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BRIEF REPORT

TITLE

The effects of dietary supplementation with inulin and inulin-propionate ester on hepatic steatosis in adults with non-alcoholic fatty liver disease

SHORT RUNNING TITLE

Inulin, inulin-propionate ester and liver fat

AUTHORS AND AFFILIATIONS

Edward S. Chambers[1], Claire S. Byrne[1], Annette Rugyendo[1], Douglas J. Morrison[2], Tom Preston[2], M. Catriona Tedford[3], Jimmy D. Bell[4], E. Louise Thomas[4], Arne N. Akbar[5], Natalie E. Riddell[6], Rohini Sharma[7], Mark R. Thursz[7], Pinelopi Manousou[8], Gary Frost[1*].

[1]Section for Nutrition Research, Faculty of Medicine, Imperial College London, 6th Floor Commonwealth Building, Hammersmith Hospital, London, UK
[2]Stable Isotope Biochemistry Laboratory, Scottish Universities Environmental Research Centre, University of Glasgow, East Kilbride, Glasgow, Scotland
[3]School of Science, University of the West of Scotland, Hamilton, Scotland
[4]Research Centre for Optimal Health, Department of Life Sciences, Faculty of Science and Technology, University of Westminster.
[5]Division of Infection and Immunity, University College London, London, UK
[6]Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
[7]Department of Surgery and Cancer, Imperial College London, UK.
[8]Liver Unit, St Mary's Hospital, Imperial College NHS Trust, London, UK

Corresponding Author: Prof. Gary Frost
Section for Nutrition Research
Faculty of Medicine
Imperial College London
6th Floor Commonwealth Building
Hammersmith Hospital
London W12 0NN
UK
Email: g.frost@imperial.ac.uk
Tel: +44 (0)20 3313 8037

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ABSTRACT: 170 words (180 Max)

MAIN BODY: 2071 words (1800 Max)

REFERENCES: 21 (15 Max)

TABLES AND FIGURES: 2 (2 Max)

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ABSTRACT

The short chain fatty acid (SCFA) propionate, produced through fermentation of dietary fibre by the gut microbiota, has been shown to alter hepatic metabolic processes that reduce lipid storage. We aimed to investigate the impact of raising colonic propionate production on hepatic steatosis in adults with non-alcoholic fatty liver disease (NAFLD). Eighteen adults were randomised to receive 20g/day of an inulin-propionate ester (IPE), designed to deliver propionate to the colon, or an inulin-control for 42-days in a parallel design. The change in intrahepatocellular lipid (IHCL) following the supplementation period was not different between groups (P=0.082), however IHCL significantly increased within the inulin-control group (20.9±2.9 to 26.8±3.9%; *P*=0.012; *n*=9), which was not observed within the IPE group (22.6±6.9 to 23.5±6.8%; *P*=0.635; *n*=9). The predominant SCFA from colonic fermentation of inulin is acetate, which in a background of NAFLD and a hepatic metabolic profile that promotes fat accretion, may provide surplus lipogenic substrate to the liver. The increased colonic delivery of propionate from IPE appears to attenuate this acetate-mediated increase in IHCL.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a condition characterized by the accumulation of fat within the liver, is regarded as a major risk factor in the development of Type 2 diabetes[1]. The prevalence of NAFLD is strongly associated with obesity[1], thus current guidelines for the prevention and management of NAFLD are based solely on weight loss through diet and exercise[1]. Whilst lifestyle modifications are successful in reducing body weight in the short-term, numerous studies demonstrate that long-term maintenance of weight loss in obese individuals is very poor[2]. Lifestyle modifications alone are therefore unlikely to reduce the growing prevalence of NAFLD and there is an urgent need to develop therapeutic interventions that can safely be applied at the population level.

Recent investigations suggest that diet, the gut microbiota and liver fat storage could be linked through a mechanism involving short chain fatty acids (SCFA), the major products of dietary fibre fermentation in the colon. It has been repeatedly observed that when animals are fed fermentable fibre they are protected against steatosis induced by high fat diets[3-5]. This effect may be due to the SCFA propionate, as ~90% of propionate produced in the colon is extracted from the portal vein by the liver[6], which has been shown to alter hepatic metabolic processes to reduce lipid content[7,8]. To augment colonic propionate production we have developed an inulin-propionate ester (IPE), whereby the SCFA propionate is bound to the dietary fibre inulin, which is released through microbial hydrolysis in the colon[9]. Our recent first-in-human studies provided preliminary evidence that supplementing the diet with 10g/day IPE for 24 weeks reduced liver fat content in adults with NAFLD[9]. These volunteers were identified as having NAFLD on the basis of an elevated intrahepatocellular lipid (IHCL) content from magnetic resonance imaging.

The aim of the current study was to develop *in vivo* proof-of-concept for IPE as a therapeutic to reduce hepatic steatosis in volunteers with a histological confirmation of NAFLD, which is considered the gold-standard to establish diagnosis. We hypothesised that the addition of 20 g IPE to the diet of adults with NAFLD for 42 days would significantly reduce IHCL compared to 20 g of an inulin-control.

METHODS

All volunteers provided informed, written consent prior to the clinical trial which was approved by the London Brent Research Ethics Committee (14/LO/0645). The study was carried out in accordance with the Declaration of Helsinki and is registered with the ISRCTN registry (ISRCTN71814178).A detailed methodology is presented in the Supplementary Material. Men and women aged 18-65 years, with a body mass index (BMI) of 20-40 kg/m^2 were recruited from liver clinics at St Mary's Hospital, Imperial College Healthcare National Health Service Trust. Potential

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volunteers were eligible if they had a confirmation of NAFLD by liver biopsy within the previous five years and controlled blood glucose levels (HbA1c <48 mmol/mol). The study was conducted using a randomised, double-blind, placebo controlled, parallel design. Subjects received either 20 g/day of inulin-control or IPE for 42 days. The 20 g dose of IPE would have provided 14.6 g of inulin (and 5.4 g bound propionate) to the diet[9]. Inulin was therefore chosen as a positive control to account for any effects that may derive from fermentation of this substrate by the gut microbiota. The supplements were provided to volunteers in 10 g ready-to-use sachets and they were instructed to mix the contents into their habitual diet twice a day. Participants were required to attend the NIHR Imperial Clinical Research Facility pre- (day 0) and post-supplementation (day 42) to determine outcome measures.

RESULTS AND DISCUSSION

Of 20 volunteers that were randomised and enrolled into the study, data were analysed from the 18 volunteers that completed the supplementation period (Figure S1). The characteristics of these volunteers are presented in Table 1 and Table S1. Estimated compliance was similar in the supplementation groups (inulin-control: 90±7% vs. IPE: 95±2%; P=0.213). The changes in IHCL (Figure 1A-C and Table 1) were unexpected, as we observed an increase in IHCL post-supplementation in both groups (main effect for time; P=0.020). The change in IHCL was not significantly different between supplementation groups (Figure 1A; P=0.082), however, within-group analysis showed that IHCL was significantly increased within the inulin-control group (Figure 1B; P=0.012) and not the IPE group (Figure 1C; P=0.635). Analysis of metabolic and inflammatory responses (Figures 1D-I and Tables S2-S4) highlight that the change in insulin resistance (HOMA-IR) was significantly different between groups (Figure 1D; P=0.046), with a non-significant increase in the inulin-control group (Figure 1E; P=0.060) and decrease in the IPE group (Figure 1E; P=0.389), respectively. There were no within- or between-group differences in body composition (Table 1), self-reported food intake or physical activity following the supplementation period (Table S5). Our hypothesis was that IPE supplementation would decrease IHCL in adults with NAFLD, as observed in our previous study[9]; however, IPE supplementation did not reduce liver fat content. The disparate outcome may be explained by methodological differences in IPE dose (10g/day vs. 20g/day) and exposure (6 weeks vs. 24 weeks) in the two studies. Furthermore, volunteers in the present study had a confirmation of NAFLD by liver biopsy, which is considered gold-standard to establish diagnosis, and metabolic parameters would indicate these individuals had poorer glycaemic control compared to the volunteers from our previous work (fasting glucose: 5.0 mmol/L vs. 6.1 mmol/L; HbA1c: 38 mmol/mol vs. 42 mmol/mol).

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Whilst breath hydrogen, a marker of colonic fermentation, was elevated in both groups post-supplementation (Table S3), the impact on SCFAs measured in peripheral blood was limited, as we observed that IPE supplementation only reduced levels of butyrate in fasting samples compared to the inulin-control group (Table S3). The blood samples were collected >12 hours after volunteers were requested to ingest their final supplement, which may explain why we were unable to detect large differences in circulating SCFAs post-supplementation. Nevertheless, previous research using stable isotope methodology has demonstrated that inulin is predominantly fermented in the human colon into acetate (82%), with considerably less propionate and butyrate produced (6% and 12%, respectively)[10]. Dietary supplementation with inulin-type fructans (ITF) has generally been associated with positive effects on metabolic health. The evidence for this beneficial effect is primarily derived from rodent studies, where dietary supplementation with ITF has consistently been shown to prevent the accumulation of liver fat and metabolic dysregulation induced by a high fat diet[3-5]. However, to the best of our knowledge, ITF have not been shown to reduce liver fat when added to the diet of rodents with pre-existing steatosis. Studies investigating the effect of ITF on metabolic health in humans are equivocal with a recent meta-analysis reporting no association between ITF supplementation and fasting glucose and insulin levels[11]. Fewer studies have quantified the impact of ITF supplementation on liver fat content in humans. Our previous work demonstrated that 30g/day ITF supplemented into the habitual diet of overweight adults with normal glycaemic control had no effect on IHCL[12]. In contrast, a superior reduction in IHCL in adults with pre-diabetes was found when a weight-loss diet was combined with 30g/day ITF supplementation[13]. The serendipitous observation in the current study is that supplementing 20g/day inulin into a habitual weight-maintaining diet raises IHCL and further exacerbates glucose homeostasis in adults with NAFLD. Taken together, our data suggests that ITF supplementation does not have a homogenous impact on hepatic lipid content in humans and its effects may depend on the pre-existing metabolic health of the individual and the energy-balance promoted by the background diet.

Previous research would suggest that the acetate derived from inulin fermentation would have contrasting metabolic fates depending on hepatic lipid metabolism in different physiological conditions. For example, it has previously been reported that greater amounts of exogenous acetate are used for hepatic *de novo* lipogenesis (DNL) in obese compared to lean individuals. This metabolic response was associated with higher insulin levels in the obese group, which is the chief regulator of hepatic DNL[14]. Previous work has also demonstrated that chronic intragastric acetate infusion in rats promotes postprandial hyperinsulineamia and increases liver triglyceride content[15]. The conversion of SCFAs into metabolic intermediates is initially determined by the acyl-CoA synthetase short-chain family members (ACSS)[16]. Human hepatocytes express the cytosolic isoform *ACSS2*, which has high specificity for acetate and increases the availability of acetyl-CoA for lipid synthesis[16]. Evidence highlights that, together with higher insulin levels[17], humans with

NAFLD have an elevated expression of hepatic genes that favour fat accumulation, with increased expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), which are key enzymes in hepatic DNL[18]. Consequently, NAFLD patients are reported to have rates of DNL up to 3-fold higher compared to BMI-matched controls[17]. The current data suggests that in weight-stable individuals with NAFLD, an increased supply of acetate to the liver from the colonic fermentation of inulin provides surplus acetyl-CoA for DNL and hepatic lipid accretion. Interestingly, diet-induced weight loss in mice has been shown to markedly reduce insulin levels and rates of hepatic DNL[19], whilst the expression of hepatic DNL-related genes are also reduced by states of chronic negative energy balance[20]. This may explain the disparate effect of inulin supplementation on liver fat content in the present study compared to our previous investigation when inulin intervention was added to a hypo-caloric diet that achieved a ~5% reduction in body weight[13].

IPE supplementation did not significantly raise IHCL content, as observed within the inulin-control group. The contrasting outcome could be due to differences in amounts of acetate derived from inulin fermentation throughout the supplementation period, as the inulin-control group were provided with a greater amount of inulin compared to IPE (20 g/day vs 14.6 g/day). *In vitro* faecal fermentation profiles have previously demonstrated, however, that comparable quantities of acetate are produced from equivalent doses of inulin and IPE[9]. Interestingly, IPE does substantially alter the proportion of SCFAs produced, as the molar ratio of acetate, propionate and butyrate changes from 74:16:10 with inulin to 25:69:6 with an equivalent amount of IPE[9]. It could be suggested that the elevated ratio of colonic propionate:acetate promoted by IPE supplementation may have prevented the accumulation of liver fat observed in the inulin-control group by impairing hepatic acetate metabolism. This proposed mechanism is concordant with the results from Wolever and colleagues who demonstrated using stable isotope technology that propionate inhibits incorporation of colonic [1,2-^{13}C] acetate into plasma lipids in humans[7]. Studies using rat hepatocytes have also highlighted that propionate inhibits lipid synthesis when acetate is a major source of acetyl-CoA[8]. Recent evidence has demonstrated a third ACSS isoform, ACSS3, for which propionate is the preferred substrate over acetate, and which is highly expressed in the mitochondrial matrix of hepatocytes[16]. *ACSS3* converts propionate to propionyl-CoA allowing it to enter mitochondrial respiration through succinate and the TCA cycle[16]. Elevating hepatic propionate metabolism would therefore increase competition with acetate for conversion into their CoA adducts at tissue level, which may reduce cytosolic acetyl-CoA availability for DNL. This potential mechanism is supported by a recent observation that exposing HepG2 cells to elevated ratios of propionate:acetate increases the formation of heptadecanoic acid derived from propionyl-CoA, which inhibits the synthesis of palmitate from acetyl-CoA[21].

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The present study has a number of potential limitations, chiefly, the considerable variability in metabolic health of the recruited volunteers. Nevertheless, the individual change in IHCL post-supplementation were not associated with any baseline metabolic variable (Table S6) and significant differences between-groups at baseline were only found in two outcome measures (cholesterol and LDL-cholesterol). In addition, the inclusion criteria permitted a histological diagnosis of NAFLD within the previous 5 years, thus a volunteer's histological characterisation could have changed in the timeframe between initial diagnosis and recruitment into the study. However, all volunteers exhibited a raised IHCL (>5%) when assessed at baseline (Table S1).

In conclusion, inulin consumed at 20g/day increased IHCL in weight-stable adults with NAFLD, an effect not observed with IPE supplementation. We speculate that in the context of NAFLD and a hepatic metabolic profile that stimulates DNL, the acetate derived from colonic fermentation of inulin could provide additional lipogenic precursor to the liver. The increased colonic delivery of propionate from IPE appears to attenuate this acetate-mediated increase in IHCL, possibly by interfering with the availability of acetate-derived acetyl-CoA for DNL. Further work is warranted to explore how altering colonic SCFA production profiles modulates the metabolic pathways that govern hepatic lipid storage in humans. In particular, future research should determine how the hepatic metabolic processing of acetate and propionate changes in different states of energy balance and to determine distinctions between NAFLD patients and healthy controls.

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TABLE

Table 1. Baseline characteristics of volunteers and changes in intrahepatocellular lipid and body composition following 42 days of inulin-control or inulin-propionate ester (IPE) supplementation. Data are expressed as mean ± SEM.

	Inulin-Control (N = 9)			IPE (N = 9)			Mixed ANOVA	
							Time	Group×Time
Variable	**Pre**	**Post**	**P Value**	**Pre**	**Post**	**P Value**	**P Value**	**P Value**
Sex (N)								
Male	5			4				
Female	4			5				
Race or ethnicity (N)								
White	5			7				
Asian	4			2				
Age (years)	49 ± 4			51 ± 4				
Diabetes (Y/N)	3/6			2/7				
Dyslipidaemia (Y/N)	5/4			5/4				
Hypertension (Y/N)	2/7			2/7				
Liver Biopsy Histology (NAFLD/NASH)	6/3			7/2				
IHCL (%)	20.9 ± 2.9	26.8 ± 3.9	0.012	22.6 ± 6.9	23.5 ± 6.8	0.635	0.020	0.082
Weight (kg)	83.3 ± 4.4	83.2 ± 4.0	0.914	93.6 ± 7.6	93.9 ± 7.4	0.556	0.438	0.578
BMI (kg/m2)	29.5 ± 1.4	29.5 ± 1.4	0.966	31.5 ± 1.9	31.6 ± 1.9	0.377	0.696	0.620
Fat Mass (kg)	26.8 ± 3.4	27.1 ± 3.3	0.524	35.3 ± 5.2	34.9 ± 5.3	0.485	0.931	0.302
Fat Free Mass (kg)	56.5 ± 3.7	56.1 ± 3.3	0.631	58.3 ± 5.0	59.1 ± 5.2	0.055+	0.748	0.341

+ = non-parametric statistical analysis. Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Intrahepatocellular lipid (IHCL), body mass index (BMI). Detailed volunteer characteristics are presented in Table S1.

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FIGURE LEGEND

Figure 1. Effects of 42 days of inulin-control and inulin-propionate ester (IPE) supplementation on liver fat and glucose homeostasis *A.-C.* Intrahepatocellular lipid (IHCL) *D.-F.* Homeostatic model assessment of insulin resistance (HOMA-IR) and *C.* Glycosylated haemoglobin (HbA1c) Group data (A., D. and G.) expressed as mean ± SEM (n=9).

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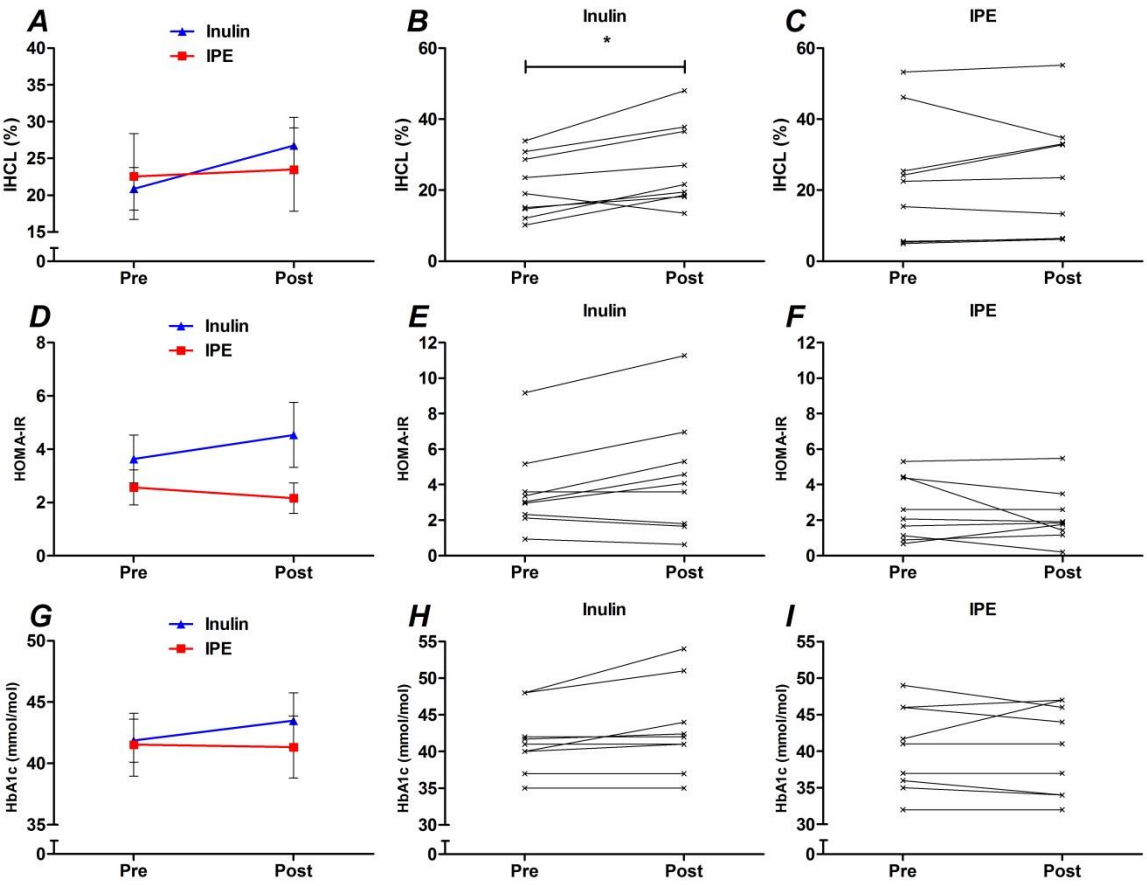
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SUPPLEMENTARY MATERIAL

METHODS

Potential participants were excluded if they met any of the following criteria: diagnosis of cirrhosis, other clinically significant illness, started new medication or changed the dose of existing medication in the preceding 3 months likely to interfere with energy metabolism, a weight loss of 3 kg or greater in the preceding two months, smoking, substance abuse, psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes and thyroid function). Women were ineligible if they were pregnant or breast-feeding. A urinary pregnancy test was conducted at screening and before the collection of experimental data at the two study visits.

A previous investigation found that 20 g/day IPE improved measures of metabolic health (fasting insulin and HOMA-IR) in overweight and obese adults at the end of a 42 day intervention (unpublished data). Liver fat content was not assessed in this investigation, but it was postulated the improvements in glucose homeostasis would be related to reductions in intrahepatocellular lipid (IHCL), as observed in previous work[1]. The same dose (20 g/day) and supplementation period (42 days) was therefore used in the current study.

Two strata were defined according to gender and randomisation sequences and allocation was conducted via a remote internet-based service (www.sealedenvelope.com). The supplement sachets were prepared by DJM and labelled 'A' and 'B'. DJM held the key to the allocations for the duration of the trial. Throughout the trial, none of the participants or investigators involved in the trial had complete information on the randomisation allocations.

All subjects were instructed to maintain their usual dietary and activity habits during the study period and regular communication between subjects and study investigators encouraged good compliance. Subjects returned all their used and unused sachets to estimate compliance.

The day prior to the study visits, participants were requested to refrain from strenuous exercise and alcohol prior to fasting overnight for >12 hours. Participants were asked to eat the same ready-made or shop-prepared meal the evening before each study visit. Participants were free to choose their meal and compliance was assessed by requesting that volunteers bring the outer packaging of the meal and the receipt of purchase to the study visit. Participants were requested to ingest their final supplement sachet with their evening meal.

Liver fat, body weight and composition

Liver fat content was assessed using MRS, as previously described[2]. Body weight, fat mass (FM) and fat free mass (FFM) with bioelectrical impedance (Tanita BC-418MA, Japan). Subjects were

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asked to change into lightweight hospital scrubs and to void their bladder before measurements were taken.

Mixed meal test (MMT)

A cannula was inserted into an antecubital vein and two fasting blood samples were collected >5 min apart to assess plasma concentrations of glucose and serum concentrations of insulin and short chain fatty acids (SCFA). At 0 min, subjects were served a standard liquid meal (Ensure Plus, Abbott, UK: 660 kcal; 88.9 g carbohydrate, 21.6 g fat, 27.5 g protein) that was ingested within 10 min. Postprandial blood samples were taken at 10, 20, 30, 45, 60, 90, 120 and 180 min and collected into sodium fluoride-coated tubes and serum-separating tubes. Plasma glucose was measured using an Abbott Architect ci8200 analyser (Abbott Diagnostics, USA). Insulin-like immunoreactivity was measured using an ultra-sensitive human insulin radioimmunoassay (Millipore, USA). SCFA were measured in fasting and the 60 min samples with the use of an Agilent 7000C Triple Quadrupole GC/MS System according to a previously published method [3]. Fasting breath hydrogen concentrations, a marker of colonic fermentation [4], were measured using a handheld breath hydrogen analyser (EC60 Gastrolyser Breath Hydrogen Monitor, Bedfont Scientific, Upchurch, Kent, UK).

Cardiovascular disease, diabetes risk factors and inflammatory markers.

A fasting blood sample was collected and analysed for levels of triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, glycosylated HbA1c, alanine transaminase and C-reactive protein. All analytes were measured by the Department of Chemical Pathology, Imperial College Healthcare National Health Service Trust. IL-6, IL-8, IL-10, IL-12 and IL-17A were measured in fasting serum using the Cytometric Bead Array (BD Biosciences, UK), according to the manufacturer's protocol. IL-17A results are not shown, as only two volunteers had detectable values for this analyte.

Self-reported food intake, physical activity and gastrointestinal adverse events

Energy and macronutrient intake was recorded with food diaries during the final 3-days of each supplementation period (Dietplan 6.0; Forestfield Software Ltd, UK). Physical activity was assessed during the final 7 days of each supplementation period using the short self-administered format of the International Physical Activity Questionnaire (IPAQ)[5]. Ratings of gastrointestinal side-effects were made using 100 mm visual analogue scales (VAS). Subjects were asked to rate the occurrence of each side effect with extreme statements anchored at each end of the rating scale (0 mm Never, 100 mm All the time) [1].

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Calculations and statistical analysis

Data from our previous study was used to estimate the required sample size [1]. A power calculation confirmed that 16 participants (8 per group) would be sufficient to detect a mean 8% difference between groups in the baseline change in IHCL, with a common standard deviation (SD) of 5% (α=0.05, power=0.80). 20 volunteers were recruited to allow an estimated attrition rate of 20%. Time course data from the MMT were analysed by calculating areas under the curve (AUC) using the trapezoid rule and dividing by 180 to generate a mean postprandial value. Insulin resistance was assessed by homeostatic model assessment (HOMA-IR)[6].

Mixed analysis of variance was conducted to assess if the change in outcome measures was different over time (pre-supplementation, post-supplementation) between the two supplementation groups (inulin-control, IPE). The interaction (Group×Time) and main effect for time are presented. Levene's Test and Box's Test were performed to confirm homogeneity of variances. Within-group differences were compared using paired t tests. Data were checked for normality using the Shapiro-Wilk Test. Non-parametric data were log transformed prior to carrying out parametric statistical tests. Non-parametric within-group analysis (Wilcoxon signed rank test) was performed on data when log transformed values were not normally distributed. Between-group differences at baseline were assessed using unpaired t tests. Data were checked for normality using the Shapiro-Wilk Test. Non-parametric data were log transformed prior to carrying out parametric statistical tests. Non-parametric between-group analysis (independent samples Mann-Whitney U Tests) was performed on data when log transformed values were not normally distributed. Correlation analysis was performed to assess if the delta change (Δ) in liver fat content was related to baseline variables. Pearson correlation coefficients or Spearman's rank correlation coefficients were calculated All statistical analyses were carried out with SPSS version 23.0 for Windows (SPSS Inc, USA). Data are presented as means ± SEM or 95% CI. n = 9 per supplementation group, unless otherwise state. Due to difficulties cannulating two volunteers at post-supplementation visits, postprandial values from the mixed-meal test MMT were analysed from 16 volunteers (8 per supplementation group). P<0.05 was considered significant.

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FIGURES

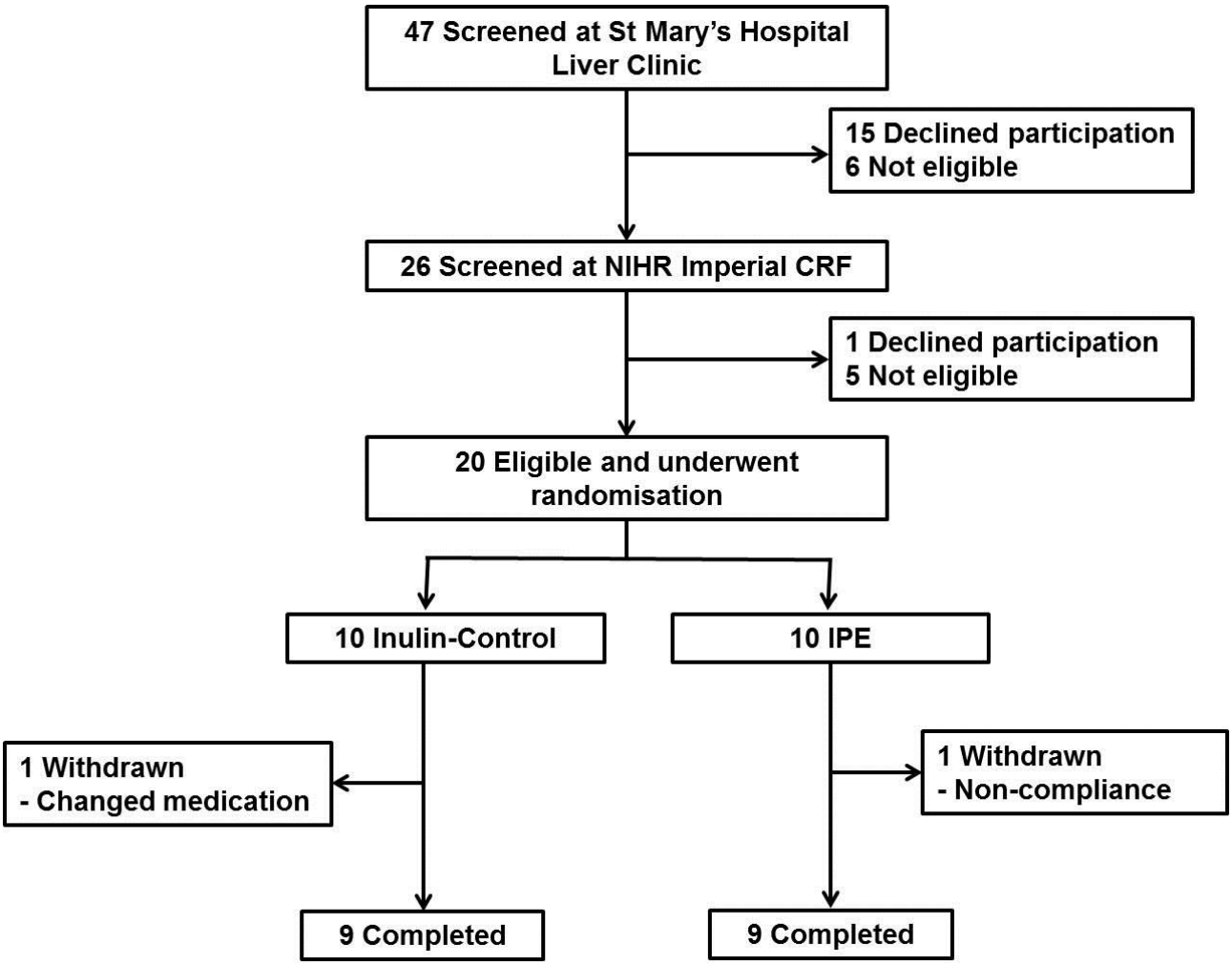

Figure S1. Recruitment and retention in the study. Inulin-propionate ester (IPE).

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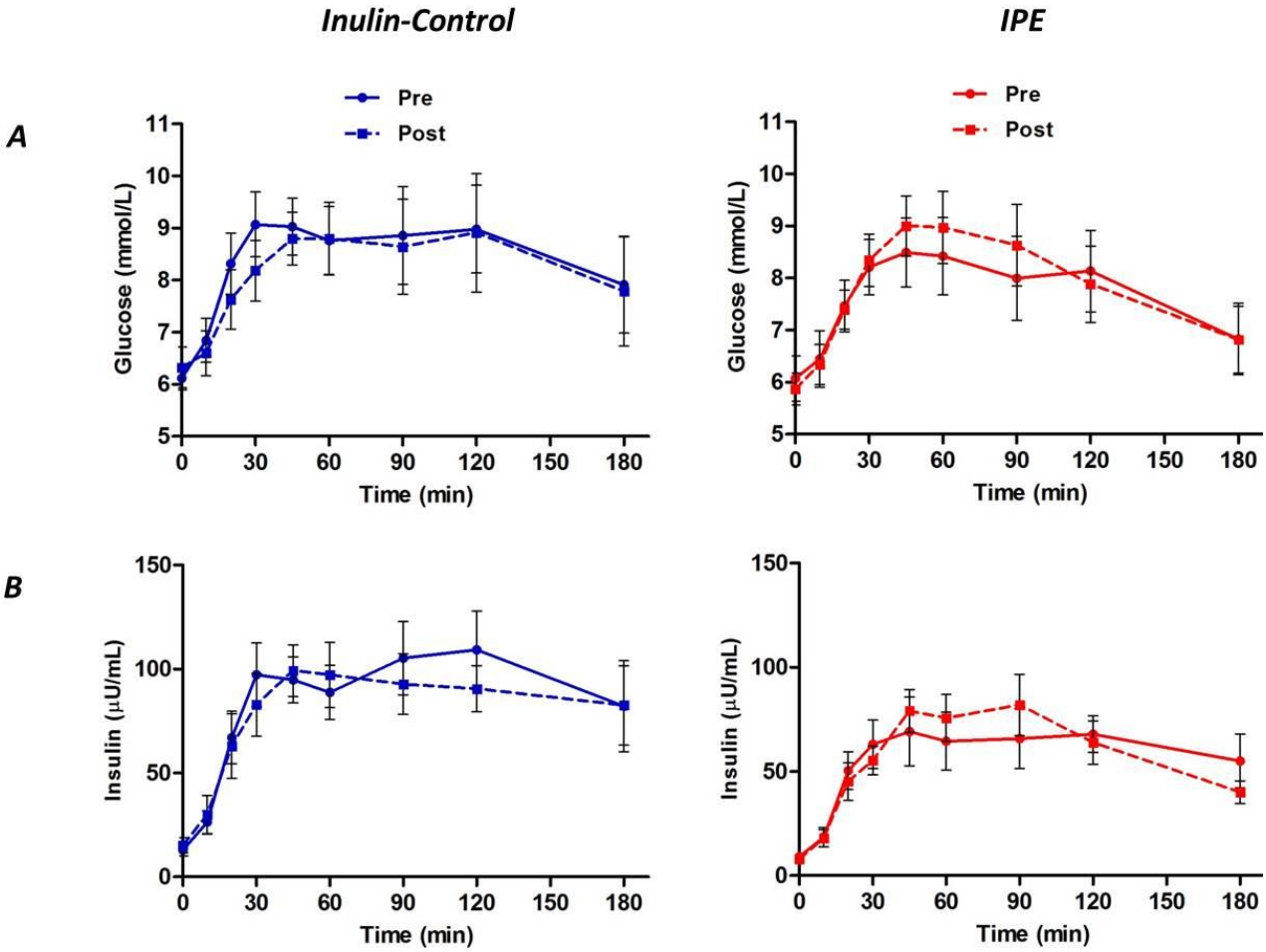

Figure S2. The effects of 42 days of inulin control and inulin propionate ester (IPE) supplementation on postprandial *A.* glucose and *B.* insulin responses (Data are expressed as mean ± SEM (n = 8 each group).

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TABLES

Table S1. Volunteer characteristics. Histological assessment of the liver from biopsy. Intra hepatocellular lipid (IHCL) content, alanine transaminase, HbA1c, metabolic comorbidities and medications at baseline.

Volunteer	Group	Steatosis Grade^	Diagnostic Classification	Fibrosis	Years Between Biopsy and Recruitment	IHCL (%)	Alanine Transaminase (IU/L)	HbA1c (mmol/mol)	Type 2 Diabetes - Medication	Dyslipidaemia - Medication	Hypertension - Medication
1	IC	1	NAFLD	None	4	19	61	40	Y- Metformin	Y- Statins	Y- Bendroflumethiazide
2	IC	2	NASH	Mild	4	24	47	42	N	N	N
3	IC	1	NASH	None	2	34	123	42	N	N	N
4	IC	2	NAFLD	Not Specified	5	10	97	40	N	Y- Statins	N
5	IC	1	NAFLD	None	4	12	36	35	N	N	Y- Amlodipine
6	IC	2	NASH	Mild	3	29	47	48	Y- Metformin	Y- Statins	N
7	IC	1	NAFLD	None	4	15	30	37	N	N	N
8	IC	1	NAFLD	None	2	15	59	41	N	Y- Statins	N
9	IC	1	NAFLD	None	2	31	33	48	Y- Metformin	Y- Statins	N
10	IPE	1	NAFLD	None	3	24	30	32	N	N	N
11	IPE	1	NAFLD	Not Specified	4	25	57	37	N	Y- Statins	N
12	IPE	1	NASH	Mild	3	23	58	48	Y- Metformin	Y- Statins	Y- Amlodipine
13	IPE	1	NAFLD	None	2	5	29	35	N	N	N
14	IPE	2	NASH	None	4	53	122	41	N	N	N
15	IPE	1	NAFLD	None	3	5	19	42	N	Y- Statins	Y- Ramipril
16	IPE	1	NAFLD	Not Specified	5	6	11	36	N	N	N
17	IPE	1	NAFLD	None	3	15	25	46	Y- Metformin	Y- Statins	N
18	IPE	2	NASH	None	3	46	37	48	N	Y- Statins	N

Inulin-control (IC), inulin-propionate ester (IPE), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Glycosylated haemoglobin (HbA1c).
^Steatosis graded: 0=<5%, 1=5-33%, 2=33-66%, 3=>66%[7].

Table S2. Changes in fasting and postprandial metabolic responses following 42 days of inulin-control or inulin propionate ester (IPE) supplementation. Data are expressed as mean ± SEM or 95% CI.

	Inulin-Control (N = 9)			IPE (N = 9)			Mixed ANOVA	
							Time	Group×Time
Variable	**Pre**	**Post**	**P Value**	**Pre**	**Post**	**P Value**	**P Value**	**P Value**
Fasting Glucose (mmol/L)	6.1 ± 0.2	6.3 ± 0.4	0.343	6.1 ± 0.4	5.9 ± 0.3	0.211	0.943	0.190
Postprandial Glucose^ (mmol/L)	8.5 ± 0.7	8.3 ± 0.8	0.610	7.8 ± 0.7	7.9 ± 0.6	0.431	0.897	0.416
Fasting Insulin (µU/mL)	12.9 ± 2.8	15.3 ± 3.5	0.102	9.1 ± 2.0	7.9 ± 1.7	0.496	0.616	0.115
Postprandial Insulin^ (µU/mL)	89.9 ± 12.5	83.67 ± 11.2	0.409	59.4 ± 9.7	59.2 ± 7.7	0.967	0.558	0.494
HOMA-IR	3.6 ± 0.9	4.5 ± 1.2	0.060	2.6 ± 0.7	2.2 ± 0.6	0.389	0.418	0.046
HbA1c (mmol/mol)	41.9 ± 3.7	43.5 ± 2.3	0.056	41.5 ± 2.6	41.3 ± 2.5	0.821	0.203	0.113
Triglycerides (mmol/L)	1.1 ± 0.1	1.9 ± 0.9	0.148+	1.4 ± 0.3	1.5 ± 0.2	0.842	0.227	0.564
Cholesterol† (mmol/L)	4.0 ± 0.3	4.0 ± 0.4	0.957	5.4 ± 0.3	5.3 ± 0.4	0.510	0.650	0.597
LDL Cholesterol† (mmol/L)	2.4 ± 0.2	2.1 ± 0.3	0.303	3.5 ± 0.3	3.4 ± 0.4	0.181	0.143	0.606
HDL Cholesterol (mmol/L)	1.1 ± 0.1	1.1 ± 0.1	0.778	1.2 ± 0.1	1.2 ± 0.1	0.813	0.989	0.715
Alanine Transaminase (IU/L)	59.2± 10.5	67.6 ± 9.4	0.290	43.1 ± 11.2	44.0 ± 11.1	0.718	0.235	0.382
Breath Hydrogen (ppm)	10.5± 2.9	21.1 ± 5.4	0.014	3.7 ± 0.8	11.4 ± 3.8	0.028	0.001	0.651

+ = non-parametric statistical analysis. ^ =statistical analysis performed on n=8 each group. † = Significant difference between groups at baseline. Homeostatic model assessment of insulin resistance (HOMA-IR), Glycosylated haemoglobin (HbA1c), Low density lipoprotein (LDL), High density lipoprotein (HDL).

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Table S3. Changes in fasting and postprandial SCFA following 42 days of inulin-control or inulin propionate ester (IPE) supplementation. Data are expressed as mean ± SEM or 95% CI.

	Inulin-Control (N = 9)			IPE (N = 9)			Mixed ANOVA	
							Time	Group×Time
Variable	**Pre**	**Post**	**P Value**	**Pre**	**Post**	**P Value**	**P Value**	**P Value**
Fasting Acetate (μmol/L)	16.5 ± 2.5	19.7 ± 3.7	0.562	25.4 ± 2.8	35.8 ± 5.5	0.157	0.128	0.409
Postprandial Acetate^ (μmol/L)	20.4 ± 2.0	24.0 ± 4.1	0.376	25.5 ± 4.3	25.7 ± 4.4	0.955	0.475	0.522
Fasting Propionate (μmol/L)	2.1 ± 0.1	2.4 ± 0.3	0.336	2.6 ± 0.3	2.5 ± 0.3	0.847	0.605	0.425
Postprandial Propionate^ (μmol/L)	2.5 ± 0.3	2.4 ± 0.4	0.640	2.5 ± 0.2	3.2 ± 0.4	0.117	0.262	0.155
Fasting Butyrate (μmol/L)	1.7 ± 0.2	1.9 ± 0.2	0.506	2.5 ± 0.3	2.0 ± 0.2	0.003	0.214	0.023
Postprandial Butyrate^ (μmol/L)	2.1± 0.2	2.2 ± 0.2	0.891	1.9 ± 0.2	2.2 ± 0.3	0.548	0.529	0.640

^ =statistical analysis performed on n=8 each group.

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Table S4. Changes in inflammatory markers following 42 days of inulin-control or inulin propionate ester (IPE) supplementation. Data are expressed as mean ± SEM or 95% CI.

	Inulin-Control (N = 9)			IPE (N = 9)			Mixed ANOVA	
							Time	Group×Time
Variable	**Pre**	**Post**	**P Value**	**Pre**	**Post**	**P Value**	**P Value**	**P Value**
IL-6 (pg/mL)	5.4 ± 3.7	3.0 ± 1.2	0.438+	1.9 ± 0.5	1.7 ± 0.3	0.500+	0.293	0.541
IL-8 (pg/mL)	7.7 ± 1.6	11.0 ± 4.6	0.610	6.1 ± 1.1	5.8 ± 1.0	0.650	0.942	0.973
IL-10 (pg/mL)	2.0 ± 0.7	3.0 ± 1.2	0.393+	1.6 ± 0.3	1.4 ± 0.1	0.351+	0.592	0.269
IL-12 (pg/mL)	28.8 ± 6.8	39.5 ± 11.6	0.098+	18.4 ± 3.0	19.6 ± 3.4	0.513	0.168	0.342
C Reactive Protein (mg/L)	2.3 ± 0.7	3.1 ± 1.5	0.672+	5.8 ± 2.8	5.7 ± 2.9	0.867+	0.839	0.920

+ = non-parametric statistical analysis

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Table S5. Changes in self-reported food intake, physical activity and gastrointestinal side-effects following 42 days of inulin-control or propionate ester (IPE) supplementation. Data are expressed as mean ± SEM or 95% CI.

	Inulin-Control (N = 9)			IPE (N = 9)			Mixed ANOVA	
							Time	Group×Time
Variable	**Pre**	**Post**	**P Value**	**Pre**	**Post**	**P Value**	**P Value**	**P Value**
Energy Intake (kcal/day)	2418 ± 381	2044 ± 235	0.242	2233 ± 254	2268 ± 141	0.878	0.367	0.281
Carbohydrate (g/day)	275 ± 40	237 ± 25	0.216	248 ± 32	255 ± 24	0.796	0.521	0.185
Fat (g/day)	112 ± 23	89 ± 13	0.284	93 ± 14	94 ± 8	0.945	0.378	0.338
Protein (g/day)	95 ± 11	85 ± 9	0.260	101 ± 8	98 ± 5	0.709	0.281	0.589
Fibre (NSP) (g/day)	17 ± 3	14 ± 2	0.334	14 ± 1	13 ± 1	0.321	0.178	0.689
Total Physical Activity (MET-h/week)	13 ± 3	19 ± 6	0.412	19 ± 6	21 ± 5	0.673	0.349	0.636
Stomach Discomfort (mm)	25 ± 10	28 ± 9	0.818	9 ± 7	6 ± 2	0.638	0.864	0.909
Nausea (mm)	11 ± 6	24 ± 10	0.358	11 ± 7	3 ± 1	0.234	0.849	0.154
Bloating (mm)	22 ± 11	33 ± 12	0.519	9 ± 4	16 ± 5	0.352	0.282	0.949
Flatulence (mm)	20 ± 10	59 ± 8	0.006	17 ± 8	25 ± 10	0.591	0.020	0.094
Heartburn (mm)	13 ± 6	5 ± 3	0.292	12 ± 5	3 ± 1	0.145	0.091	0.742
Belching (mm)	18 ± 10	17 ± 9	0.968	7 ± 4	4 ± 2	0.643	0.870	0.956

Energy intake was recorded with 3-day food diaries. Physical activity was measured using the short self-administered format of the International Physical Activity Questionnaire (IPAQ) [5]. Ratings of gastrointestinal side-effects were made using 100 mm visual analogue scales (VAS). Subjects were asked to rate the occurrence of each side effect with extreme statements anchored at each end of the rating scale (0 mm Never, 100 mm All the time).

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Table S6. Correlations between baseline variables and the delta change (Δ) in IHCL following 42 days of inulin-control or propionate ester (IPE) supplementation.

Baseline Variable	ΔIHCL (%)					
	Inulin-Control (N=9)		IPE (N=9)		All (N=18)	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
IHCL (%)	0.295	0.440	-0.259	0.501	0.138	0.624
Fasting Glucose (mmol/L)	0.186	0.186	-0.247	0.556	-0.071	0.802
Postprandial Glucose (mmol/L)^	-0.076	0.858	-0.178	0.674	-0.095	0.736
Fasting Insulin (µU/mL)	-0.291	0.484	0.192	0.650	0.062	0.825
Postprandial Insulin (µU/mL)^	-0.014	0.974	0.488	0.220	0.075	0.790
HOMA-IR	-0.206	0.625	0.048	0.910	0.050	0.860
HbA1c (mmol/mol)	0.089	0.819	-0.592	0.093	-0.170	0.545
Triglycerides (mmol/L)	-0.447	0.227	-0.053	0.893	-0.211+	0.451
Cholesterol (mmol/L)	-0.207	0.593	-0.453	0.221	-0.266	0.338
LDL Cholesterol (mmol/L)	-0.106	0.786	-0.323	0.397	-0.156	0.579
HDL Cholesterol (mmol/L)	-0.216	0.576	-0.276	0.473	-0.185	0.510
Alanine Transaminase (IU/L)	0.385	0.306	0.134	0.731	0.206	0.462
Breath Hydrogen (ppm)	0.490	0.218	-0.481	0.227	0.074+	0.794
Fasting Acetate (µmol/L)	0.053	0.901	0.128	0.763	0.082+	0.771
Postprandial Acetate (µmol/L)^	0.614	0.105	0.297	0.475	0.303	0.273
Fasting Propionate (µmol/L)	-0.086	0.839	-0.412	0.310	-0.258	0.353
Postprandial Propionate (µmol/L)^	0.601	0.115	0.207	0.623	0.416	0.109
Fasting Butyrate (µmol/L)	-0.174	0.728	-0.266	0.524	-0.411	0.128
Postprandial Butyrate (µmol/L)^	-0.338	0.413	0.642	0.086	-0.041	0.885
IL-6 (pg/mL)	-0.050+	0.898	-0.365	0.374	-0.279+	0.315
IL-8 (pg/mL)	0.007	0.986	-0.193	0.647	-0.161	0.566
IL-10 (pg/mL)	0.067+	0.864	-0.578+	0.133	-0.153+	0.586
IL-12 (pg/mL)	0.337	0.318	-0.051	0.904	-0.088+	0.756
C Reactive Protein (mg/L)	0.567	0.112	0.233	0.615	0.323	0.022
Energy Intake (kcal/day)	0.556	0.153	0.037	0.930	0.411	0.128
Carbohydrate (g/day)	0.512	0.195	0.452+	0.260	0.439+	0.101
Fat (g/day)	0.552	0.156	-0.034	0.937	0.411	0.128
Protein (g/day)	0.478	0.231	-0.090	0.832	0.232	0.406
Fibre (NSP) (g/day)	0.243	0.563	0.162	0.701	0.276	0.319
Total Physical Activity (MET-h/week)	-0.405	0.280	-0.076	0.846	-0.428	0.111

+ = non-parametric Spearman's Rank correlation coefficient. ^ =statistical analysis performed on n=8/n=16.

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