

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

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Response to the reviewer's comments on manuscript DOM-18-0527-BR

We are grateful to the referees for reviewing the manuscript. Below are listed the changes we have made in response to specific comments from the reviewers.

Referee: 1

I am concerned that the presentation of results could be improved. The results in the abstract compare before and after values for the two groups individually and not the two groups (e.g., delta values for the two groups). Both groups show an increase in intrahepatic fat, but the difference between the groups is not statistically significant.

The between group analysis has been added to the Abstract (Page 10; Line 13).

It would be interesting if the authors could find room to elaborate on their potential explanations for this finding.

Further references and information that detail potential explanations for our findings have been added to the Results and Discussion (Page 13; Lines 15-29 / Page 14; Lines 48-52)

Using parametric analyses in such a small dataset may not be the best option. If investigators choose this strategy, tests for normal distribution should be presented. I would suggest that authors could consider presenting non-parametric analyses.

As stated in the Statistical Analysis section in the Supplementary Material (Page 23), parametric analysis was only performed on data that was normally distributed. Data were first checked for normality using the Shapiro-Wilk Test. Data that were not normally distributed was then log transformed prior to carrying out parametric statistical tests.

A number of the within-group data sets were still not normally distributed following log-transformation. These were analysed by Wilcoxon signed rank test and have been highlighted by the '+' symbol in the tables in throughout the manuscript.

Included participants have histologically verified NAFLD. We know that it is fibrosis which is the main driver of liver related disease and the main prognostic factor. I would therefore recommend including information about the number of patients with NASH, the diagnostic criteria used, and the degree of fibrosis.

Table S1 has been added to the Supplementary Material providing this information.

The registered protocol gives the impression that appetite suppression is the primary objective and that the effects of propionate ester on glucose and lipid metabolism (added 24/1/18) and hepatic steatosis are secondary endpoints. The paper does not include information about appetite suppression. Are these data available?

The registered protocol involves two separate studies from the same ethics application (14/LO/0645). The primary outcome from 'Study 1' was the change in glucose homeostasis in overweight/obese adults (no diagnosis of NAFLD) following IPE supplementation, with appetite regulation measured as a secondary objective. 'Study 2' had a primary outcome of change in liver fat in adults with NAFLD. Appetite regulation was not a secondary outcome in this study and was not measured.

Referee: 2

The contrasting results between this study and the previous one using Inulin alone need to be discussed more thoroughly with references or possibly just on speculative ground.

Further discussion comparing our findings with previous research using inulin has been added to the Results and Discussion (Page 13; Lines 14-39).

For Review Only

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Referee: 3

Table 1 and supplementary tables: How was the IPE-Inulin-control column calculated? Since these were two different groups of people how can statistics be done? I do not understand these data.

Table 1 and supplementary tables have been amended following comments from Referee 5. The between-group analysis has now been conducted using a mixed ANOVA. The group (inulin and IPE) × time (pre- and post-supplementation) design assesses if the change in each outcome measure was different between groups over time. The P value for this Group×Time interaction is presented together with the main effect for time in the tables for each outcome measure.

This analysis is also explained in the Statistical Analysis section in the Supplementary Material (Page 23),

Page 7, line 9: The authors refer to a number of rodent studies where insulin type fructans prevented the accumulation of liver fat. But they cite only one over 5 year old study dealing mainly with hypothalamic action. They should provide more adequate references.

Further references and information that detail potential explanations for our findings have been added to the Results and Discussion (Page 13; Line 14-39). The recent publication, suggested by the Referee, highlighting the effects of propionate and acetate on liver metabolism has also been added (Page 14; Line 48-52).

Preview Only

Referee: 4

It is not reported whether this RCT had a priori been registered in a database for clinical trials (i.e., ClinicalTrials.gov or other).

Please refer to response to Referee 1. The current study was registered on the ICRTN database, as stated in the Methods section (Page 11, Line 53).

There is no specific information for allocation concealment, blinding process and the production of the placebo.

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.

This information has been added to the Supplementary Material (Page 21; Line 29-35).

Placebo was not well prepared, since it contained 20 g/d inulin, whereas the IPE contains 14.6 gr/d inulin.

Inulin was used as a positive control to account for any effects that may derive from SCFA production from the inulin contained in the IPE, rather than the esterified propionate.

An equivalent total amount of inulin and IPE (20 g/d) was used rather than "inulin matched" (14.6g inulin vs. 20 g IPE), as our previous in vitro fermentation data showed that comparable quantities of acetate and butyrate are produced from matched quantities of inulin and IPE.

This information is provided in the Results and Discussion section (Page 14; Lines 24-27).

The analysis was not intention-to-treat.

ITT was not performed as attrition in the study was low (10%). Furthermore, outcome measures were only collected pre- and post-supplementation, thus only baseline measurements would be carried over into an ITT analysis, rather than any interim assessments.

ITT cannot be performed on any of the metabolic outcomes, as biochemical analysis was only performed on the 18 volunteers that completed the study.

The potential comorbidities and medications of both groups at baseline, especially those closely related to MetS, i.e., diabetes, dyslipidemia, cardiovascular disease, hypertension, were not presented.

This has been added to Table 1. Table S1 has also been added to the Supplementary Material providing this information.

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Statistical analysis: A 2x2 ANOVA would be more appropriate analysis than independent t-test and paired t-test for between and within group comparisons, respectively. A paired t-test on the differences (Δ) between groups (baseline-day 42) should be avoided.

A mixed ANOVA has now been conducted to assess between group changes for each outcome measure. The group (inulin and IPE) \times time (pre- and post-supplementation) design assesses if the change in each outcome measure was different between groups over time. The P value for this Group \times Time interaction is presented together with the main effect for time in the tables for each outcome measure.

This analysis is also explained in the Statistical Analysis section in the Supplementary Material (Page 23).

Statistical analysis: A $p < 0.10$ was considered as a trend, but this should be avoided.

This has been removed throughout the manuscript.

No baseline comparisons between groups were provided in the tables.

Baseline comparisons between groups were performed using unpaired t tests or independent samples Mann-Whitney U Tests, where appropriate. The only outcome measures that were significantly different between groups at baseline were fasting cholesterol and LDL cholesterol. This information has been added to the Results and Discussion section (Page 15; Lines 5-7), Supplementary Material (Page 23) and Table S2.

The authors should explain why they had specified the duration of the study to 42 days.

A 42 day supplementation period was chosen as a prior unpublished study observed that 20 g day IPE for 42 days improved measures of glucose homeostasis (fasting insulin, HOMA-IR) in overweight and obese adults. This information has been added to the Supplementary Material (Page 21; Lines 19-26).

Page 5, line 3: 5 years is a long period for the histological confirmation; the diagnosis may possibly have changed in the meanwhile in some or most of the participants.

This has been added as a possible limitation of the study in the Results and Discussion section (Page 15; Lines 8-12). The information regarding the time period between histological confirmation and study recruitment has been added to Table S1. Whilst diagnosis may have possibly changed in this timeframe, all volunteers were still attending a liver clinic for fatty liver disease and all had substantial liver fat (>5%) when scans were performed at baseline.

Page 5, lines 40-45 and page 6, line 19: These were not shown; HOMA-IR, HbA1c and IL-12 provided non-significant results. Furthermore, no association was shown in this study between IHCL and HOMA-IR, HbA1c and IL-12.

This has been removed.

Page 5, lines 45-49: This was not shown; it required a 2x2 ANCOVA.

This has been removed.

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2 *Page 7, lines 35-37: This was not shown in this study.*
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5 This sentence has been slightly amended, to clarify that this was not shown in the study, but is a
6 speculative mechanism to explain the results in the current study. Results and Discussion section
7 (Page 15; Lines 16-19).
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10 *Figure 1: A line graph (with lines connecting individual values pre and post treatment) would be*
11 *more appropriate than the dot graph.*
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13 Figure 1 has been amended to include this information.
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16 *The limitations of the study should be summarized in a separate paragraph before the paragraph*
17 *of conclusions*
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19 This paragraph has been added to the Results and Discussion section (Page 15; Lines 1-12).
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23 *Table S1: Inulin-control group has higher insulin and HOM-IR, but lower triglycerides, which is a*
24 *seemingly paradox.*
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26 There was no significant difference between groups in these measurements at baseline.
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Referee: 5

The authors state several times that they investigated colonic propionate. The authors also state in the abstract that "The increased colonic propionate from IPE appears to prevent this acetate-mediated increase in IHCL". However, I do not find the data on colonic propionate or acetate in the manuscript. Was colonic SCFA content measured? If not, please adjust these statements accordingly.

This has been amended to "the increased colonic propionate delivery" throughout the manuscript, which is a more accurate description of the effects of the IPE. We have also demonstrated in our previous studies using stable isotope labelling that the release of esterified propionate from IPE occurs mainly (~80%) in the colon (Chambers et al, Gut 2015; Polyviou et al, APT, 2017).

Were the subjects matched on any variable during the randomization, such as body mass index or age? Please provide the information.

Please refer to response to point 2 from Referee 4.

Please provide the medication data of the subjects in the groups. Did any of the subjects use thiazolidinediones, metformin, sulfonylureas or insulin which may have had an impact on hepatic metabolism? How many subjects in the groups had diabetes?

Table S1 has been added to the Supplementary Material providing this information.

One subject was excluded because of poor compliance. This was estimated by used and unused sachets. How was poor compliance defined, and was there any variation in compliance among subjects? Please provide data.

Compliance data has been added to the Results and Discussion (Page 12; Lines 26-27).

The authors write that a liver enzyme profile promotes fat accretion. They also emphasize de novo lipogenesis in the discussion. It is well established that rather than liver enzymes, it is insulin which is the key regulator of hepatic DNL. Please discuss.

This information has been added to the Results and Discussion (Page 13; Lines 47-49).

The inulin-control and the IPE groups seem to have significant differences at baseline. Most importantly (see above), postprandial insulin was 51% higher and HOMA-IR 38% higher in the inulin-control group as compared to the IPE group. In addition, ALT was 37% higher and breath hydrogen almost 3-fold higher in the inulin-control than the IPE group. The inulin-control group was also less physically active and had more gastrointestinal side-effects at the baseline.

Baseline comparisons between groups were performed using unpaired t tests or independent samples Mann-Whitney U Tests, where appropriate. The only outcome measures that were significantly different between groups at baseline were fasting cholesterol and LDL cholesterol. This information has been added to the Results and Discussion (Page 15; Lines 3-8) and highlighted in Table S2.

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2 *Does any baseline characteristic, such as postprandial insulin or HOMA-IR explain the variation in*
3 *the change in IHCL? Please provide analysis and discuss.*
4

5 Correlations analysis has been performed on baseline variables and the delta change in IHCL.
6 Pearson correlation (or Spearman correlation if a variable was not normally distributed) were
7 performed for individual supplementation groups and for all volunteers. This analysis did not
8 identify a single baseline characteristic that significantly correlated with the individual change in
9 IHCL.

10 These correlations are presented in Table S6 in the Supplementary Material and discussed in the
11 Results and Discussion section (Page 15; Lines 1-6).
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14 *There seems to be large variation in the change in IHCL, HOMA-IR and HbA1c within the groups,*
15 *i.e. there were responders and non-responders in both groups. Because of this within-group*
16 *variation, please provide individual values for IHCL, HOMA-IR and HbA1c before and after the*
17 *intervention for the subjects in the two groups (similar as Figure 2B in your previous paper*
18 *Chambers et al Gut 2015).*
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21 Figure 1 has been amended to include this information.
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23 *In addition, please provide individual data on energy intake, because it seems to paradoxically*
24 *decrease in the inulin-control group.*
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26 The non-significant reduction in energy intake in the inulin group is primary driven by a single
27 volunteer whose self-reported intake was reduced from 4471 to 2120 kcal. Removal of this
28 volunteer shows that energy intake changed from 2125±741 to 2034±718 (P=0.341, n=8) in the
29 inulin group.
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31 *Please provide explanation for the abbreviation ICHNHST.*
32

33 Added (Supplementary material Page 22; Line 35).
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35 *The subjects had a histological confirmation of NAFLD. Please provide the full histological*
36 *characterization of the subjects (steatosis, necroinflammation and fibrosis) in the inulin-control and*
37 *the IPE groups.*
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40 Table S1 has been added to the Supplementary Material providing this information.
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2 **BRIEF REPORT**

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4 **TITLE**

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6 *The effects of dietary supplementation with inulin and inulin-propionate ester on hepatic steatosis*
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8 *in adults with non-alcoholic fatty liver disease*
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11 **SHORT RUNNING TITLE**

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13 *Inulin, inulin-propionate ester and liver fat*
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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 $26.8\pm 3.9\%$; $P=0.012$; $n=9$), which was not observed within the IPE group (22.6 ± 6.9 to $23.5\pm 6.8\%$; $P=0.635$; $n=9$). 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.

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 diabetes¹. The prevalence of NAFLD is strongly associated with obesity¹, thus current guidelines for the prevention and management of NAFLD are based solely on weight loss through diet and exercise¹. 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 poor². 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 diets³⁻⁵. 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 liver⁶, 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 colon⁹. 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 NAFLD⁹. 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/m² were recruited from liver clinics at St Mary's Hospital, Imperial College Healthcare National Health Service Trust. Potential

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2 volunteers were eligible if they had a confirmation of NAFLD by liver biopsy within the previous five
3 years and controlled blood glucose levels (HbA1c <48 mmol/mol). The study was conducted using
4 a randomised, double-blind, placebo controlled, parallel design. Subjects received either 20 g/day
5 of inulin-control or IPE for 42 days. The 20 g dose of IPE would have provided 14.6 g of inulin (and
6 5.4 g bound propionate) to the diet⁹. Inulin was therefore chosen as a positive control to account
7 for any effects that may derive from fermentation of this substrate by the gut microbiota. The
8 supplements were provided to volunteers in 10 g ready-to-use sachets and they were instructed to
9 mix the contents into their habitual diet twice a day. Participants were required to attend the NIHR
10 Imperial Clinical Research Facility pre- (day 0) and post-supplementation (day 42) to determine
11 outcome measures.
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21 **RESULTS AND DISCUSSION**

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24 Of 20 volunteers that were randomised and enrolled into the study, data were analysed from the 18
25 volunteers that completed the supplementation period (Figure S1). The characteristics of these
26 volunteers are presented in Table 1 and Table S1. Estimated compliance was similar in the
27 supplementation groups (inulin-control: 90±7% vs. IPE: 95±2%; P=0.213). The changes in IHCL
28 (Figure 1A-C and Table 1) were unexpected, as we observed an increase in IHCL post-
29 supplementation in both groups (main effect for time; P=0.020). The change in IHCL was not
30 significantly different between supplementation groups (Figure 1A; P=0.082), however, within-
31 group analysis showed that IHCL was significantly increased within the inulin-control group (Figure
32 1B; P=0.012) and not the IPE group (Figure 1C; P=0.635). Analysis of metabolic and inflammatory
33 responses (Figures 1D-I and Tables S2-S4) highlight that the change in insulin resistance (HOMA-
34 IR) was significantly different between groups (Figure 1D; P=0.046), with a non-significant increase
35 in the inulin-control group (Figure 1E; P=0.060) and decrease in the IPE group (Figure 1E;
36 P=0.389), respectively. There were no within- or between-group differences in body composition
37 (Table 1), self-reported food intake or physical activity following the supplementation period (Table
38 S5). Our hypothesis was that IPE supplementation would decrease IHCL in adults with NAFLD, as
39 observed in our previous study⁹; however, IPE supplementation did not reduce liver fat content.
40 The disparate outcome may be explained by methodological differences in IPE dose (10g/day vs.
41 20g/day) and exposure (6 weeks vs. 24 weeks) in the two studies. Furthermore, volunteers in the
42 present study had a confirmation of NAFLD by liver biopsy, which is considered gold-standard to
43 establish diagnosis, and metabolic parameters would indicate these individuals had poorer
44 glycaemic control compared to the volunteers from our previous work (fasting glucose: 5.0 mmol/L
45 vs. 6.1 mmol/L; HbA1c: 38 mmol/mol vs. 42 mmol/mol).
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2 Whilst breath hydrogen, a marker of colonic fermentation, was elevated in both groups post-
3 supplementation (Table S3), the impact on SCFAs measured in peripheral blood was limited, as
4 we observed that IPE supplementation only reduced levels of butyrate in fasting samples
5 compared to the inulin-control group (Table S3). The blood samples were collected >12 hours after
6 volunteers were requested to ingest their final supplement, which may explain why we were unable
7 to detect large differences in circulating SCFAs post-supplementation. Nevertheless, previous
8 research using stable isotope methodology has demonstrated that inulin is predominantly
9 fermented in the human colon into acetate (82%), with considerably less propionate and butyrate
10 produced (6% and 12%, respectively)¹⁰. Dietary supplementation with inulin-type fructans (ITF)
11 has generally been associated with positive effects on metabolic health. The evidence for this
12 beneficial effect is primarily derived from rodent studies, where dietary supplementation with ITF
13 has consistently been shown to prevent the accumulation of liver fat and metabolic dysregulation
14 induced by a high fat diet³⁻⁵. However, to the best of our knowledge, ITF have not been shown to
15 reduce liver fat when added to the diet of rodents with pre-existing steatosis. Studies investigating
16 the effect of ITF on metabolic health in humans are equivocal with a recent meta-analysis reporting
17 no association between ITF supplementation and fasting glucose and insulin levels¹¹. Fewer
18 studies have quantified the impact of ITF supplementation on liver fat content in humans. Our
19 previous work demonstrated that 30g/day ITF supplemented into the habitual diet of overweight
20 adults with normal glycaemic control had no effect on IHCL¹². In contrast, a superior reduction in
21 IHCL in adults with pre-diabetes was found when a weight-loss diet was combined with 30g/day
22 ITF supplementation¹³. The serendipitous observation in the current study is that supplementing
23 20g/day inulin into a habitual weight-maintaining diet raises IHCL and further exacerbates glucose
24 homeostasis in adults with NAFLD. Taken together, our data suggests that ITF supplementation
25 does not have a homogenous impact on hepatic lipid content in humans and its effects may
26 depend on the pre-existing metabolic health of the individual and the energy-balance promoted by
27 the background diet.

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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 DNL¹⁴. Previous work has also demonstrated that chronic intragastric
acetate infusion in rats promotes postprandial hyperinsulinaemia and increases liver triglyceride
content¹⁵. 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 synthesis¹⁶. Evidence highlights that, together with higher insulin levels¹⁷, humans with

1
2 NAFLD have an elevated expression of hepatic genes that favour fat accumulation, with increased
3 expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), which are key
4 enzymes in hepatic DNL¹⁸. Consequently, NAFLD patients are reported to have rates of DNL up to
5 3-fold higher compared to BMI-matched controls¹⁷. The current data suggests that in weight-stable
6 individuals with NAFLD, an increased supply of acetate to the liver from the colonic fermentation of
7 inulin provides surplus acetyl-CoA for DNL and hepatic lipid accretion. Interestingly, diet-induced
8 weight loss in mice has been shown to markedly reduce insulin levels and rates of hepatic DNL¹⁹,
9 whilst the expression of hepatic DNL-related genes are also reduced by states of chronic negative
10 energy balance²⁰. This may explain the disparate effect of inulin supplementation on liver fat
11 content in the present study compared to our previous investigation when inulin intervention was
12 added to a hypo-caloric diet that achieved a ~5% reduction in body weight¹³.

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21 IPE supplementation did not significantly raise IHCL content, as observed within the inulin-control
22 group. The contrasting outcome could be due to differences in amounts of acetate derived from
23 inulin fermentation throughout the supplementation period, as the inulin-control group were
24 provided with a greater amount of inulin compared to IPE (20 g/day vs 14.6 g/day). *In vitro* faecal
25 fermentation profiles have previously demonstrated, however, that comparable quantities of
26 acetate are produced from equivalent doses of inulin and IPE⁹. Interestingly, IPE does substantially
27 alter the proportion of SCFAs produced, as the molar ratio of acetate, propionate and butyrate
28 changes from 74:16:10 with inulin to 25:69:6 with an equivalent amount of IPE⁹. It could be
29 suggested that the elevated ratio of colonic propionate:acetate promoted by IPE supplementation
30 may have prevented the accumulation of liver fat observed in the inulin-control group by impairing
31 hepatic acetate metabolism. This proposed mechanism is concordant with the results from
32 Wolever and colleagues who demonstrated using stable isotope technology that propionate inhibits
33 incorporation of colonic [1,2-¹³C] acetate into plasma lipids in humans⁷. Studies using rat
34 hepatocytes have also highlighted that propionate inhibits lipid synthesis when acetate is a major
35 source of acetyl-CoA⁸. Recent evidence has demonstrated a third ACSS isoform, ACSS3, for
36 which propionate is the preferred substrate over acetate, and which is highly expressed in the
37 mitochondrial matrix of hepatocytes¹⁶. ACSS3 converts propionate to propionyl-CoA allowing it to
38 enter mitochondrial respiration through succinate and the TCA cycle¹⁶. Elevating hepatic
39 propionate metabolism would therefore increase competition with acetate for conversion into their
40 CoA adducts at tissue level, which may reduce cytosolic acetyl-CoA availability for DNL. This
41 potential mechanism is supported by a recent observation that exposing HepG2 cells to elevated
42 ratios of propionate:acetate increases the formation of heptadecanoic acid derived from propionyl-
43 CoA, which inhibits the synthesis of palmitate from acetyl-CoA²¹.

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

10
11 In conclusion, inulin consumed at 20g/day increased IHCL in weight-stable adults with NAFLD, an
12 effect not observed with IPE supplementation. We speculate that in the context of NAFLD and a
13 hepatic metabolic profile that stimulates DNL, the acetate derived from colonic fermentation of
14 inulin could provide additional lipogenic precursor to the liver. The increased colonic delivery of
15 propionate from IPE appears to attenuate this acetate-mediated increase in IHCL, possibly by
16 interfering with the availability of acetate-derived acetyl-CoA for DNL. Further work is warranted to
17 explore how altering colonic SCFA production profiles modulates the metabolic pathways that
18 govern hepatic lipid storage in humans. In particular, future research should determine how the
19 hepatic metabolic processing of acetate and propionate changes in different states of energy
20 balance and to determine distinctions between NAFLD patients and healthy controls.
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35 **ACKNOWLEDGEMENTS**

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42 are those of the authors and not necessarily those of the NHS, the NIHR or the Department of
43 Health. A patent application for "Compounds and their effects on appetite control and insulin
44 sensitivity" surrounding the use of inulin-propionate ester has been filed by Prof Frost and Dr
45 Morrison. (WO2014020344). None of the other authors reported a conflict of interest related to the
46 study.
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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 \pm SEM.

Variable	Inulin-Control (N = 9)			IPE (N = 9)			Mixed ANOVA	
	Pre	Post	P Value	Pre	Post	P Value	Time P Value	Group \times Time P Value
Sex (N)								
Male	5			4				
Female	4			5				
Race or ethnicity (N)								
White	5			7				
Asian	4			2				
Age (years)	49 \pm 4			51 \pm 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 \pm 2.9	26.8 \pm 3.9	0.012	22.6 \pm 6.9	23.5 \pm 6.8	0.635	0.020	0.082
Weight (kg)	83.3 \pm 4.4	83.2 \pm 4.0	0.914	93.6 \pm 7.6	93.9 \pm 7.4	0.556	0.438	0.578
BMI (kg/m²)	29.5 \pm 1.4	29.5 \pm 1.4	0.966	31.5 \pm 1.9	31.6 \pm 1.9	0.377	0.696	0.620
Fat Mass (kg)	26.8 \pm 3.4	27.1 \pm 3.3	0.524	35.3 \pm 5.2	34.9 \pm 5.3	0.485	0.931	0.302
Fat Free Mass (kg)	56.5 \pm 3.7	56.1 \pm 3.3	0.631	58.3 \pm 5.0	59.1 \pm 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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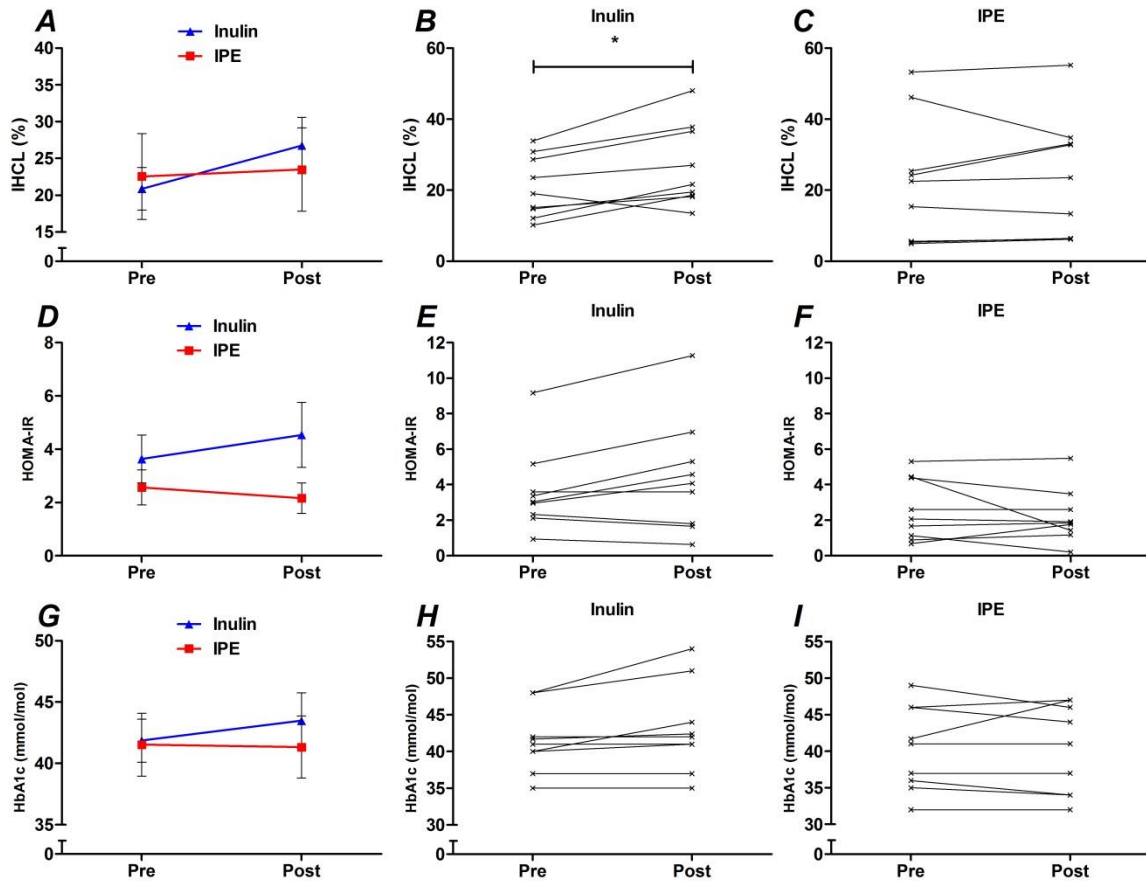
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1
2 **FIGURE LEGEND**
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4 **Figure 1. Effects of 42 days of inulin-control and inulin-propionate ester (IPE) supplementation on**
5 **liver fat and glucose homeostasis A.-C. Intrahepatocellular lipid (IHCL) D.-F. Homeostatic model**
6 **assessment of insulin resistance (HOMA-IR) and G. Glycosylated haemoglobin (HbA1c) Group data**
7 **(A., D. and G.) expressed as mean \pm SEM (n=9).**
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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 work¹. 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 described². Body weight, fat mass (FM) and fat free mass (FFM) with bioelectrical impedance (Tanita BC-418MA, Japan). Subjects were

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2 asked to change into lightweight hospital scrubs and to void their bladder before measurements
3 were taken.
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5 6 ***Mixed meal test (MMT)***

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

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32 A fasting blood sample was collected and analysed for levels of triglycerides, total cholesterol, low-
33 density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, glycosylated
34 HbA1c, alanine transaminase and C-reactive protein. All analytes were measured by the
35 Department of Chemical Pathology, Imperial College Healthcare National Health Service Trust. IL-
36 6, IL-8, IL-10, IL-12 and IL-17A were measured in fasting serum using the Cytometric Bead Array
37 (BD Biosciences, UK), according to the manufacturer's protocol. IL-17A results are not shown, as
38 only two volunteers had detectable values for this analyte.
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45 ***Self-reported food intake, physical activity and gastrointestinal adverse events***

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47 Energy and macronutrient intake was recorded with food diaries during the final 3-days of each
48 supplementation period (Dietplan 6.0; Forestfield Software Ltd, UK). Physical activity was
49 assessed during the final 7 days of each supplementation period using the short self-administered
50 format of the International Physical Activity Questionnaire (IPAQ)⁵. Ratings of gastrointestinal side-
51 effects were made using 100 mm visual analogue scales (VAS). Subjects were asked to rate the
52 occurrence of each side effect with extreme statements anchored at each end of the rating scale (0
53 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 size¹. 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% ($\alpha=0.05$, 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 \times 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 \pm 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.

FIGURES

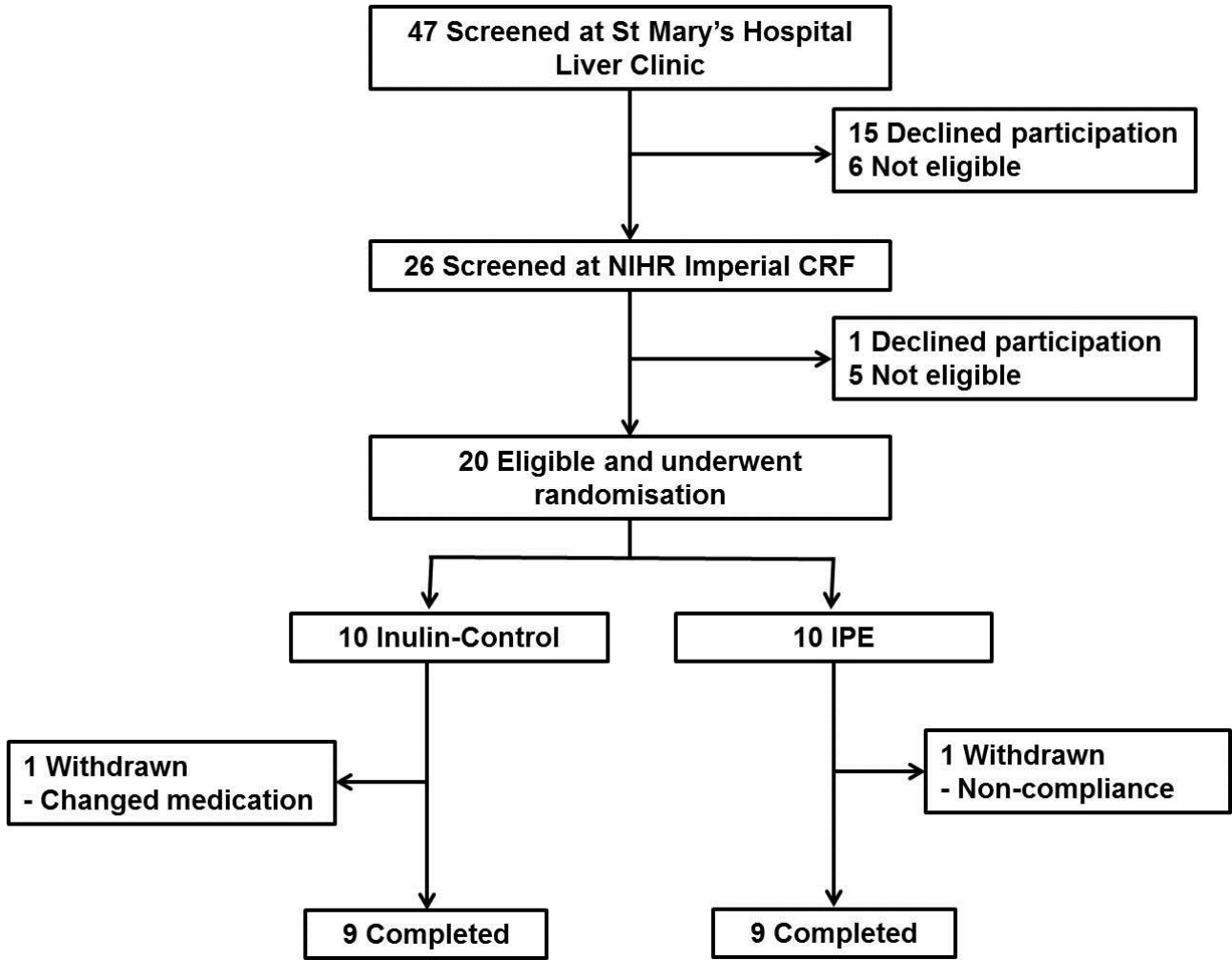


Figure S1. Recruitment and retention in the study. Inulin-propionate ester (IPE).

Only

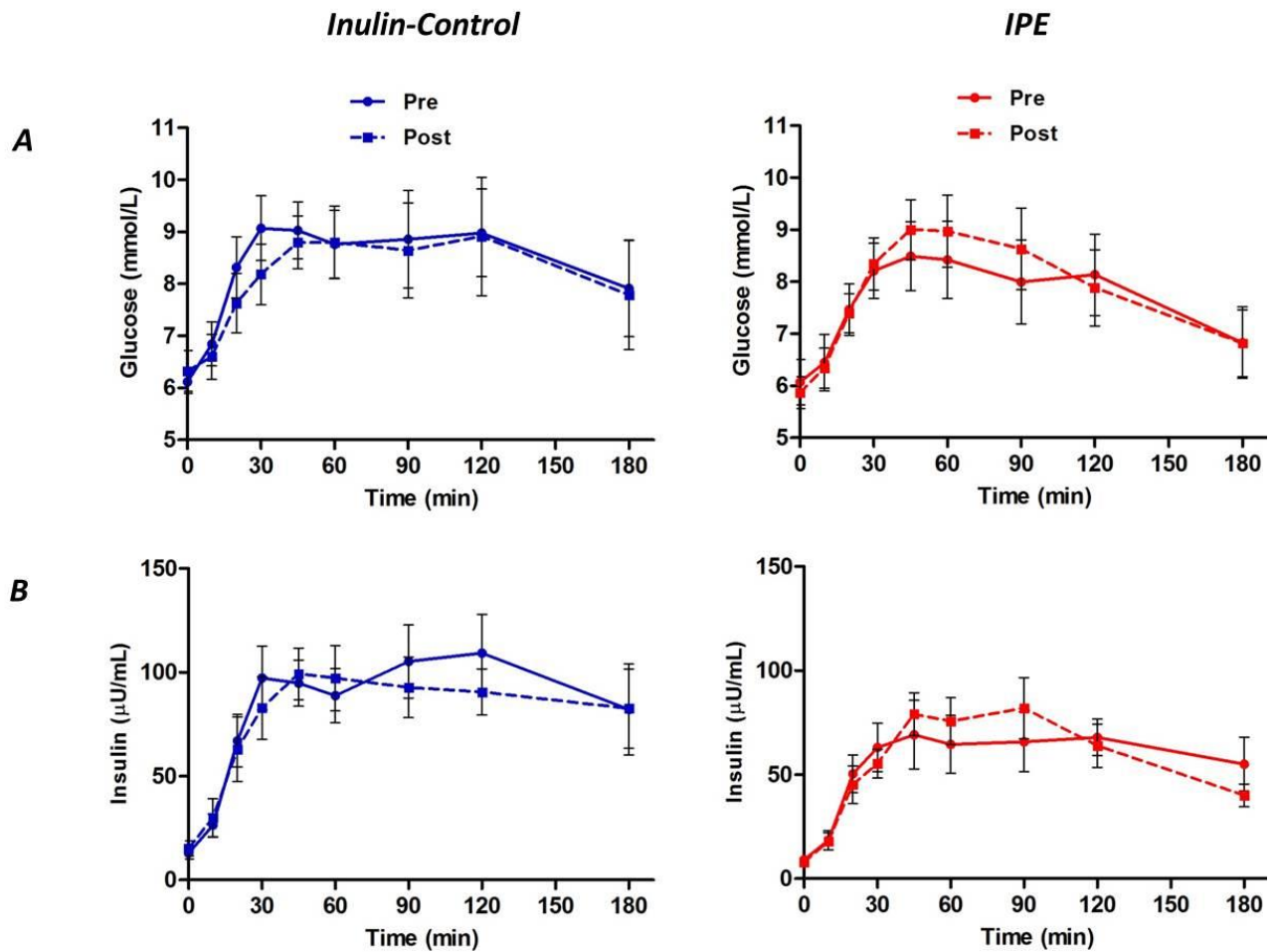


Figure S2. The effects of 42 days of inulin control and inulin propionate ester (IPE) supplementation on postprandial **A.** glucose and **B.** insulin responses (Data are expressed as mean \pm SEM ($n = 8$ each group)).

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%.

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 \pm SEM or 95% CI.

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

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 \pm SEM or 95% CI.

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

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

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

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

+ = non-parametric statistical analysis

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 \pm SEM or 95% CI.

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

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	Δ IHCL (%)					
	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 (μ U/mL)	-0.291	0.484	0.192	0.650	0.062	0.825
Postprandial Insulin (μ U/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 (μ mol/L)	0.053	0.901	0.128	0.763	0.082+	0.771
Postprandial Acetate (μ mol/L) [^]	0.614	0.105	0.297	0.475	0.303	0.273
Fasting Propionate (μ mol/L)	-0.086	0.839	-0.412	0.310	-0.258	0.353
Postprandial Propionate (μ mol/L) [^]	0.601	0.115	0.207	0.623	0.416	0.109
Fasting Butyrate (μ mol/L)	-0.174	0.728	-0.266	0.524	-0.411	0.128
Postprandial Butyrate (μ mol/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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