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ORIGINAL ARTICLE

The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population

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INTRODUCTION AND OBJECTIVES: An obese-type human microbiota with an increased *Firmicutes:Bacteroidetes* ratio has been described that may link the gut microbiome with obesity and metabolic syndrome (MetS) development. Dietary fat and carbohydrate are modifiable risk factors that may impact on MetS by altering the human microbiome composition.
We determined the effect of the amount and type of dietary fat and carbohydrate on faecal bacteria and short chain fatty acid (SCFA) concentrations in people 'at risk' of MetS.
DESIGN: A total of 88 subjects at increased MetS risk were fed a high saturated fat diet (HS) for 4 weeks (baseline), then randomised onto one of the five experimental diets for 24 weeks: HS; high monounsaturated fat (MUFA)/high glycemic index (GI) (HM/HGI); high MUFA/low GI (HM/LGI); high carbohydrate (CHO)/high GI (HC/HGI); and high CHO/low GI (HC/LGI). Dietary intakes, MetS biomarkers, faecal bacteriology and SCFA concentrations were monitored.
RESULTS: High MUFA diets did not affect individual bacterial population numbers but reduced total bacteria and plasma total and LDL-cholesterol. The low fat, HC diets increased faecal *Bifidobacterium* ($P = 0.005$, for HC/HGI; $P = 0.052$, for HC/LGI) and reduced fasting glucose and cholesterol compared to baseline. HC/HGI also increased faecal *Bacteroides* ($P = 0.038$), whereas HC/LGI and HS increased *Faecalibacterium prausnitzii* ($P = 0.022$ for HC/HGI and $P = 0.018$, for HS). Importantly, changes in faecal *Bacteroides* numbers correlated inversely with body weight ($r = -0.64$). A total bacteria reduction was observed for high fat diets HM/HGI and HM/LGI ($P = 0.023$ and $P = 0.005$, respectively) and HS increased faecal SCFA concentrations ($P < 0.01$).
CONCLUSION: This study provides new evidence from a large-scale dietary intervention study that HC diets, irrespective of GI, can modulate human faecal saccharolytic bacteria, including bacteroides and bifidobacteria. Conversely, high fat diets reduced bacterial numbers, and in the HS diet, increased excretion of SCFA, which may suggest a compensatory mechanism to eliminate excess dietary energy.

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INTRODUCTION

The prevalence of both obesity and metabolic syndrome (MetS) is rising exponentially on a global scale.[1] MetS is a constellation of characteristics (dyslipidaemia: elevated plasma triacylglycerol (TAG) and low HDL-cholesterol levels, hypertension, central adiposity, insulin resistance),[2–4] which confers increased coronary heart disease risk. Diet has an important role in MetS pathogenesis. There is evidence that the amount and type of dietary fats and carbohydrates (CHO) can modify dyslipidaemia, insulin sensitivity, endothelial dysfunction and blood pressure.[5,6] Recently, the human gut microbiota has been implicated in MetS risk.[7,8] The gut microbiota appears to differ between lean and obese animals, whether obesity is diet induced[9–11] or genetic.[12–14] Obese-type gut microbiota, characterised by a higher *Firmicutes*:*Bacteroidetes* ratio in obese as opposed to lean, have been observed both in murine models of obesity and humans. Moreover, germ-free mice colonised with the microbiota from obese mice display increased body fat, higher faecal total energy content (by bomb calorimetry) and higher concentrations of faecal short chain fatty acids (SCFA) compared with their conventionally fed lean counterparts, indicating that the microbiota of obese animals may have an increased capacity to harvest energy.[15] In addition, weight loss in humans induced by CHO- or fat-restricted diets has been associated with a change in gut microbial composition, resembling the microbiota of lean individuals (that is, increased *Bacteroidetes*).[16]

The colonic fermentation of carbohydrate and fibre produces SCFA, which may impact on a number of physiological processes related to human energy metabolism, including satiety, hepatic lipogenesis, adipocyte fat deposition and thermogenesis.[7] Although studies have examined the impact of selected fibres and prebiotics on the human gut microbiota, few studies have examined the impact of whole dietary carbohydrate load on intestinal bacteria, especially in individuals at MetS risk.[17,18] Data from human studies show higher faecal SCFA concentrations in overweight and obese humans compared with their lean

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counterparts on a similar Western-style diet. This might suggest the existence of some compensatory mechanism to prevent weight gain or uncontrolled energy intake via the excretion of excess energy in the form of end-products of fermentation.[19] Dietary supplementation studies with the prebiotics inulin or oligofructose in humans and animals have shown that changes in colonic SCFA are accompanied by reduced plasma lipids, particularly in those with hyperlipidaemia and hypercholesterolaemia.[20–22] Cani *et al*.[9] reported that high-fat diets significantly lowered the levels of dominant members of the gut microbiota in mice (that is, *Bacteroides* spp., mouse intestinal bacteria, *Eubacterium rectale/Clostridium coccoides* population and *Bifidobacterium* spp.) and induced insulin resistance, a low grade systemic inflammation and higher plasma endotoxin concentrations, defined as metabolic endotoxaemia. Restoration of bifidobacteria levels in high-fat fed mice through dietary supplementation with the prebiotic oligofructose significantly reduced metabolic endotoxaemia and MetS development. This may have occurred by a mechanism involving the increased production, via prebiotic fermentation, of endogenous glucagon-like peptide-2, which improved epithelial barrier function and reduced intestinal permeability.[22,23]

Although very little is known about the impact of high fat diets on the gut microbiota, high dietary fat intake may increase the quantities of fat and bile acids reaching the colon. It has been suggested that the gut microbiota may metabolise dietary fats (producing diacylglycerols from polyunsaturated fats), convert primary bile acids into secondary bile acids and impact on the enterohepatic circulation of bile acids and fat absorption from the small intestine. However, few studies have investigated the effect of high-fat diets on the levels of dominant members of the human gut microbiota or SCFA output,[18,24] especially in individuals with increased obesity and type 2 diabetes risk.

In the present study, we tested the hypothesis that the type and quantity of dietary fat and carbohydrate significantly affect the gut microbiota and colonic fermentation in human subjects 'at risk' or suffering from the MetS. In addition, we investigated the impact of any gut microbial changes on metabolic and cardiovascular disease biomarkers. This was achieved by measuring fecal microbial composition and SCFA in participants undergoing a 6-month dietary intervention.

METHODS

Study design

This study was part of a five-centred intervention study, the 'RISCK' trial, funded by the UK's Food Standards Agency,[25,26] and describes the analysis of faecal microbiology on samples collected at one site (University of Reading). The study was conducted according to the Declaration of Helsinki guidelines and was given a favourable ethical opinion by the University of Reading Research Ethics Committee and the Local Research Ethics Committee. RISCK was registered as a clinical trial (ISRCTN29111298). The study was a randomised, controlled, single blind, parallel design and was carried out at the Hugh Sinclair Unit of Human Nutrition, University of Reading, UK. A total of 130 age and sex matched, free-living volunteers were recruited, of which 88 participants completed the 24 weeks dietary intervention period and provided faecal samples.

Participant recruitment

Participants were recruited at the Hugh Sinclair Unit of Human Nutrition at the University of Reading on the basis of their increased risk for developing the MetS. A weighted scoring system, which ensured that volunteers expressed a minimum of two features of the MetS (score $\geqslant 4$), was employed. In brief, participants were scored on their BMI or waist circumference, plasma insulin or glucose, plasma TAG and HDL cholesterol concentrations and hypertension, which is detailed in Jebb *et al*.[25] The study inclusion criteria included men and women, aged between 30 and 65 years, with normal hepatic and renal function.

Dietary intervention

The RISCK food exchange model was developed to achieve the dietary targets of the RISCK intervention that has been reported in detail previously.[26,27] Participants followed a 4-week run-in reference diet that was a high saturated fat diet (HS; saturated fatty acids, SFA)- high glycemic index (GI) diet (38%E fat), after which they were randomly assigned to either continue with the reference diet or one of four experimental diets (HM/HGI:high monounsaturated fat (MUFA)/high GI; HM/LGI:high MUFA/low GI; HC/HGI:high carbohydrate (CHO)/high GI; HC/LGI:high CHO/low GI) for 24 weeks. All groups were matched for age (mean ± s.d., 54 ± 9.5 years), gender, BMI (28.8 ± 4.9 kg m^{-2}) and HDL cholesterol concentration (1.6 ± 0.4 mmol l^{-1}). Target nutrients intakes (expressed as percentage of total energy intake) for the five isocaloric diets are summarised as follows: HS: total fat 38%E, SFA 18%E, MUFA 12%E, PUFA 6%E, CHO 45%E, GI 64%; HM/HGI: total fat 38%E, SFA 10%E, MUFA 20%E, PUFA 6%E, CHO 45%E, GI 64%; HM/LGI: total fat 38%E, SFA 10%E, MUFA 20%E, PUFA 6%E, CHO 45%E, GI 53%; HC/HGI: total fat 28%E, SFA 10%E, MUFA 11%E, PUFA 6%E, CHO 55%E, GI 64%; HC/LGI: total fat 28%E, SFA 10%E, MUFA 11%E, PUFA 6%E, CHO 55%E, GI 51%. Dietary calculations were based on the National Diet and Nutrition Survey and National Food Survey habitual intake estimates.[28,29] Dietary manipulation has been described in detail by Moore *et al*.[26] and was achieved by replacing all exchangeable fats and CHO in the habitual diets of each participant with products specifically chosen or formulated to provide the required fat/carbohydrate intake and composition in each of the intervention diets.

Analysis of food diaries

The 4-day food diaries (including 1 weekend day) were analysed by the Nutrition Epidemiology Group at Human Nutrition Research, Department of Nutrition and Health Research, Cambridge, as previously described.[25]

Faecal samples collection and analysis

Faecal samples were collected within 2 h of defaecation, diluted 1/10 (wt/vol) with sterile 1 M phosphate buffered solution pH 7.2, then homogenized in a stomacher (Seward STOMACHER 400 Lab System, Seward Ltd, Thetford, Norfolk, UK) for 2 min at normal speed. Glass beads (5 mm diameter) were added, samples were vortexed and centrifuged at 100 *g* for 2.5 min. Supernatants containing bacterial cells were fixed in 4% (wt/vol) paraformaldehyde solution (pH 7.2) at 4 °C overnight, washed with phosphate buffered solution, re-suspended in phosphate buffered solution and ethanol for storage at −20 °C and used for fluorescent *in situ* hybridisation as previously described.[30] Nine genus- and group-specific 16S rRNA-targeted and 5′-Cy3-labelled oligonucleotide probes (MGW-Biotech, Milton Keynes, UK) were employed: EREC482, specific for most bacteria within the clostridial cluster XIVa; Bac303, specific for most *Bacteroides* and *Prevotella* spp., *Barnesiella* spp. and *Oridobacter splanchnicus*; Fpra655, specific for *Faecalibacterium prausnitzii*; Bif164, specific for bifidobacteria and *Parascardovia denticolens*; Ato291, specific for all *Atopobium*, *Cryptobacterium*, *Collinsella*, *Eggerthella* and *Olsenella*; Lab158, specific for most lactobacilli, *Leuconostoc* and *Weissella*, and all *Enterococcus*, *Vagococcus*, *Melisococcus*, *Tetragenococcus*, *Paralactobacillus*, *Pediococcus*, *Oenococcus* and *Catelliococcus* spp. and *Lactococcus lactis*; Chis150, specific for most bacteria within clostridial cluster I and all members of clostridial cluster II; SRB687, specific for *Desulfovibrioaceae*; Enterobacteriaceae probe D, specific for most *Enterobacteriaceae*.[31–39] 4′-6-diamidino-2-phenylindole (DAPI) was employed for total bacterial cell enumeration.

Faecal SCFA analysis by gas chromatography

Samples were acidified to pH 2–3 with 6 M HCl, centrifuged at 13 000 *g* for 5 min and filtered through 0.2 μm. polycarbonate syringe filter. Standard solutions containing 20, 10, 5, 1 and 0.5 mM external standards and 2 mM of internal standard (2-ethylbutyirc acid) were used. Fatty acids were determined by gas-liquid chromatography on a Hewlett Packard (Agilent) 5890 Series II GC system (HP, Crawley, West Sussex, UK) fitted with a FFAP column (30 m × 0.53 mm, diameter = 0.50 μm, J&W Scientific, Agilent

Technologies Ltd, South Queensferry, West Lothian, UK) a flame-ionisation detector and with glass wool inserted in the injection port. The injected sample volume was 1 μl. The carrier gas helium was delivered at a flow rate of 14 ml min^{-1}. The head pressure was set at 10 psi and the split ratio was 10:1. The total flow was 140 ml min^{-1}. Injector and detector temperature were set at 280 °C and 300 °C, respectively. The initial oven temperature was 100 °C, maintained for 0.5 min, raised to 150 °C at 8 °C per min, then increased to 250 °C at 50 °C per min and finally held at 250 °C for 2 min. Fatty acid concentrations were calculated by peak integration using Atlas Lab managing software (Thermo Lab Systems, Mainz, Germany) and expressed as mmol g^{-1} of faeces.

Blood samples collection and analysis

Blood samples were collected from participants after an overnight fast into tubes appropriate for the collection of plasma (TAG, total cholesterol, HDL-cholesterol and NEFA), plain serum (LDL-cholesterol concentration and serum C-reactive protein), citrated plasma (PAI-1 and ICAM-1), fluoride/oxalate (glucose) and heparinised plasma (leptin and insulin). Plasma TAG, glucose, total cholesterol and HDL-cholesterol concentrations were determined using commercially available kits on a Monarch Automatic Analyzer ILab 600 (Instrumentation Laboratories Ltd, Warrington, UK). LDL-cholesterol was isolated from the serum samples by selective precipitation (Randox CH1350, Randox Laboratories Ltd, Crumlin, Co. Antrim, UK). Plasma insulin was determined by a specific commercial enzyme-linked immunosorbent assay (ELISA) kit (DAKO Diagnostic Ltd, Cambridgeshire, UK). Plasma NEFA concentration was measured by a commercially available assay (Wako NEFA C kit; Alpha Laboratories Ltd, Hampshire, UK). Plasma PAI-1 (plasminogen activator inhibitor-1) and ICAM-1 (intercellular adhesion molecule 1) were determined by a chromogenic assay (Chromogenix AB, Mölndal, Sweden). Circulating soluble ICAM-1 was measured by quantitative sandwich enzyme immunoassay (R&D Systems Inc., Minneapolis, MN, USA, Cat n. BBE 1B). Serum C-reactive protein was determined by ELISA (Wako, Neuss, Germany), plasma leptin by a commercially available ELISA (Quantikine Human Leptin Kit, R&D Systems Europe Ltd, Abingdon, UK).

Intravenous glucose tolerance test

Intravenous glucose tolerance test was performed as previously described by Jebb *et al*.[25] Glucose effectiveness (Sg) and insulin sensitivity (Si) were estimated with the MINMOD Millennium program (6.02; MINMOD Inc., Pasadena, CA, USA).

Statistical analysis

Normal distribution of data was tested by calculating the skewness and kurtosis and by employing the Kolmogorov–Smirnov test. Data that were not normally distributed were Log_{10} transformed (that is, bacterial numbers) before parametric statistical analysis. Statistical significance of differences between diets was analysed by the analysis of variance (ANOVA) followed by *post hoc* Bonferroni's multiple comparison test. The difference between the values measured after treatment and at baseline was introduced in the ANOVA as a dependent variable. Paired Student's *t*-test was used to analyze changes within each experimental group after treatment compared to baseline. Correlation between changes in each parameter were measured using Pearson's regression analysis. The confidence interval was 95%.

RESULTS

Participant characteristics

The characteristics of the participants at screening are shown in Table 1. The participants were classified as being 'at risk' of developing the MetS when they scored $\geqslant 4$ points, Jebb *et al*.[25] There was no significant difference between men and women recruited to the Reading cohort of the RISCK study.

Table 1. Characteristics of study participants at screening

Characteristics	*M* (n = *43*)	*F* (n = *45*)
Age (y)	52.7 ± 9.8	59.1 ± 9.0
Postmenopause (*n* (%))	–	28 (62.2)
BMI (kg m^{-2})	28.2 ± 4.0	29.4 ± 5.5
Waist circumference (cm)	98.5	90.9
Total cholesterol (mmol l^{-1})	5.6 ± 0.7	5.7 ± 0.9
LDL-cholesterol (mmol l^{-1})	3.5 ± 0.7	3.3 ± 0.9
TAG (mmol l^{-1})	1.5 ± 0.7	1.3 ± 0.6
Glucose (mmol l^{-1})	5.0 ± 0.4	4.8 ± 0.4
Insulin (pmol l^{-1})	62.6 ± 28.0	60.3 ± 29.6
Systolic BP (mm Hg)	137.4 ± 14.9	134.5 ± 16.8
Diastolic BP (mm Hg)	84.1 ± 10.4	81.5 ± 9.6
Cigarettes smokers (*n* (%))	2 (4.7)	2 (4.4)
BP medication (*n* (%))	10 (23.3)	9 (20)
Metabolic score	5.7 ± 1.2	5.2 ± 1.2

Abbreviations: BMI, body mass index; BP, blood pressure; TAG, triacylglycerol.

Nutrient intake

The mean daily intake of macronutrients is given in Table 2. The dietary targets of the RISCK study were broadly met. The two high-MUFA diets showed a significantly lower saturated fat intake compared with the reference diet ($P<0.01$), and a concomitant increase in monounsaturated fat ($P<0.01$). Similarly, the two high-CHO diets achieved the target values for reduced fat intake ($P<0.01$), which was replaced by increased carbohydrate intake ($P<0.01$). The manipulation of GI was also successful, with a lower GI in both low GI diets ($P<0.01$), and higher GI in both high GI diets ($P=0.022$) compared to baseline and between each diet ($P<0.001$). The two high-CHO diets differed in total starch intake ($P=0.017$), as starch increased in HC/LGI compared to baseline, whereas it decreased in HC/HGI. In both low GI diets, non-starch polysaccharide consumption increased compared to baseline, also reaching statistical significance in the HM/LGI group ($P<0.01$). Despite the diets being designed as iso-energetic, total energy intake decreased after the intervention with both high CHO diets compared to baseline ($P=0.002$) and also compared with the other intervention groups (HC/HGI vs control HS: $P=0.0087$; HC/LGI vs control HS: $P=0.017$; HC/HGI vs HM/HGI $P=0.0396$; HC/LGI vs HM/HGI: $P=0.0522$; HC/HGI vs HM/LGI: $P=0.0069$; HC/LGI vs HM/LGI $P=0.0048$). There were no changes in protein intake between diets. However, there was a small increase in protein intake in both high CHO diets compared to baseline ($P=0.002$ and $P=0.011$, respectively).

Anthropometric measures, biochemical characteristics and intravenous glucose tolerance test

No significant changes in anthropometric and blood pressure measurements were observed between the five diets at the end of intervention (Table 3). A small, but statistically significant, decrease in body fat percentage was observed with HC/HGI compared to baseline ($P=0.010$) also accompanied by a slight decrease in body weight ($P=0.068$). A significant increase in waist circumference compared to baseline was observed in the HM/LGI group after treatment ($P=0.001$).

Multiple comparisons between the dietary groups showed no significant differences in the measured biochemical parameters (Table 3). The only difference observed was a significant decrease in NEFA concentration after intervention with HC/LGI compared to the control HS ($P=0.023$) and to HC/HGI ($P=0.014$). However, some changes were observed when comparing the five interventions with the relative baseline. Plasma total and LDL cholesterol were significantly decreased compared to baseline in all intervention groups ($P<0.05$), while HDL cholesterol was slightly decreased after HC/HGI diet compared to the baseline ($P=0.013$) (Table 3). The concentration of NEFA increased after intervention

Table 2. Daily macronutrients intake estimated by analysis of 4 day food diaries collected from volunteers after the 4 weeks run-in diet (baseline, B) and after 24 weeks dietary intervention (treatment, T) with the five experimental diets (HS; HM/HGI; HM/LGI; HC/HGI; HC/LGI)

	HS (n = *11*)	*HM/HGI* (n = *17*)	*HM/LGI* (n = *22*)	*HC/HGI* (n = *21*)	*HC/LGI* (n = *17*)
Energy (kcal)					
B	2153 ± 443	2136 ± 499	2005 ± 578	1920 ± 569	2212 ± 426
T	2127 ± 470	2056 ± 418	2019 ± 591	1645 ± 479*	1854 ± 314**
Fat (%)					
B	39.4 ± 3.1	39.9 ± 5.3	36.4 ± 4.5	35.9 ± 4.9	37.7 ± 5.5
T	37.7 ± 65.3	37.7 ± 6.3	35.3 ± 4.0	26.5 ± 7.3**	22.7 ± 3.6**
SFA (%)					
B	17.2 ± 1.2	17.6 ± 2.6	16.3 ± 2.6	15.3 ± 2.4	15.5 ± 2.9
T	17.5 ± 6.1	9.5 ± 1.7**	8.7 ± 1.8**	9.1 ± 3.3**	6.9 ± 2.0**
MUFA (%)					
B	12.0 ± 1.4	12.3 ± 2.0	11.0 ± 1.7	11.0 ± 1.9	11.8 ± 2.5
T	10.9 ± 3.8	18.2 ± 5.1**	17.3 ± 3.3**	9.6 ± 2.6*	8.2 ± 1.9**
PUFA (%)					
B	6.1 ± 1.4	6.1 ± 1.1	5.1 ± 1.1	5.9 ± 1.1	6.3 ± 1.8
T	5.5 ± 0.7	6.7 ± 1.6	6.2 ± 1.2**	4.8 ± 1.3**	4.7 ± 1.5**
CHO (%)					
B	43.8 ± 4.7	41.2 ± 6.2	44.4 ± 5.6	43.6 ± 6.4	42.5 ± 6.0
T	42.9 ± 10.5	42.7 ± 6.0	45.6 ± 5.5	50.6 ± 8.3**	54.7 ± 6.5**
Starch (g)					
B	139.7 ± 37.1	132.4 ± 52.3	129.6 ± 52.7	128.1 ± 51.4	143.2 ± 22.4
T	142.9 ± 49.9	136.4 ± 35.1	137.5 ± 48.4	121.8 ± 41.3	158.0 ± 36.1
Sugar (g)					
B	108.0 ± 25.3	103.3 ± 30.8	107.8 ± 34.8	93.8 ± 35.2	102.8 ± 36.3
T	101.1 ± 32.3	97.6 ± 44.3	104.4 ± 37.5	96.9 ± 36.7	110.3 ± 31.8
NSP (g)					
B	18.4 ± 3.9	17.7 ± 6.1	16.7 ± 8.2	16.5 ± 6.4	19.9 ± 3.7
T	17.5 ± 5.2	18.5 ± 6.5	19.6 ± 7.2**	17.1 ± 5.7	21.5 ± 4.8
Protein (%)					
B	14.7 ± 2.0	15.7 ± 2.0	16.1 ± 3.0	17.0 ± 2.5	15.7 ± 2.1
T	16.1 ± 3.2	16.2 ± 2.0	16.8 ± 2.6	19.5 ± 3.8**	17.9 ± 2.8*
GI					
B	63.2 ± 2.9	64.0 ± 3.0	62.4 ± 2.8	65.6 ± 3.9	62.8 ± 3.7
T	65.6 ± 2.9	65.7 ± 2.3*	54.1 ± 3.6**	66.0 ± 2.9	55.8 ± 3.34**

Abbreviations: CHO, carbohydrate; HC, high carbohydrate; HGI, high glycemic index; HM, high monounsaturated fatty acid; HS: high saturated fatty acid; LGI, low glycaemic index; MUFA, high monounsaturated fat; NSP, non-starch polysaccharide; PUFA, polyunsaturated fatty acid; SFA, high saturated fat. Values are expressed as mean ± s.d. *, **Indicate significant differences with baseline values of the same diet (*$P \leqslant 0.05$; **$P \leqslant 0.01$).

Table 3. Metabolic score at screening, anthropometric measurements, biochemical data and IVGTT after the 4 weeks run-in diet (baseline, B) and after 24 weeks dietary intervention (treatment, T) with the five experimental diets (HS; HM/HGI; HM/LGI; HC/HGI; HC/LGI)

	HS (n = *11*)	*HM/HGI* (n = *17*)	*HM/LGI* (n = *22*)	*HC/HGI* (n = *21*)	*HC/LGI* (n = *17*)
MS	5.0 ± 0.9	5.4 ± 1.1	5.3 ± 1.3	5.9 ± 1.2	5.3 ± 1.5
BMI (Kg/m2)					
B	27.1 ± 2.4	27.4 ± 4.0	29.1 ± 4.8	30.4 ± 7.4	28.6 ± 3.3
T	27.1 ± 2.4	27.5 ± 3.9	29.1 ± 4.7	29.7 ± 6.3	28.3 ± 3.0
WT (kg)					
B	75.4 ± 11.7	78.9 ± 13.9	82.9 ± 16.1	89.4 ± 20.2	81.3 ± 10.2
T	75.2 ± 11.1	79.3 ± 13.9	83.1 ± 16.1	87.6 ± 17.8	80.5 ± 9.8
WC (cm)					
B	89.7 ± 8.7	92.8 ± 9.5	93.8 ± 13.3	98.1 ± 12.4	92.8 ± 8.3
T	88.9 ± 7.0	93.4 ± 9.5	95.9 ± 12.6**	97.6 ± 10.7	92.5 ± 9.0
SBP (mm Hg)					
B	127 ± 12	127 ± 16	128 ± 13	136 ± 14	127 ± 14
T	129 ± 12	130 ± 16	127 ± 14	136 ± 14	126 ± 19
DBP (mm Hg)					
B	78 ± 8	82 ± 9	78 ± 7	83 ± 8	79 ± 11
T	78 ± 8	84 ± 9.63	78 ± 11	83 ± 8	78 ± 12
Body fat %					
B	30.5 ± 8.2	32.5 ± 9.5	34.3 ± 8.6	33.9 ± 10.4	31.5 ± 7.3
T	30.3 ± 7.8	32.7 ± 8.7	33.9 ± 8.3	32.7 ± 10.2**	31.1 ± 7.1
Total cholesterol (mmol l^{-1})					
B	5.9 ± 1.1	6.0 ± 0.9	5.9 ± 1.1	5.9 ± 0.8	5.9 ± 0.9
T	5.4 ± 1.6	5.7 ± 0.7*	5.5 ± 0.9**	5.6 ± 0.9*	5.6 ± 0.9*
HDL-cholesterol (mmol l^{-1})					
B	1.5 ± 0.3	1.4 ± 0.3	1.5 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
T	1.4 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.4*	1.4 ± 0.3
LDL-cholesterol (mmol l^{-1})					
B	3.6 ± 0.8	4.0 ± 0.8	3.7 ± 0.8	3.8 ± 0.7	3.8 ± 0.9
T	3.2 ± 1.2	3.7 ± 0.5*	3.4 ± 0.7*	3.6 ± 0.8*	3.5 ± 0.8*
TAG (mmol l^{-1})					
B	1.5 ± 0.7	1.4 ± 0.5	1.6 ± 0.7	1.6 ± 0.8	1.5 ± 0.4
T	1.5 ± 0.8	1.4 ± 0.4	1.5 ± 0.6	1.6 ± 0.7	1.6 ± 0.8
NEFA (mmol l^{-1})					
B	558 ± 134	682 ± 242	622 ± 236	538 ± 129	642 ± 193
T	621 ± 202†	573 ± 144	626 ± 184	617 ± 165*‡	558 ± 137*†‡
Glucose (mmol l^{-1})					
B	5.2 ± 0.4	5.7 ± 0.5	5.7 ± 0.5	5.7 ± 0.6	5.8 ± 0.5
T	5.2 ± 0.3	5.6 ± 0.5	5.5 ± 0.6	5.5 ± 0.5*	5.6 ± 0.6*
Insulin (ρmol l^{-1})					
B	77.6 ± 93.8	59.8 ± 21.1	67.8 ± 25.2	79.0 ± 35.2	73.3 ± 37.7
T	72.8 ± 80.4	61.5 ± 25.4	68.1 ± 32.1	67.9 ± 29.4*	70.6 ± 35.4
Leptin (ng ml^{-1})					
B	15.8 ± 14.0	20.0 ± 18.1	20.3 ± 13.6	29.7 ± 33.8	16.6 ± 12.2
T	17.0 ± 14.9	19.1 ± 17.0	20.1 ± 14.6	20.0 ± 24.1	17.4 ± 10.4
PAI-1 (μ ml^{-1})					
B	11.4 ± 4.9	16.0 ± 6.9	14.5 ± 8.1	14.8 ± 6.5	13.6 ± 8.6
T	11.7 ± 4.4	15.9 ± 7.2	13.4 ± 8.4	13.7 ± 5.7	14.1 ± 8.3
ICAM-1 (μ ml^{-1})					
B	232.2 ± 65.4	238.6 ± 48.0	259.9 ± 45.1	235.7 ± 53.0	246.2 ± 56.3
T	250.4 ± 35.9	275.8 ± 59.4*	251.6 ± 58.1	259.3 ± 39.3	255.8 ± 29.7
CRP (mg l^{-1})					
B	1.6 ± 2.3	2.3 ± 2.7	1.1 ± 1.6	2.9 ± 5.9	1.3 ± 1.8
T	1.8 ± 2.3	1.7 ± 2.3	1.8 ± 2.4	3.3 ± 7.0	1.1 ± 2.3
IS ($\times 10^{-4}$ ml μU-1 min^{-1})					
B	3.58 ± 1.67	3.49 ± 2.85	3.78 ± 2.17	2.75 ± 1.55	2.86 ± 1.21
T	3.21 ± 1.38	3.02 ± 1.28	3.13 ± 1.97	2.93 ± 1.48	3.45 ± 2.12

with HC/HGI compared to the baseline ($P = 0.013$), while it decreased after intervention with HC/LGI (558 ± 137 vs 642 ± 193, $P = 0.044$, mmol l^{-1}, mean ± s.d.). There were no significant changes in plasma TAG compared to baseline for any of the five diets. Fasting plasma glucose concentrations were significantly lower after intervention with both HC diets compared to baseline ($P = 0.015$ and $P = 0.034$, respectively, for HC/HGI and HC/LGI) and HC/HGI also decreased plasma insulin concentrations ($P = 0.028$). Soluble ICAM-1 was higher after HM/HGI compared to baseline values (275.8 ± 59.4 vs 238.6 ± 48.0, $P = 0.045$, μml^{-1}, mean ± s.d.) (Table 3). Comparisons between diets showed no significant effect of the dietary interventions on insulin sensitivity parameters calculated from the intravenous glucose tolerance test data (Si and Sg, Table 3). Statistical conclusions did not change following adjustment for weight loss.

Table 3 (*Continued*)

	HS (n = 11)	*HM/HGI* (n = 17)	*HM/LGI* (n = 22)	*HC/HGI* (n = 21)	*HC/LGI* (n = 17)
Sg ($\times 10^{-3}$ per min)					
B	16.2 ± 4.6	14.7 ± 2.9	17 ± 4.6	17.4 ± 6.4	15.6 ± 3.7
T	15.8 ± 3.5	17.6 ± 6.3	15.9 ± 5	17.2 ± 7.5	19.4 ± 7

Abbreviations: BMI, body mass index; DBS, diastolic blood pressure; HC, high carbohydrate; HGI, high glycemic index; HM, high monounsaturated fatty acid; HS, high saturated fatty acid; HT, height; IS, insulin sensitivity; IVGTT, intravenous glucose tolerance test; LGI, low glycaemic index; MS, metabolic score at screening; SBP, systolic blood pressure; Sg, insulin-independent glucose disposal; WC, waist circumference; WT, weight. †, ‡ Indicate significant differences between diets ($P \leqslant 0.05$). *, **Indicate significant differences compared to baseline values of the same diet (*$P \leqslant 0.05$; **$P \leqslant 0.01$).

Microbial enumeration in faecal samples by FISH

Significant changes were observed in total bacteria number at the end of the study compared to baseline in some of the experimental groups (Table 4). Total bacteria decreased after intervention in the three diets with the highest fat content. Total bacterial numbers of both high MUFA- diets were significantly lower than HC/HGI ($P = 0.0022$ and $P = 0.0148$, respectively, for HM/HGI and HM/LGI) and in comparison with baseline values (10.6 ± 0.2 vs 10.5 ± 0.2, $P = 0.023$ and 10.7 ± 0.2 vs 10.5 ± 0.2, $P = 0.005$, Log_{10}[bacterial cells per g faecal contents], mean ± s.d., for HM/HGI and HM/LGI, respectively). Volunteers on HS also showed some decrease in total bacteria levels at the end of the study compared to baseline (10.7 ± 0.2 vs 10.6 ± 0.2, Log_{10} [bacterial cells per g faecal contents], mean ± s.d., $P = 0.105$), although this change was not significant in comparison with other diets. In contrast, the number of total bacteria remained unchanged in the two diets with a lower fat content (10.7 ± 0.2 vs 10.7 ± 0.3, $P = 0.659$ and 10.6 ± 0.3 vs 10.6 ± 0.2, $P = 0.863$, Log_{10}[bacterial cells per g faecal contents], mean ± s.d., respectively, for HC/HGI and HC/LGI).

Interestingly, the two HC diets showed the largest increase in *Bifidobacterium* spp. population levels compared to baseline values (9.2 ± 0.6 vs 8.8 ± 0.8, mean ± s.d., $P = 0.005$ and 9.2 ± 0.6 vs 9.0 ± 0.5, $P = 0.052$, Log_{10}[bacterial cells per g faecal contents], respectively, for HC/HGI and HC/LGI), though only the increase observed in the HC/HGI group was significant compared to control HS ($P = 0.0214$). Moreover, participants on the HC/HGI diet also had significantly higher levels of *Bacteroides* spp. compared to baseline (9.9 ± 0.4 vs 9.7 ± 0.2 Log_{10}[bacterial cells per g faecal contents], mean ± s.d., $P = 0.038$), but not compared to the other experimental diets. This increase in bacteroides numbers after HC/HGI diet was associated with decreases in body weight, BMI and waist circumference (Pearson's correlation: $r = -0.64$, $r = -0.64$, $r = -0.45$, respectively) (Figure 1).

Numbers of *Faecalibacterium prasnutzii* increased after intervention with HS compared to baseline (9.5 ± 0.3 vs 9.8 ± 0.3 Log_{10}[bacterial cells per g faecal contents], mean ± s.d., $P = 0.018$) and HC/LGI (9.6 ± 0.3 vs 9.7 ± 0.3 Log_{10}[bacterial cells per g faecal contents], mean ± s.d., $P = 0.022$).

Faecal SCFA concentrations

The result of faecal SCFA concentrations analysis are shown in Table 5. No differences in SCFA levels were observed when comparing the five intervention diets. However, the fecal concentrations of acetate, propionate and n-butyrate all increased after the high saturated fat, control diet (HS) compared to baseline (30.6 ± 11.9 vs 25.2 ± 10.2, $P = 0.011$; 7.6 ± 4.0 vs 6.3 ± 3.9, $P = 0.005$; 7.8 ± 4.8 vs 6.0 ± 3.4, $P = 0.009$, mmol l^{-1}, mean ± s.d., respectively, for acetate, propionate and n-butyrate).

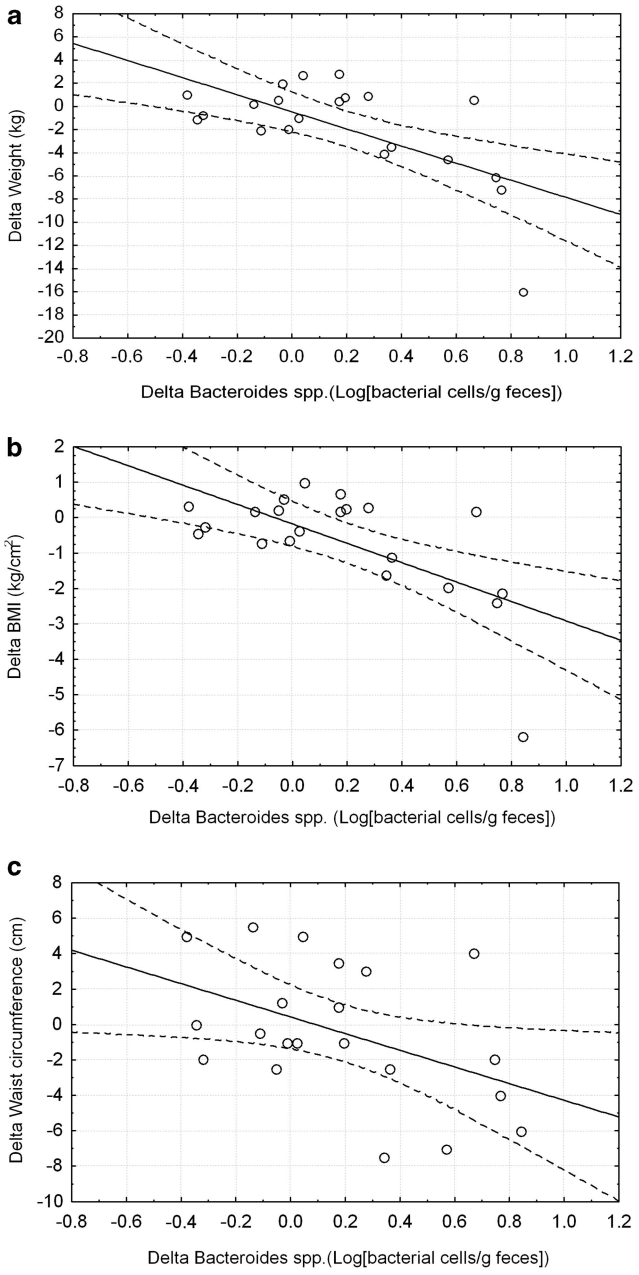

Figure 1. Significant correlation between changes in *Bacteroides* spp. faecal numbers and body weight (**a**), BMI (**b**) and waist circumference (**c**) in the HC/HGI intervention group. Pearson's correlation, $r = -0.64$ (**a**), $r = -0.64$ (**b**) and $r = -0.45$ (**c**).

DISCUSSION

Dietary intervention with both high-MUFA diets and with both low-fat/high-CHO diets decreased plasma total and LDL-cholesterol concentrations in comparison with baseline. The decrease in LDL-cholesterol was statistically significant for both low-fat/high-CHO diets, regardless of the GI, while it was significant only in the high MUFA- diet with the lower GI- (HM/LGI). These results are in accordance with those reported previously for the entire RISCK study population ($n = 640$) and confirm dietary compliance.[25] Replacement of dietary fat for carbohydrate had a positive impact on fasting glycemia (HC/HGI and HC/LGI) and decreased fasted insulin levels (HC/HGI) compared to baseline, although did not affect insulin sensitivity measures. However, in this sub-group of

Table 4. Microbial enumeration by FISH in faecal samples collected after the 4 weeks run-in diet (baseline, B) and after 24 weeks dietary intervention (treatment, T) with the five experimental diets (HS; HM/HGI; HM/LGI; HC/HGI; HC/LGI)

Probe/DNA stain	*HS (n = 11)*	*HM/HGI (n = 17)*	*HM/LGI (n = 22)*	*HC/HGI (n = 21)*	*HC/LGI (n = 17)*
DAPI					
B	10.7 ± 0.2	10.6 ± 0.2	10.7 ± 0.2	10.7 ± 0.3	10.6 ± 0.2
T	10.6 ± 0.2	10.5 ± 0.2*†	10.5 ± 0.3**‡	10.7 ± 0.2	10.6 ± 0.3†‡
EREC482					
B	10.0 ± 0.3	10.0 ± 0.3	10.2 ± 0.2	10.1 ± 0.3	10.0 ± 0.4
T	10.1 ± 0.3	10.0 ± 0.3	10.1 ± 0.3	10.1 ± 0.3	10.0 ± 0.3
Bac303					
B	9.7 ± 0.3	9.7 ± 0.3	9.7 ± 0.2	9.7 ± 0.2	9.6 ± 0.5
T	9.8 ± 0.3	9.7 ± 0.3	9.8 ± 0.3	9.9 ± 0.4*	9.8 ± 0.3
Ato291					
B	9.2 ± 0.5	9.2 ± 0.4	9.3 ± 0.4	9.3 ± 0.5	9.2 ± 0.4
T	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.4	9.4 ± 0.4	9.2 ± 0.4
Fpra655					
B	9.5 ± 0.3	9.5 ± 0.4	9.8 ± 0.2	9.7 ± 0.3	9.6 ± 0.3
T	9.8 ± 0.3*	9.5 ± 0.5	9.7 ± 0.3	9.8 ± 0.3	9.7 ± 0.3*
Bif164					
B	8.7 ± 0.7	8.6 ± 0.9	8.8 ± 0.8	8.8 ± 0.8	9.0 ± 0.5
T	8.7 ± 0.7†	8.9 ± 0.5	8.9 ± 0.5	9.2 ± 0.6**	9.2 ± 0.6*†
Lab158					
B	8.6 ± 0.3	8.5 ± 0.4	8.5 ± 0.3	8.4 ± 0.5	8.5 ± 0.3
T	8.7 ± 0.4	8.5 ± 0.3	8.5 ± 0.4	8.6 ± 0.4	8.5 ± 0.6
Chis150					
B	8.4 ± 0.4	8.1 ± 0.7	8.5 ± 0.6	8.4 ± 0.7	8.2 ± 0.5
T	8.4 ± 0.6	8.2 ± 0.4	8.2 ± 0.6	8.4 ± 0.6	8.3 ± 0.4

Abbreviations: DAPI, 4′-6-diamidino-2-phenylindole; HC, high carbohydrate; HGI, high glycemic index; HM, high monounsaturated fatty acid; HS: high saturated fatty acid; LGI, low glycaemic index. Bacterial numbers are expressed as $\log_{10}$ [cells per g of faecal content wet weight], mean ± s.d. †, ‡ Indicate significant differences between diets ($P \leqslant 0.05$). *, **Indicate significant differences with baseline values of the same diet (*$P \leqslant 0.05$; **$P \leqslant 0.01$).

Table 5. Faecal SCFA (mmol l^{-1}, mean ± s.d.), measured by gas chromatography after the 4 weeks run-in diet (baseline, B) and after 24 weeks dietary intervention (treatment, T) with the five experimental diets (HS; HM/HGI; HM/LGI; HC/HGI; HC/LGI)

	HS (n = 11)	*HM/HGI (n = 17)*	*HM/LGI (n = 22)*	*HC/HGI (n = 21)*	*HC/LGI (n = 17)*
Acetate					
B	25.21 ± 10.15	31.36 ± 12.49	33.98 ± 10.56	32.97 ± 14.70	29.21 ± 12.17
T	30.61 ± 11.93*	31.04 ± 13.79	32.57 ± 14.04	34.79 ± 20.69	31.14 ± 10.60
Propionate					
B	6.28 ± 3.90	7.84 ± 3.09	9.38 ± 4.00	9.21 ± 6.84	7.31 ± 3.79
T	7.57 ± 3.96**	7.32 ± 3.99	9.19 ± 4.72	7.87 ± 4.30	7.72 ± 3.95
i-Butyrate					
B	0.56 ± 0.34	0.56 ± 0.48	0.77 ± 0.46	0.42 ± 0.25	0.51 ± 0.46
T	0.55 ± 0.31	0.44 ± 0.67	0.58 ± 0.35	0.61 ± 0.43	0.52 ± 0.42
n-Butyrate					
B	6.02 ± 3.43	7.92 ± 5.32	8.60 ± 4.60	8.45 ± 6.17	7.84 ± 5.61
T	7.76 ± 4.79**	7.12 ± 4.62	9.52 ± 8.99	8.87 ± 7.80	8.13 ± 3.77
i-Valerate					
B	1.01 ± 0.49	0.92 ± 0.74	1.34 ± 0.79	0.79 ± 0.47	0.88 ± 0.74
T	0.99 ± 0.44	0.94 ± 1.11	1.00 ± 0.54	1.05 ± 0.67	0.85 ± 0.77
n-Valerate					
B	0.82 ± 0.60	0.89 ± 0.65	0.98 ± 0.78	0.73 ± 0.60	0.76 ± 0.69
T	0.84 ± 0.63	0.90 ± 0.69	0.89 ± 0.65	0.88 ± 0.71	0.76 ± 0.46
n-Caproate					
B	1.28 ± 0.59	0.72 ± 0.43	0.70 ± 0.84	0.65 ± 0.39	1.20 ± 1.40
T	1.16 ± 0.75	0.49 ± 0.34	0.71 ± 0.81	0.86 ± 0.75	1.04 ± 0.84

Abbreviations: HC, high carbohydrate; HGI, high glycemic index; HM, high monounsaturated fatty acid; HS, high saturated fatty acid; LGI, low glycaemic index; SCFA, short chain fatty acid. *, **Indicate significant differences with baseline values of the same diet (*$P \leqslant 0.05$; **$P \leqslant 0.01$).

RISCK study participants ($n = 88$) no changes between diets were observed.

We investigated the long-term impact of different amounts and quality of dietary fat and carbohydrate on gut microbial composition and fermentation activities in a population 'at MetS risk'. Reducing dietary fat intake and increasing dietary carbohydrate consumption increased both faecal *Bacteroides* and *Bifidobacterium* spp., groups of bacteria that have been independently linked to improved body energy regulation and reduced risk factors of MetS.[16,22] Increased *Bacteroides* numbers after the HC/HGI diet directly and significantly correlated with a modest decrease in body weight, waist circumference and BMI (Figure 1). A lower prevalence of *Bacteroidetes* has been reported for both animal models of obesity (ob/ob mice) and in obese humans compared with their lean counterparts.[40,12,15] Weight loss following 1-year fat/CHO-restricted diets was shown to correlate with increased *Bacteroidetes* in humans.[16] However, no details were given on dietary design. In the present study, the diets were designed to maintain body weight. Nevertheless, when a small loss of weight was observed, there was a concomitant increase in *Bacteroides*. Participants on both low-fat high-CHO diets also had significant increase in *Bifidobacterium* and showed a modest increase in *Atopobium* numbers, both within the *Actinobacteria* phylum. These dominant members of the human gastrointestinal microbiota are important degraders of carbohydrate, and their growth may have been stimulated by the increased bioavailability of dietary carbohydrate. Studies in ileostomy patients have shown that the amount of starch reaching the colon is directly proportional to the quantity of starch ingested.[41] This is important, as starch is the major constituent of most human carbohydrate-rich foods. Other complex carbohydrate common in the human diet include pectin, inulin, arabinoxylan and â-glucan, which to a large degree escape digestion in the upper gut and reach the colon, where they are readily fermented. In the RISCK study, there were no significant differences in the total starch or non-starch polysaccharide intakes, although the resistant starch intake or percentage of fermentable fibre were undetermined. Non-starch polysaccharide, one measure of dietary fibre, was significantly higher in HM/LGI but not in HC/LGI compared to baseline. While it might be expected that low-GI diets are enriched in fibres, it should be considered that GI is a measure of a physiological response to a food (that is, the glycemic response) and not an inherent chemical property of a food (that is, the fibre content).

A significant decrease in fasted blood glucose was observed after both high-carbohydrate/low-fat diets compared to baseline. Moreover, a trend towards decreased body weight and BMI was observed after HC/HGI and HC/LGI. Moreover, a trend towards decreased body weight and BMI was observed after HC/HGI and HC/LGI. Interestingly, in those participants with a significant and marked faecal bifidobacteria increase (HC/HGI), significant insulin and percentage of body fat decreases were observed relative to baseline. Although mean counts of faecal bifidobacteria increased by 0.3 Log_{10}[bacterial cells per g faecal contents] in the HM/HGI diet group, this increase was not significant and from the data analysis only occurred in a minority of individuals. Bifidobacteria are beneficial members of the gastrointestinal microbiota,[42] and can be augmented with prebiotic fibres ingestion.[43] Cani *et al*.[22] showed an association between the bifidogenesis following dietary supplementation with oligofructose and improved MetS

biomarkers (improved glucose tolerance, insulin sensitivity, body weight gain, fat mass development and inflammatory tone) in high-fat fed mice.[22]

Despite the observed changes in microbial population levels, no significant changes were observed in faecal SCFA after any of the experimental diets except the HS control diet. These findings were unexpected, as acetate, propionate and butyrate are mainly microbial saccharolytic end products. Recent studies have shown that caecal SCFA levels are higher in obese animals compared with lean,[15] despite a lower prevalence of the major polysaccharide degrading gut bacteria. Schwiertz *et al*.[19] also reported higher SCFA in obese humans compared with lean. Higher faecal SCFA levels might be due to decreased intestinal absorption or a modulation of the gut microbiota activity or composition (for example, a decreased bacterial populations that utilise these fermentation products as a source of energy).[43] Vogt and Wolever demonstrated that the faecal acetate percentage inversely correlates with the absorbed acetate percentage after rectal infusion.[44] Higher SCFA observed in our HS group might reflect lower absorption, rather than higher colonic fermentation. The high fat diets (HS, HM/HGI, HM/LGI) in this study were accompanied by decreased total bacteria compared to baseline, without a concomitant decrease in any of the FISH-enumerated bacteria, suggesting that the employed FISH probes did not cover all the faecal bacterial populations and that a broader spectrum technique (that is, high throughput sequencing) might be more appropriate to monitor subtle gut microbial changes.

In conclusion, this study provides evidence that both the type and quantity of dietary carbohydrate and fat impact on gut microbial composition and activity in people at MetS risk. We report a significant association between faecal bacteroides and weight loss and present data to show that HC diets stimulate faecal bifidobacteria, an established gut health biomarker. We also observed increased faecal SCFA after a HS, a finding that may implicate an aberrant production or absorption of SCFA in response to a modern diet in people at increased metabolic risk.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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