI.

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/m^2, 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.

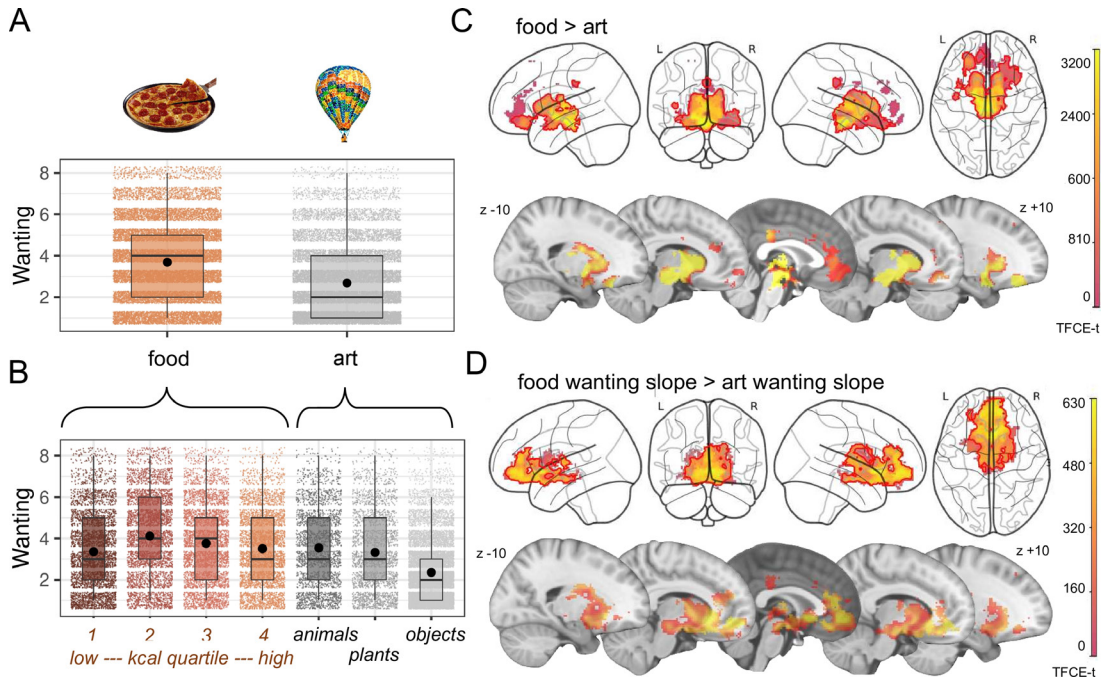

Figure 3 Behavioural (A, B) and neural response (C, D) to food and art stimuli in overweight adults during decision-making. Participants responded to food with higher wanting scores compared with art (n_{obs}=32 111, n_{subj}=59) (A), showing highest mean values for moderately high caloric stimuli, and 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/m^2±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/m^2)	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;

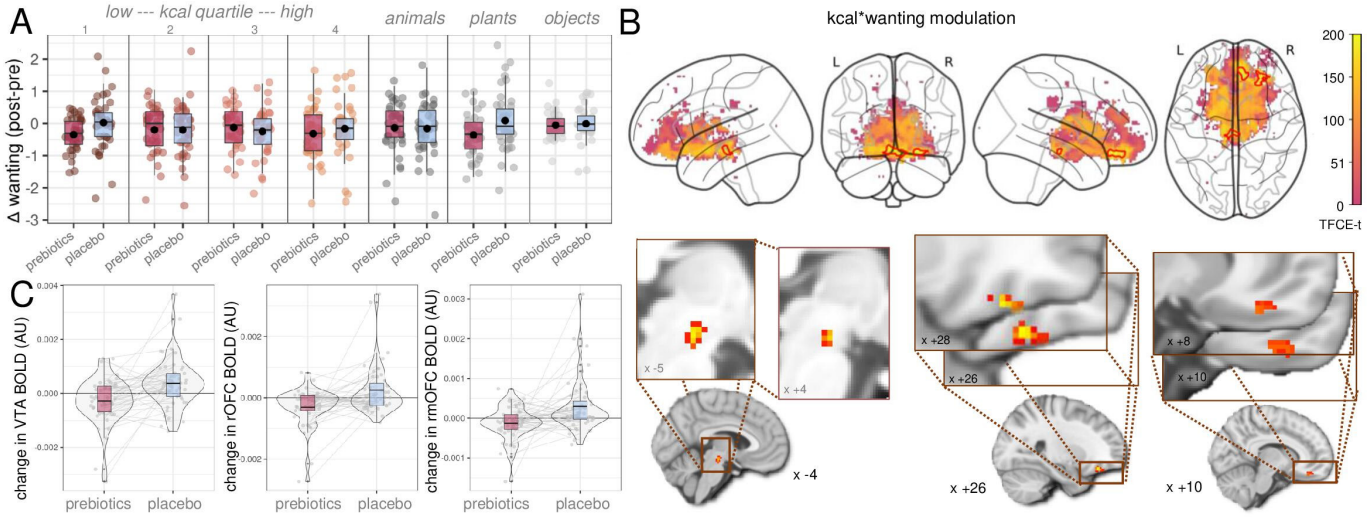

Figure 4 Effects of prebiotic intervention on food decision-making. After the intervention, participants decreased wanting scores for food from caloric quartiles 1 and 4 as well as animals (exploratory analysis, n_{subj}=59, (A). At the neural level, brain activation decreased in the ventral tegmental 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 ($p_{FWE\text{-}corr}$=0.042), in the right OFC (rOFC, $p_{FWE\text{-}corr}$<0.05) and in the right medial OFC (rmOFC, $p_{FWE\text{-}corr}$<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–Donline 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.

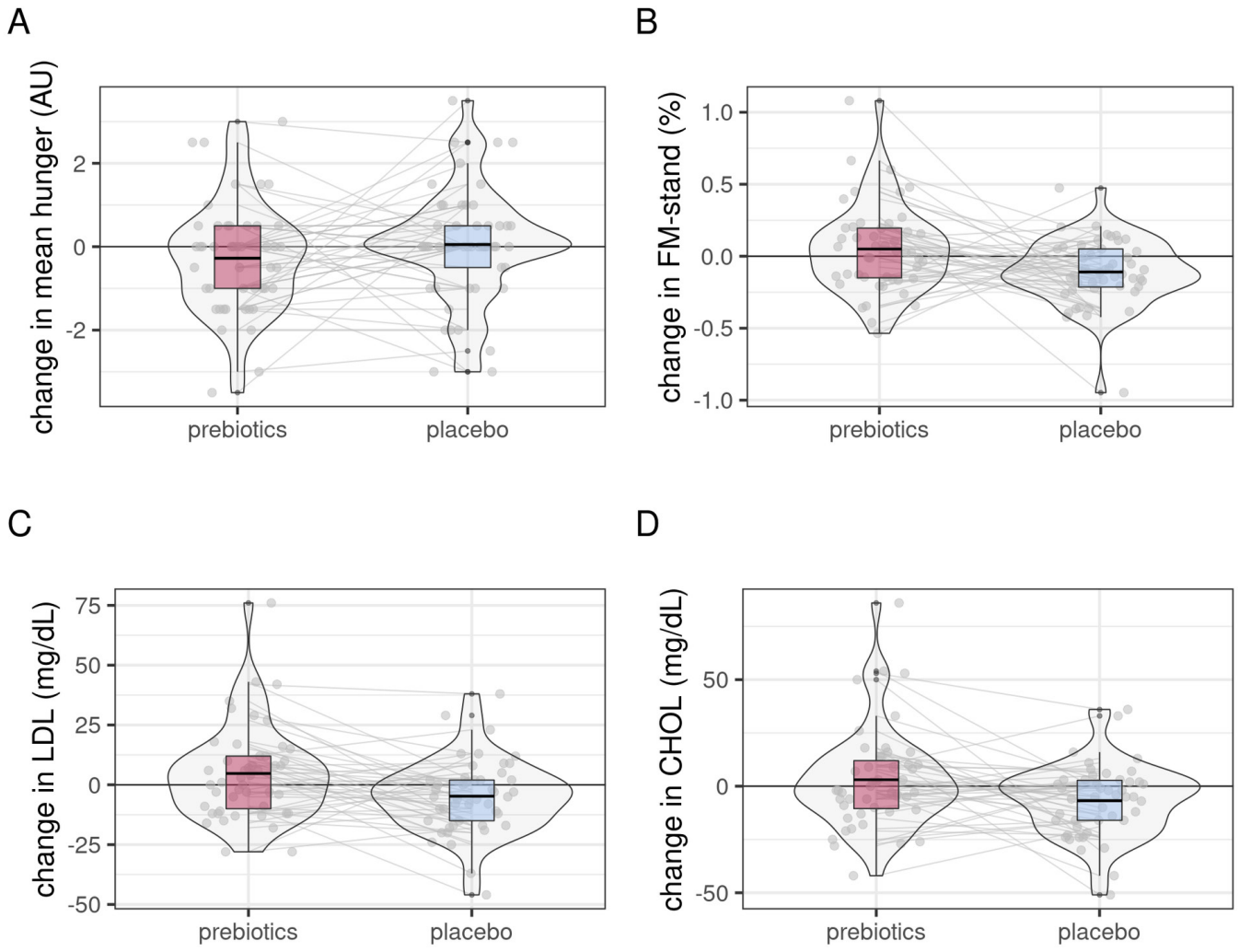

Figure 5 Changes in secondary eating behaviour-related outcomes after prebiotic (red) compared with placebo condition (light blue). Hunger ratings during fMRI significantly decreased after prebiotics (A), while gender-standardised body fat mass (FM-stand, B), serum lipid markers low-density lipoprotein (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,

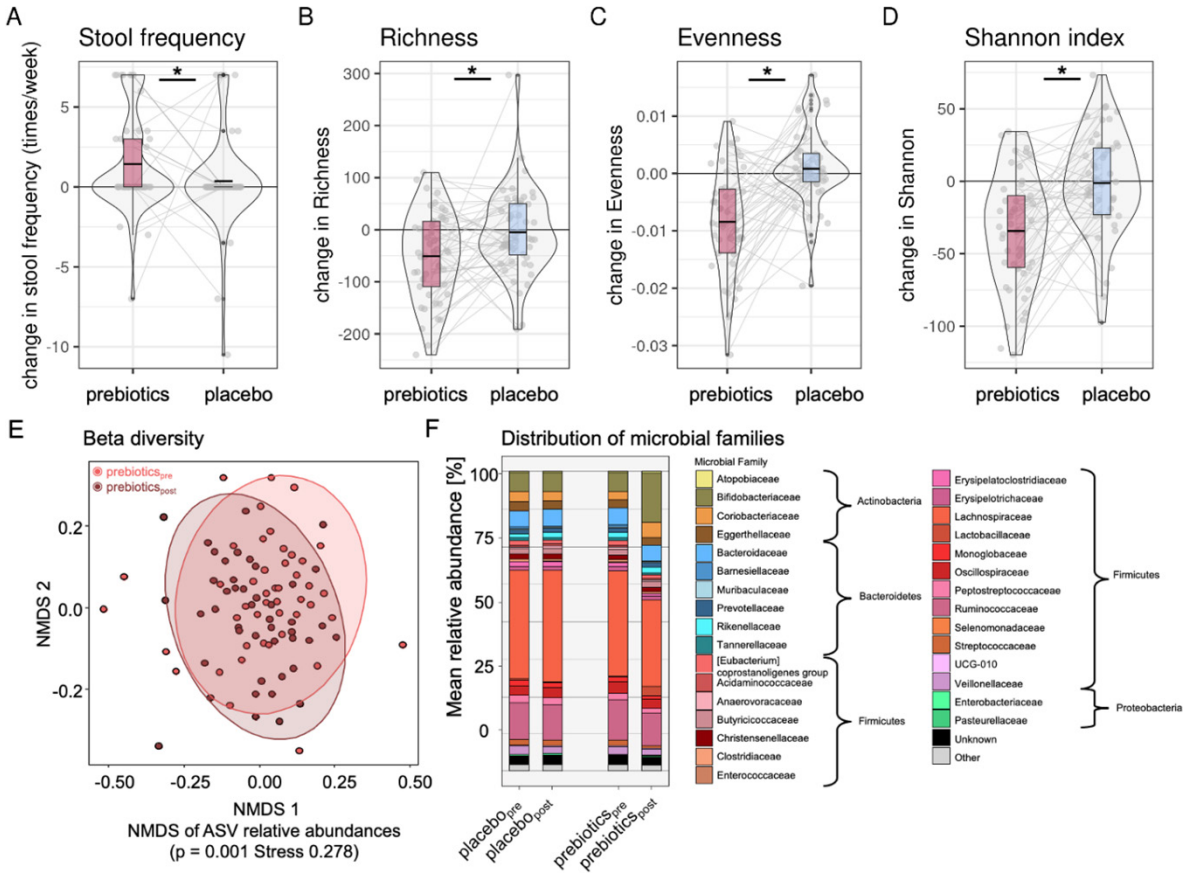

Figure 6 Microbiota-related shifts after 2-week prebiotic intervention (exploratory analyses). Increases in stool frequency (A) and decreases in (B) microbiota richness, (C) evenness, (D) Shannon index, (E) beta diversity changes compared by dissimilarity gradients according to group and timepoint after 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, 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.[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	42	7.9±2.7	41	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

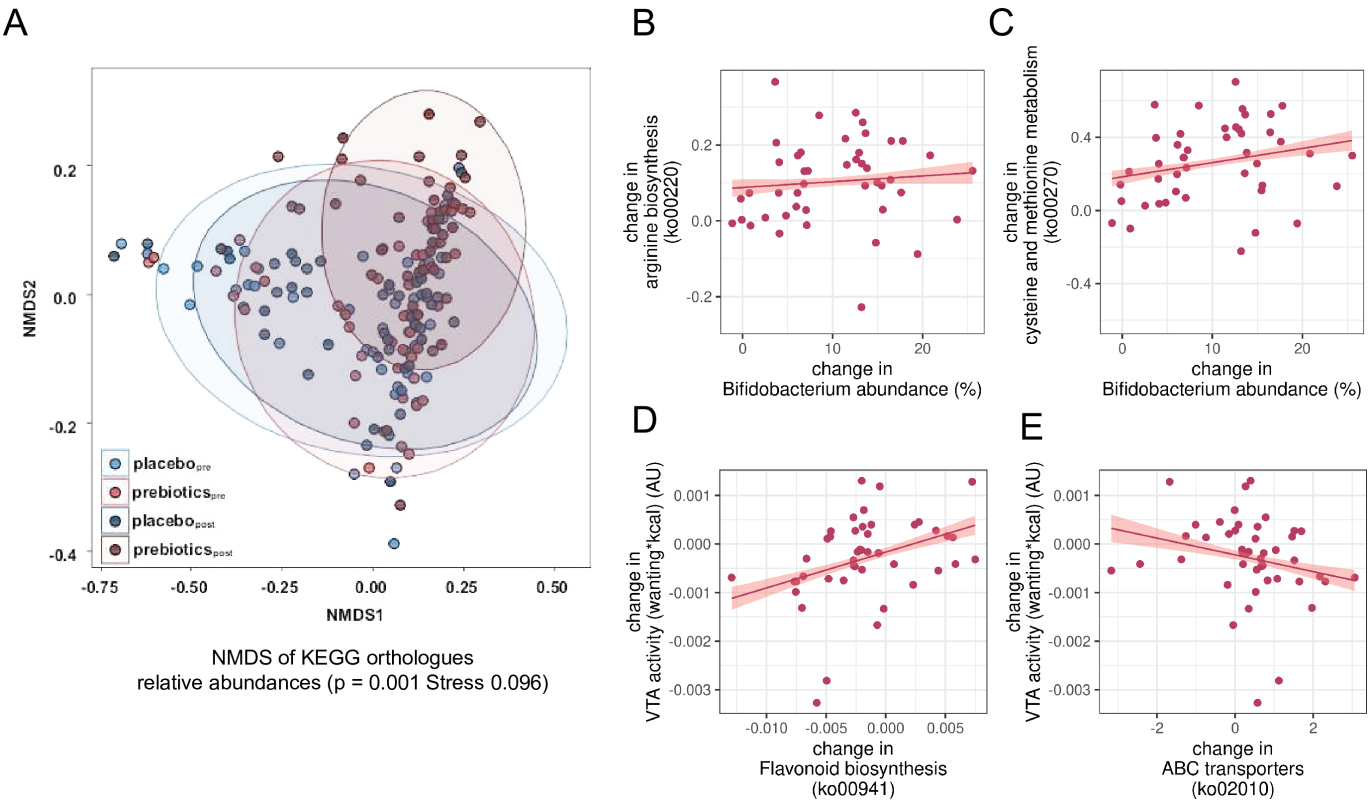

Figure 7 Predicted functional shifts and their correlations with changes in microbiota genera and in reward-related brain activation after prebiotic intervention (exploratory analyses). (A) Dissimilarity of functional composition of microbiome preprebiotic to versus postprebiotic intervention based on 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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Study details

All participants reported omnivorous, non-restrictive eating habits.
At all four test measurements, time-of-day for each participant was standardized to 07:15AM, 08:00AM, 09:15AM, 10:30AM or 11:15AM, respectively. Participants were instructed to come in overnight fasted.
Participants underwent task-based fMRI in a semi-satiated state after receiving a standardized protein shake (mean 159 kcal ± 10 SD for women, mean 206 kcal ± 16 SD for men) subsequent to overnight fasting (12.5 h ± 2.2 SD).
The protein shake comprised a plant-based drink with 10% of energy need [1] based on protein powder (Vegan Protein Neutral, Foodspring, Berlin, Germany) and oat drink (EDEKA BIO Hafer Drink Classic Vegan, Germany). The following formulas were used to provide the drink:

Energy basal metabolic rate(men) =
66.473+13.752xbodyweight[kg]+5.3xbodyheight[cm]-6.755xage[years]

Energy basal metabolic rate (women) =
655.96+9.563xbodyweight[kg]+1.85xbodyheight[cm]-4.676xage[years]

Intervention details

Participants were instructed to take one sachet in the morning and one at lunchtime in any preferred form. Compliance scores were not different for each of the supplements over two weeks or 48h before the follow-up appointment (full-null model comparison, both $b < -0.5$, $p > .09$).

Power analysis and sample size rationale

We did not find directly comparable studies in the literature. Two human studies reported changes in microbial composition due to a dietary change within 3-10 days in n = 11 and n = 22 participants, respectively [2, 3]. To simulate a dietary intervention effect on food wanting measured using task-based fMRI with a Likert scale, we used the effect size of the significant interaction effect of insulin-resistance vs. non-resistance on response to stimulus type (food vs. non-food) as a basis for a power calculation (Fig. 2a: $F(1,46)=5.49$; $p=0.02$, $\eta^2 = 0.12$, n = 48, rmANOVA; n = 48 young adults, [4]), comparing to an effect size of f = 0.37. According to outputs of the software G*Power, with a repeated measures ANOVA design to detect a significant difference of pre vs. post (2 measures) in the intervention compared to the placebo condition (2 groups) and a power of 0.95, alpha of 5% as well as conservative zero correlation between measures and no non-spheric correction, this yielded a sample size of n = 50. With estimating a 20% dropout-rate, we aimed to include 60 participants.

Blood parameters

Blood drawing was done using safety-multifly needles (21G, 200 mm) and BD Vacutainer Multiple Sample Luer Adapter and different monovettes (2x S-Monovette 9 ml Z-Gel, S-Monovette 2.7 ml FE for glucose, S-Monovette 2.7 ml K3E for whole blood, Greiner VACUETTE® TUBE 2.5 ml CAT Serum Separator Clot Activator for gut hormones). Gut hormones were collected in a 2.5 ml tube with instantly added inhibitors (25 µl DPP-IV inhibitor, Merck, Germany; 25 µl of dissolved Pefabloc ® SC (AEBSF), Roche, Germany), 30 min waiting time and then centrifuged with the other tubes. Blood samples were centrifuged at 3500 rpm at 7° C for 6 min and serum was aliquoted within 1 h of obtainment. Processed aliquots were stored at -80° C within 1 h of collection and further analyzed in one batch per marker. Analyses were conducted at Synevo Studien Service Labor GmbH c/o IMD Institut für Medizinische Diagnostik Berlin-Potsdam GbR, Berlin, Germany and the Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics (ILM) Leipzig University, Leipzig, Germany. Measurements beyond the lower detection threshold were set to half of the value of the lower bound (e.g. for hCRP if lower bound is <0.30, then value set to 0.15). Biologically implausible values were excluded from the analysis (in total 3 values: TMAO > 1000 ng/ml, ghrelin > 1250 pg/ml, CRP > 85 mg/l).

Medawar E, *et al. Gut* 2023;0:1–13. doi: 10.1136/gutjnl-2023-330365

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Baseline characteristics

While participants were considered healthy without clinical diagnosis of metabolic disorder at screening, a moderate proportion showed signs of impaired glucose tolerance (n = 10, insulin > 25mIU/l or glucose > 5.6mmol/l or glycated hemoglobin A1c, HbA1c, > 5.7) or hyperlipidemia (n = 17, total cholesterol > 250 mg/dl or low-density lipoprotein, LDL, > 130 mg/dl or triglycerides > 150 mg/dl), based on fasted blood levels at first baseline sessions (**SI Table 1** and **SI Fig. 1**). Few participants were on regular medication (anti-hypertensives: n = 1, L-thyroxine: n = 1, asthma medication: n = 1), and more than half on certain testing days only (n = 35 including painkillers (NSAIDs) or vitamins, in one case a single-dose antibiotic leading to drop-out), all females were on hormonal contraception (pill: n = 11, IUD: n = 1, vaginal ring: n = 1, NA: n = 6).

SI-Table 1: Questionnaire's baseline characteristics at study timepoint T0.

	n = 59	Mean (SD)	Median [Min, Max]
Barrat Impulsiveness Scale (BIS)	total	31.0 (5.72)	31.0 [19.0, 45.0]
	attentional	8.76 (2.17)	9.00 [5.00, 14.0]
	motor	10.8 (2.67)	10.0 [6.00, 17.0]
	non-planning	11.4 (2.60)	12.0 [6.00, 17.0]
Eating Disorder Examination Questionnaire (EDEQ)	Eating Concern	0.18 (0.27)	0.20 [0, 3.60]
	Restraint	0.52 (0.69)	0.20 [0, 3.60]
	Shape Concern	1.08 (0.98)	0.81 [0, 3.50]
	Weight Concern	0.87 (0.89)	0.60 [0, 3.60]
Three Factor Eating Questionnaire (TFEQ)	hunger	4.29 (3.23)	4.00 [0, 13.0]
	cognitive restraint	5.47 (3.68)	5.00 [0, 13.0]
	disinhibition	5.41 (2.44)	5.00 [1.00, 11.0]
Big Five Personality Questionnaire (NEO-FFI)	neuroticism	1.38 (0.60)	1.38 [0.17, 2.92]
	extraversion	2.43 (0.59)	2.46 [1.00, 3.58]
	openness	2.67 (0.49)	2.71 [1.42, 3.67]
	agreeableness	2.63 (0.53)	2.58 [1.25, 3.83]
	conscientiousness	2.67 (0.49)	2.71 [1.42, 3.75]
State-Trait Anxiety and Depression Inventory (STADI-T)	trait-dystymia	7.43 (2.42)	7.00 [5.00, 15.0]
	trait-emotionality	8.83 (2.04)	9.00 [5.00, 13.0]
	trait-euthymia	15.3 (3.28)	15.0 [6.00, 20.0]
	trait-worry	9.17 (3.08)	9.00 [5.00, 19.0]
Vienna Art Interest and Art Knowledge (VAIAK)	total	34.4 (14.5)	31.0 [13.0, 67.0]
World Health Organisation (WHO)-5 Well being	total	15.2 (4.85)	15.0 [3.00, 24.0]
Eurohis well-being	total	31.9 (5.02)	31.5 [16.0, 40.0]
Beckett Depression Inventory (BDI)	total	4.05 (4.24)	3.0 [0, 21.0]
Smoking status	Non-Smoker	51 (86.4%)	
	Smoker	7 (11.9%)	
	Missing	1 (1.7%)	
Mode of feeding as a child	Bottle-fed	5 (8.5%)	
	Brest-fed	46 (78%)	
	Unknown	7 (11.9%)	
	Missing	1 (1.7%)	
Mode of birth	Cesarian	8 (13.6%)	
	Vaginal	47 (79.7%)	
	Unknown	3 (5.1%)	
	Missing	1 (1.7%)	

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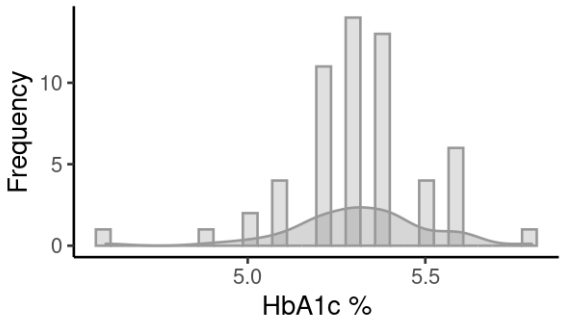

SI-Fig. 1: Distribution of serum glycated hemoglobin A1c (HbA1c) levels (%) at baseline**.**

Results – Descriptives for change in anthropometrics and biomarkers.

SI-Table 2: Anthropometric markers for all timepoints by intervention condition.

	prebiotics		placebo	
	BL	FU	BL	FU
	(N=55)	(N=47)	(N=53)	(N=49)
BMI [kg/m²]				
Mean (SD)	27.2 (1.50)	27.3 (1.62)	27.4 (1.61)	27.3 (1.67)
Median [Min, Max]	27.1 [24.5, 30.2]	27.2 [24.2, 30.6]	27.3 [25.0, 31.2]	27.1 [24.9, 31.7]
Fat mass [%]				
Mean (SD)	26.2 (6.49)	26.2 (6.24)	27.0 (6.66)	26.0 (6.46)
Median [Min, Max]	24.8 [7.59, 38.5]	24.9 [10.6, 39.0]	26.7 [9.53, 41.6]	25.2 [7.76, 38.9]
Missing	0 (0%)	0 (0%)	1 (1.9%)	0 (0%)
Fat mass gender-standardized [%]				
Mean (SD)	-0.0671 (0.975)	-0.00729 (0.942)	0.109 (1.05)	-0.0727 (1.03)
Median [Min, Max]	-0.0108 [-3.48, 2.41]	-0.154 [-2.82, 2.51]	0.0217 [-3.05, 2.54]	-0.156 [-3.44, 2.40]
Missing	0 (0%)	0 (0%)	1 (1.9%)	0 (0%)
Fat-free mass gender-standardized [kg]				
Mean (SD)	-0.0269 (1.00)	-0.00723 (0.953)	-0.0212 (0.973)	0.0546 (1.02)
Median [Min, Max]	-0.220 [-1.94, 4.47]	-0.0759 [-2.11, 3.18]	-0.198 [-2.13, 3.80]	-0.238 [-2.18, 4.02]
Missing	0 (0%)	0 (0%)	1 (1.9%)	0 (0%)
Waist-to-hip ratio				
Mean (SD)	0.820 (0.0540)	0.816 (0.0618)	0.821 (0.0563)	0.814 (0.0550)
Median [Min, Max]	0.811 [0.700, 0.942]	0.816 [0.694, 0.970]	0.824 [0.686, 0.980]	0.809 [0.712, 0.981]
10% of daily energy requirement [kcal]				
Mean (SD)	191 (26.2)	194 (26.8)	193 (26.0)	192 (26.6)
Median [Min, Max]	194 [142, 249]	196 [141, 245]	194 [141, 246]	194 [141, 247]
Missing	0 (0%)	1 (2.1%)	0 (0%)	0 (0%)

SI-Table 3: Serum markers for all timepoints by intervention condition.

	prebiotics		placebo	
	BL	FU	BL	FU
	(N=55)	(N=47)	(N=53)	(N=49)

Time fasted [h]				
Mean (SD)	12.5 (2.25)	12.3 (1.70)	12.5 (2.25)	12.3 (1.70)
Median [Min, Max]	12.3 [6.00, 18.0]	12.0 [6.50, 15.0]	12.3 [6.00, 18.0]	12.0 [6.50, 15.0]
Missing	1 (1.8%)	0 (0%)	1 (1.8%)	0 (0%)
Triglycerides [mg/dl]				
Mean (SD)	103 (47.3)	98.0 (47.5)	103 (47.3)	98.0 (47.5)
Median [Min, Max]	92.0 [40.0, 285]	89.0 [26.0, 229]	92.0 [40.0, 285]	89.0 [26.0, 229]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cholesterol [mg/dl]				
Mean (SD)	170 (27.9)	173 (41.8)	170 (27.9)	173 (41.8)
Median [Min, Max]	166 [120, 259]	164 [112, 345]	166 [120, 259]	164 [112, 345]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
LDL [mg/dl]				
Mean (SD)	97.7 (24.5)	102 (33.9)	97.7 (24.5)	102 (33.9)
Median [Min, Max]	94.0 [35.0, 160]	97.0 [44.0, 236]	94.0 [35.0, 160]	97.0 [44.0, 236]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
HDL [mg/dl]				
Mean (SD)	50.3 (11.0)	49.9 (12.9)	50.3 (11.0)	49.9 (12.9)
Median [Min, Max]	51.0 [25.0, 77.0]	48.0 [27.0, 79.0]	51.0 [25.0, 77.0]	48.0 [27.0, 79.0]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Leptin [ng/ml]				
Mean (SD)	11.6 (11.7)	11.8 (11.3)	11.6 (11.7)	11.8 (11.3)
Median [Min, Max]	6.10 [0.100, 51.9]	7.40 [0.100, 40.7]	6.10 [0.100, 51.9]	7.40 [0.100, 40.7]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Insulin [uU/ml]				
Mean (SD)	10.5 (6.64)	10.5 (7.94)	10.5 (6.64)	10.5 (7.94)
Median [Min, Max]	8.80 [3.20, 34.2]	8.50 [3.20, 54.4]	8.80 [3.20, 34.2]	8.50 [3.20, 54.4]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glucose [mmol/ml]				
Mean (SD)	4.97 (0.457)	5.02 (0.444)	4.97 (0.457)	5.02 (0.444)
Median [Min, Max]	4.96 [4.05, 6.54]	4.97 [4.03, 6.67]	4.96 [4.05, 6.54]	4.97 [4.03, 6.67]
Missing	0 (0%)	2 (4.3%)	0 (0%)	2 (4.3%)
Ghrelin [pg/ml]				
Mean (SD)	147 (107)	148 (102)	147 (107)	148 (102)
Median [Min, Max]	132 [4.15, 505]	144 [4.83, 393]	132 [4.15, 505]	144 [4.83, 393]
Missing	2 (3.6%)	2 (4.3%)	2 (3.6%)	2 (4.3%)
GLP-1 [pg/ml]				
Mean (SD)	117 (51.5)	116 (49.1)	117 (51.5)	116 (49.1)
Median [Min, Max]	110 [1.30, 234]	108 [39.8, 245]	110 [1.30, 234]	108 [39.8, 245]
Missing	2 (3.6%)	2 (4.3%)	2 (3.6%)	2 (4.3%)
PYY [pg/ml]				
Mean (SD)	56.3 (59.9)	58.9 (58.3)	56.3 (59.9)	58.9 (58.3)
Median [Min, Max]	45.5 [6.80, 235]	46.5 [6.80, 299]	45.5 [6.80, 235]	46.5 [6.80, 299]
Missing	2 (3.6%)	2 (4.3%)	2 (3.6%)	2 (4.3%)
IL-6 [pg/ml]				
Mean (SD)	1.56 (2.08)	1.11 (0.360)	1.56 (2.08)	1.11 (0.360)
Median [Min, Max]	1.00 [1.00, 12.0]	1.00 [1.00, 2.30]	1.00 [1.00, 12.0]	1.00 [1.00, 2.30]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TNF-alpha [pg/ml]				
Mean (SD)	5.94 (1.79)	6.12 (1.84)	5.94 (1.79)	6.12 (1.84)
Median [Min, Max]	6.00 [2.00, 10.2]	6.10 [2.00, 9.90]	6.00 [2.00, 10.2]	6.10 [2.00, 9.90]
Missing	0 (0%)	0 (0%)	0 (0%)	0 (0%)
HCRP [mg/l]				
Mean (SD)	3.13 (4.82)	2.62 (3.92)	3.13 (4.82)	2.62 (3.92)
Median [Min, Max]	1.39 [0.150, 27.6]	1.51 [0.150, 24.3]	1.39 [0.150, 27.6]	1.51 [0.150, 24.3]
Missing	1 (1.8%)	0 (0%)	1 (1.8%)	0 (0%)
TMAO [ng/ml]				

Mean (SD)	281 (210)	234 (231)	281 (210)	234 (231)
Median [Min, Max]	216 [54.0, 944]	191 [14.0, 1130]	216 [54.0, 944]	191 [14.0, 1130]
Missing	0 (0%)	1 (2.1%)	0 (0%)	1 (2.1%)
Tryptophan [umol/l]				
Mean (SD)	33.1 (7.66)	33.9 (8.48)	33.1 (7.66)	33.9 (8.48)
Median [Min, Max]	31.7 [20.2, 58.4]	32.5 [20.9, 58.7]	31.7 [20.2, 58.4]	32.5 [20.9, 58.7]
Missing	2 (3.6%)	3 (6.4%)	2 (3.6%)	3 (6.4%)
Tyrosine [umol/l]				
Mean (SD)	52.6 (11.8)	54.6 (10.4)	52.6 (11.8)	54.6 (10.4)
Median [Min, Max]	53.1 [25.5, 79.1]	55.2 [27.7, 73.3]	53.1 [25.5, 79.1]	55.2 [27.7, 73.3]
Missing	0 (0%)	3 (6.4%)	0 (0%)	3 (6.4%)
ALAT [ukat/l]				
Mean (SD)	0.381 (0.136)	0.473 (0.312)	0.381 (0.136)	0.473 (0.312)
Median [Min, Max]	0.370 [0.190, 0.790]	0.395 [0.200, 1.96]	0.370 [0.190, 0.790]	0.395 [0.200, 1.96]
Missing	0 (0%)	1 (2.1%)	0 (0%)	1 (2.1%)
ASAT [ukat/l]				
Mean (SD)	0.395 (0.0769)	0.425 (0.132)	0.395 (0.0769)	0.425 (0.132)
Median [Min, Max]	0.390 [0.260, 0.640]	0.400 [0.220, 0.970]	0.390 [0.260, 0.640]	0.400 [0.220, 0.970]
Missing	0 (0%)	1 (2.1%)	0 (0%)	1 (2.1%)
TSH [mU/l]				
Mean (SD)	62.5 (223)	131 (290)	62.5 (223)	131 (290)
Median [Min, Max]	1.94 [1.01, 959]	2.13 [1.09, 956]	1.94 [1.01, 959]	2.13 [1.09, 956]
Missing	0 (0%)	1 (2.1%)	0 (0%)	1 (2.1%)
Creatinine [umol/l]				
Mean (SD)	81.4 (12.3)	82.2 (13.5)	81.4 (12.3)	82.2 (13.5)
Median [Min, Max]	82.0 [56.0, 104]	82.5 [54.0, 109]	82.0 [56.0, 104]	82.5 [54.0, 109]
Missing	0 (0%)	1 (2.1%)	0 (0%)	1 (2.1%)

Anthropometric markers did not significantly change across measurement timepoints, i.e. BMI, gender-standardized waist-to-hip ratio, fat-free mass and blood pressure (interaction timepoint*intervention, $p_{all} > 0.05$), except for gender-standardized fat mass (%), which increased significantly after prebiotic intake (interaction timepoint*intervention, b = 0.16, p = 0.001). All models were adjusted for age, gender, and person and intervention*timepoint as random factors. Both intervention and placebo supplements contained the same amounts of calories and participants reported equally high compliance in taking the daily supplements. Blood marker analyses were adjusted for age, gender, individual and intervention*timepoint as random factors, time of day at blood withdrawal and time fasted.

Results – Linear mixed model results for changes in anthropometric biomarkers.

SI-Table 4: Mixed effects linear model results on anthropometric markers for post-prebiotic intervention.

	n_{obs}	n_{subj}	fixed effects	estimate	SE	t-value	full-null model comparison p
BMI	204	59	(intercept)	28.02	0.88	31.83	
			time (follow-up)	-0.09	0.06	-1.61	
			intervention (prebiotic)	-0.11	0.07	-1.57	
			age	-0.04	0.03	-1.21	
			gender (male)	0.55	0.43	1.28	
			time (follow-up) * intervention (prebiotic)	0.16	0.08	2.05	0.06
Waist-to-hip ratio	204	59	(intercept)	0.68	0.02	29.68	
			time (follow-up)	-0.01	0.01	-1.61	

		intervention (prebiotic)	0.00	0.01	-0.43	
		age	0.00	0.00	3.80	
		gender (male)	0.08	0.01	7.18	
		time (follow-up) * intervention (prebiotic)	0.00	0.01	0.13	0.89
% Fat mass gender-standardized	203 59	(intercept)	0.01	0.56	0.01	
		time (follow-up)	-0.11	0.04	-2.93	
		intervention (prebiotic)	-0.11	0.05	-2.35	
		age	0.00	0.02	0.09	
		gender (male)	0.05	0.02	0.09	
		time (follow-up) * intervention (prebiotic)	**0.16****	**0.05**	**2.92**	**0.005**
Fat mass gender-standardized [kg]	203 59	(intercept)	0.07	0.56	0.13	
		time (follow-up)	-0.09	0.03	-3.03	
		intervention (prebiotic)	-0.10	0.04	-2.62	
		age	0.00	0.02	-0.08	
		gender (male)	0.05	0.27	0.18	
		time (follow-up) * intervention (prebiotic)	**0.13****	**0.04**	**3.28**	**0.001**
Fat-free mass gender-standardized [kg]	203 59	(intercept)	0.32	0.56	0.58	
		time (follow-up)	0.04	0.04	1.10	
		intervention (prebiotic)	0.02	0.04	0.63	
		age	-0.02	0.02	-0.75	
		gender (male)	0.03	0.28	0.09	
		time (follow-up) * intervention (prebiotic)	-0.04	0.05	-0.84	0.39
10% of daily energy requirement	203 59	(intercept)	180.89	7.64	23.69	
		time (follow-up)	-1.35	0.76	-1.78	
		intervention (prebiotic)	-0.82	0.87	-0.94	
		age	-0.86	0.27	-3.18	
		gender (male)	50.94	3.76	13.57	
		time (follow-up) * intervention (prebiotic)	1.63	0.94	1.74	0.08

*Formula: variable_of_interest ~ intervention * timepoint + (1+(timepoint+intervention)|subject)+ age + gender. REML criterion at convergence > 158. Significance, *** p < 0.001.*

SI-Table 5: Mixed effects linear model results on serum markers for post-prebiotic intervention.

	n_{obs}	n_{subj}	fixed effects	estimate	SE	t-value	full-null model comparison p
hsCRP	195	58	(intercept)	6.80	2.27	3.00	
			time (follow-up)	0.39	0.60	0.64	
			intervention (prebiotic)	0.47	0.60	0.78	
			age	-0.11	0.07	-1.61	
			gender (male)	-3.90	0.95	-4.12	
			time of day (8:00 AM)	-1.54	1.826	-0.84	
			time of day (9:15 AM)	-0.06	1.02	-0.06	
			time of day (10:30 AM)	-1.56	1.85	-0.84	
			time of day (11:15 AM)	0.92	1.24	0.74	
			time fasted (hours)	0.16	0.12	1.30	
			time (follow-up) * intervention (prebiotic)	-0.80	0.67	-1.19	0.26
IL-6	196	58	(intercept)	1.26	0.85	1.49	
			time (follow-up)	0.21	0.30	0.70	
			intervention (prebiotic)	0.27	0.29	0.92	
			age	-0.004	0.02	-0.21	
			gender (male)	0.28	0.28	0.99	
			time of day (8:00 AM)	-0.86	0.53	-1.63	
			time of day (9:15 AM)	-0.52	0.29	-1.78	
			time of day (10:30 AM)	-0.50	0.59	-0.85	
			time of day (11:15 AM)	-0.73	0.38	-1.92	
			time fasted (hours)	0.03	0.06	0.59	
			time (follow-up) * intervention (prebiotic)	-0.71	0.38	-1.89	0.06
TNF	196	58	(intercept)	3.13	1.09	2.86	
			time (follow-up)	0.01	0.21	0.05	
			intervention (prebiotic)	0.04	0.26	0.16	
			age	0.04	0.03	1.24	
			gender (male)	0.68	0.45	1.52	
			time of day (8:00 AM)	0.87	0.85	1.03	
			time of day (9:15 AM)	-0.06	0.48	-0.12	
			time of day (10:30 AM)	-0.10	0.89	-0.11	
			time of day (11:15 AM)	0.25	0.60	0.42	
			time fasted (hours)	0.09	0.06	1.60	
			time (follow-up) * intervention (prebiotic)	0.18	0.29	0.62	0.53
HDL	197	58	(intercept)	62.06	6.21	9.99	
			time (follow-up)	-0.27	0.93	-0.29	
			intervention (prebiotic)	0.69	0.80	0.87	
			age	-0.22	0.21	-1.03	
			gender (male)	-11.61	2.86	-4.07	
			time of day (8:00 AM)	0.66	5.45	0.12	
			time of day (9:15 AM)	1.51	3.11	0.48	
			time of day (10:30 AM)	-2.46	5.52	-0.45	

			time of day (11:15 AM)	2.89	3.58	0.81	
			time fasted (hours)	0.04	0.21	0.18	
			time (follow-up) * intervention (prebiotic)	-0.36	1.02	-0.35	0.73
LDL	197	58	(intercept)	87.13	17.34	5.03	
			time (follow-up)	-4.63	2.64	-1.75	
			intervention (prebiotic)	-1.74	2.08	-0.84	
			age	0.21	0.590	0.36	
			gender (male)	3.85	8.00	0.48	
			time of day (8:00 AM)	1.34	15.26	0.09	
			time of day (9:15 AM)	9.36	8.68	1.08	
			time of day (10:30 AM)	12.39	15.47	0.80	
			time of day (11:15 AM)	12.99	9.84	1.32	
			time fasted (hours)	-0.24	0.55	-0.43	
			time (follow-up) * intervention (prebiotic)	**10.30****	**2.96**	**3.48**	**0.00059**
LDL/ HDL	197	58	(intercept)	1.10	0.49	2.26	
			time (follow-up)	-0.08	0.05	-1.61	
			intervention (prebiotic)	-0.07	0.05	-1.34	
			age	0.03	0.02	1.73	
			gender (male)	0.48	0.23	2.08	
			time of day (8:00 AM)	-0.30	0.44	-0.67	
			time of day (9:15 AM)	-0.04	0.25	-0.15	
			time of day (10:30 AM)	0.20	0.45	0.45	
			time of day (11:15 AM)	0.03	0.28	0.10	
			time fasted (hours)	-0.01	0.01	-0.67	
			time (follow-up) * intervention (prebiotic)	**0.24*****	**0.07**	**3.54**	**0.0005**
Triglycerides	197	58	(intercept)	69.26	32.55	2.13	
			time (follow-up)	-9.48	6.96	-1.36	
			intervention (prebiotic)	-4.52	6.90	-0.66	
			age	1.76	0.95	1.86	
			gender (male)	-12.28	12.95	-0.95	
			time of day (8:00 AM)	-33.64	24.54	-1.37	
			time of day (9:15 AM)	-19.30	14.07	-1.37	
			time of day (10:30 AM)	-13.25	26.05	-0.51	
			time of day (11:15 AM)	-33.43	17.54	-1.91	
			time fasted (hours)	1.16	1.60	0.73	
			time (follow-up) * intervention (prebiotic)	4.34	9.56	0.45	0.64
Cholesterol	197	58	(intercept)	154.21	20.13	7.66	
			time (follow-up)	-6.80	3.06	-2.22	
			intervention (prebiotic)	-1.34	2.73	-0.49	
			age	0.67	0.67	1.01	
			gender (male)	-10.10	9.11	-1.11	
			time of day (8:00 AM)	2.01	17.31	0.12	
			time of day (9:15 AM)	8.99	9.91	0.91	

			time of day (10:30 AM)	5.16	17.75	0.29	
			time of day (11:15 AM)	3.55	11.57	0.31	
			time fasted (hours)	0.02	0.68	0.02	
			time (follow-up) * intervention (prebiotic)	**10.81****	**3.75**	**2.88**	**0.004**
Cholesterol (without sub-47)	196	58	(intercept)	141.17	18.84	7.49	
			time (follow-up)	-7.14	2.78	-2.57	
			intervention (prebiotic)	-0.66	2.70	-0.24	
			age	0.91	0.62	1.48	
			gender (male)	-6.07	8.39	-0.72	
			time of day (8:00 AM)	-0.69	15.74	-0.04	
			time of day (9:15 AM)	3.21	9.13	0.35	
			time of day (10:30 AM)	4.20	16.17	0.26	
			time of day (11:15 AM)	1.37	10.64	0.13	
			time fasted (hours)	0.36	0.67	0.53	
			time (follow-up) * intervention (prebiotic)	**9.37***	**3.70**	**2.53**	**0.01**
Insulin	197	58	(intercept)	13.54	3.28	4.14	
			time (follow-up)	-0.20	0.73	-0.27	
			intervention (prebiotic)	0.81	0.81	1.00	
			age	-0.19	0.10	-1.85	
			gender (male)	0.24	1.39	0.17	
			time of day (8:00 AM)	-2.45	2.49	-0.94	
			time of day (9:15 AM)	-0.81	1.48	-0.55	
			time of day (10:30 AM)	-0.18	2.73	-0.07	
			time of day (11:15 AM)	-0.50	1.76	-0.28	
			time fasted (hours)	0.16	0.17	0.96	
			time (follow-up) * intervention (prebiotic)	-0.01	1.02	-0.01	0.997
Ghrelin	193	58	(intercept)	217.05	60.04	3.62	
			time (follow-up)	15.34	14.29	1.07	
			intervention (prebiotic)	-8.71	13.56	-0.64	
			age	1.01	1.66	0.61	
			gender (male)	-91.16	23.06	-3.95	
			time of day (8:00 AM)	94.14	42.97	2.19	
			time of day (9:15 AM)	27.56	24.43	1.13	
			time of day (10:30 AM)	-17.24	46.70	-0.37	
			time of day (11:15 AM)	11.70	30.61	0.38	
			time fasted (hours)	-3.94	3.16	-1.25	
			time (follow-up) * intervention (prebiotic)	-8.75	18.97	-0.46	0.64
GLP-1	194	58	(intercept)	145.20	29.69	4.89	
			time (follow-up)	-0.47	6.00	-0.08	
			intervention (prebiotic)	2.98	6.64	0.45	
			age	-0.97	0.88	-1.11	
			gender (male)	26.64	12.00	2.22	
			time of day (8:00 AM)	-59.91	22.67	-2.64	

			time of day (9:15 AM)	-33.30	12.91	-2.58	
			time of day (10:30 AM)	-28.88	23.95	-1.21	
			time of day (11:15 AM)	-22.93	15.91	-1.44	
			time fasted (hours)	0.26	1.50	0.17	
			time (follow-up) * intervention (prebiotic)	-2.68	8.55	-0.31	0.75
PYY	194	58	(intercept)	35.52	30.47	1.17	
			time (follow-up)	-2.61	6.50	-0.40	
			intervention (prebiotic)	1.65	8.04	0.21	
			age	1.01	0.88	1.16	
			gender (male)	1.43	12.02	0.12	
			time of day (8:00 AM)	-39.52	22.63	-1.75	
			time of day (9:15 AM)	-14.45	12.80	-1.13	
			time of day (10:30 AM)	0.92	24.17	0.04	
			time of day (11:15 AM)	-26.48	15.87	-1.67	
			time fasted (hours)	0.07	1.66	0.04	
			time (follow-up) * intervention (prebiotic)	4.57	8.40	0.54	0.58
Glucose	195	58	(intercept)	4.61	0.25	18.71	
			time (follow-up)	-0.04	0.05	-0.85	
			intervention (prebiotic)	0.04	0.05	0.68	
			age	0.01	0.01	1.33	
			gender (male)	0.18	0.11	1.73	
			time of day (8:00 AM)	-0.02	0.20	-0.12	
			time of day (9:15 AM)	-0.14	0.11	-1.23	
			time of day (10:30 AM)	0.03	0.21	0.13	
			time of day (11:15 AM)	-0.16	0.14	-1.14	
			time fasted (hours)	0.00	0.01	0.01	
			time (follow-up) * intervention (prebiotic)	0.05	0.07	0.71	0.47
Leptin	197	58	(intercept)	38.51	4.51	8.55	
			time (follow-up)	-0.10	0.81	-0.13	
			intervention (prebiotic)	-0.52	0.76	-0.68	
			age	-0.32	0.15	-2.20	
			gender (male)	-18.59	2.03	-9.17	
			time of day (8:00 AM)	-4.16	3.84	-1.08	
			time of day (9:15 AM)	1.10	2.22	0.50	
			time of day (10:30 AM)	-5.80	3.92	-1.48	
			time of day (11:15 AM)	-0.24	2.59	-0.09	
			time fasted (hours)	-0.31	0.18	-1.72	
			time (follow-up) * intervention (prebiotic)	1.01	1.08	0.94	0.34
Betain	195	58	(intercept)	1.95	0.65	3.00	
			time (follow-up)	-0.02	0.12	-0.18	
			intervention (prebiotic)	0.15	0.12	1.23	
			age	0.03	0.02	1.36	
			gender (male)	1.83	0.28	6.52	

			time of day (8:00 AM)	0.35	0.53	0.66	
			time of day (9:15 AM)	-0.27	0.31	-0.89	
			time of day (10:30 AM)	-1.12	0.55	-2.03	
			time of day (11:15 AM)	-0.19	0.37	-0.51	
			time fasted (hours)	-0.04	0.03	-1.33	
			time (follow-up) * intervention (prebiotic)	-0.05	0.15	-0.35	0.71
Carnitin	195	58	(intercept)	3.76	0.82	4.58	
			time (follow-up)	0.14	0.14	0.97	
			intervention (prebiotic)	-0.01	0.15	-0.01	
			age	0.05	0.03	1.89	
			gender (male)	2.51	0.35	7.26	
			time of day (8:00 AM)	-0.30	0.66	-0.46	
			time of day (9:15 AM)	-0.13	0.38	-0.34	
			time of day (10:30 AM)	-0.49	0.69	-0.71	
			time of day (11:15 AM)	-0.84	0.46	-1.81	
			time fasted (hours)	-0.02	0.03	-0.53	
			time (follow-up) * intervention (prebiotic)	0.03	0.20	0.13	0.88
Cholin	195	58	(intercept)	0.74	0.11	6.52	
			time (follow-up)	-0.02	0.03	-0.72	
			intervention (prebiotic)	0.02	0.03	0.81	
			age	0.005	0.003	1.74	
			gender (male)	0.10	0.05	2.25	
			time of day (8:00 AM)	-0.06	0.09	-0.72	
			time of day (9:15 AM)	-0.07	0.05	-1.37	
			time of day (10:30 AM)	-0.16	0.09	-1.78	
			time of day (11:15 AM)	0.01	0.06	0.06	
			time fasted (hours)	-0.003	0.01	-0.57	
			time (follow-up) * intervention (prebiotic)	0.02	0.04	0.43	0.67
TMAO	194	58	(intercept)	69.92	96.55	0.72	
			time (follow-up)	16.48	30.85	0.53	
			intervention (prebiotic)	81.23	33.58	2.42	
			age	1.53	2.54	0.60	
			gender (male)	-22.01	34.24	-0.64	
			time of day (8:00 AM)	9.28	61.65	0.15	
			time of day (9:15 AM)	-34.20	35.82	-0.96	
			time of day (10:30 AM)	-188.38	69.32	-2.72	
			time of day (11:15 AM)	25.92	45.09	0.58	
			time fasted (hours)	9.90	6.39	1.55	
			time (follow-up) * intervention (prebiotic)	-71.71	42.44	-1.69	0.09
Tryptophan	188	57	(intercept)	30.33	4.56	6.66	
			time (follow-up)	-0.65	1.04	-0.63	
			intervention (prebiotic)	-1.06	1.07	-0.99	
			age	0.10	0.14	0.73	

			gender (male)	2.73	1.86	1.47	
			time of day (8:00 AM)	7.88	3.46	2.28	
			time of day (9:15 AM)	0.21	1.98	0.11	
			time of day (10:30 AM)	10.01	3.63	2.76	
			time of day (11:15 AM)	-0.49	2.49	-0.20	
			time fasted (hours)	-0.17	0.25	-0.68	
			time (follow-up) * intervention (prebiotic)	1.51	1.45	1.05	0.28
Tryptophan/LNAA	187	57	(intercept)	110.5	17.25	6.405	
			time (follow-up)	3.447	3.17	1.086	
			intervention (prebiotic)	2.380	3.33	0.714	
			age	0.333	0.56	0.596	
			gender (male)	-12.36	7.65	-1.616	
			time of day (8:00 AM)	39.95	14.27	2.800	
			time of day (9:15 AM)	6.486	8.18	0.793	
			time of day (10:30 AM)	61.77	14.79	4.178	
			time of day (11:15 AM)	7.551	9.927	0.761	
			time fasted (hours)	0.049	0.077	0.063	
			time (follow-up) * intervention (prebiotic)	-4.704	4.25	-1.108	0.27
Tyrosine	193	58	(intercept)	60.59	5.98	10.14	
			time (follow-up)	-0.46	1.55	-0.30	
			intervention (prebiotic)	-2.06	1.56	-1.32	
			age	0.28	0.16	1.74	
			gender (male)	10.78	2.21	4.87	
			time of day (8:00 AM)	-0.05	4.21	-0.01	
			time of day (9:15 AM)	-4.34	2.40	-1.81	
			time of day (10:30 AM)	-7.59	4.49	-1.69	
			time of day (11:15 AM)	-7.91	3.07	-2.58	
			time fasted (hours)	-1.41	0.34	-4.14	
			time (follow-up) * intervention (prebiotic)	1.63	2.15	0.76	0.44
Tyrosine/LNAA	187	57	(intercept)	0.230	0.022	10.608	
			time (follow-up)	0.003	0.005	0.677	
			intervention (prebiotic)	-0.002	0.005	-0.442	
			age	0.002	0.001	2.289	
			gender (male)	-0.003	0.009	-0.335	
			time of day (8:00 AM)	0.020	0.018	1.152	
			time of day (9:15 AM)	-0.003	0.010	-0.250	
			time of day (10:30 AM)	-0.013	0.018	-0.714	
			time of day (11:15 AM)	-0.011	0.012	-0.906	
			time fasted (hours)	-0.004	0.001	-4.031	
			time (follow-up) * intervention (prebiotic)	-0.004	0.006	-0.604	0.55
ASAT	196	58	(intercept)	0.39	0.08	5.08	
			time (follow-up)	0.06	0.03	1.95	
			intervention (prebiotic)	-0.004	0.03	-0.13	

		age	-0.001	0.002	-0.49	
		gender (male)	0.08	0.03	2.85	
		time of day (8:00 AM)	0.02	0.05	0.43	
		time of day (9:15 AM)	-0.02	0.03	-0.53	
		time of day (10:30 AM)	0.06	0.05	1.16	
		time of day (11:15 AM)	-0.002	0.04	-0.05	
		time fasted (hours)	-0.001	0.01	-0.22	
		time (follow-up) * intervention (prebiotic)	-0.03	0.04	-0.88	0.37
ALAT	196 58	(intercept)	0.26	0.10	2.59	
		time (follow-up)	0.003	0.03	0.012	
		intervention (prebiotic)	-0.02	0.03	-0.78	
		age	0.0002	0.003	0.07	
		gender (male)	0.20	0.04	5.19	
		time of day (8:00 AM)	0.03	0.07	0.40	
		time of day (9:15 AM)	-0.02	0.04	-0.44	
		time of day (10:30 AM)	0.11	0.08	1.49	
		time of day (11:15 AM)	-0.05	0.05	-0.96	
		time fasted (hours)	0.001	0.01	0.16	
		time (follow-up) * intervention (prebiotic)	**0.09**	**0.04**	**2.47**	**0.013**

*Formula: marker_of_interest ~ intervention * timepoint + (1 + (timepoint+intervention)| subject) + age + gender + time_of_day + time_fasted. REML criterion at convergence > 700. Significance, *** p < 0.001.*

References

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Supplementary Information on fMRI results

MRI assessments

MRI acquisition. Anatomical MRI was acquired using a T1-weighted MPRAGE sequence using the ADNI protocol with the following parameters: TR = 2300ms; TE = 2.98ms; flip angle = 9°; FOV: (256 mm)²; voxel size: (1.0mm)³; 176 slices. Task-based fMRI was acquired using T2*-weighted images: EPI BOLD: repetition time TR = 2000ms, echo time TE = 23.60ms, flip angle FA = 80°C, field-of-view FOV = (204 mm)^2; voxel size 2 x 2 x 2 mm^3; 60 slices; gap 0.26mm; orientation T>C -15°; multi-band = 3, interleaved. Field maps and ap/pa were acquired to be used for correcting scanner inhomogeneities in the preprocessing pipeline.

Stimuli and sessions. Stimuli for the fMRI task (wanting) and subsequent behavioral tasks (liking) were taken from validated databases, including food and art databases [1, 2, 3]. In total, for the wanting task 640 stimuli (320 food, 320 art) were chosen and split to 4 sets, in a randomized order over four sessions within each participant, matched by calorie content quartiles. Also, the four parallel versions of image sets matched in number of animals, plants, object stimuli and in low-level image characteristics (red, blue, green, object size, contrast: pall > 0.05). All stimuli were also included in the liking task. Stimuli size was normed to 600x450 pixels and presented on white background in Presentation (R) version 16.2 0.13.17 (Windows XP) using a mirror mounted display. Stimuli order was limited to maximally three stimuli of one group (food or art) in a row, and order was randomized per subject across all sessions.

Stimuli characteristics were derived from the databases including low-level characteristics, nutrient content (kcal / 100g for food only) and high-level characteristics based on normative ratings (i.e. population craving). We extended nutritional information and added (grams of fiber / 100g) based on the mean of four independent raters (inter-rater reliability ICC 0.76) and item type (1=Dairy & Eggs, 2=Fruits, 3=Vegetables, 4=Confectionery & Sweets, 5=Bakery Wares & Cereals, 6=Meat, 7=Fish, 8=Beverages, 9=Ready-to-eat Savories, 10=Prepared) (for details see [4]).

One session consisted of one block (with two 30s breaks, about 31 min), each with 160 stimuli, i.e. 80 food and 80 art images. The original German rating questions were: “Wie sehr möchten Sie dies jetzt haben?”. Ratings were acquired using a two-button box with the index and middle finger to move a cursor with a trial-by-trial random position. Orientation of the rating scale was randomized across subjects to left-to-right (1-8) or right-to-left (8-to-1). The original German label for 1 was “überhaupt nicht” and for 8 “unbedingt”. The reward item was randomly chosen for all equally high rated stimuli across one domain for maximal ratings by the study staff.

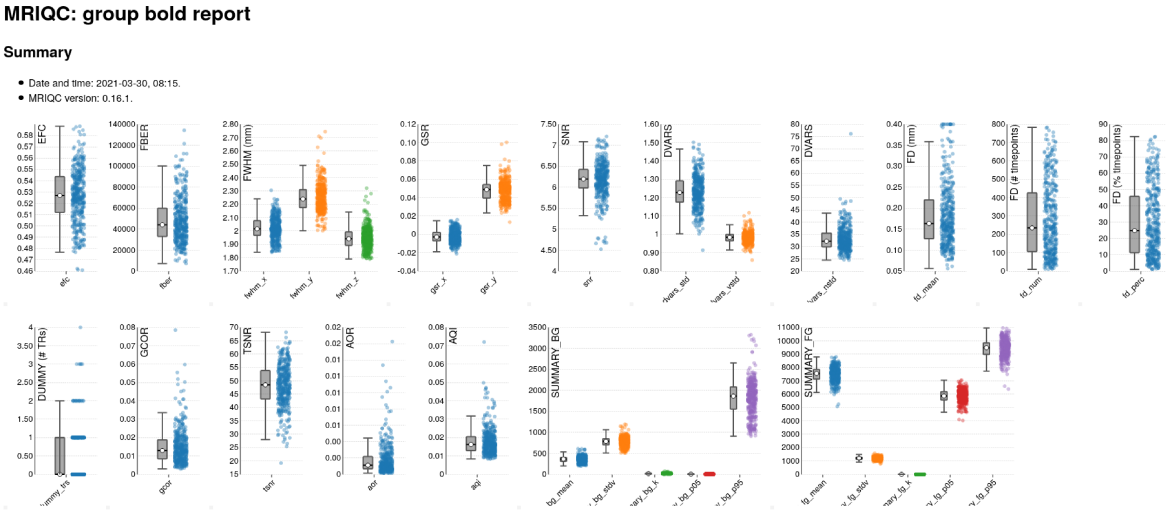
Task instructions were given verbally outside the scanner, followed by a test task inside the scanner (only on the first testing day), where participants were able to ask comprehension questions, followed by written instructions right before the task. [Detailed instructions in German: "Gleich startet die Bewertungsaufgabe. Zur Erinnerung: Eines der am höchsten bewerteten Lebensmittel bekommen Sie zum DIREKTEN Verzehr. Einen der am höchsten bewerteten Kunstdrucke bekommen Sie als Ausdruck zum Mitnehmen. Beachten Sie: 1. Ihre Antwort wird nur gezählt, wenn Sie den grünen Balken MINDESTENS EINMAL bewegen. 2. Merken Sie sich die Bilder so gut wie möglich. Im Anschluss fragen wir ab, ob Sie sich an diese erinnern können."]

MRI Quality Controls

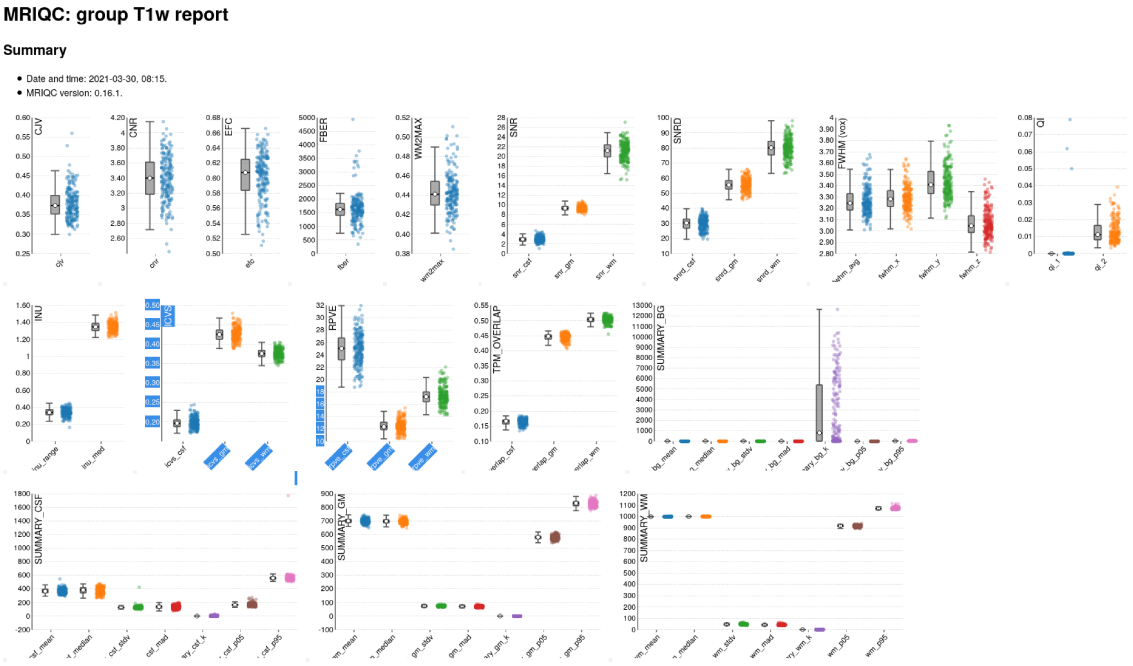
Visual examination of the fMRIprep generated reports was done, checking for the correct extraction of the brainmask, correct surface reconstruction, for unusual artefacts and correct coregistration between fieldmap to EPI and EPI to T1 images. Smoothing with FWHM 6mm was done in SPM12. Slice Timing was not used. MRIQC was used for the individual and group level for both EPI BOLD and T1w sequences [5].

Medawar E, *et al. Gut* 2023;0:1–13. doi: 10.1136/gutjnl-2023-330365

Supplementary Information on fMRI results



SI Figure 1: MRIQC report for BOLD sequences on the group level.



SI Figure 2: MRIQC report for T1w on the group level.

First-level fMRI analysis specifications

Parameter settings. SPM default parameters were used, and implicit masking was set to 0.4 to include all within-skull voxels. From picture onset, picture duration was modelled to 4000ms.

Dataset exclusion. Pre-defined exclusion criteria were employed: a) manual exclusion in case of severe brain pathology (based on T1w/FLAIR image) leading to errors in coregistration by

Supplementary Information on fMRI results

visual inspection of study doctors (0 cases), b) fMRI data was excluded if there was an erroneous co-registration of EPI to T1w (quality assessment done on fmriprep outputs) (0 cases). In addition, timepoints in which participants showed no interest in the stimuli at all (if all stimuli are ranked 1 out 8 for either food or art) had to be excluded from the SPM analysis as the parametric modulator is collinear to the onset regressor (1 participant at 2 sessions).

fMRI trial exclusion. Missed trials during wanting fMRI (i.e. missed response) were handled in two ways according to the randomness of those. Trials missing at random vs. non-random trials: if missed trials occurred in blocks, those were considered non-random and likely due to inattentiveness or sleep of the participants and therefore excluded from the analysis (occurred in 10 subject-session cases), in contrast to missed trials scattered in time, which might have been randomly missed and therefore, worthwhile to impute the concurrent wanting rating to allow inferences about BOLD activity whilst picture evaluation (occurred in 24 subject-session cases). Visual inspection of logfiles were performed by one rater and if the decision is unclear, then group consensus decisions was made (not necessary). Imputation of single-item ratings was done only if missed items were up to 10% of all 160 stimuli of one session (up to 16 missed stimuli) and replaced with the individual average of the respective stimuli type, food or art, respectively, of that session. Missed trials during the behavioural liking task were treated likewise: if >=10% items (72 out 720 stimuli) were missed or not available due to a missing follow-up appointment, then the respective session of that participant was excluded from analysis for model C1/C2.

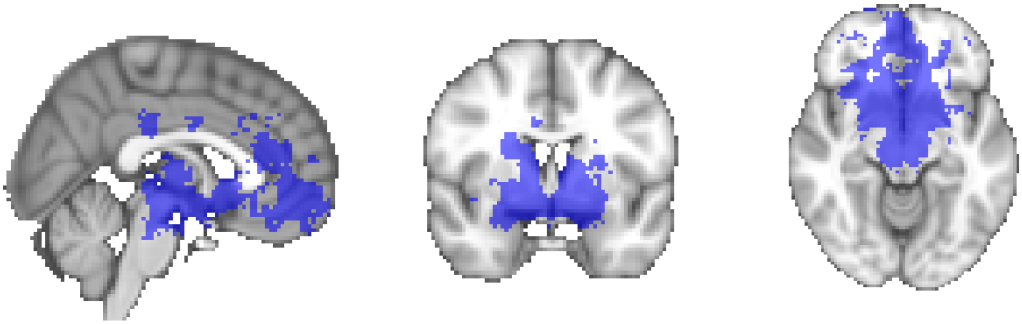
Motion scrubbing. In all contrasts, the six rigid motion parameters derived by fmriprep, as well as a binary regressor for TRs exceeding a motion threshold of 0.9mm were included (derived by fsl_motion_outliers), as recommended by [6].

First-level contrasts were done in SPM12 with four predictors (placebo_BL, placebo_FU, verum_BL, verum_FU) and three design matrices. Design matrix A: food > art [1 0 -1 0], art > food [-1 0 1 0], food > art wanting slope [0 1 0 -1], art > food wanting slope [0 -1 0 1] and wanting modulation [0 0.5 0 0.5]; Design Matrix B1: kcal*wanting [0 0 1 0 0]; Design Matrix B2: fiber*wanting [0 0 1 0 0]; Design Matrix C1 for liking as parametric modulator of no interest: food > art [1 0 -1 0 0], art > food [-1 0 1 0 0], food > art wanting slope [0 1 0 -1 0], art > food wanting slope [0 -1 0 1 0] and wanting modulation [0 0.5 0 0.5 0]; Design Matrix C2 for liking as parametric modulator of interest: food > art liking slope [0 1 0 -1], art > food liking slope [0 -1 0 1] and liking modulation [0 0.5 0 0.5 0]. Orthogonalization in SPM was set to 1 is case of one parametric modulator per condition (model A and C2) and in case of more than one parametric modulator, orthogonalization was set to 0 (models B1, B2 and C1).

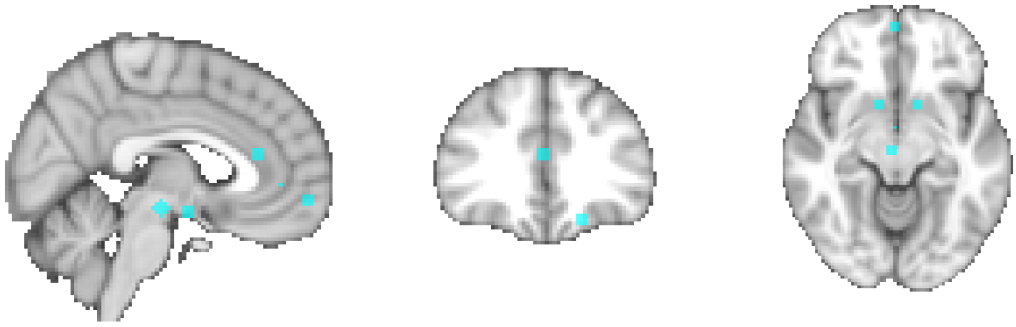
Brain mask: A region-of-interest (ROI) mask was created using 3D-volumes provided by neurosynth.org, from a meta-analysis based on 922 studies for “reward” as search term in combination with a meta-analysis based on 98 studies for “hypothalamus” (accessed on 19 April 2021). The reward-mask was thresholded at zmin= 1.96 (corresponding to alpha<0.05, uncorr.) and combined with the hypothalamus-mask thresholded at z=10 (chosen to get a constrained mask of the hypothalamus), to create a bilateral ROI mask for voxel-wise primary analysis. Secondary analyses were performed in two ways: (i) constrained to a pre-specified second mask comprising 4mm spheres around peak voxel activations according to the above described neurosynth meta-analyses of “reward” and “hypothalamus” (see revised SI Fig. 4), and (ii) at the whole-brain level. With (i) we intended to offer insights on BOLD-activation in the very core activation peaks of the reward network (as indicated by neurosynth-metaanalysis). With (ii) we aimed to provide an unconstrained activation map covering all areas of the brain.

Supplementary Information on fMRI results

Brain mask:



SI Figure 3: Brain region-of-interest mask used for main fMRI analyses, defined by combined meta-analyses of 922 and 98 studies, respectively, on neurosynth.org using the keywords “hypothalamus” and “reward” in April 2019.



SI Figure 4: Brain region-of-interest mask used for secondary fMRI analyses, defined by 4mm spheres around peak voxels according to a combined meta-analyses of 922 and 98 studies, respectively, on neurosynth.org using the keywords “hypothalamus” and “reward” in April 2019.

Whole-brain analysis. Whole-brain results are also reported using MNI152 T1 2mm brain mask as explicit mask.

fMRI 2nd level specifications

Hypothesis testing. The modified SwE approach was selected, with all subjects modeled as one group assuming that all subjects share a common covariance matrix. Visits (1,2,3,4) and subjects (1 to 60) were entered according to SwE structure, and four binary covariates for intervention*timepoint were modeled (placebo_BL, placebo_FU, verum_BL, verum_FU) matching the input order of the images. Type 3 small sample bias adjustment and for estimating the degrees of freedom the “pprox. II” option to account for missing data without assuming a missing-data-bias were used.

Contrast matrices. Main effects were modelled as [0.25 0.25 0.25 0.25] and interaction effects as [-1 1 1 -1] with the predictors [placebo_BL, placebo_FU, verum_BL, verum_FU].

Thresholding. Repeated measures accounted for by within-subject correlation were estimated at TFCE-p-FEW<0.05 for masked brain areas using SwE default parameters (E = 0.5, H = 2). Additionally, results with TFCE-p<0.001 uncorrected are reported. Before non-parametric wild

Supplementary Information on fMRI results

bootstrap resampling (999 permutations), residuals were small sample adjusted with “type C2” (referred to as type 3). TFCE is used to avoid a priori definition of a threshold, however, coming at the cost of a spatial bias [7].

Results – fMRI 2nd level main analyses.

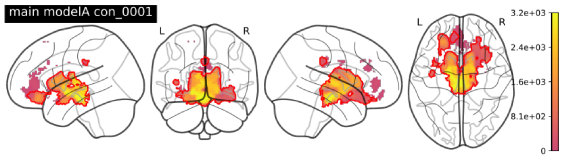
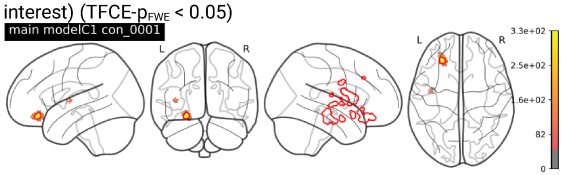
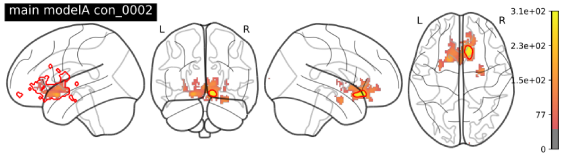
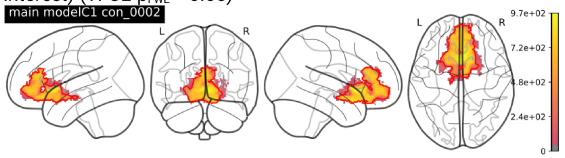
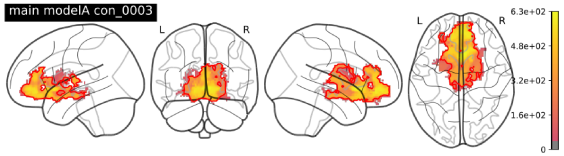
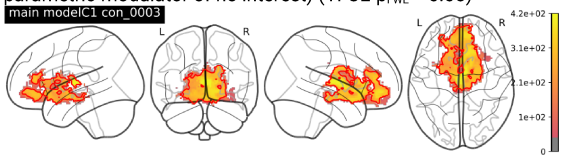
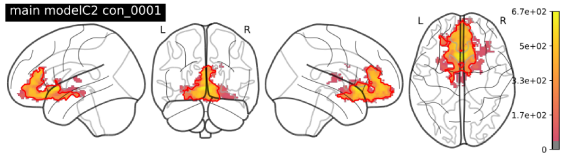
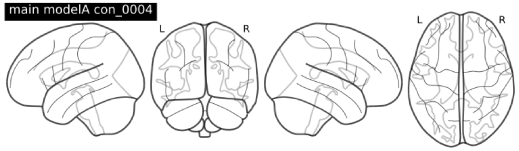
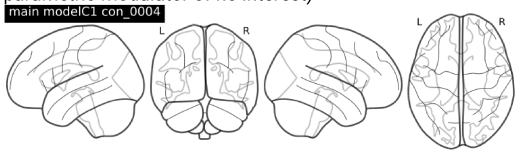
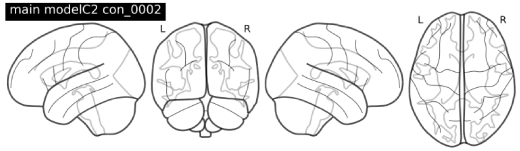
Out of 59 included participants, fMRI data from 57 participants, from at least one and up to four timepoints passed quality control, summing up to in total 200 neuroimaging measurements for inclusion into main analyses.

Results are reported for primary analysis using the thresholded Neurosynth mask (left) only.

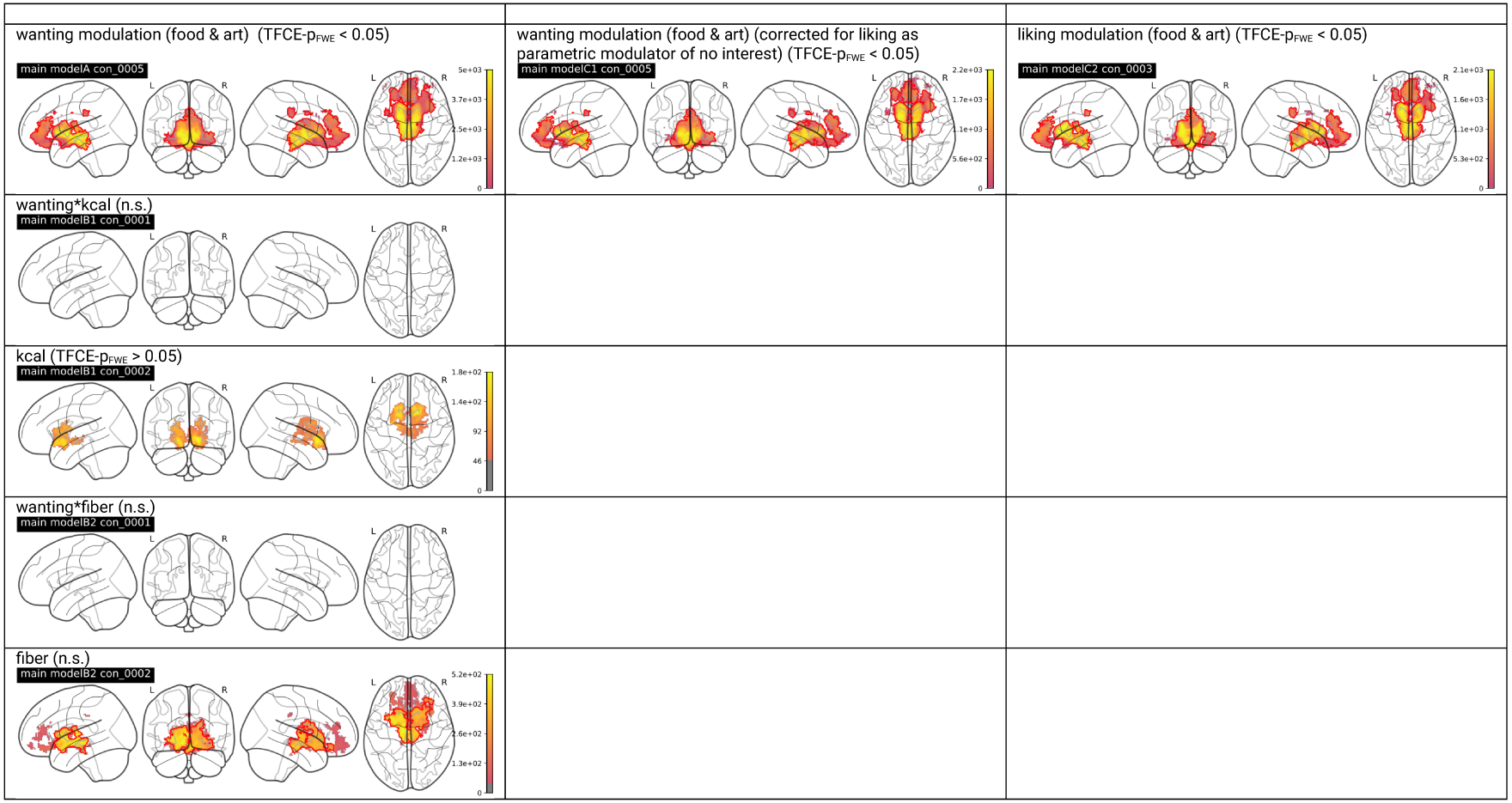
model A	con_0001	food > art
model A	con_0002	art > food
model A	con_0003	food wanting slope > art wanting slope
model A	con_0004	art wanting slope > food wanting slope
model A	con_0005	wanting modulation
model B1	con_0001	wanting*kcal
	con_0002	kcal
model B2	con_0001	wanting*fiber
	con_0002	fiber
model C1	con_0001	food > art
	con_0002	art > food
	con_0003	food wanting slope > art wanting slope
	con_0004	art wanting slope > food wanting slope
	con_0005	wanting modulation
model C2	con_0001	food liking slope > art liking slope
	con_0002	art liking slope > food liking slope
	con_0003	liking modulation

Supplementary Information on fMRI results

Primary analysis (pre-registered):

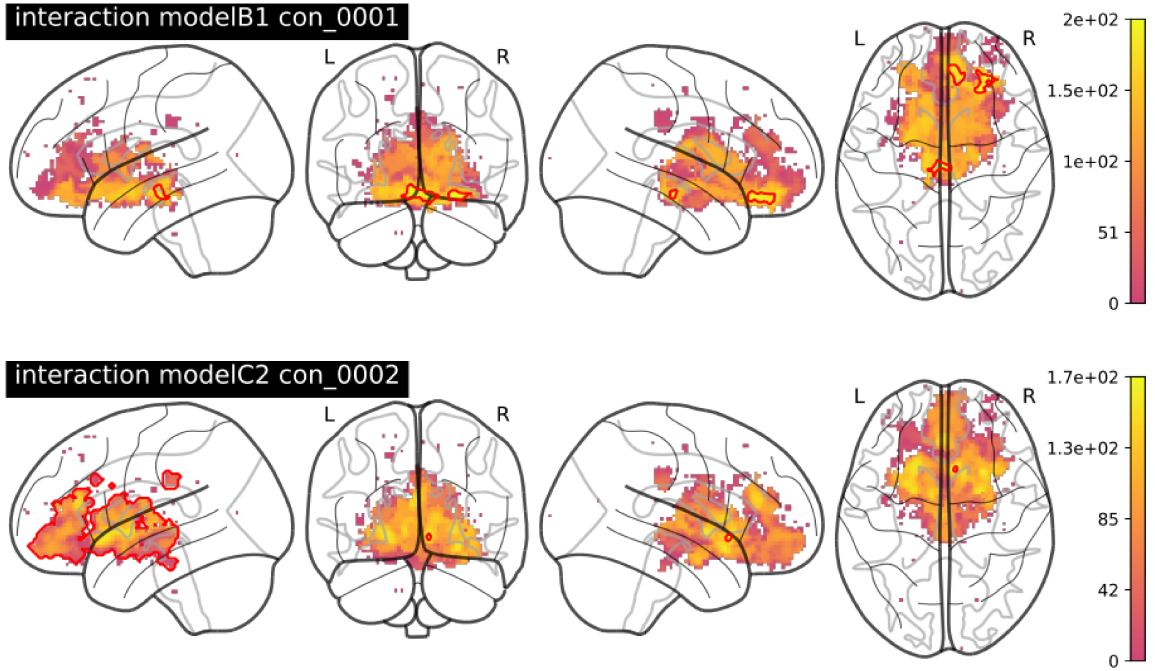
Models considering parametric wanting ratings models A, B1, B2	Models considering parametric wanting and liking ratings model C1	Models considering parametric liking ratings model C2
food > art (TFCE-p_{FWE} < 0.05) 	food > art (corrected for liking as parametric modulator of no interest) (TFCE-p_{FWE} < 0.05) 	
art > food (TFCE-p_{FWE} < 0.05) 	art > food (corrected for liking as parametric modulator of no interest) (TFCE-p_{FWE} < 0.05) 	
food wanting slope > art wanting slope (TFCE-p_{FWE} < 0.05) 	food wanting slope > art wanting slope (corrected for liking as parametric modulator of no interest) (TFCE-p_{FWE} < 0.05) 	food liking slope > art liking slope (TFCE-p_{FWE} < 0.05) 
art wanting slope > food wanting slope (n.s.) 	art wanting slope > food wanting slope (corrected for liking as parametric modulator of no interest) 	art liking slope > food liking slope (n.s.) 

Supplementary Information on fMRI results



SI Figure 3: Main effects for food wanting-related activity. *Statistically significant BOLD activity depicting parametric TFCE t-statistic (min = 50 for visualizing purposes) and wild-boot strapped $p\text{-}_{FWE} < 0.05$ (p_{FWE}-corrected permutation test results delineated as red contour). Column 1 depicts all statistical models related to wanting ratings only, column 2 depicts models as in column 1 but additionally with liking ratings added as a confounder of no interest, and column 3 reflects certain models of column 1 only including parametric liking ratings only as a parametric modulator of interest. All models were run on the 2nd level using Neurosynth mask raw as shown before. Input images computed with SwE toolbox and plotted with nilearn.*

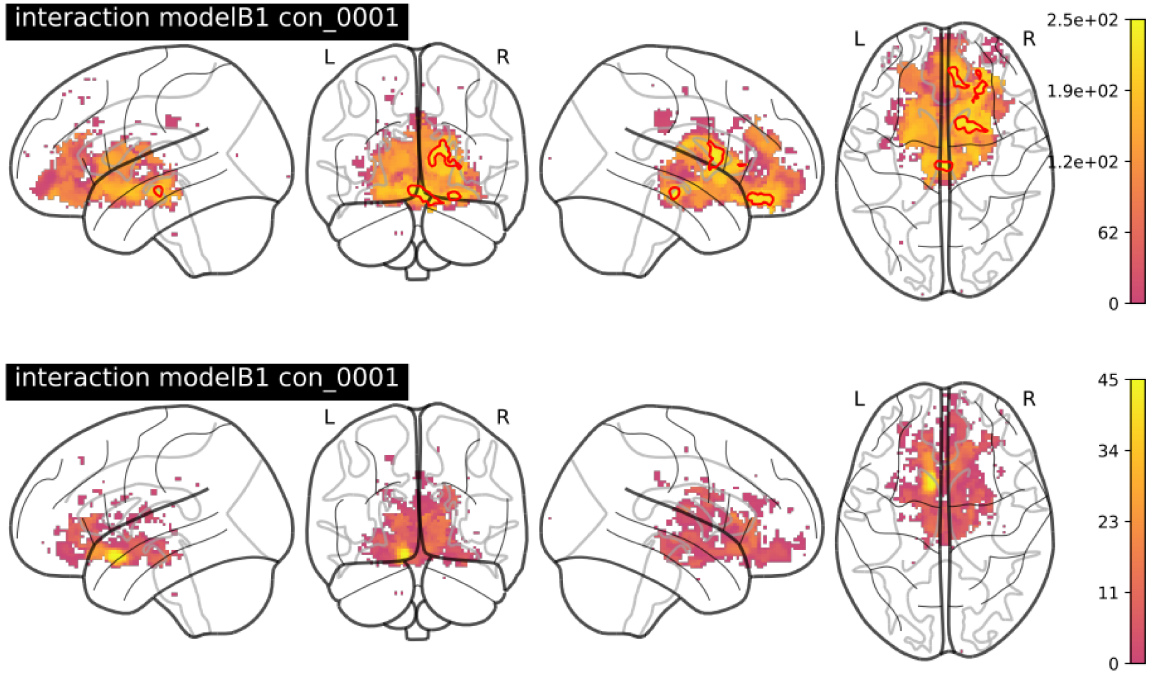
Supplementary Information on fMRI results



SI Figure 4: Prebiotic diet induced changes in food wanting-related activity contrasted to post-placebo intervention. *Statistically significant BOLD activity depicting parametric TFCE t-statistic (min = 0) and wild-boot strapped* $p\text{-}_{FWE} < 0.05$ *(*p_{FWE}*-corrected permutation test results delineated as red contour) for post-prebiotic intervention compared to post-placebo intervention for the activation contrast for wanting*kcal for all available MR datapoints (n=200 in 57 individuals). Neurosynth raw mask used as 2nd level brain mask. Input images computed with SwE toolbox and plotted with nilearn.*

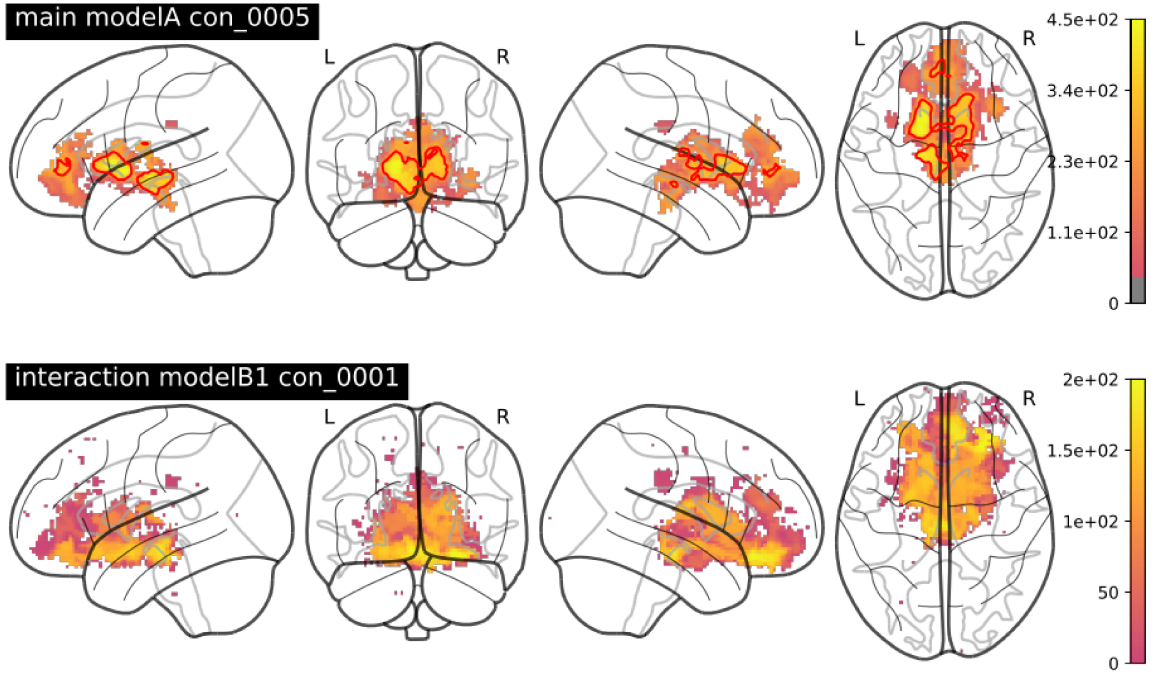
Supplementary Information on fMRI results

Secondary sensitivity analysis (pre-registered):



SI Figure 5: Sex-stratified prebiotic diet induced changes in food wanting-related activity contrasted to post-placebo intervention. *Top: Male only (n = 38), Bottom: Female only (n = 19).*

Supplementary Information on fMRI results



SI Figure 6: Main results corrected for confounding factors age, sex and SES index. *Top: Main effect of wanting modulation (model A), Bottom: Interaction effect of timepoint*intervention showing prebiotic diet induced changes in food wanting-related activity contrasted to post-placebo intervention (model B1).*

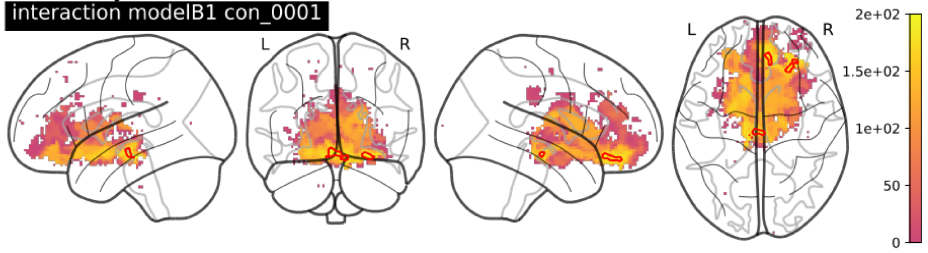
Supplementary Information on fMRI results

Sensitivity analyses for model B1 – kcal*wanting interaction effects

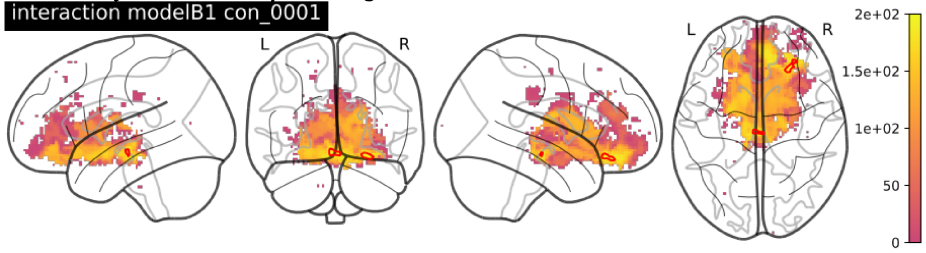
Medawar E, *et al. Gut* 2023;0:1–13. doi: 10.1136/gutjnl-2023-330365

Supplementary Information on fMRI results

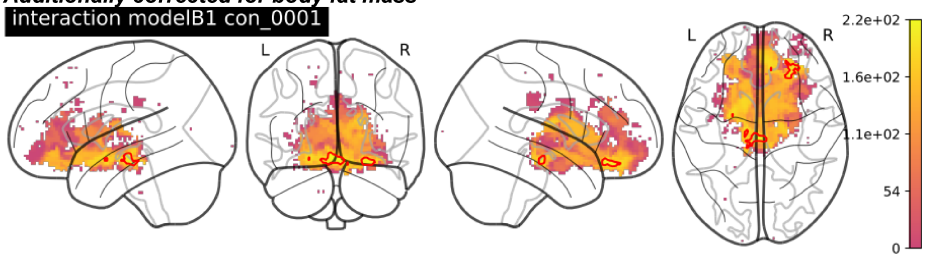
Additionally corrected for fiber intake



Additionally corrected for post-hunger



Additionally corrected for body fat mass



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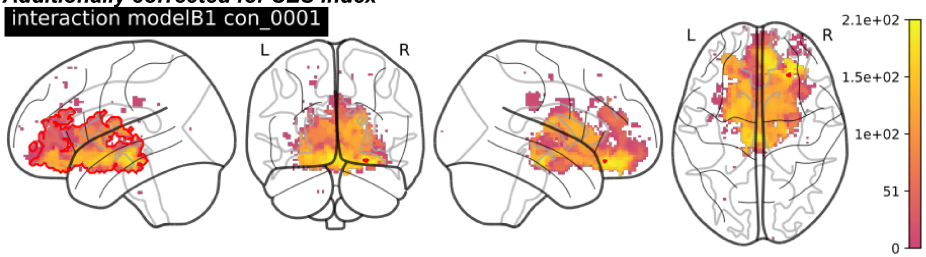

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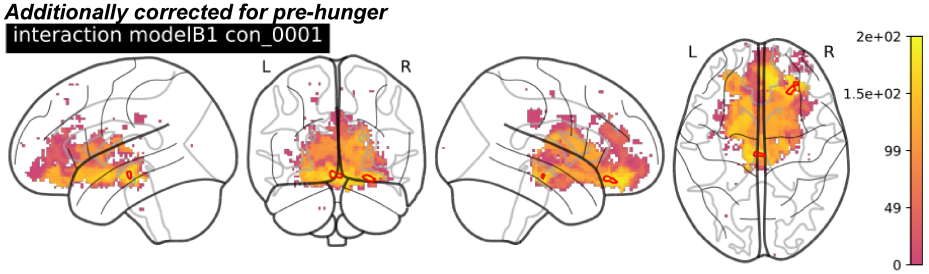
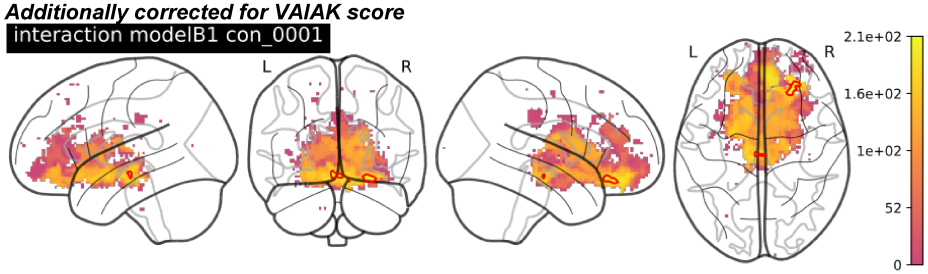
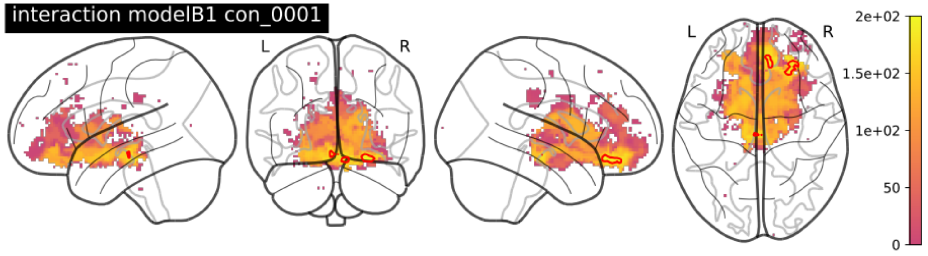


Additionally corrected for VAIAK score



Additionally corrected for well-being during scanning

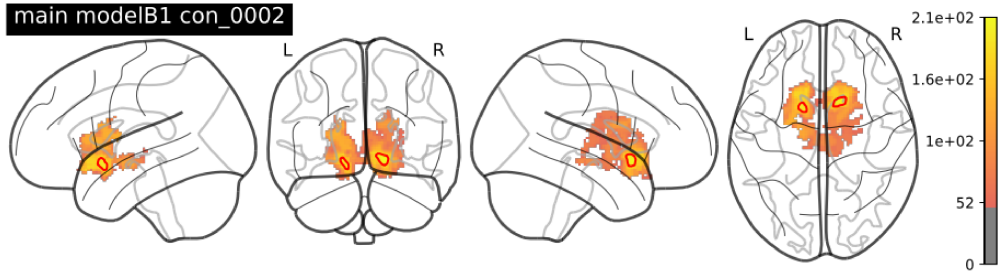
Supplementary Information on fMRI results



SI Figure 7: Main results corrected for confounding factors age, sex and SES index. *Top: Main effect of wanting modulation (model A), Bottom: Interaction effect of timepoint*intervention showing prebiotic diet induced changes in food wanting-related activity contrasted to post-placebo intervention (model B1).*

Model B1 – kcal only (con_0002) main effects

Additionally corrected for body fat

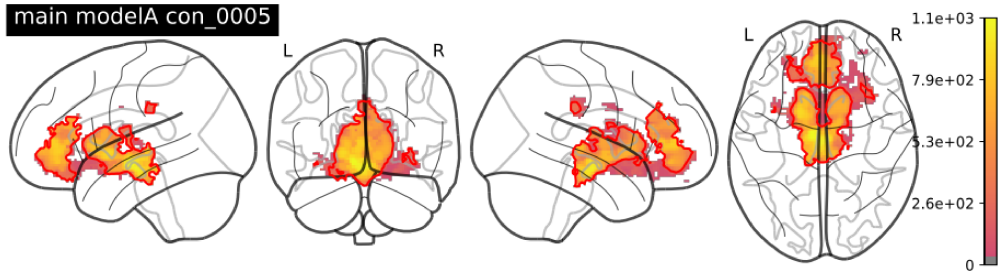


SI Figure 8: Main results (model B1, kcal only) corrected for confounding factor body fat mass.

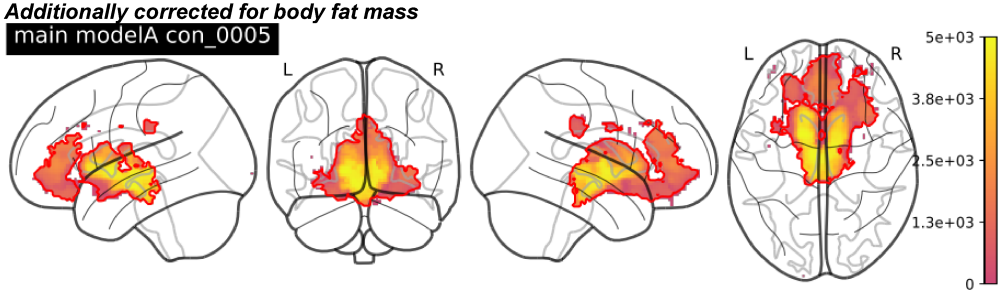
Supplementary Information on fMRI results

Model A – wanting modulation (food & art) effects

Additionally corrected for fiber intake



Additionally corrected for body fat mass



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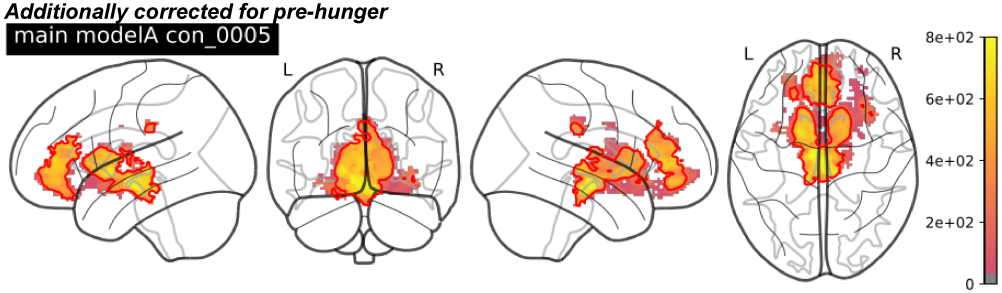
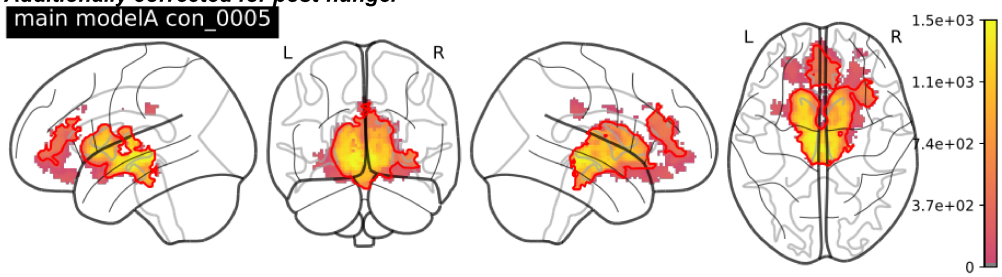
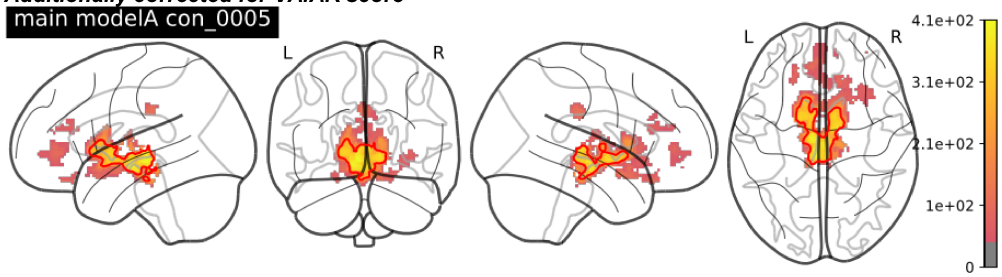


Supplementary Information on fMRI results

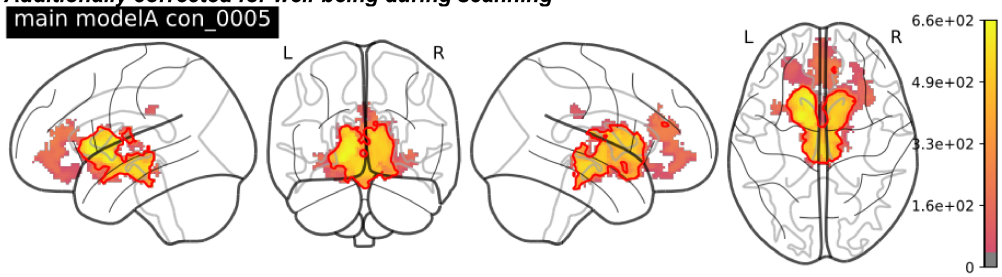
Additionally corrected for post-hunger



Additionally corrected for VAIAK score

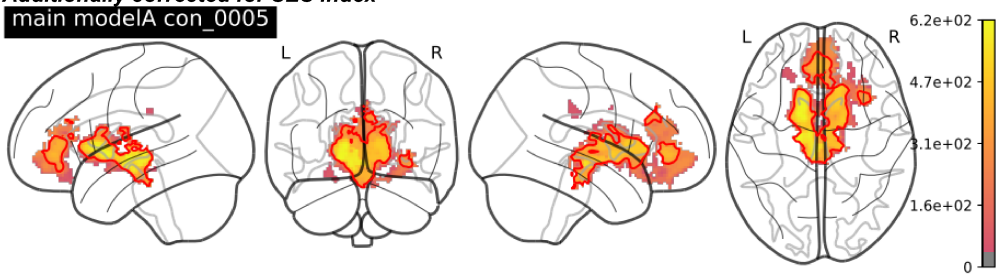


Additionally corrected for well-being during scanning



Supplementary Information on fMRI results

Additionally corrected for SES index



SI Figure 9: Main results (model A (food and art wanting) corrected for various confounding factors.

Supplementary Information on fMRI results

Sensitivity and exploratory fMRI analyses

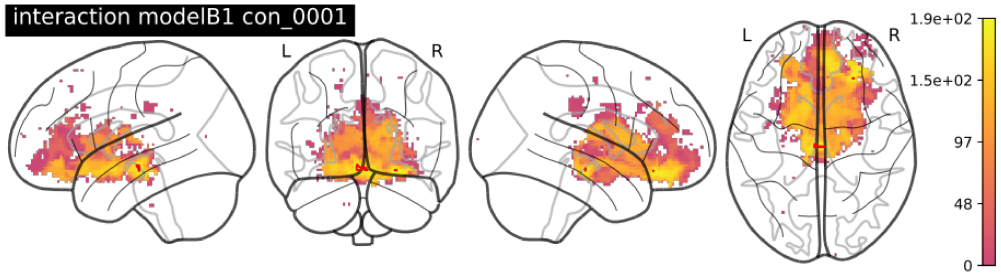
To explore differences in fMRI results with regard to gender, we conducted gender-stratified analyses (19 women and 38 men, respectively) as pre-registered. Overall, results were similar to the whole-sample analysis, yet with one more cluster emerging in the male sample, and results in the female sample only did not survive pFWE-correction.

Further, we repeated analyses adding age, gender and SES index as confounding factors of no interest to the models. Here, the interaction effect of timepoint and intervention did not survive pFWE-correction, yet activation patterns were comparable to the uncorrected analysis (**SI_fMRI Figures 6-7**). See **SI_fMRI** and **SI_behav** for further constrained ROI-based and whole-brain as well as behavioral analyses according to the preregistration. All unthresholded TFCE maps are available on neurovault.org (https://identifiers.org/neurovault.collection:14111).

Note that deviant to the preregistration, we restricted further sensitivity analyses to the main contrast of interest that yielded significant results (i.e., food wanting by calorie content). As preregistered, we corrected interaction effects for *a priori* known confounders of interest (dietary fiber intake, gender-standardized % body fat, subjective hunger ratings, SES index, VAIAK score, well-being inside of scanner). Correcting for habitual fiber intake and well-being yielded the same three significant clusters, whereas when correcting for body fat mass, hunger or VAIAK only the VTA and the rOFC clusters survived p-FWE-correction. When correcting for SES index, only the clusters in the rOFC survived thresholding (**SI_fMRI Fig. 8**).

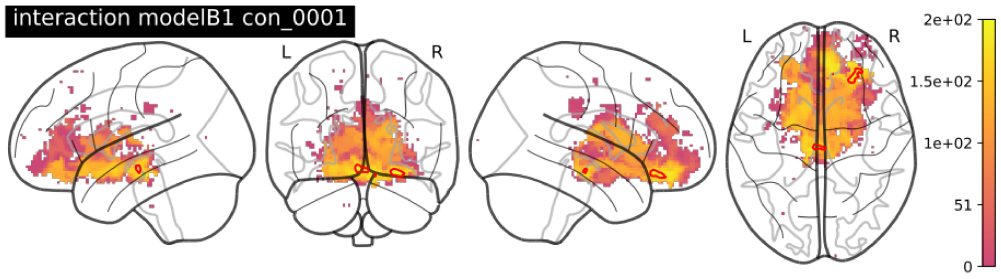
Also, when excluding one participant who reported depressive symptoms based on questionnaires or one participant who reported to not have taken the supplement for 48h (4 sachets), analyses showed similar results (VTA cluster, rOFC partly) (**see SI_fMRI Fig. 10 and SI_fMRI Table 1**).

excluded sub-30 (depressive symptoms):

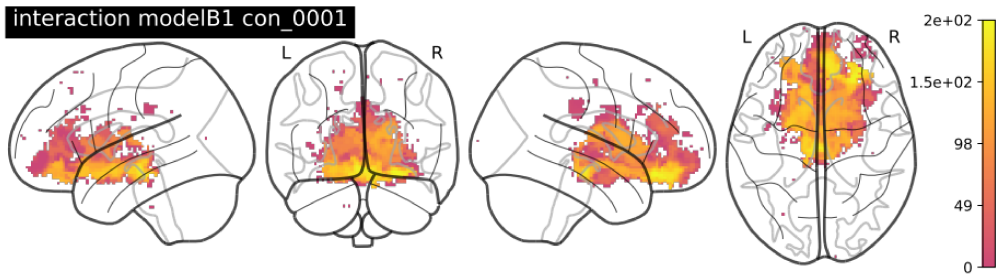


excluded sub-47 (>48h no supplement):

Supplementary Information on fMRI results



both excluded (n.s.):

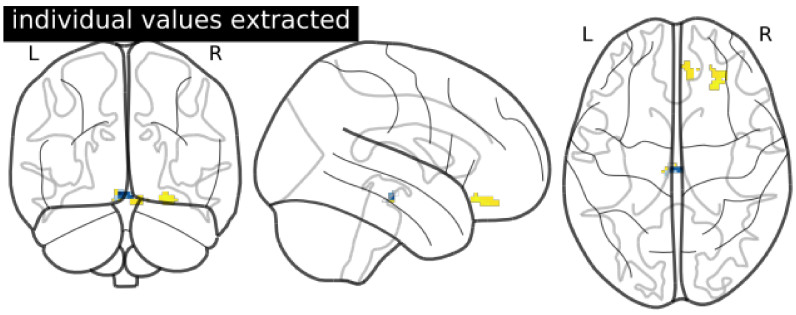


SI Figure 10: Interaction results (model B1, kcal*wanting) regarding excluded participants.

SI Table 1: Interaction results (model B1, kcal*wanting) regarding excluded participants.

Model	TFCE_ p(FWE-corr)	TFCE_ cluster size	peak_ p(FWE-corr)	peak_ p(FDR-corr)	peak_Z	peak_ p(unc)	x {mm}	y {mm}	z {mm}	assigned region
Model B1 (wanting*kcal) - act Excluded sub-30 ses-01	0.049	1	0.18	0.171	3.509	0.001	26	32	-16	right OFC
	0.047	10	0.239	0.171	3.406	0.001	-4	-20	-14	VTA
Model B1 (wanting*kcal) - act Excluded sub-47 ses-04	0.038	21	0.132	0.128	3.595	0.001	26	32	-16	right OFC
	0.04	15	0.238	0.128	3.388	0.001	-4	-20	-14	VTA
	0.05	2	0.44	0.128	3.075	0.001	10	36	-20	right OFC
Model B1 (wanting*kcal) - act Excluded sub-30 ses-01 and sub-47 ses-04	-	-	-	-	-	-	-	-	-	-

Extracted brain activation clusters used for network analysis



Supplementary Information on fMRI results

Using a secondary mask based on peak voxel activity of meta-analytic neurosynth maps, no significant clusters emerged for all models, except for model C2 (art liking slope > food liking slope), which resulted in one significant cluster in mPFC/ACC.

TFCE_p(FWE-corr)	TFCE_	TFCE_equivk	TFCE_	peak_p(FWE-corr)	peak_p(FDR-corr)	peak_Z	peak_p(unc)	_x	y	z {mm}
0.011	12			0.017	0.041	3.338	0.001	0	34	16

For whole-brain analysis, no clusters survived in all models, except for model B1 (wanting*kcal), which resulted in four significant clusters in cerebellar regions.

TFCE_p(FWE-corr)	TFCE_	TFCE_equivk	TFCE_	peak_p(FWE-corr)	peak_p(FDR-corr)	peak_Z	peak_p(unc)	_x	y	z {mm}
0.036	187			0.071	0.128	4.226	0.001	-4	-52	-12
				0.445	0.128	3.635	0.001	6	-44	-16
				0.483	0.142	3.582	0.002	10	-38	-20
0.029	742			0.13	0.128	4.049	0.001	-26	-80	-44
				0.154	0.128	4.002	0.001	-24	-60	-36
				0.254	0.128	3.85	0.001	-20	-72	-30
0.037	124			0.343	0.128	3.74	0.001	-38	-54	-44
				0.566	0.128	3.481	0.001	-36	-50	-36
0.05	5			0.956	0.15	2.868	0.003	-16	-70	-44

fMRI 2nd level masks. Clusters used for brain activity extraction and in network analysis. Interaction effects in VTA, rOFC and rmOFC (left), peak voxel spheres reward and hypothalamus network (right), main effect of food wanting (model A, con 01, 03, 05) (not shown).

References

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Supplementary Information - Behavioural results **Medawar et al.**

Additional behavioral assessments

Liking ratings of food and art stimuli were collected similar to wanting ratings, yet outside the MR scanner and after all pre- and post-intervention visits. In one session (about 1h30min), 720 stimuli were presented on a computer screen under standardized conditions (all from the wanting task across all sessions, plus additional ones). Participants were asked "How much do you like this in general" (German original: "Wie sehr mögen Sie dies generell?") and responded by moving a trial-by-trial randomly placed cursor on a 8-point Likert scale (1 = "not at all", 8 = "absolutely"; German original: 1 = "überhaupt nicht", 8 = "äußert gern") with arrow buttons. Diverging from the MR setting, here each stimuli was presented up to 10s and participants could actively confirm their rating choice by clicking the space bar. Participants were explicitly instructed to report general liking, and that no post-experiment reward was provided. Time of day was not standardized and fasted state not acquired for this day.

[Detailed instructions: "Sie können eine Wertung zwischen 1 und 8 auswählen. 1 bedeutet, dass Sie das Lebensmittel so abstoßend finden, dass Sie es unter keinen Umständen essen würden, und 8 bedeutet, dass Sie das Lebensmittel so lecker finden, dass Sie es jederzeit sehr gerne essen würden. Für die Kunstbilder bedeutet 1, dass Sie das Bild so hässlich finden, dass Sie es nicht ansehen möchten, und 8 bedeutet, dass Sie es so schön finden, dass Sie den Blick nicht abwenden möchten. Zum Fortfahren bitte LEERTASTE drücken."]

Food intake. The DEGS-1 German Food Frequency Questionnaire (FFQ) [1] was used to assess habitual dietary intake for the last 24h and the last 7 days at each timepoint. We developed a pipeline to compute daily nutrient intake based on self-reported dietary habits [2]. We did this by combining computed mean daily portion [g] based on DEGS-1 FFQ and corresponding nutrient information based on reference nutrient data (using the German Nutrient Reference Database "Bundeslebensmittelschlüssel" version 3.02) for each of the 53 items. This resulted in mean daily intake of macro- and micronutrients, e.g. daily fiber intake in grams.

Traits. The following questionnaires were administered once for each individual at the pre-baseline assessment: personality traits (NEOFFI-30) [3], Three-Factor Eating Questionnaire (TFEQ) [4], Eating Disorder Examination Questionnaire (EDEQ) [5], art knowledge (VAIAK) [6], physical activity (IPAQ), general well-being (8-item Eurohis QoL and 5-item WHO-5), trait anxiety (STADI-T) [7] and impulsivity (BIS-15) [8].

States. The following questionnaires were administered at each intervention visit: sleep quality of the last 24h and last 7 days (SF-A/R, SF-B/R) [9], gastrointestinal quality of life (GIQLI) [10], personality states (BFMM), changes to physical activity, depressive symptoms (Beck Depression Inventory, BDI) [11], well-being (WHO-5), state anxiety (STADI-S) [7], mood (POMS) [12], affect (PANAS) [13].

Behavioral hypotheses and codes. Please see preregistration and code at https://osf.io/f6qz5 and https://gitlab.gwdg.de/gut_brain_study/food-wanting/task-fmri-behavior-analysis for details on hypotheses and according set-up of statistical models.

Supplementary Information - Behavioural results **Medawar et al.**

Behavioral Results

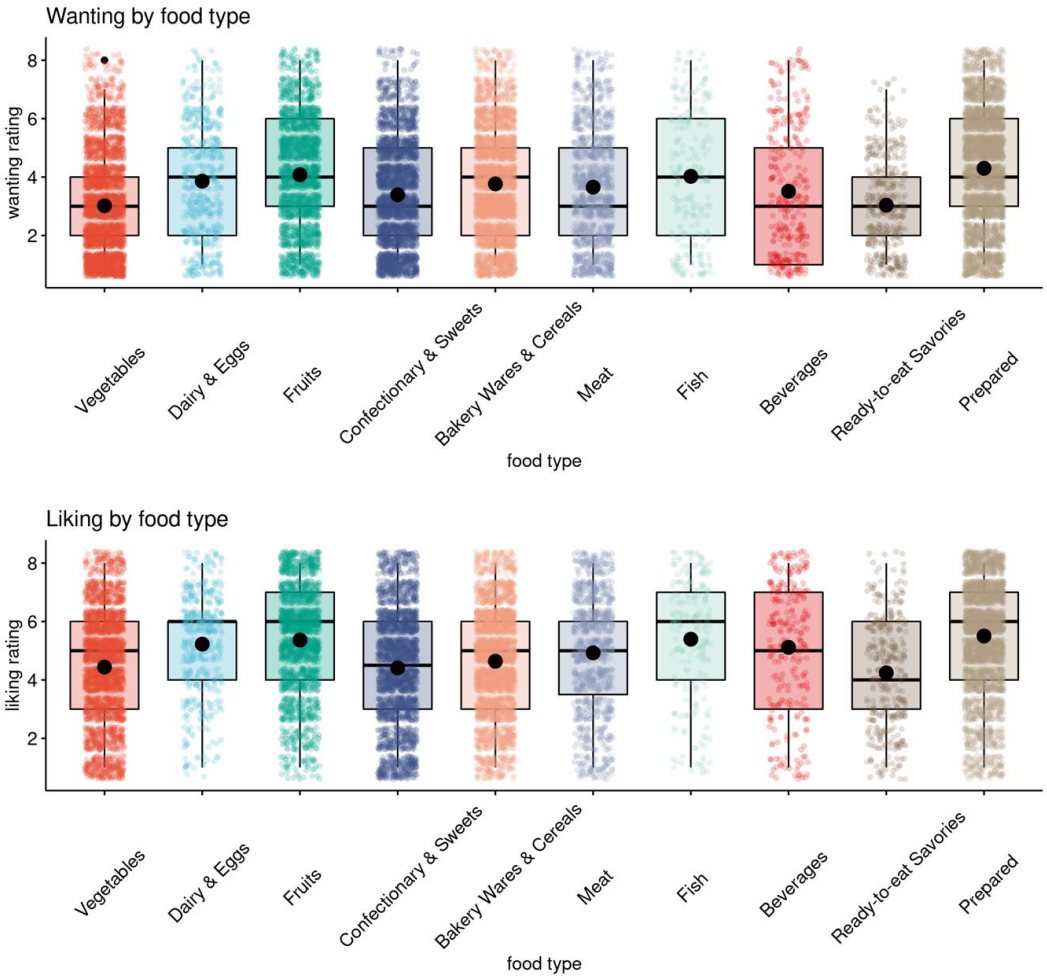
SI-Table 1: Wanting ratings by stimulus category and stimulus type by timepoint for each intervention arm. Based on means of individuals for each stimulus type. sd = standard deviation.

timepoint	intervention	stim_category	stim_type	variable	n	mean	sd
BL	placebo	F	cal1	wanting	53	3.394	1.15
BL	placebo	F	cal2	wanting	53	4.173	1.078
BL	placebo	F	cal3	wanting	53	3.844	1.107
BL	placebo	F	cal4	wanting	53	3.521	1.155
BL	placebo	NF	animals	wanting	53	3.662	1.164
BL	placebo	NF	plants	wanting	53	3.245	1.181
BL	placebo	NF	objects	wanting	53	2.401	0.87
BL	fiber	F	cal1	wanting	55	3.532	1.235
BL	fiber	F	cal2	wanting	55	4.204	1.221
BL	fiber	F	cal3	wanting	55	3.797	1.21
BL	fiber	F	cal4	wanting	55	3.724	1.168
BL	fiber	NF	animals	wanting	55	3.608	1.323
BL	fiber	NF	plants	wanting	55	3.562	1.378
BL	fiber	NF	objects	wanting	55	2.399	0.91
FU	placebo	F	cal1	wanting	49	3.294	1.286
FU	placebo	F	cal2	wanting	49	3.991	1.288
FU	placebo	F	cal3	wanting	49	3.653	1.059
FU	placebo	F	cal4	wanting	49	3.391	1.112
FU	placebo	NF	animals	wanting	49	3.422	1.217
FU	placebo	NF	plants	wanting	49	3.329	1.412

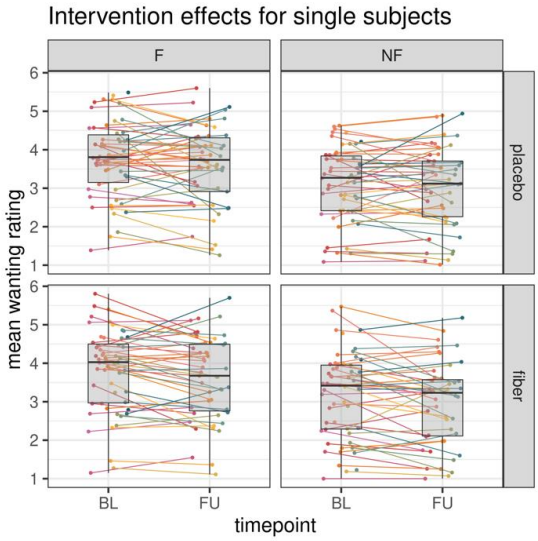
FU	placebo	NF	objects	wanting	49	2.369	947
FU	fiber	F	cal1	wanting	47	3.212	1.218
FU	fiber	F	cal2	wanting	47	4.074	1.34
FU	fiber	F	cal3	wanting	47	3.755	1.186
FU	fiber	F	cal4	wanting	47	3.398	1.116
FU	fiber	NF	animals	wanting	47	3.447	1.338
FU	fiber	NF	plants	wanting	47	3.165	1.328
FU	fiber	NF	objects	wanting	47	2.259	906

BL baseline, F Food, FU follow-up, NF Non-food

Preregistered linear models for model 1/A (food vs. art), model 2/A (intervention effect) for different stimulus classes (stimulus category, stimulus type) for either average across class or stimulus-by-stimulus values (note number of observations: $n\_obs_{category} > 1{,}470$, $n\_obs_{stimulus} > 32{,}000$) are reported here.

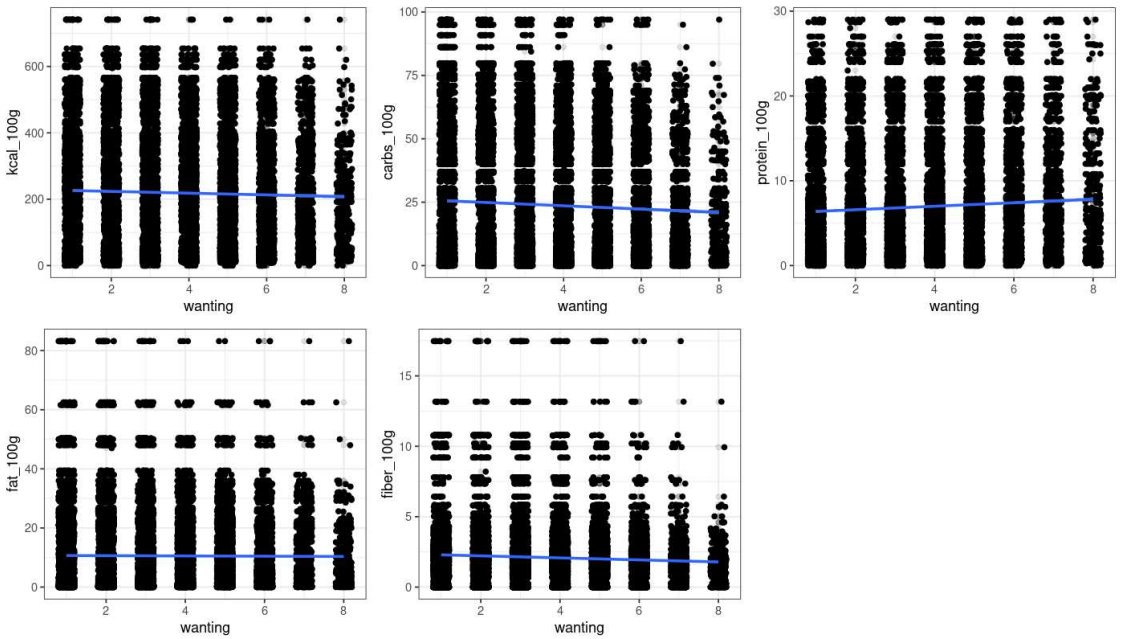


SI-Fig. 2: Distribution of food wanting and liking ratings by food type.



SI-Fig. 3: Intervention effects on wanting ratings by stimulus category and timepoint. Average and individual ratings by timepoint and by intervention depicting inter-individual variability in wanting ratings.

Food items higher in protein/100g (b = 0.02, t = 3.35), lower in fiber/100g (b = -0.06, t = -3.51), and to a lesser extent, lower in carbohydrates/100g (b = -0.004, t = -2.22) were more wanted ($p_{all} < .03$).



SI-Fig. 4: Food wanting ratings correlate with nutrient content.

Model 1/A: Main effect of stimulus category (food vs. art)

H_behav_0.1: Individual wanting is higher for food compared to art wanting for between-subject analysis (b = 1.03, t = 7.78, null model comparison p < 0.001).

SI-Table 2: Mixed effects linear model results on the subjective wanting for food and art stimuli on the level of stimulus category.

random effects	**variance**	**SD**	
subject (intercept)	0.73	0.86	
stim_category (food)	1.00	1.00	
residual	2.47	1.57	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	2.77	0.11	23.94
stim_category (food)	**1.03*** **	**0.13**	**7.78**
time (follow-up)	-0.05	0.03	-2.05
intervention (prebiotic)	0.10	0.03	4.03
time (follow-up) * intervention (prebiotic)	-0.13	0.04	-3.58

***Formula**: wanting ~ stim_category + timepoint * intervention + (stim_category | subject). **REML** criterion at convergence: 120620, n_{obs} = 32,111, groups: n_{subj} = 59. **Significance**, *** p < 0.001*

Additional analysis for stimulus type

SI-Table 3: Mixed effects linear model results on the subjective wanting for food and art stimuli on the level of stimulus type.

random effects	**variance**	**SD**	
subject (intercept)	0.57	0.75	
residual	2.54	1.60	
fixed effects	**estimate**	**SE**	**t-value**
(Intercept)	3.41	0.10	33.24
stim_typecal2	**0.75*** **	**0.04**	**20.99**
stim_typecal3	**0.40*** **	**0.04**	**11.08**
stim_typecal4	**0.14*** **	**0.04**	**4.02**
stim_typeanimals	**0.18*** **	**0.04**	**4.31**
stim_typeplants	**-0.04*** **	**0.04**	**-0.91**
stim_typeobjects	**-1.01*** **	**0.03**	**-34.36**
time (follow-up)	-0.05	0.03	-2.00
intervention (prebiotic)	0.10	0.03	4.00
time (follow-up) * intervention (prebiotic)	-0.13	0.04	-3.52

*Formula: wanting ~ stim_type + timepoint*intervention + (1 | subject). REML criterion at convergence: 121425, n_{obs} = 32,111, groups: n_{subj} = 59. Significance, *** p < 0.001.*

Note: No random slope "stimulus type" as model couldn't converge -> only random intercept "subject".

Additional analysis for food type (10 types)

All types of food are more liked than vegetables with fruits, fish and prepared most liked (between-subject) (b_{all} > 0.03, t_{all} > 0.38, null model comparison p < 0.001).

SI-Table 4: Mixed effects linear model results on the subjective wanting for food stimuli on the level of food-pics type (food only).

random effects	**variance**	**SD**	
subject (intercept)	0.90	0.95	
residual	2.58	1.61	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	3.08	0.13	23.82
type1 - Dairy & eggs	**0.84*** **	**0.07**	**11.29**
type2 - Fruits	**1.06*** **	**0.05**	**22.90**
type4 - Confectionary & sweets	**0.38*** **	**0.04**	**8.77**
type5 - Bakery wares & cereals	**0.75*** **	**0.04**	**17.72**
type6 - Meat	**0.63*** **	**0.06**	**11.08**
type7 - Fish	**1.01*** **	**0.11**	**9.48**
type8 - Beverages	**0.50*** **	**0.10**	**5.15**
type9 - Ready-to-eat savories	**0.03*** **	**0.09**	**0.38**
type10 - Prepared	**1.28*** **	**0.04**	**30.51**

time (follow-up)	-0.11	0.04	-2.94
intervention (prebiotic)	0.10	0.04	2.73
time (follow-up) * intervention (prebiotic)	-0.09	0.05	-1.71

*Formula: wanting ~ food_pics_type + timepoint * intervention + (1 | subject), data = data_F_only. REML criterion at convergence: 61111, n_{obs} = 16,071, groups: n_{subj} = 59. Significance, *** p < 0.001.*

No random slope "food pics type" as model couldn't converge -> only random intercept "subject".

Additional analysis for nutrient content (macronutrients)

SI-Table 5: Mixed effects linear model results on the subjective wanting for stimuli per nutrient content (food only). Less fiber content and higher amounts of protein and carbohydrates related to higher wanting.

fixed effects	estimate	SE	t-value
(Intercept)	3.79	0.14	27.16
kcal_100g	-0.00	0.00	-0.86
time (follow-up)	-0.11	0.04	-3.04
intervention (prebiotic)	0.10	0.03	2.90
time (follow-up) * intervention (prebiotic)	-0.09	0.05	-1.80
(Intercept)	3.88	0.14	28.45
fiber_100g	**-0.06*** **	**0.02**	**-3.51**
time (follow-up)	-0.11	0.04	-3.04
intervention (prebiotic)	0.10	0.03	2.91
time (follow-up) * intervention (prebiotic)	-0.09	0.05	-1.80
(Intercept)	3.62	0.14	26.37
protein_100g	**0.02*** **	**0.01**	**3.35**
time (follow-up)	-0.11	0.04	-3.04
intervention (prebiotic)	0.10	0.03	2.91
time (follow-up) * intervention (prebiotic)	-0.09	0.05	-1.81
(Intercept)	3.75	0.13	27.79
fat_100g	0.00	0.00	0.10
time (follow-up)	-0.11	0.04	-3.04
intervention (prebiotic)	0.10	0.03	2.91
time (follow-up) * intervention (prebiotic)	-0.09	0.05	-1.80
(Intercept)	3.83	0.14	28.11
carbs_100g	**-3.5*10-3***	**1.5*10-3**	**-2.22**
time (follow-up)	-0.11	0.04	-3.04
intervention (prebiotic)	0.10	0.03	2.91
time (follow-up) * intervention (prebiotic)	-0.09	0.05	-1.80

*Formula: wanting ~ nutrient_of_interest_pics_type + timepoint * intervention + (1 | subject) + (1 | image_number), data = data_F_only. For each model n_{obs} = 16,071, groups: n_{subj} = 59, n_{images} = 410. Significance, * / *** ANOVA null-full model comparison p < 0.05 / p < 0.001*

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Additional analysis for H_behav_0.1 with hunger rating as covariate

SI-Table 6: Mixed effects linear model results on the subjective wanting for subjective hunger rating on wanting by stimulus category.

random effects	**variance**	**SD**	
subject (intercept)	0.72	0.85	
stim_category (food)	0.96	0.98	
residual	2.46	1.57	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	2.59	0.13	19.96
stim_category (food)	0.82	0.16	5.16
hunger (mean pre-/post-wanting task)	0.03	0.02	2.01
time (follow-up)	-0.06	0.03	-2.17
intervention (prebiotic)	0.08	0.03	3.05
time (follow-up) * intervention (prebiotic)	-0.11	0.04	-3.05
stim_category (food) * hunger (mean)	**0.05***	**0.02**	**2.28**

*Formula: wanting ~ stim_category * hunger_mean_wanting + stim_category + hunger_mean_wanting + timepoint*intervention + (stim_category | subject). REML criterion at convergence: 120603, n_{obs} = 32,111, groups: n_{subj} = 59. Significance, * p < 0.05*

Model 2/A: Intervention effect

H_behav_A0: Individual food wanting compared to art wanting is not significantly different after a two-week high-fiber intervention, when looking at stimulus category (R1 with timepoint*intervention*stim_category vs. R0, p = 0.317, **SI-Table 7**), but for stimulus type (R1 with timepoint*intervention*stim_type vs. R0, p = 0.002, **SI-Table 8**).

SI-Table 7a: Mixed effects linear model results on the subjective wanting for post-intervention by stimulus category. Alternative model (H1) including triple interaction (time (follow-up) * intervention (prebiotic) * stim_category (food)).

random effects	**variance**	**SD**	
subject (intercept)	0.69	0.83	
time (follow-up)	0.07	0.27	
stim_category (food)	1.00	1.00	
intervention (prebiotic)	0.13	0.36	
residual	2.41	1.55	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	2.69	0.11	24.03
time (follow-up)	0.01	0.05	-0.17
intervention (prebiotic)	0.10	0.06	1.69
stim_category (food)	1.06	0.14	7.82
time (follow-up) * intervention (prebiotic)	-0.15	0.05	-2.94
time (follow-up) * stim_category (food)	-0.11	0.05	-2.14
intervention (prebiotic) * stim_category (food)	-0.002	0.05	-0.05
time (follow-up) * intervention (prebiotic) * stim_category (food)	0.07	0.07	1.00

*Formula: wanting ~ timepoint*intervention*stim_category+ timepoint*stim_category+ timepoint*intervention+ stim_category*intervention+ timepoint+ stim_category+i ntervention+(1+timepoint*intervention|subject). REML criterion at convergence: 120207, n_{obs} = 32,111, groups: n_{subj} = 59. Significance, p = 0.317*

SI-Table 7b: Mixed effects linear model results on the subjective wanting for post-intervention by stimulus category. Null model (H0) withouth triple interaction (time (follow-up) * intervention (prebiotic) * stim_category (food)).

random effects	**variance**	**SD**	
subject (intercept)	0.69	0.83	
time (follow-up)	0.07	0.27	
stim_category (food)	1.00	1.00	
intervention (prebiotic)	0.13	0.36	
residual	2.41	1.55	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	2.70	0.11	24.20
time (follow-up)	0.01	0.05	-0.18
intervention (prebiotic)	0.09	0.06	1.47
stim_category (food)	1.04	0.13	7.76
time (follow-up) * intervention (prebiotic)	**-0.11****	**0.04**	**-3.14**
time (follow-up) * stim_category (food)	-0.07	0.04	-2.03
intervention (prebiotic) * stim_category (food)	-0.03	0.04	-0.90

*Formula: wanting ~ timepoint*stim_category+ timepoint*intervention+ stim_category*intervention+ timepoint+ stim_category+intervention+(1+timepoint*intervention|subject). REML criterion at convergence: 120205, n_{obs} = 32,111, groups: n_{subj} = 59.*

SI-Table 8: Mixed effects linear model results on the subjective wanting for post-intervention by stimulus type.

random effects	**variance**	**SD**	
subject (intercept)	0.53	0.73	
time (follow-up)	0.07	0.27	
intervention (prebiotic)	0.13	0.36	
residual	2.50	1.58	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	3.39	0.11	31.34
time (follow-up)	-0.03	0.08	-0.41
intervention (prebiotic)	0.19	0.07	2.15
stim_type (cal2)	0.78	0.07	11.24
stim_type (cal3)	0.45	0.07	6.47
stim_type (cal4)	0.13	0.07	1.80
stim_type (animals)	0.28	0.08	3.50
stim_type (plants)	-0.15	0.08	-1.87
stim_type (objects)	-0.99	0.06	-17.33
time (follow-up) * intervention (prebiotic)	-0.25	0.10	-2.47
time (follow-up) * stim_type (cal2)	-0.09	0.10	-0.88
time (follow-up) * stim_type (cal3)	-0.10	0.10	-1.00
time (follow-up) * stim_type (cal4)	-0.04	0.10	-0.43
time (follow-up) * stim_type (animals)	-0.16	0.12	-1.40
time (follow-up) * stim_type (plants)	0.17	0.12	1.50

time (follow-up) * stim_type (objects)	0.05	0.08	0.56
intervention (prebiotic) * stim_type (cal2)	-0.10	0.10	-1.06
intervention (prebiotic) * stim_type (cal3)	-0.18	0.10	-1.88
intervention (prebiotic) * stim_type (cal4)	0.06	0.10	0.66
intervention (prebiotic) * stim_type (animals)	-0.20	0.11	-1.83
intervention (prebiotic) * stim_type (plants)	0.18	0.11	1.59
intervention (prebiotic) * stim_type (objects)	-0.14	0.08	-1.74
time (follow-up) * intervention (prebiotic) * stim_type (cal2)	**0.27****	**0.14**	**1.94**
time (follow-up) * intervention (prebiotic) * stim_type (cal3)	**0.37****	**0.14**	**2.61**
time (follow-up) * intervention (prebiotic) * stim_type (cal4)	**0.03****	**0.14**	**0.21**
time (follow-up) * intervention (prebiotic) * stim_type (animals)	**0.33****	**0.16**	**2.02**
time (follow-up) * intervention (prebiotic) * stim_type (plants)	**-0.24****	**0.16**	**-1.49**
time (follow-up) * intervention (prebiotic) * stim_type (objects)	**0.13****	**0.12**	**1.10**

*Formula: wanting ~ timepoint * intervention * stim_type + timepoint * stim_type + timepoint * intervention + stim_type * intervention + timepoint + stim_type + intervention + (1+(timepoint+intervention)|subject). REML criterion at convergence: 121445, n_{obs} = 32,111, groups: n_{subj} = 59. Significance, ** p < 0.01*

*Note: No random slope "stimulus type * timepoint * intervention" as model didn't converge -> random slopes "timepoint * intervention" but random effects too small (error: isSingular) -> random slopes therefore chosen as "timepoint + intervention".*

Model 2: Impact of hunger on intervention effect

SI-Table 9: Mixed effects linear model results on the subjective wanting for post-intervention by stimulus category dependent on hunger rating.

random effects	**variance**	**SD**	
subject (intercept)	1.45	0.70	
time (follow-up)	0.04	0.21	
intervention (prebiotic)	0.14	0.37	
mean hunger rating	0.04	0.20	
residual	2.64	1.62	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	3.07	0.22	13.82
time (follow-up)	-0.54	0.15	-3.72
intervention (prebiotic)	-0.25	0.17	-1.45
stim_category (food)	0.48	0.10	4.58
mean hunger rating	-0.08	0.04	-1.96
time (follow-up) * intervention (prebiotic)	0.56	0.18	3.07
time (follow-up) * stim_category (food)	-0.10	0.15	-0.67
intervention (prebiotic) * stim_category (food)	-0.19	0.15	-1.26
time (follow-up) * mean hunger rating	0.13	0.02	4.04
intervention (prebiotic) * mean hunger rating	0.08	0.03	2.29
stim_category (food) * mean hunger rating	0.12	0.02	5.44
time (follow-up) * intervention (prebiotic) * stim_category (food)	0.03	0.22	0.13
time (follow-up) * intervention (prebiotic) * mean hunger rating	-0.17	0.04	-4.43
time (follow-up) * stim_category (food) * mean hunger rating	0.00	0.03	0.06

intervention (prebiotic) * stim_category (food) * mean hunger rating	0.04	0.03	1.25
time (follow-up) * intervention (prebiotic) * stim_category (food) * mean hunger rating	0.02	0.05	0.40

*Formula: wanting ~ timepoint * intervention * stim_category * hunger_mean_wanting + ntervention * stim_category * hunger_mean_wanting + timepoint * stim_category * hunger_mean_wanting + timepoint * intervention * hunger_mean_wanting + timepoint * intervention * stim_category + timepoint * intervention + timepoint * stim_category + timepoint * hunger_mean_wanting + stim_category * intervention + intervention * hunger_mean_wanting + stim_category * hunger_mean_wanting + imepoint + stim_category + intervention + hunger_mean_wanting + (1 + (timepoint + intervention + hunger_mean_wanting) | subject). REML criterion at convergence: 122827, n_{obs} = 32,110 , groups: n_{subj} = 59. Significance, p=0.69*

*Note: No random slope "stimulus category * time * intervention * hunger" as random effects too small (error: isSingular) -> random slopes "time * intervention * hunger" or " stimulus category + time + intervention + hunger" model did not converge -> random slopes "time + intervention + hunger""*

H_behav_3/B: Nutrient content effect on wanting (food only)

H_behav_B1: Individual food wanting is not different for kcal_100g content after a two-week high-fiber intervention across all food stimuli (null model comparison p = 0.85).

SI-Table 10: Mixed effects linear model results on the subjective wanting for post-intervention dependent on caloric content (kcal / 100g).

random effects	**variance**	**SD**	
subject (intercept)	0.85	0.92	
time (follow-up)	0.12	0.35	
intervention (prebiotic)	0.18	0.43	
residual	2.70	1.64	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	3.85	0.13	29.71
time (follow-up)	$-5.63*10^{-2}$	0.08	-0.70
intervention (prebiotic)	$6.43*10^{-2}$	0.08	0.77
kcal_100g	$-3.48*10^{-4}$	$1.45*10^{-4}$	-2.40
session (session 2, 3, 4)	$-7.01*10^{-2}$	0.05	-1.42
time (follow-up) * intervention (prebiotic)	$-6.63*10^{-2}$	0.08	-0.76
time (follow-up) * kcal_100g	$-5.61*10^{-5}$	$2.08*10^{-4}$	-0.27
intervention (prebiotic) * kcal_100g	$1.91*10^{-4}$	$2.02*10^{-4}$	0.94
time (follow-up) * intervention (prebiotic) * kcal_100g	$-5.55*10^{-5}$	$2.95*10^{-4}$	-0.19

*Formula: wanting ~ timepoint * intervention * kcal_100g + intervention * kcal_100g + timepoint * kcal_100g + timepoint * intervention + intervention + timepoint + kcal_100g + (1 + (intervention + timepoint) | subject) + session_1_2, data: data_F_only). REML criterion at convergence: 62061, n_{obs} = 16,071, groups: n_{subj} = 59.*

H_behav_B2*:* Individual food wanting is not different for fiber_100g content after a two-week high-fiber intervention across all food stimuli (null model comparison p = 0.32).

SI-Table 11: Mixed effects linear model results on the subjective wanting for post-intervention dependent on fiber content (fiber / 100g).

random effects	**variance**	**SD**	
subject (intercept)	0.85	0.92	
time (follow-up)	0.12	0.35	

intervention (prebiotic)	0.18	0.43	
residual	2.70	1.64	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	3.91	0.13	30.54
time (follow-up)	-0.08	0.08	-1.00
intervention (prebiotic)	0.07	0.08	0.85
fiber_100g	-0.07	0.01	-5.45
session (session 2, 3, 4)	-0.07	0.05	-1.45
time (follow-up) * intervention (prebiotic)	-0.03	0.08	-0.30
time (follow-up) * fiber_100g	0.005	0.02	0.29
intervention (prebiotic) * fiber_100g	0.02	0.02	1.08
time (follow-up) * intervention (prebiotic) * fiber_100g	-0.02	0.03	-0.99

*Formula: wanting ~ timepoint * intervention * fiber_100g + intervention * fiber_100g + timepoint * fiber_100g + timepoint * intervention + intervention + timepoint + fiber_100g + session_1_2 + (1 + (intervention + timepoint) | subject), data: data_F_only). REML criterion at convergence: 61946, n_{obs} = 16,071, groups: n_{subj} = 59. Significance, *** p < 0.001.*

Model C: Liking as a potential confounding variable on wanting ratings

Test if subjective liking is a confounding variable for subjective wanting. Note that less datapoints could be included due to incomplete liking ratings.

SI-Table 12: Mixed effects linear model results on the subjective wanting for post-intervention dependent on subjective liking ratings.

random effects	**variance**	**SD**	
subject (intercept)	0.35	0.60	
time (follow-up)	0.08	0.28	
intervention (prebiotic)	0.13	0.36	
stim_category (food)	0.75	0.87	
residual	1.71	1.31	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	1.14	0.09	12.27
time (follow-up)	-0.04	0.05	-0.79
intervention (prebiotic)	0.07	0.06	1.07
stim_category (food)	0.09	0.13	0.70
liking	**0.50*** **	**0.005**	**103.22**
time (follow-up) * intervention (prebiotic)	-0.13	0.05	-2.81
time (follow-up) * stim_category (food)	-0.09	0.05	-2.03
intervention (prebiotic) * stim_category (food)	0.02	0.05	0.41
time (follow-up) * intervention (prebiotic) * stim_category (food)	0.04	0.06	0.70

*Formula: wanting ~ timepoint * intervention * stim_category + timepoint * stim_category + timepoint * intervention + stim_category * intervention + liking + timepoint + stim_category + intervention + (1 + (stim_category + timepoint + intervention + stim_category)| subject), (data = data_liking_only). REML criterion at convergence: 96357, n_{obs} = 27,445, groups: n_{subj} = 45. Significance, *** p < 0.001.*
*Note: No random slope "stimulus category * timepoint * intervention" as model wouldn't converge -> only random slopes "stimulus category + timepoint + intervention"*

Interpretation: Yes, subjective liking has a significant positive impact on wanting ratings ($p < 0.001$).

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Effects of subjective hunger

Wanting ratings for food were significantly higher, when subjective hunger was higher (b = 0.05 ± 0.02, t = 2.38, p < 0.05, **SI_behav Table 6**). Individuals' subjective hunger ratings during fMRI sessions were diverse and significantly lower after prebiotic intervention compared to placebo (interaction b = -0.39, t = -39.65; null model comparison p < 0.001, **SI_behav Table 18-19**).

SI-Table 18: Subjective hunger ratings by timepoint for each intervention arm. Ratings were measured using a Likert scale inside MR scanner at 10 and 40 min after 10% energy intake using a breakfast shake, with a scale from 1 (not at all) to 8 (extremely).

Timepoint	Intervention	n	hunger rating			
			10 min postprandial	40 min postprandial	mean 10-40 min postprandial	
			mean ± SD	mean ± SD	mean SD	change± SD
BL	prebiotics	55	4.25 ± 1.76	5.16 ± 1.71	4.71 ± 1.66	-
FU	prebiotics	48	4.13 ± 1.66	4.72 ± 1.73	4.43 ± 1.60	-0.28 ± 1.32
BL	placebo	53	3.77 ± 1.69	4.79 ± 1.65	4.28 ± 1.58	-
FU	placebo	49	3.92 ± 1.68	4.67 ± 1.72	4.30 ± 1.54	0.05 ± 1.37

SI-Table 19: Mixed effects linear model results on the effects of prebiotic intervention on subjective hunger ratings (average). Ratings were measured using a Likert scale inside MR scanner at 10 and 40 min after 10% energy intake and averaged, with a scale from 1 (not at all) to 8 (extremely). Model comparison, p < 0.001.

random effects	**variance**	**SD**	
subject (intercept)	2.33	1.53	
time (follow-up)	1.08	1.04	
intervention (prebiotic)	1.12	1.06	
residual	0.18	0.42	
fixed effects	**estimate**	**SE**	**t-value**
(intercept)	4.03	0.83	4.86
time (follow-up)	0.10	0.15	0.67
intervention (prebiotic)	0.46	0.15	3.07
age	0.02	0.03	0.52
gender (male)	-0.27	0.41	-0.66
time (follow-up) * intervention (prebiotic)	**-0.39*** **	**0.28*** **	**-39.65*** **

***Formula**: hunger ~ timepoint * intervention + age + gender + (1+(timepoint + intervention) | subject). **REML** criterion at convergence: 37696, Number of observations: 204, groups: participants, 59. Significance, *** p < 0.001*

*No random slope "timepoint * intervention" as model did not converge -> only random slopes "timepoint" and "intervention".*

Additional wanting models with "true wanting" models considering weighted ratings.

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Deviant to the preregistration, we did not further explore interaction effects for wanting ratings modelled as dependent outcome variable in three different ways (1, individual wanting – individual liking; 2, individual wanting - individual liking - population mean of wanting; 3 individual wanting * population mean of wanting) per item, to simplify results.

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Methods

Feces parameters

Fecal samples were collected independently at home, where they were frozen and stored at -15 to -20°C. Samples were delivered to the institute in isolated boxes until they were stored at -80°C. Samples were aliquoted in frozen state. For microbial measurements, DNA was extracted and further analysis was done by GENEWIZ Germany GmbH, Leipzig, Germany. For metabolomics (SCFA measurements), samples were analyzed by the Center for Environmental Research (UfZ), Leipzig, Germany.

SCFA measurements in blood and feces.

Metabolite extraction:

Chemicals: Acetonitrile, formic acid and methanol were purchased from Sigma Aldrich (St Louis, MO, USA). D7-butyric acid was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). All short chain fatty acids standards (SCFAs) used for linear regression and quantitation were purchased from Sigma Aldrich (St Louis, MO, USA). All solvents for MS were of analytical grade purity. Experimental water (resistivity of 18.2 MΩ cm) was purified using a Milli-Q system (Millipore, Milford, MA, USA).

For SCFAs the method of Han et al. (2015) was modified. First, 100 mg feces were mixed with 500 µl ACN:Water (1:1, v/v) and homogenized using a TissueLyser II (30 Hz, 10 min; Retsch Qiagen). After short centrifugation (2 min, 14000 rpm) 100 µl of the supernatant were added to 500 µl ACN:Water:methanol (3:1:2, v/v/v) and the sample was vortexed for 5 min. After sonication (5 min) and centrifugation (14.000 rpm, 4°C, 5 min) 550 µl of the supernatant were transferred into a new tube and evaporated to dryness. Pellet was reconstituted in 100 µl 50% and 38 µl used for further derivatization. Next, 20 µl serum and 2 µl of standards were diluted with 18 µl and 38 µl 100% ACN, respectively. For derivatization, both specimen, serum and feces supernatant, were combined with 2 µl D7-butyric acid (2 mM) used as internal standard, 20 µl 3-nitrophenylhydrazine in 50% ACN (200 mM) and 20 µl N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride in 50% ACN with 6% pyridine (120 mM). Incubation of the mixture was done for 30 min at 40 °C in a thermomixer (Eppendorf, Hamburg, Germany).

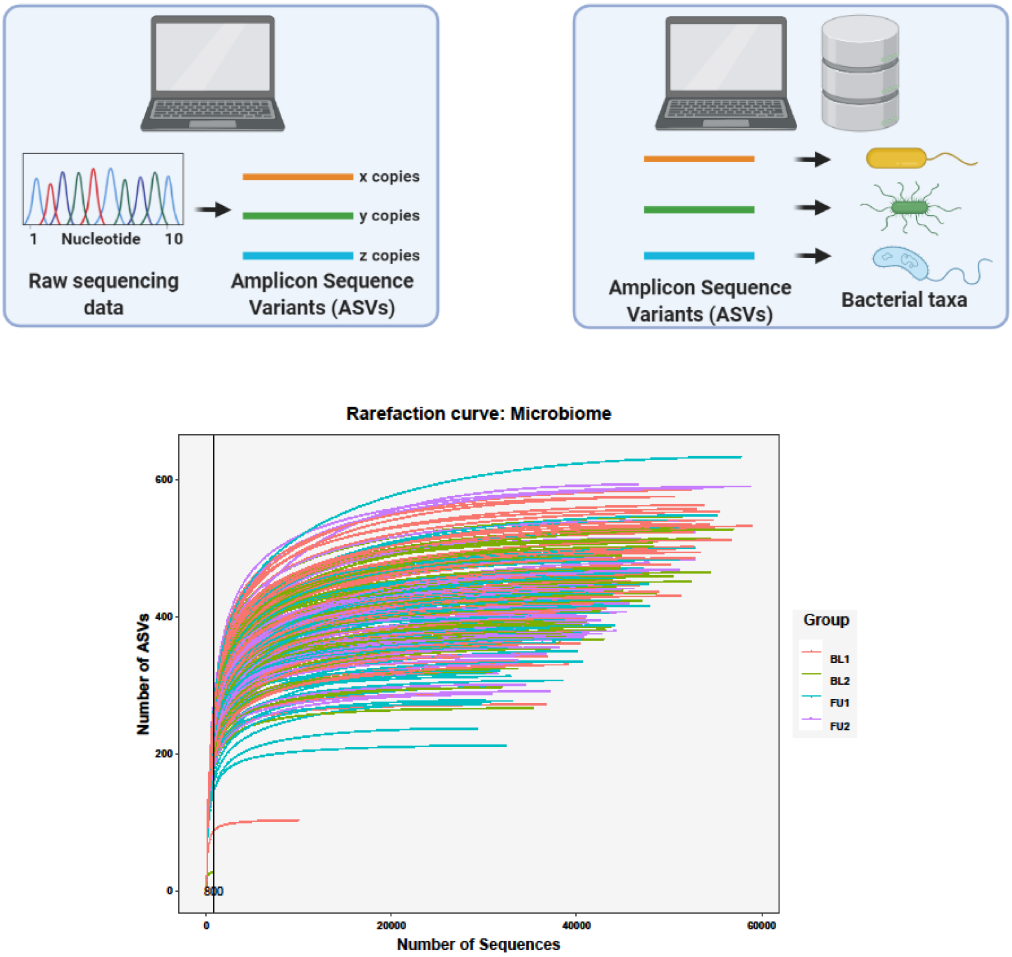
Prior to measurement, the resulting derivative was diluted 1:50 in 10% ACN. Of each sample 10 µl were injected into the UltiMate 3000 HPLC system (ThermoFisher Scientific™, Waltham, MA, USA) coupled online to a QTRAP® 5500 mass spectrometer (Sciex, Framingham, USA). Chromatographic separation of SCFAs was performed on an Acquity UPLC BEH C18 column (1.7µm, 2.1 x 100 mm) with H2O + 0.01 % formic acid and ACN + 0.01% formic acid as mobile phases. Constant flow rate was set to 0.35 ml and linear LC gradient was as follows: 0-2 min at 15% B, 2-17 min 15-50% B, 17-18 min 100 % B, 18-18.1 min 100-15% B, 18.1 -21 min 15 % B. Mass spectrometric measurement was performed in negative ionization mode. For identification and quantitation, a scheduled multiple reaction monitoring (MRM) method was used, with specific transitions for every SCFA. Peak areas of all samples and standards for linear regression were determined in Analyst® Software (v. 1.6.2, AB Sciex) and areas for single SCFAs were exported. Normalization and statistics were performed with in-house written R scripts.

Note that fecal concentrations were higher compared to serum concentrations, and that the number of available samples dropped considerably due to methodological challenges in SCFA

measurements, including lack of remaining blood and feces material after the preceding analyses.

16S rRNA sequencing.

Sample preparation and sequencing was performed by GENEWIZ (GENEWIZ Germany GmbH, Leipzig) for sequencing. Briefly, following GENEWIZ standardized workflow. For each sample, paired-end reads were joined, low-quality reads were removed, reads were corrected, chimeras removed and Amplicon Sequence Variants (ASVs) were obtained. Taxonomy was annotated to the ASVs using the RDP database 95. The read counts per ASV with taxonomic annotation were normalized and relative abundances of each ASV and taxa were calculated using the R scripts Rhea. Visualization of all library-indexed genera was done as in 96 by inhouse written R-tools using ggplot2. Group statistics (4 groups: intervention, timepoint) consists of paired ANOVA Benjamini-Hochberg adjusted with pairwise post hoc Games-Howell.



SI Figure 1: 16S-rRNA sequencing to ASV extraction.

Supplementary Information on Gut Microbiome **Medawar et al.**

Results – Microbiotal outcome measures after intervention.

SI Table 1: Significant shifts in gut-related variables of interest after prebiotic intervention, according to linear effect modelling.

	intervention*timepoint effects				ANOVA null model comparison
	n_{subj}	n_{obs}	b	t	p
Stool frequency	59	201	**1.24***	**2.05***	**0.04**
Bristol Stool Scale	57	196	-0.26	-0.88	0.38
Richness	58	200	**-51.63***	**-3.23**	**0.001**
Evenness	58	200	**-0.0085***	**-5.12**	**<0.001**
Linear mixed modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with p < 0.05): with the Formula: variable_of_interest ~ timepoint * intervention + timepoint + intervention + (1 + (timepoint+intervention) | subject) + age + gender.					
Shannon Effective	58	200	**-34.68***	**-4.81**	**<0.001**
Simpson Effective	58	200	**-28.63***	**-5.34**	**<0.001**
Linear mixed modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with p < 0.05): with the Formula: variable_of_interest ~ timepoint * intervention + timepoint + intervention + (1 + (timepoint+intervention) | subject) + age + gender + time_of_day.					

SI-Table 2: Significant shifts in microbiota relative abundances on the genera level after prebiotic intervention, according to 16S-rRNA sequencing and linear mixed effects modelling.

	Interaction effect time (follow-up) * intervention (prebiotic)		**ANOVA null model comparison**	
increased abundance:	**b**	**t**	**p**	**p_{adj}**
Akkermansia	0.20	2.19	0.029	0.16
Anaerostipes	**0.73**	**3.01**	**0.003**	**0.017**
Bifidobacterium	**9.82**	**10.42**	**< 0.001**	**< 0.001**
Catenibacterium	0.11	2.19	0.029	0.16
Collinsella	**2.66**	**4.96**	**< 0.001**	**< 0.001**
Defluviitaleaceace UCG 011	0.02	2.12	0.034	0.19
Epulopiscium	0.01	1.96	0.049	0.27
Hafnia Obseumbacterium	0.03	0.02	0.047	0.26
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[1]	**2.08**	**2.65**	**0.008**	**0.045**
Libanicoccus	0.20	2.35	0.019	0.11
Ligilactobacillus	**0.28**	**2.67**	**0.008**	**0.045**
Limosilactobacillus	**0.28**	**5.10**	**< 0.001**	**< 0.001**
Neisseria	<0.01	2.01	0.043	0.24
Weissella	0.08	2.05	0.041	0.23
decreased abundance:				
Acetanaerobacterium	-0.01	-2.14	0.032	0.18
Actinomyces	-0.11	-2.38	0.018	0.10
Anaerofustis	-0.02	-2.22	0.027	0.15
Anaerotruncus	-0.02	-2.17	0.031	0.17
Bilophila	-0.10	-2.25	0.025	0.14
Blautia	-1.85	-2.14	0.033	0.18
Candidatus Saccharimonas	-0.01	-1.97	0.048	0.27
Catenibacillus	-0.01	-2.08	0.039	0.21

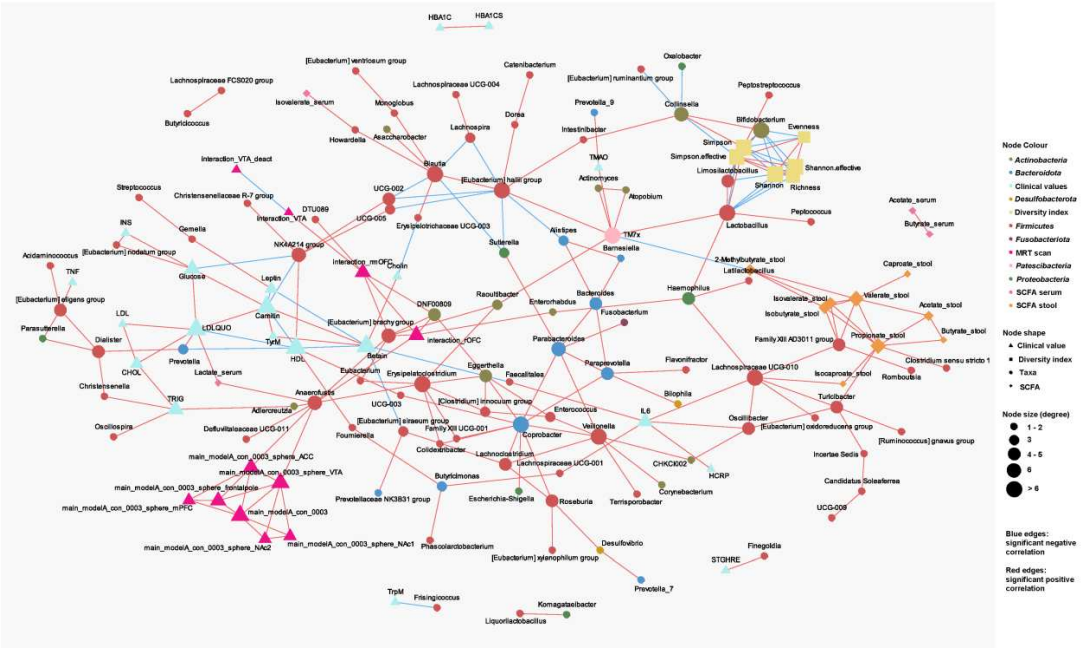
Clostridium innocuum group	-0.05	-2.06	0.042	0.23
Corynebacterium	-0.01	-2.56	0.011	0.06
Desulfovibrio	**-0.20**	**-3.41**	**0.001**	**0.006**
Eggerthella	**-0.33**	**-3.46**	**0.001**	**0.006**
Erysipelatoclostridium	-0.19	-2.20	0.028	0.16
Eubacterium brachy group	**-0.11**	**-3.18**	**0.002**	**0.011**
Eubacterium eligens group	**-0.21**	**-2.76**	**0.006**	**0.033**
Eubacteirum ventriosum group	-0.18	-2.00	0.046	0.26
Faecalitalea	-0.08	-2.12	0.029	0.16
Family XIII AD3011 group	-0.28	-2.50	0.013	0.07
Family XIII UCG 001	-0.08	-2.32	0.021	0.12
Gemella	-0.02	-2.26	0.025	0.14
Gordonibacter	-0.10	-2.49	0.013	0.07
Holdemania	-0.02	-2.34	0.019	0.11
Incertae Sedis	-0.35	-2.08	0.037	0.21
Lachnospira	-0.16	-1.99	0.047	0.26
Lachnospiraceae NK4A136 group	-0.45	-2.56	0.011	0.06
Levilactobacillus	-0.01	-2.23	0.026	0.15
Natranaerovirga	-0.01	-2.06	0.039	0.22
Roseburia	**-1.10**	**-3.86**	**< 0.001**	**0.001**
Rothia	-0.01	-1.97	0.049	0.27
Ruminococcus gauvreauii group	**-0.69**	**-3.86**	**< 0.001**	**0.001**
Ruminococcus torques group	-0.87	-2.63	0.009	0.05
Shuttleworthia	**-0.08**	**-2.78**	**0.006**	**0.033**
Subdoligranulum	**-1.30**	**-2.82**	**0.005**	**0.028**
Tyzzerella	-0.23	-2.37	0.019	0.10
UCG 003	**-0.16**	**-3.42**	**< 0.001**	**0.004**

*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 ~ timepoint * intervention + timepoint + intervention + (1 + (intervention + timepoint) | subject). All models run on $n_{obs} = 204$ in $n_{subj} = 58$ and listed in alphabetical order of genera of interest. 1, statistics refer to models without random slopes due to non-convergence.*

Exploratory weighted network analysis (WGNCA)

Using weighted network analysis we clustered microbiota genera to modules. In detail, data from participants with complete measures from all four timepoints (n_{subj} = 35) entered these network analyses and 4 out of 13 taxa modules were significantly correlated to prebiotic intervention (M05 r = 0.51, p < 0.001; M06 r = -0.23, p = 0.006; M08 r = -0.22, p = 0.007; M09 r = -0.20, p = 0.018). However, none of those 4 clusters correlated with prebiotic-induced changes in brain activation during decision-making. Similarly, neither hubs nor clusters of microbiota abundance differences before compared to after prebiotic intervention, nor hubs nor clusters of the microbiota pattern after prebiotic per se, correlated significantly with brain activation.

Results – Network analysis.



SI Figure 2: Network analysis.

KEGG analysis

We conducted functional capacity prediction of 16S-rRNA gene profiling data using the Tax4fun R-package and the Kyoto Encyclopedia of Genes and Genomes (KEGG) [2].This resulted in 8800 KEGG functional orthologues.

SI Table 3: KEGG pathway relative abundance group posthoc pairwise PERMANOVA test (p-adjusted Benjamini Hochberg).

Pairs	**F.Model**	**R2**	**p.value**	**p.adjusted**	**sign.**
BL_placebo vs. BL_prebiotics	0.625	0.007	0.517	0.777	ns
BL_placebo vs. FU_placebo	0.105	0.001	0.987	0.987	ns
BL_placebo vs. FU_prebiotics	**12.76**	**0.127**	**0.001**	**0.002**	**
BL_prebiotics vs. FU_placebo	0.473	0.005	0.655	0.786	ns
BL_prebiotics vs. FU_prebiotics	**11.459**	**0.115**	**0.001**	**0.002**	**
FU_placebo vs. FU_prebiotics	**14.433**	**0.141**	**0.001**	**0.002**	**

SI Table 4: Significant shifts in functional pathway capacity after prebiotic intervention, according to KEGG analysis and linear effect modelling.

KEGG pathway	**intervention*timepoint effects** (prebiotics * follow-up)		**ANOVA null model comparison**	
increased post-prebiotic intervention:	**b**	**t**	**p**	**p_{adj}**
ABC transporters (ko02010)	**0.55**	**2.77**	**0.01**	**0.029**
Acarbose and validamycin biosynthesis (ko00525)	**0.01**	**3.12**	**0.004**	**0.014**
Alanine aspartate and glutamate metabolism (ko00250)	**0.12**	**5.39**	**<0.001**	**<0.001**
Aminoacyl tRNA biosynthesis (ko00970)	**0.10**	**2.66**	**0.011**	**0.03**

Arginine biosynthesis (ko00220)	**0.10**	**4.19**	**<0.001**	**0.001**
Carbapenem biosynthesis (ko00332)	**0.02**	**4.14**	**<0.001**	**0.001**
Cyanoamino acid metabolism (ko00460)	**0.03**	**2.43**	**0.019**	**0.045**
Cysteine and methionine metabolism (ko00270)	**0.25**	**5.39**	**<0.001**	**<0.001**
D Glutamine and D glutamate metabolism (ko00471)	**0.01**	**2.69**	**0.008**	**0.025**
DNA replication (ko03030)	**0.06**	**2.61**	**0.012**	**0.032**
Ferroptosis (ko04216)	**0.03**	**3.79**	**<0.001**	**0.002**
Galactose metabolism (ko00052)	**0.09**	**2.44**	**0.02**	**0.046**
Glucosinolate biosynthesis (ko00966)	**0.004**	**2.53**	**0.022**	**0.049**
Glycine serine and threonine metabolism (ko00260)	**0.09**	**5.63**	**<0.001**	**<0.001**
Homologous recombination (ko03440)	**0.11**	**3.85**	**<0.001**	**0.002**
Isoquinoline alkaloid biosynthesis (ko00950)	**0.05**	**5.36**	**<0.001**	**<0.001**
Lysine biosynthesis (ko00300)	0.05	2.26	0.029	0.062
Mismatch repair (ko03430)	**0.06**	**3.04**	**0.004**	**0.015**
Nicotinate and nicotinamide metabolism (ko00760)	**0.08**	**6.79**	**<0.001**	**<0.001**
Nucleotide excision repair (ko03420)	**0.08**	**3.89**	**<0.001**	**0.001**
Phenylalanine tyrosine and tryptophan biosynthesis (ko00400)	**0.10**	**2.97**	**0.006**	**0.019**
Phenylpropanoid biosynthesis (ko00940)	**0.03**	**2.66**	**0.011**	**0.029**
Polyketide sugar unit biosynthesis (ko00523)	**0.02**	**2.07**	**0.057**	**0.11**
Primary bile acid biosynthesis (ko00120)	**0.01**	**3.35**	**0.001**	**0.003**
Proteasome (ko03050)	**0.01**	**3.79**	**<0.001**	**0.002**
Purine metabolism (ko00230)	**0.19**	**4.33**	**<0.001**	**0.001**
Pyrimidine metabolism (ko00240)	**0.12**	**3.34**	**0.002**	**0.008**
Quorum sensing (ko02024)	**0.19**	**2.62**	**0.014**	**0.035**
Ribosome (ko03010)	**0.27**	**3.08**	**0.003**	**0.014**
RNA degradation (ko03018)	**0.04**	**2.81**	**0.007**	**0.022**

RNA polymerase (ko03020)	**0.03**	**3.39**	**0.001**	**0.006**
Secondary bile acid biosynthesis (ko00121)	**0.01**	**3.09**	**0.002**	**0.008**
Selenocompound metabolism (ko00450)	**0.08**	**6.61**	**<0.001**	**<0.001**
Starch and sucrose metabolism (ko00500)	**0.18**	**2.98**	**0.005**	**0.017**
Taurine and hypotaurine metabolism (ko00430)	**0.03**	**7.04**	**<0.001**	**<0.001**
Terpenoid backbone biosynthesis (ko00900)	**0.04**	**2.71**	**0.009**	**0.027**
Thiamine metabolism (ko00730)	**0.05**	**3.21**	**0.002**	**0.007**
Tropane piperidine and pyridine alkaloid biosynthesis (ko00960)	**0.04**	**4.78**	**<0.001**	**<0.001**
Valine leucine and isoleucine biosynthesis (ko00290)	**0.04**	**2.86**	**0.008**	**0.026**
Vitamin B6 metabolism (ko00750)	**0.02**	**5.86**	**<0.001**	**<0.001**
Zeatin biosynthesis (ko00908)	0.004	2.28	0.029	0.063
decreased post-prebiotic intervention:	**b**	**t**	**p**	**p_{adj}**
Amino sugar and nucleotide sugar metabolism (ko00520)	**-0.07**	**-2.63**	**0.011**	**0.030**
Arachidonic acid metabolism (ko00590)	**-0.01**	**-2.90**	**0.005**	**0.017**
Atrazine degradation (ko00791)	**-0.01**	**-3.33**	**0.002**	**0.008**
Basal transcription factors (ko03022)	**-0.004**	**-3.41**	**0.001**	**0.005**
beta Alanine metabolism (ko00410)	-0.04	-2.19	0.039	0.080
Biofilm formation - Pseudomonas aeruginosa (ko02025)	-0.09	-2.83	0.005	0.17
Biosynthesis of siderophore group nonribosomal peptides (ko01053)	**-0.04**	**-2.47**	**0.019**	**0.045**
Biosynthesis of terpenoids and steroids (ko01062)	**0.00**	**-3.97**	**< 0.001**	**< 0.001**
Biosynthesis of type II polyketide products (ko01057)	**-0.004**	**-2.84**	**0.009**	**0.027**
Biosynthesis of unsaturated fatty acids (ko01040)	**-0.03**	**-2.70**	**0.010**	**0.028**

Biotin metabolism (ko00780)	**-0.08**	**-3.52**	**0.001**	**0.004**
Caprolactam degradation (ko00930)	**-0.01**	**-2.53**	**0.014**	**0.034**
Carotenoid biosynthesis (ko00906)	**-0.004**	**-3.07**	**< 0.001**	**0.001**
Cell cycle - Caulobacter (ko04112)	-0.06	-2.13	0.033	0.069
Citrate cycle - TCA cycle (ko00020)	**-0.05**	**-4.54**	**< 0.001**	**< 0.001**
Fatty acid biosynthesis (ko00061)	**-0.05**	**-3.38**	**0.001**	**0.007**
Flavonoid biosynthesis (ko00941)	0.00	-2.30	0.031	0.064
Fluorobenzoate degradation (ko00364)	-0.01	-2.16	0.041	0.082
Fructose and mannose metabolism (ko00051)	**-0.16**	**-2.47**	**0.017**	**0.042**
Glycerophospholipid metabolism (ko00564)	-0.02	-2.12	0.048	0.09
Glycolysis - Gluconeogenesis (ko00010)	-0.08	-2.59	0.010	0.28
Indole alkaloid biosynthesis (ko00901)	**0.00**	**-3.09**	**0.004**	**0.014**
Inositol phosphate metabolism (ko00562)	**-0.02**	**-4.14**	**< 0.001**	**0.001**
Methane metabolism (ko00680)	**-0.06**	**-4.55**	**< 0.001**	**< 0.001**
Nitrotoluene degradation (ko00633)	**-0.02**	**-3.14**	**0.004**	**0.015**
Non homologous end joining (ko03450)	**-0.003**	**-4.30**	**< 0.001**	**0.001**
Nonribosomal peptide structures (ko01054)	**-0.01**	**-2.37**	**0.021**	**0.047**
Oxidative phosphorylation (ko00190)	**-0.10**	**-4.95**	**< 0.001**	**< 0.001**
Phosphonate and phosphinate metabolism (ko00440)	-0.01	-2.28	0.027	0.059
Polycyclic aromatic hydrocarbon degradation (ko00624)	**-0.003**	**-2.73**	**0.010**	**0.028**
Porphyrin and chlorophyll metabolism (ko00860)	**-0.16**	**-7.03**	**< 0.001**	**< 0.001**
Pyruvate metabolism (ko00620)	**-0.10**	**-4.58**	**< 0.001**	**< 0.001**
RNA transport (ko03013)	**-0.02**	**-6.00**	**< 0.001**	**< 0.001**
Steroid degradation (ko00984)	**-0.01**	**-4.06**	**< 0.001**	**0.001**
Stilbenoid. diarylheptanoid and gingerol biosynthesis (ko00945)	0.00	-2.30	0.031	0.064
Styrene degradation (ko00643)	**-0.01**	**-2.94**	**0.006**	**0.019**
Sulfur metabolism (ko00920)	-0.08	-2.44	0.024	0.054

Sulfur relay system (ko04122)	**-0.04**	**-3.96**	**< 0.001**	**0.001**
Tryptophan metabolism (ko00380)	-0.04	-2.15	0.043	0.085
Two component system (ko02020)	**-1.01**	**-5.55**	**< 0.001**	**< 0.001**
Xylene degradation (ko00622)	**-0.02**	**-3.04**	**0.005**	**0.017**

Linear mixed modelling outcome compared to null model and model of interest as follows (ANOVA model comparison with $p < 0.05$ (uncorrected) and $p_{adj} < 0.05$ (FDR corrected, marked in bold)): with the Formula: pathyway_of_interest ~ timepoint * intervention + timepoint + intervention + (1 + (timepoint+intervention)| subject). All models on n = 205 observations in n = 58 individuals and listed in alphabetical order of genera of interest.

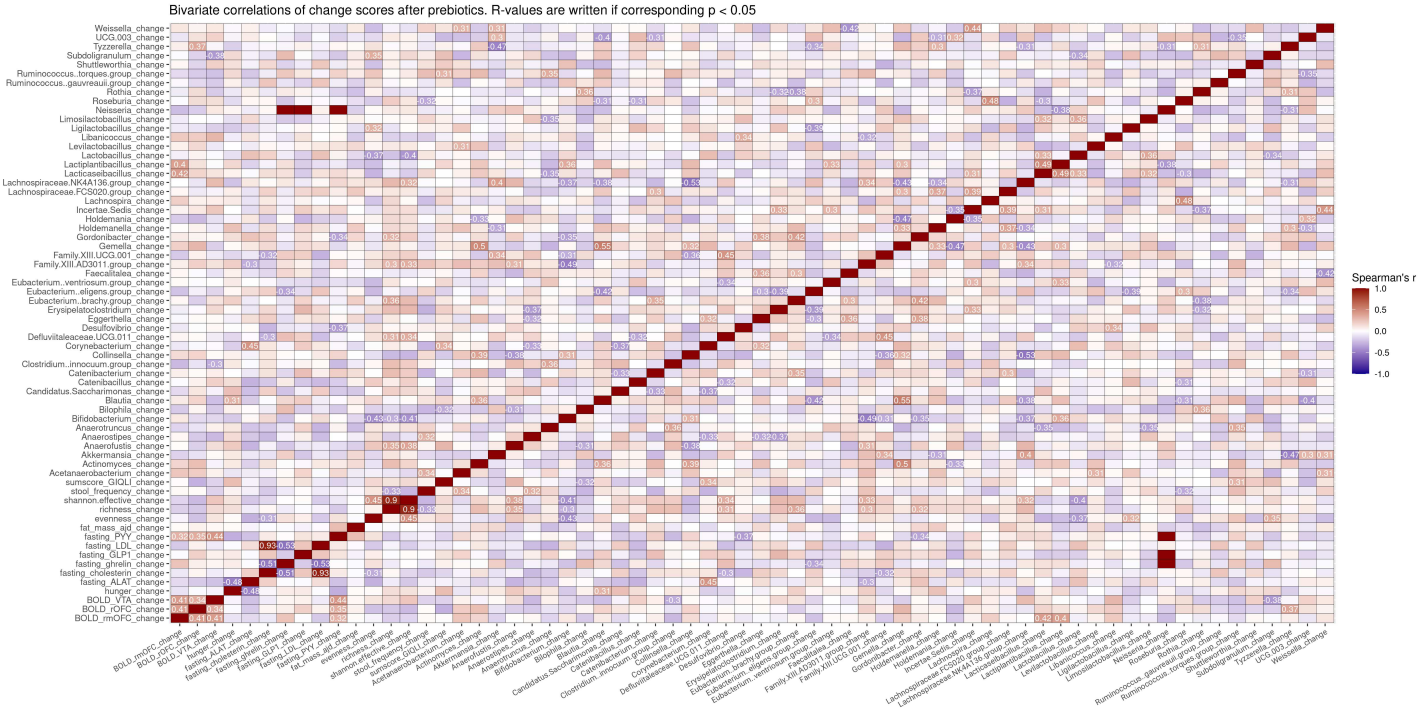
SI Figure 2: Heatmap of bivariate correlations between significant changes in reward-related brain activation and changes in microbial markers after prebiotics. **A:** brain activation, blood markers and microbiota genera. **B:** brain activation, blood markers and predicted microbial functional pathways. Color according to Spearman's r, red, positive correlations, blue, negative correlations. Written R values relate to corresponding p-values of $p < 0.05$. VTA, ventral tegmental are, OFC, orbitofrontal cortex, r, right, m, middle.

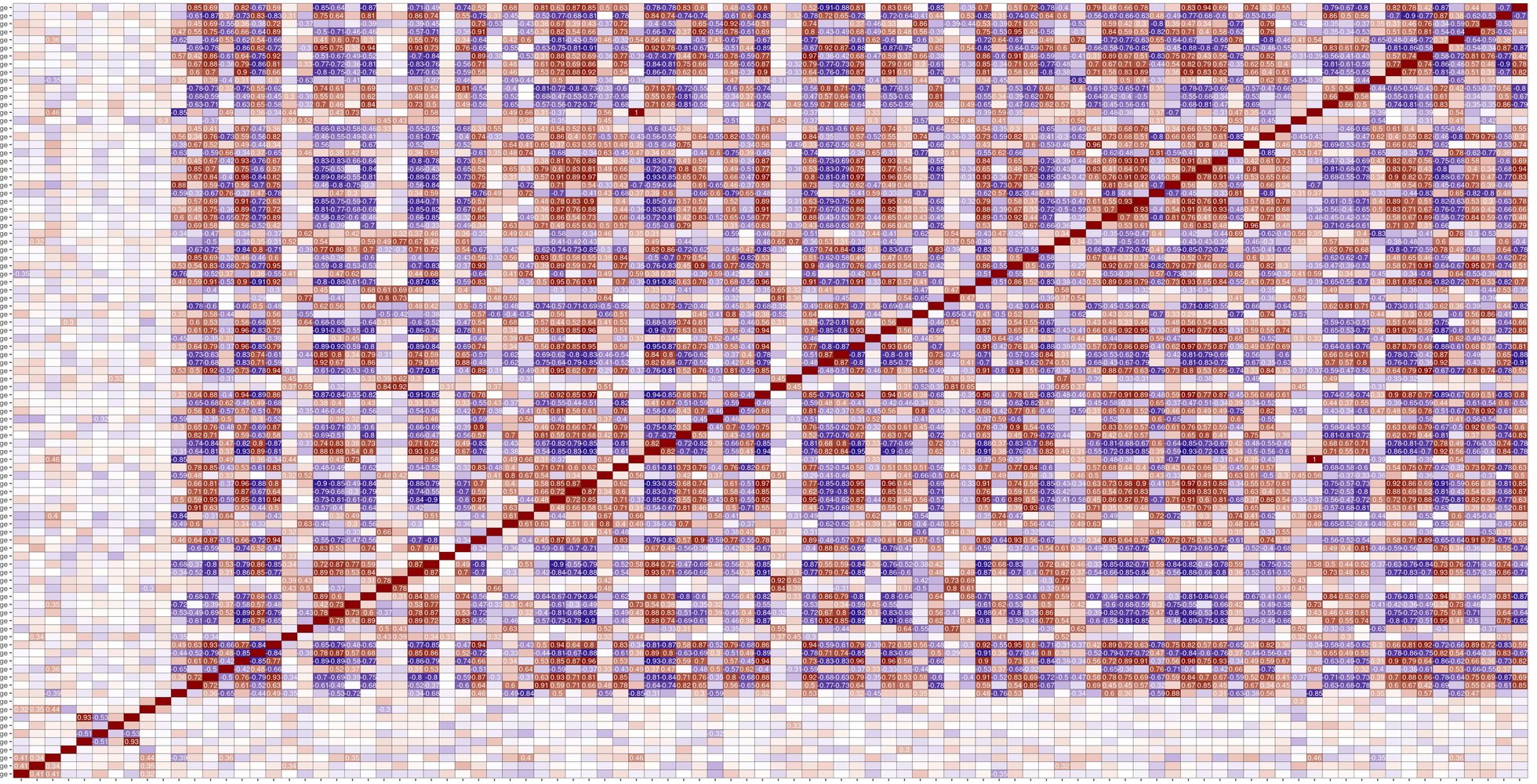
------ see supplementary files SI_Figure2A/B.tiff-----

References.

1 Han J, Lin K, Sequeira C, *et al.* An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography–tandem mass spectrometry. *Anal Chim Acta* 2015;**854**:86–94.

2 Ogata H, Goto S, Fujibuchi W, *et al.* Computation with the KEGG pathway database. *BioSystems* 1998;**47**:119–28. doi:10.1016/S0303-2647(98)00017-3


Bivariate correlations of change scores after prebiotics. R-values are written if corresponding p < 0.05
Spearman's r
1.0
0.5
0.0
-0.5
-1.0


Bivariate correlations of change scores after prebiotics. R-values are written if corresponding p < 0.05
Spearman's r
1.0
0.5
0.0
-0.5
-1.0