

Supplementary Information – General

Medawar et al.

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 \pm 10 SD for women, mean 206 kcal \pm 16 SD for men) subsequent to overnight fasting (12.5 h \pm 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:

$$\text{Energy basal metabolic rate(men)} = 66.473 + 13.752 \times \text{bodyweight[kg]} + 5.3 \times \text{bodyheight[cm]} - 6.755 \times \text{age[years]}$$

$$\text{Energy basal metabolic rate (women)} = 655.96 + 9.563 \times \text{bodyweight[kg]} + 1.85 \times \text{bodyheight[cm]} - 4.676 \times \text{age[years]}$$

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 μ l DPP-IV inhibitor, Merck, Germany; 25 μ l 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).

Supplementary Information – General

Medawar et al.

Baseline characteristics

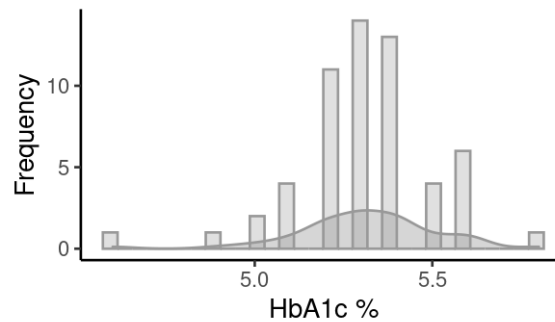
While participants were considered healthy without clinical diagnosis of metabolic disorder at screening, a moderate proportion showed signs of impaired glucose tolerance ($n = 10$, insulin $> 25\text{mIU/l}$ or glucose $> 5.6\text{mmol/l}$ or glycated hemoglobin A1c, HbA1c, > 5.7) or hyperlipidemia ($n = 17$, total cholesterol $> 250\text{mg/dl}$ or low-density lipoprotein, LDL, $> 130\text{mg/dl}$ or triglycerides $> 150\text{mg/dl}$), 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%)	

Supplementary Information – General

Medawar et al.



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)

Supplementary Information – General

Medawar et al.

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]				

Supplementary Information – General

Medawar et al.

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	0.06
			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	
Waist-to-hip ratio	204	59	(intercept)	0.68	0.02	29.68	
			time (follow-up)	-0.01	0.01	-1.61	

Supplementary Information – General

Medawar et al.

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

Supplementary Information – General

Medawar et al.

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

	n _{obs}	n _{su} n _{bj}	fixed effects	estimate	SE	t-value	full-null model compariso n p
hsCRP	195	58	(intercept)	6.80	2.27	3.00	0.26
			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	
			IL-6	196	58	(intercept)	
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	
TNF	196	58				(intercept)	3.13
			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	
			HDL	197	58	(intercept)	62.06
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	

Supplementary Information – General

Medawar et al.

		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	

Supplementary Information – General

Medawar et al.

		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	

Supplementary Information – General

Medawar et al.

			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	

Supplementary Information – General

Medawar et al.

		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	

Supplementary Information – General

Medawar et al.

			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/LNA A	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	

Supplementary Information – General

Medawar et al.

		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

- 1 Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. Proc Natl Acad Sci U S A 1918;**4**:370-3.
- 2 Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, *et al.* Enterotypes of the human gut microbiome. Nature 2011;**473**:174-80.
- 3 David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, *et al.* Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;**505**:559-63.
- 4 Tiedemann LJ, Schmid SM, Hettel J, Giesen K, Francke P, Buchel C, Brassens S. Central insulin modulates food valuation via mesolimbic pathways. Nat Commun 2017;**8**:16052.