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ORIGINAL ARTICLE

Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults

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ABSTRACT

Objective The colonic microbiota ferment dietary fibres, producing short chain fatty acids. Recent evidence suggests that the short chain fatty acid propionate may play an important role in appetite regulation. We hypothesised that colonic delivery of propionate would increase peptide YY (PYY) and glucagon like peptide-1 (GLP-1) secretion in humans, and reduce energy intake and weight gain in overweight adults.

Design To investigate whether propionate promotes PYY and GLP-1 secretion, a primary cultured human colonic cell model was developed. To deliver propionate specifically to the colon, we developed a novel inulin-propionate ester. An acute randomised, controlled cross-over study was used to assess the effects of this inulin-propionate ester on energy intake and plasma PYY and GLP-1 concentrations. The long-term effects of inulin-propionate ester on weight gain were subsequently assessed in a randomised, controlled 24-week study involving 60 overweight adults.

Results Propionate significantly stimulated the release of PYY and GLP-1 from human colonic cells. Acute ingestion of 10 g inulin-propionate ester significantly increased postprandial plasma PYY and GLP-1 and reduced energy intake. Over 24 weeks, 10 g/day inulin-propionate ester supplementation significantly reduced weight gain, intra-abdominal adipose tissue distribution, intrahepatocellular lipid content and prevented the deterioration in insulin sensitivity observed in the inulin-control group.

Conclusions These data demonstrate for the first time that increasing colonic propionate prevents weight gain in overweight adult humans.

Trial registration number NCT00750438.

INTRODUCTION

Evidence published over the last 25 years demonstrates that hormonal and neuronal signals from the GI tract play an important role in appetite regulation.¹ There is increasing evidence that the gut microbiota influences energy regulation and can be a major determinant in the development of obesity.² Recent investigations suggest that diet, the gut microbiota and fat storage can be linked

Significance of this study

What is already known about this subject?

- Increased intake of dietary fibre has been associated with reduced appetite and weight loss.
- The short chain fatty acids (SCFAs) produced by microbial fermentation of dietary fibre in the colon stimulate the release of the anorectic gut hormones peptide YY (PYY) and glucagon like peptide-1 (GLP-1) from rodent enteroendocrine L cells via activation of the G protein coupled free fatty acid receptor (FFAR) 2.
- Of the SCFAs produced by colonic fermentation of dietary fibre, propionate has the highest affinity for FFAR 2.
- Mice receiving a faecal transplant from a donor with a gut microbiota composition that produces elevated levels of propionate in the colon have reduced weight gain and adiposity.

What are the new findings?

- Propionate stimulates the release of PYY and GLP-1 from primary cultured human colonic cells.
- This first-in-human study demonstrates that delivery of propionate directly to the colon, acutely increases the release of PYY and GLP-1 and reduces energy intake.
- Long-term colonic propionate delivery prevents body weight gain and reduces intra-abdominal fat accretion in overweight adults.
- Long-term colonic propionate delivery significantly reduces intrahepatocellular lipid content in adults that meet the diagnostic criteria for non-alcoholic fatty liver disease.

How might it impact on clinical practice in the foreseeable future?

- Optimising colonic propionate production through selection of propiogenic dietary fibres may represent a novel route to prevent weight gain throughout life and improve public health.

through a molecular mechanism involving short chain fatty acids (SCFAs), the major products of dietary fibre fermentation by the gut microbiome.^{3–5}

A major public health challenge is the development of effective strategies that can prevent the increased prevalence in obesity and the reported average 0.3–0.8 kg/year weight gain in adults.^{6–9} Such gradual long-term weight gain can be the result of a small habitual positive energy balance of 50–100 kcal/day.¹⁰ Interventions that can be safely applied at the population level to reverse this minor energy imbalance and prevent weight gain throughout life would therefore have substantial benefits to public health. Increased intake of dietary fibre has been associated with reduced appetite and weight loss.^{11–13} In particular, evidence suggests that the fermentable component of dietary fibre is critical in mediating these satiating effects.¹⁴ Feeding rodents a high level of fermentable dietary fibre protects against high-fat, diet-induced increases in body weight and fat mass.^{15–16} There is also evidence that fermentable dietary fibre can suppress appetite and decrease body weight in humans.^{17–19} However, large amounts of dietary fibre (>30 g/day) are required for these beneficial effects, and compliance with high fibre diets is poor, due to GI side effects, which may also explain the inconsistent reports regarding their effects on appetite and body weight.²⁰ Targeting the mechanisms by which fermentable dietary fibre suppresses appetite may thus provide a more effective approach to weight control than the use of high fibre diets.

The SCFAs produced by microbial fermentation of dietary fibre in the colon have been shown to stimulate the release of the anorectic gut hormones peptide YY (PYY) and glucagon like peptide-1 (GLP-1) from rodent enteroendocrine L cells.^{21–23} These hormones are released from the gut and are involved in the short-term signal of satiation and satiety to the appetite centres of the brain.²⁴ Peripheral administration of PYY_{3–36} or GLP-1 enhances satiety and reduces food intake in animals and man.^{25–27} Recent evidence suggests that SCFAs stimulate GLP-1 release in rodents via stimulation of the G protein coupled free fatty acid receptor (FFAR) 2 on colonic L cells.²¹ Of the SCFAs produced by colonic fermentation of dietary fibre, propionate has the highest affinity for FFAR 2.^{28–29} Furthermore, propionate is an end product of bacterial metabolism, and thus, unlike acetate, does not undergo conversion to other SCFAs.³⁰ Intriguingly, Roux-en-Y gastric bypass, which results in weight loss and reduced adiposity, promotes increased levels of propionate in the colon.⁵ In keeping with these findings, a significant negative correlation between adiposity and caecal propionate concentrations has also been reported in germ-free mice receiving faecal transplants from human twin donors discordant for obesity.⁴

Increasing colonic propionate is therefore an attractive target for appetite modulation. However, orally administered SCFAs are unpalatable and are rapidly absorbed in the small intestine where L cells are sparse. Furthermore, supplementing diets with mixed high fibre does not predictably or reliably increase colonic production or circulating levels of propionate in all human populations because of the variability in gut microbial activity.³¹ To overcome the unpalatably high levels of fermentable dietary fibre needed to significantly increase colonic propionate levels, and the unpredictability in the production of the resulting SCFAs, we have developed a novel delivery system targeting the release of gram quantities of propionate in the proximal colon. We hypothesised that propionate would stimulate anorectic gut hormone release from the colon and that targeted delivery of propionate to the colon would decrease appetite and prevent long-term weight gain in humans.

METHODS

In vitro gut hormone secretion experiments

The effect of propionate on PYY and GLP-1 release from human colonic crypts was determined using a modified version of an established method³² (see online supplementary material).

Inulin-propionate ester for colonic delivery of propionate in humans

We developed a novel carrier molecule whereby propionate is chemically bound by an ester bond to inulin, a natural polymer composed mainly of fructose. This inulin-propionate ester was synthesised, as detailed in the online supplementary material. The majority of propionate chemically bound to inulin should only be released when the inulin polymer is fermented by the colonic microbiota, thus providing targeted colonic delivery. Isotope labelling studies were conducted to assess the stability of the molecule through the stomach and small intestine, and to provide information about site and extent of propionate release, as described in the online supplementary information. In addition, the effects of inulin-propionate ester on fermentation profiles and gut microbial populations were studied using an *in vitro* culture system (see online supplementary material).

Clinical studies

All subjects provided informed, written consent prior to the clinical trial which was approved by the Hammersmith and Queen Charlotte's Research Ethics Committee (08/H0707/99). All studies were carried out in accordance with the Declaration of Helsinki. All clinical trials were registered (Registration No: NCT00750438).

Investigation of acute supplementation with inulin-propionate ester on appetite regulation

In first-in-human studies, the acute effects of inulin-propionate ester on appetite regulation, hormone release and energy intake were studied in 20 volunteers. The primary outcome was energy intake, and gut hormone release was a secondary outcome. The effects on gastric emptying were examined in 14 volunteers in a separate study. Detailed inclusion and exclusion criteria and methodology for each acute study are described in the online supplementary material.

Proof-of-principle investigation of the effect of long-term supplementation with inulin-propionate ester on body weight maintenance

We hypothesised that daily intake of inulin-propionate ester over 24 weeks would decrease weight gain in overweight adults. The predefined coprimary outcomes were changes in body weight and food intake. A change in adipose tissue distribution was a secondary outcome. Men and women aged 40–65 years, with a body mass index (BMI) of 25–40 kg/m² were recruited. Potential participants were excluded if they met any of the following criteria: clinically significant illness (including type 1 or type 2 diabetes), medication known to affect appetite and/or body weight, a weight loss of 3 kg or greater in the preceding 2 months, smoking, substance abuse, psychiatric illness and any abnormalities detected on physical examination, electrocardiography or screening blood tests (measurement of complete blood count, electrolytes, fasting glucose, thyroid function and liver function). Women were ineligible if they were pregnant or breast feeding. From an initial 167 persons who responded to letters of invitation, 60 were randomly assigned to either the inulin-control or inulin-propionate ester supplementation group.

Study design

The study was conducted using a randomised, double-blind, placebo-controlled, parallel design. Two-day study visits were required at baseline (week 0) and after 24 weeks of dietary supplementation. On the day prior to each study visit, subjects were asked to consume a standard evening meal, to fast overnight from 22:00 and to avoid strenuous physical activity and alcohol. All study visits commenced between 08:00 and 09:00 and were conducted at the National Institute for Health Research (NIHR)/Wellcome Trust Imperial Clinical Research Facility. After all baseline measurements had been taken, subjects were randomly assigned to either the 10 g/day inulin-propionate ester group, or the 10 g/day inulin-control group. Subjects were randomised as described in the online supplementary material. The dietary supplement was supplied to subjects in ready-to-use sachets and they were instructed to mix the contents into their normal diet once a day during the 24-week supplementation period. All subjects were instructed to maintain their usual dietary and physical activity habits during the supplementation period. Self-reported food intake and physical activity were assessed at baseline and after 24 weeks of supplementation (see online supplementary material). Regular communication between subjects and study investigators encouraged good compliance. At week 8 and week 16 of the supplementation period, subjects attend follow-up visits to monitor compliance and adverse events. At week 24, measurements taken at baseline were repeated. Subjects returned all their used and unused sachets to estimate compliance.

Body weight and composition

Body weight was measured in all subjects to the nearest 0.1 kg (Tanita BC-418MA) while subjects were wearing light clothing. Body composition was assessed using MRI and MR spectroscopy (MRS), as previously described.³³ MRI and MRS data could not be collected in 20 subjects, due to metal implants (n=8), claustrophobia (n=9) or technical issues with the scanner (n=3).

Appetite regulation, gut hormone release and glucose homeostasis

A cannula was inserted into an antecubital vein and baseline blood samples collected at -10 min and 0 min to assess plasma concentrations of glucose, insulin, PYY and GLP-1. Following the 0 min sample, subjects were served a standardised breakfast (398 kcal; 71.2 g carbohydrate, 7.9 g fat, 10.3 g protein). At week 24, the breakfast also contained 10 g of inulin-propionate ester or 10 g inulin-control depending on supplementation group. Postprandial blood samples were taken at 15 min, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min and 300 min and collected into heparin-coated tubes containing 0.2 mL of aprotinin (Bayer, UK). GLP-1-like and PYY-like immunoreactivity were measured using established inhouse radioimmunoassay.^{34 35} Insulin and leptin were measured by radioimmunoassay using commercially available kits (Millipore, USA). Plasma glucose was measured using an Abbott Architect ci8200 analyser (Abbott Diagnostics, USA). At 300 min subjects were offered a buffet lunch with food served in excess, and asked to eat until they felt comfortably full. The amount of food was quantified and energy intake calculated. Subjective hunger, satiety and nausea were monitored with the use of 100 mm visual analogue scales (VAS).³⁶ Subjects were asked to complete the VAS before each blood sample.

Risk factors for cardiovascular disease and diabetes

A fasting blood sample was collected and analysed for levels of triglycerides, total cholesterol, low-density lipoprotein

cholesterol, high-density lipoprotein cholesterol, glycosylated haemoglobin (HbA1c), C reactive protein and liver function tests (alanine transaminase, alkaline phosphatase, aspartate transaminase). All analytes were measured by the Department of Chemical Pathology, Imperial College Healthcare National Health Service Trust. Blood pressure and pulse were also measured after subjects had been in a supine position for at least 15 min.

Statistical analysis

The treatment group size for the acute energy intake study was based on a power calculation, assuming a decrease of 15% in energy intake with a SD of 20% ($\alpha=0.05$, power=0.85), resulting in an estimated required sample size of 20 subjects. Data from the acute supplementation study suggested a sample size of 50 individuals (25 in each group) was needed for the long-term investigation. Sixty volunteers were therefore recruited to allow for an estimated attrition rate of 15%. χ^2 tests were performed to compare percentages of subjects in each group who gained $\geq 3\%$ and $\geq 5\%$ of their initial weight. For comparison of variables with a single measurement pre supplementation and post supplementation, we calculated the change from baseline at 24 weeks and compared means within groups using paired t-tests. The mean changes between groups for each of these variables were estimated using a multiple linear regression adjusted for its baseline measurement, baseline weight and randomised group. The linear model was also run on logit-transformed variables that were expressed as percentages to ensure their predictive values are within the 0–100% range.³⁷ Variables measured multiple (>2) times during the supplementation period (body weight, postprandial glucose, postprandial insulin response, PYY, GLP-1, VAS, side effects assessment) were analysed using multi-level mixed effects models to account for the variability within and between subjects. Area under the curve (AUC), unadjusted for covariates, for postprandial glucose and postprandial insulin response, was also calculated and compared between groups. In the case of data missing at random, the regression techniques described previously were applied after data were imputed using the multiple imputation by chained equations technique to account for random biases of the unobserved covariates. Data are presented as mean \pm SEM or 95% CI. p Values <0.05 were considered statistically significant.

RESULTS

Propionate stimulates PYY and GLP-1 release from human L cells *in vitro*

Propionate significantly stimulated PYY secretion from human colonic cells, with concentrations of 200 mmol/L and 400 mmol/L inducing 1.8-fold and threefold rises above basal secretion, respectively ($p<0.05$ and $p<0.001$; figure 1A). Propionate also increased GLP-1 secretion, with 200 mmol/L and 400 mmol/L inducing 1.6-fold and 2.4-fold increases in GLP-1 release, respectively ($p<0.001$; figure 1B).

Inulin-propionate ester delivers propionate to the colon

Propionate production from *in vitro* faecal fermentations was significantly higher from inulin-propionate ester compared with inulin, while no significant differences in acetate or butyrate production were observed (see online supplementary figure S1). Following ingestion of 10 g inulin-propionate ester, breath H_2 started to increase at 180 min and peaked at 240 min post ingestion. A small gradual release of ^{13}C was apparent between 0 min and 180 min post ingestion. More than 80% ($82.9 \pm 2.3\%$) of the ^{13}C recovered in breath over 24 h appeared coincident

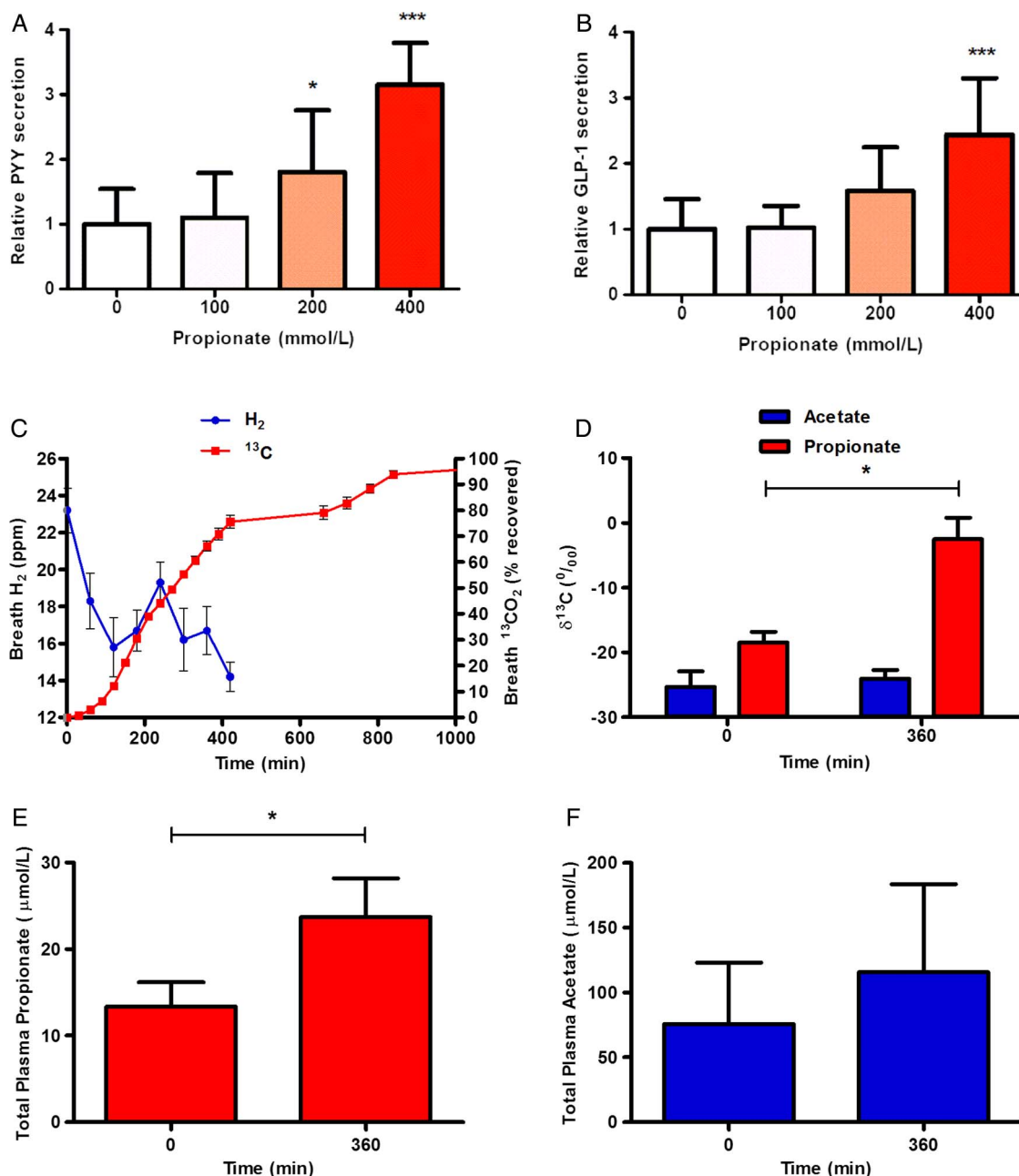


Figure 1 Propionate increases peptide YY (PYY) and glucagon like peptide-1 (GLP-1) release from primary human colonic cells and inulin-propionate ester supplementation delivers propionate to the colon in vivo. Cells isolated from human colonic tissue were incubated with increasing concentrations of propionate. (A) PYY and (B) GLP-1 levels were measured in the supernatants and lysed cells by radioimmunoassay. Percentage gut hormone release per well is expressed relative to the basal release measured ($n=4-6$). (C) The increase in breath H_2 at 240 min suggests that $>80\%$ of the labelled propionate entered the colon. (D) Plasma acetate and propionate ^{13}C enrichment ($\delta^{13}C$ per mil) at baseline and 360 min. Plasma propionate was significantly more enriched at 360 min whereas no difference was seen in acetate enrichment. Total plasma propionate (E) and acetate (F) concentrations ($\mu\text{mol/L}$) at baseline and 360 min. Data are presented as mean \pm SEM, * $p<0.05$, *** $p<0.001$.

with and after breath H_2 onset (figure 1C), suggesting delivery of the majority of the tracer to the colon. It is estimated that 10 g inulin-propionate ester delivered 2.36 g propionate to the colon after accounting for small intestinal losses (0.49 g). The isotopic data show that propionate released from the inulin-propionate ester appeared in the blood and was thus available systemically. Where circulating plasma propionate was detectable, significant increases in propionate ^{13}C enrichment and total concentration were measured in peripheral blood 360 min post ingestion compared with baseline (figure 1D, E). No

significant differences were observed in plasma acetate enrichment or total concentration (figure 1D, F). We estimate that the addition of 10 g inulin-propionate ester to the diet would lead to a 2.5-fold increase in daily colonic propionate production (see online supplementary material).

Acute supplementation with inulin-propionate ester increases PYY and GLP-1 secretion and reduces food intake

Acute supplementation with inulin-propionate ester significantly reduced food intake from 1175 kcal (95% CI 957 to

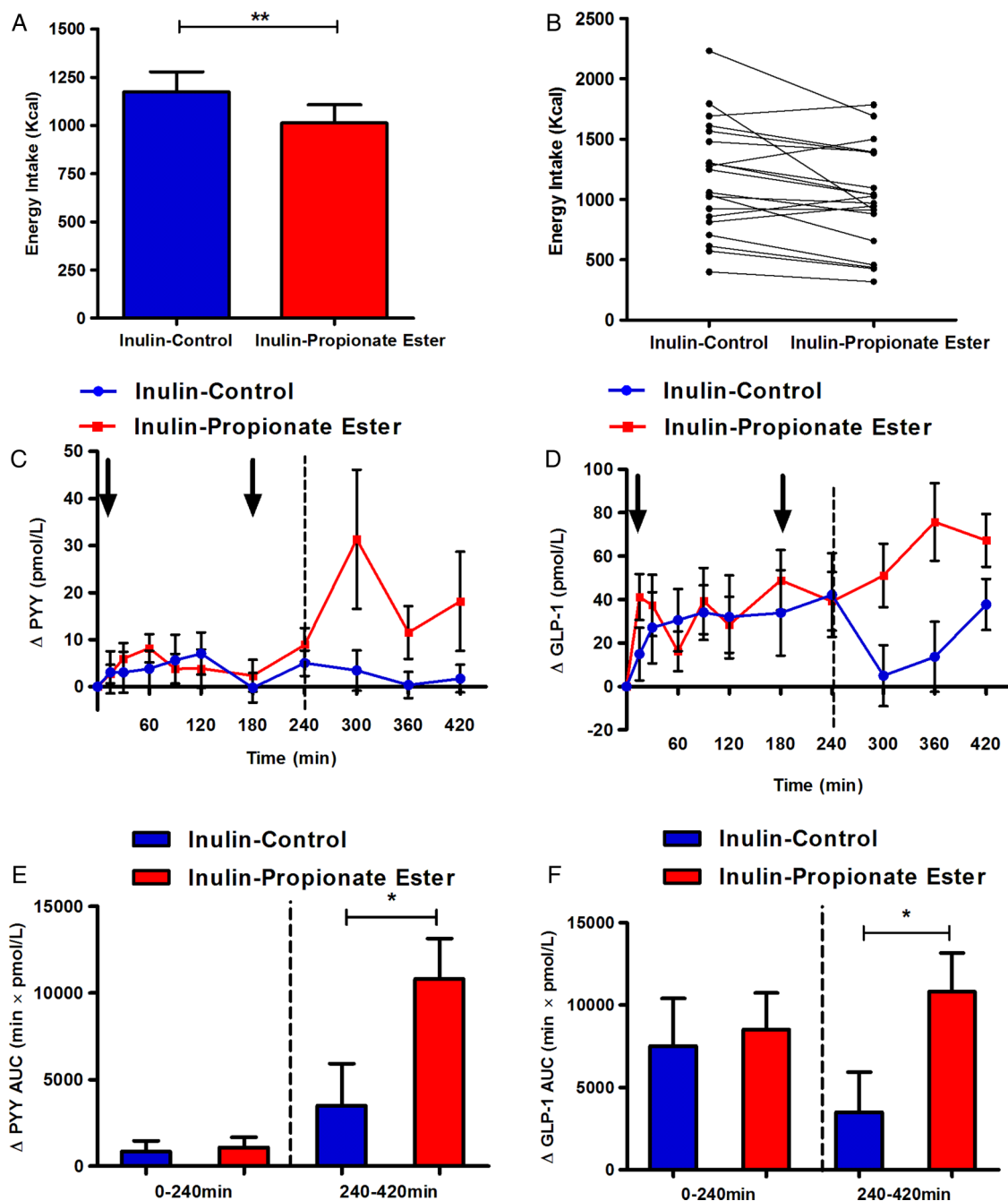


Figure 2 Acute inulin-propionate ester supplementation increases plasma peptide YY (PYY) and glucagon like peptide-1 (GLP-1) levels and reduces energy intake in humans. (A) The mean reduction in energy intake following inulin-control versus inulin-propionate ester. (B) A reduction in energy intake occurred in 16 of the 20 volunteers. (C–F) Plasma gut hormone levels following acute supplementation of inulin-control versus inulin-propionate ester. Arrows indicate standardised meals. Dotted lines signify the time point after which >80% inulin-propionate ester enters the colon as determined by the enrichment of ^{13}C in expired air and breath H_2 methodology (figure 1C). Data are presented as mean \pm SEM, * $p < 0.05$, ** $p < 0.01$. AUC, area under the curve.

1392) to 1013 kcal (95% CI 816 to 1210) (figure 2A, B; $p < 0.01$), a mean reduction of 13.8%. It takes an estimated 240 min for the inulin-propionate ester to enter the colon (figure 1C) and compared with inulin-control, inulin-propionate ester significantly increased plasma PYY ($\Delta\text{AUC}_{240-420 \text{ min}} 429 \text{ min} \times \text{pmol/L}$ (95% CI -543 to 1400) inulin-control vs 3349 min \times pmol/L (841 to 5857) inulin-propionate ester, $p < 0.05$) and GLP-1 levels ($\Delta\text{AUC}_{240-420 \text{ min}} 3495 \text{ min} \times \text{pmol/L}$ (95% CI -1567 to 8558) inulin-control vs 10801 min \times pmol/L (5897 to 15704) inulin-propionate ester,

$p < 0.05$) between 240 min and 420 min. Prior to 240 min there was no significant difference in the concentration of PYY and GLP-1 between the inulin-control and the inulin-propionate ester (figure 2D). Glucose, insulin and leptin levels and subjective ratings of appetite and nausea were not significantly different following acute inulin-propionate ester and inulin-control supplementation (see online supplementary figures S2 and S3). Acute supplementation with inulin-propionate ester did not influence the rate of gastric emptying (see online supplementary information).

Long-term supplementation with inulin-propionate ester prevents body weight gain, intra-abdominal adipose tissue accretion and reduces intrahepatocellular lipid content in those with non-alcoholic fatty liver disease

Of the 60 volunteers randomised, data were analysed from the 49 participants that completed the 24 week supplementation (table 1). Eleven participants (18%) did not complete the 24 week supplementation and there were no significant differences in attrition between the two groups (see online supplementary figure S4). Baseline and postsupplementation body composition data was collected from 17 participants in the inulin-propionate ester group and 15 participants in the inulin-control group. There was no difference between groups in compliance (95% (95% CI 92% to 98%) inulin-propionate ester vs 94% (95% CI 92% to 97%) inulin-control; $p=0.864$). Ratings of nausea were not different between supplementation groups ($p=0.736$), though there were significantly greater ratings of flatulence ($p=0.004$) in the inulin-control group during the supplementation period compared with the inulin-propionate ester group (see online supplementary table S1).

Body weight and composition

Inulin-propionate ester supplementation resulted in beneficial changes in body weight and composition. There was a significant difference in weight gain between groups. One of 25

participants gained $\geq 3\%$ of their baseline body weight following inulin-propionate ester supplementation (4%), as compared with 6 of 24 participants (25%) in the inulin-control group (figure 3A, $p=0.036$). Furthermore, none of the participants in the inulin-propionate ester group had substantial weight gain ($\geq 5\%$ baseline weight) compared with 4 of 24 (17%) following inulin-control supplementation (figure 3A; $p=0.033$). Although the primary aim of the study was to prevent weight gain, it is of interest to note that weight loss after 24 weeks was greater in the propionate ester group, though this effect was not significantly different between groups (0.38 kg (95% CI -0.95 to 1.72) inulin-control vs -1.02 kg (95% CI -2.10 to 0.04) propionate ester, $p=0.099$). Following the supplementation period, the change in the distribution of intra-abdominal adipose tissue, expressed as a percentage of total adipose tissue content, was significantly lower in the inulin-propionate ester group compared with inulin-control supplementation (table 2; $p=0.027$). Furthermore, internal adipose tissue ($p=0.002$) and the ratio of internal adipose tissue: subcutaneous adipose tissue was significantly increased within the inulin-control group ($p=0.002$), but not in the inulin-propionate ester group. There was no significant change in total adipose tissue content between groups. Within the inulin-propionate ester group there was a trend for reduced intrahepatocellular lipid (IHCL) content post supplementation ($p=0.061$). However, subjects meeting the baseline diagnostic criteria for non-alcoholic fatty liver disease (NAFLD)

Table 1 Baseline characteristics of subjects and changes in cardiovascular and diabetes risk factors following 24 weeks of inulin-control and inulin-propionate ester supplementation

Variable	Inulin-control (N=24)			Inulin-propionate ester (N=25)			Inulin-propionate Ester–inulin-control Difference (95% CI)	p Value
	Week 0	Week 24	Value	Week 0	Week 24	p Value		
Sex (% no. of subjects)								
Male	37.5 (9)			40.0 (10)				
Female	62.5 (15)			60.0 (15)				
Race or ethnicity (% no. of subjects)								
White	75.0 (18)			64.0 (16)				
Black	8.3 (2)			16.0 (4)				
Asian	17.7 (4)			20.0 (5)				
Age (years)	53.4 \pm 1.5			55.3 \pm 1.4				
Weight (kg)	91.0 \pm 2.8	91.4 \pm 3.0	0.559	88.5 \pm 2.9	87.5 \pm 3.0	0.062	-1.40 (-3.07 to 0.27)	0.099
Glucose (mmol/L)	5.1 \pm 0.1	5.0 \pm 0.1	0.624	5.0 \pm 0.1	5.0 \pm 0.1	0.898	0.05 (-0.21 to 0.32)	0.675
Insulin (μ U/mL)	11.5 \pm 1.3	10.6 \pm 0.7	0.329	9.0 \pm 1.0	8.8 \pm 0.8	0.702	-0.56 (-2.64 to 1.51)	0.582
HOMA-IR	2.7 \pm 0.3	2.3 \pm 0.2	0.372	2.0 \pm 0.2	2.0 \pm 0.2	0.644	0.14 (-0.63 to 0.34)	0.549
HbA1c (mmol/mol)	37.8 \pm 0.5	37.1 \pm 0.5	0.086	38.2 \pm 0.7	37.8 \pm 0.7	0.297	0.18 (-0.85 to 1.21)	0.729
Triglycerides (mmol/L)	1.5 \pm 0.2	1.6 \pm 0.2	0.283	1.5 \pm 0.2	1.4 \pm 0.2	0.176	-0.29 (-0.68 to 0.11)	0.148
Cholesterol (mmol/L)								
Total	5.3 \pm 0.2	5.0 \pm 0.2	0.014	5.5 \pm 0.2	5.1 \pm 0.2	<0.001	-0.10 (-0.39 to 0.19)	0.494
Low-density lipoprotein	3.3 \pm 0.2	3.1 \pm 0.2	0.132	3.5 \pm 0.2	3.2 \pm 0.2	<0.001	-0.08 (-0.34 to 0.18)	0.532
High-density lipoprotein	1.4 \pm 0.1	1.3 \pm 0.1	<0.001	1.4 \pm 0.1	1.3 \pm 0.1	0.009	0.02 (-0.06 to 0.10)	0.617
Liver function tests (IU/L)								
Alanine transaminase	32.4 \pm 3.9	25.9 \pm 3.3	0.001	29.5 \pm 3.2	23.7 \pm 2.2	0.015	-0.28 (-9.30 to 8.75)	0.949
Alkaline phosphatase	78.2 \pm 3.4	71.7 \pm 3.5	<0.001	74.6 \pm 3.7	70.5 \pm 3.3	<0.001	1.41 (-2.40 to 5.22)	0.458
Aspartate transaminase	29.8 \pm 1.6	28.7 \pm 3.0	0.543	28.9 \pm 1.3	26.2 \pm 1.0	0.007	-1.43 (-7.41 to 4.55)	0.627
Leptin (ng/mL)	31.9 \pm 4.0	33.3 \pm 4.5	0.690	25.1 \pm 3.0	24.1 \pm 2.4	0.625	-2.36 (-10.31 to 3.92)	0.551
C reactive protein (mg/L)	3.7 \pm 0.5	3.7 \pm 0.6	0.818	2.1 \pm 0.4	1.8 \pm 0.3	0.484	-0.27 (-1.39 to 0.84)	0.622
Blood pressure (mm Hg)								
Systolic	137 \pm 2	137 \pm 3	0.707	140 \pm 3	139 \pm 2	0.659	-0.83 (-4.98 to 3.81)	0.688
Diastolic	87 \pm 2	85 \pm 2	0.143	86 \pm 2	84 \pm 2	0.101	-0.86 (-4.92 to 3.19)	0.664
Pulse (bpm)	70 \pm 2	67 \pm 1	0.212	66 \pm 2	66 \pm 2	0.934	0.63 (-3.36 to 4.61)	0.763

Mean \pm SEM.

HbA1C, glycosylated haemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance.

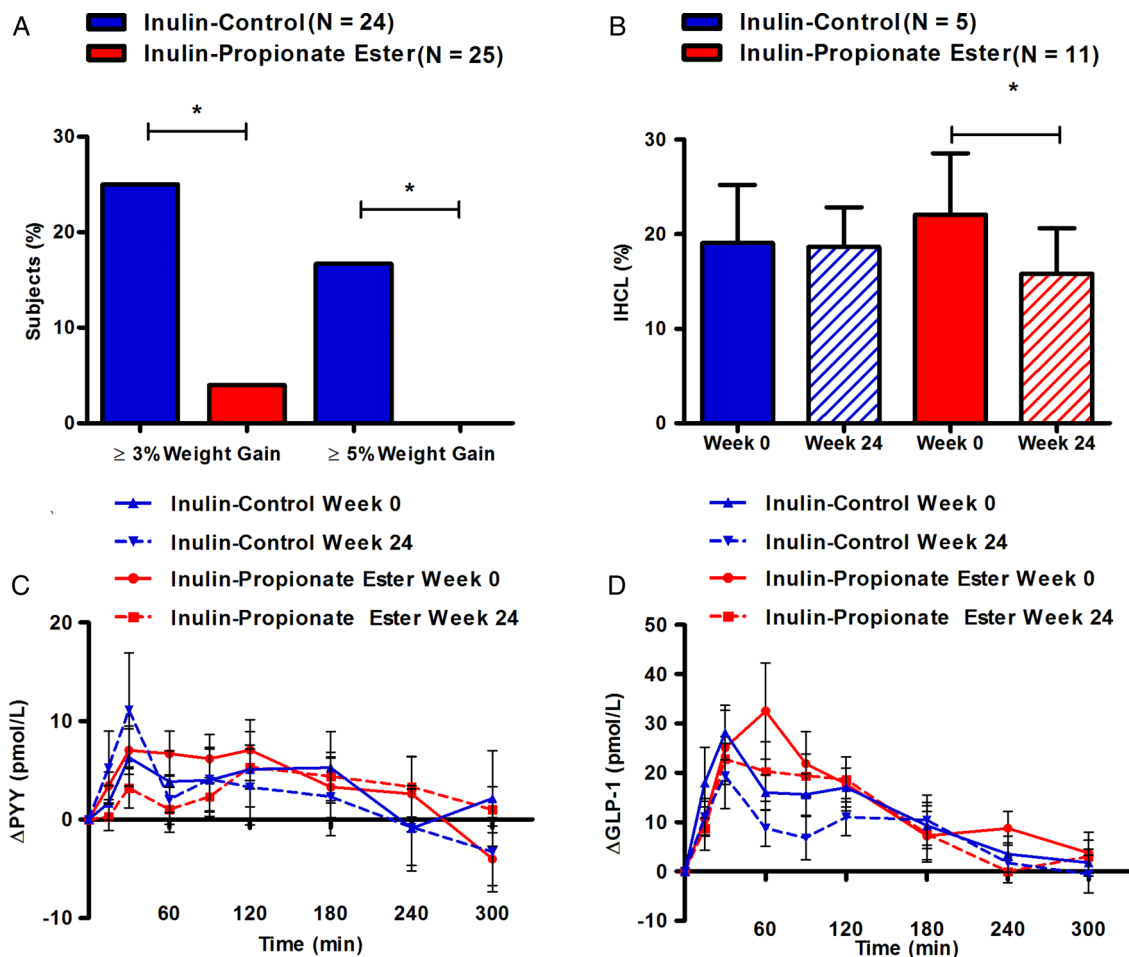


Figure 3 The effect of 24 weeks inulin-control and inulin-propionate ester supplementation on weight gain, liver fat content and gut hormone response. (A) The proportion of subjects who gained 3% or more and 5% or more of their baseline weight at 24 weeks. (B) Intrahepatocellular lipid (IHCL) content at baseline and following 24 weeks of inulin-control and inulin-propionate ester supplementation in subjects with non-alcoholic fatty liver disease (NAFLD). Subjects were identified as having NAFLD on the basis of an IHCL content >5.5% at baseline.³⁸ Postprandial plasma (C) peptide YY (PYY) and (D) GLP-1 release at baseline and following 24 weeks of inulin-control and inulin-propionate ester supplementation. Data are presented as mean±SEM, *p<0.05.

(IHCL >5.5%)³⁸ had a significant reduction in IHCL content following inulin-propionate ester supplementation (22.1% (95% CI 7.7 to 36.6) to 15.9% (95% CI 5.2 to 26.5), p=0.038, n=11; figure 3B). This effect was not observed in similar

subjects within the inulin-control group (19.1% (95% CI 2.0 to 36.1) to 18.7% (95% CI 7.1 to 30.3), p=0.576, n=5; figure 3B). *In vitro* analysis suggested that the protective effects of the inulin-propionate ester on weight gain and adipose tissue

Table 2 Body fat depots at baseline and following 24 weeks of inulin-control and inulin-propionate ester supplementation

AT distribution (% total AT)	Inulin-control (N=15)			Inulin-propionate ester (N=17)			Inulin-propionate ester–inulin-control Difference (95% CI)	p Value
	Week 0	Week 24	p Value	Week 0	Week 24	p Value		
Subcutaneous AT	81.3±1.9	80.6±1.8	0.002	76.3±1.7	76.0±1.6	0.624	−0.59 (1.69 to 0.50)	0.288
Internal AT	18.7±1.9	19.4±1.8	0.002	23.7±1.7	24.0±1.6	0.624	−0.23 (−1.09 to 0.64)	0.608
Intra-abdominal AT	10.6±1.3	11.1±1.4	<0.001	13.2±1.2	13.1±1.1	0.723	−0.46 (−0.87 to −0.05)	0.027
Abdominal subcutaneous AT	23.1±0.9	22.7±0.8	0.300	21.9±0.7	21.6±0.7	0.171	−0.23 (−0.88 to 0.42)	0.483
1H-MRS								
IHCL	7.8±2.9	7.4±2.5	0.708	15.8±5.0	11.5±3.7	0.061	−0.83 (−5.04 to 3.38)	0.699
Soleus IMCL	18.8±2.0	18.8±1.8	0.973	21.8±3.0	24.0±3.7	0.274	2.70 (−1.04 to 6.44)	0.157
Tibialis IMCL	10.0±1.5	10.3±1.1	0.869	9.1±1.0	9.5±0.9	0.701	−0.42 (−2.83 to 1.99)	0.733

Mean±SEM.

IHCL was measured relative to liver water content and IMCL was measured relative to total muscle creatine signal.³³

AT, adipose tissue; IHCL, intrahepatocellular lipid; IMCL, intramyocellular lipid; MRS, MR spectroscopy.

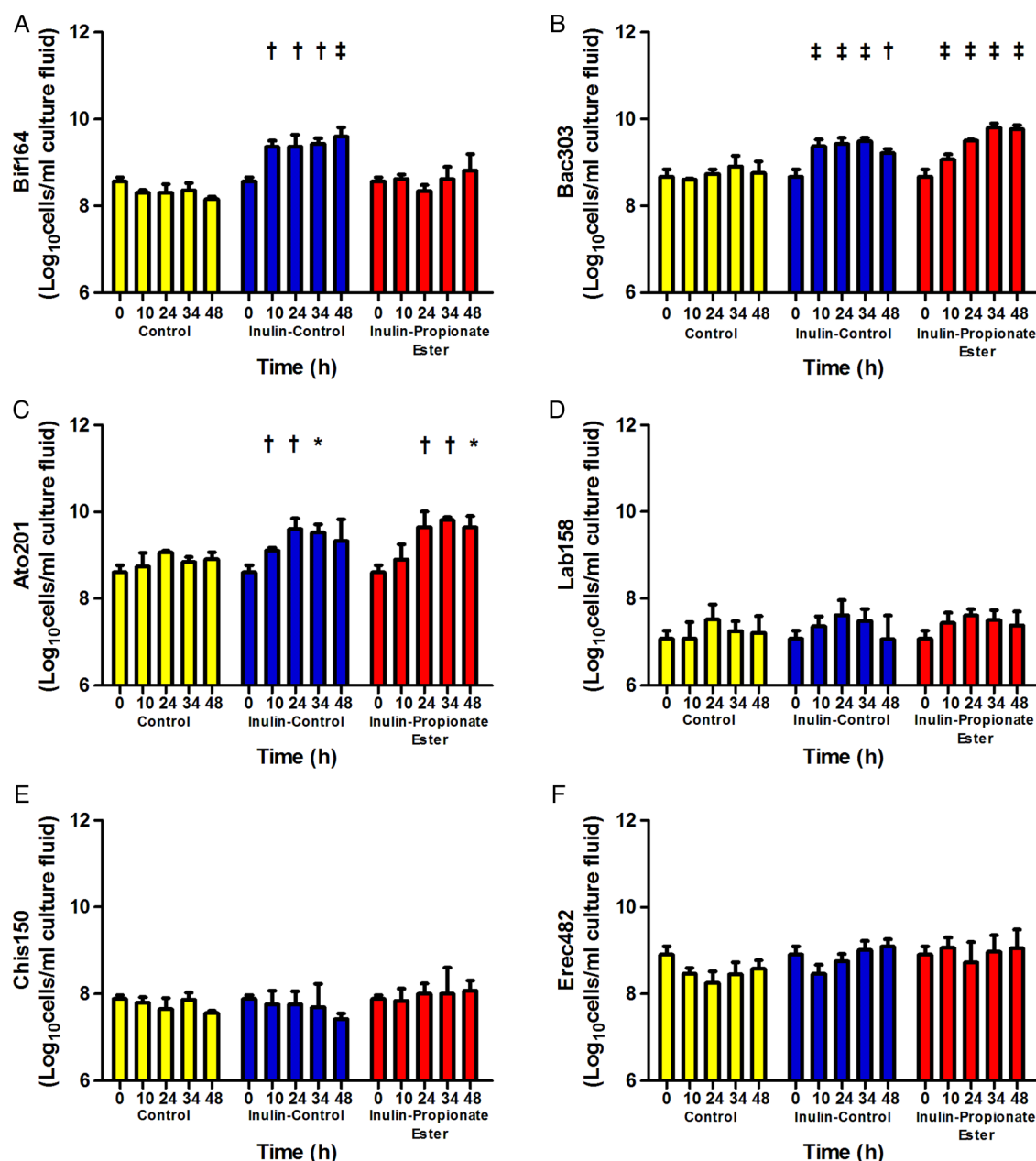


Figure 4 The effect of inulin-propionate ester on the gut microbiota. Bacterial concentrations expressed in Log₁₀ cells/mL culture fluid enumerated using fluorescent *in situ* hybridisation (FISH) targeting (A) *Bifidobacterium* spp (Bif164), (B) *Bacteroides/Prevotella* (Bac303), (C) *Atopobium* cluster (Ato291), (D) *Lactobacillus/Enterococcus* (Lab158), (E) *Clostridium histolyticum* (Chis150) and (F) *Eubacterium rectale/Clostridium coccoides* (Erec482) at 0 h, 10 h, 24 h, 34 h and 48 h anaerobic, pH controlled faecal batch culture fermentation with control (no substrate), inulin-control and inulin-propionate ester. Data are presented as mean±SEM (n=3), *<0.05, †<0.001, ‡<0.0001 with respect to the 0 h sample.

distribution were not due to changes in gut bacterial populations compared with inulin-control (see online supplementary material; figure 4A–F).

Food intake and gut hormone release following long-term supplementation with inulin-propionate ester

The change in food intake at an *ad libitum* meal following 24 weeks of supplementation was not statistically significant between groups ($p=0.972$), though long-term inulin-propionate ester intake showed a trend towards a lower food intake by 8.7% from 836 kcal (95% CI 724 to 948) to 763 kcal (95% CI 654 to 872) ($p=0.100$). Inulin-control supplementation reduced food intake from 678 kcal (95% CI 535 to 820) to

645 kcal (95% CI 514 to 776) ($p=0.197$), a mean reduction of 4.0%. Subjective ratings of appetite were significantly reduced within the inulin-propionate ester group following the supplementation period, while there were no differences in ratings of nausea (see online supplementary figure S5). Interestingly, there were no significant differences in postprandial PYY (difference -1.48%; 95% CI -6.29% to 3.33%; figure 3C; $p=0.546$) or GLP-1 secretion (difference 3.69%; 95% CI -4.48% to 12.33%; figure 3D, $p=0.361$).

Glucose homeostasis

Multilevel mixed effects models found no differences in postprandial glucose ($p=0.350$) or insulin response ($p=0.924$) between

supplementation groups. However, the change in postprandial glucose AUC to the standardised breakfast at week 24 was significantly different between groups (see online supplementary figure S6; $p=0.037$). Glycaemic response significantly deteriorated following inulin-control supplementation ($AUC_{0-300 \text{ min}}$ $1600 \text{ min} \times \text{mmol/L}$ (95% CI 1495 to 1705) to $1691 \text{ min} \times \text{mmol/L}$ (95% CI 1580 to 1802), $p=0.010$) but remained unchanged following inulin-propionate ester supplementation ($AUC_{0-300 \text{ min}}$ $1630 \text{ min} \times \text{mmol/L}$ (95% CI 1534 to 1727) to $1630 \text{ min} \times \text{mmol/L}$ (95% CI 1543 to 1717), $p=0.993$), in keeping with the differences in weight gain and body fat distribution observed within the groups. Insulin AUC was not significantly different following inulin-propionate ester ($AUC_{0-300 \text{ min}}$ $7685 \text{ min} \times \mu\text{U/mL}$ (95% CI 5858 to 9511) to $7969 \text{ min} \times \mu\text{U/mL}$ (95% CI 5955 to 9982), $p=0.612$) or inulin-control supplementation ($AUC_{0-300 \text{ min}}$ $9285 \text{ min} \times \mu\text{U/mL}$ (95% CI 7454 to 11 115) to $8781 \text{ min} \times \mu\text{U/mL}$ (95% CI 7265 to 10 297), $p=0.464$) and the change in insulin AUC was not significantly different between groups (see online supplementary figure S7; $p=0.372$), suggesting that the difference in glycaemic response reflects a difference in insulin sensitivity.

Risk factors for cardiovascular disease and diabetes

Inulin-propionate ester and inulin-control supplementation significantly reduced circulating levels of total cholesterol, high-density lipoprotein, alanine transaminase and alkaline phosphatase (table 1). Significant reductions in low-density lipoprotein ($p<0.001$) and aspartate transaminase ($p=0.007$) were only observed within the propionate ester group.

DISCUSSION

The GI tract is an important organ in the short-term control of appetite.^{1 24} The production of SCFAs by microbial fermentation of dietary fibre has been linked to positive physiological effects, including improvements in body weight, adiposity and glucose metabolism.³ Our data demonstrate that the SCFA propionate stimulates the release of the anorectic gut hormones PYY and GLP-1 from human colonic cells *in vitro*, supporting observations made in animal models.²¹ To increase colonic propionate production *in vivo*, we designed and synthesised a novel inulin-propionate ester, whereby propionate is conjugated by an ester linkage to the carrier molecule inulin. Stable isotope methodology revealed that $>80\%$ of the propionate load from the inulin-propionate ester is released in the colon coincident with or after a rise in breath H_2 . This would suggest that only a relatively small amount of the esterified propionate is released and absorbed in the small intestine. We have estimated that 10 g inulin-propionate ester ingestion leads to a 2.5-fold increase in daily colonic propionate production, a level very difficult to achieve through feeding a mixed fermentable fibre diet.²⁰ We also demonstrated that ingestion of the inulin-propionate ester increases plasma propionate levels.

We subsequently, in the first-in-human studies, demonstrated that increased delivery of propionate to the colon acutely modulates gut hormone release and reduces food intake in healthy subjects. The inulin-propionate ester did not suppress subjective appetite responses, but significantly reduced meal size, consistent with the action of a physiological satiation signal. We observed a significantly greater postprandial release of PYY and GLP-1 when a mixed calorie breakfast contained 10 g inulin-propionate ester compared with 10 g inulin-control. It has been previously shown that a sustained increase in circulating PYY and GLP-1 can influence appetite-regulating circuits of the brain and inhibit food intake.^{25 27} In this study, the rise in PYY and

GLP-1 was apparent between 240 min and 420 min following oral administration of the inulin-propionate ester, and reached levels similar to those observed following a 1000 kcal meal.³⁹ Such a rise did not occur following ingestion of the inulin-control, suggesting that it is a specific effect of the inulin-propionate ester rather than the standardised 356 kcal lunch provided in both trials. This would suggest that compared with the inulin-propionate ester, a 10 g dose of inulin-control does not raise colonic SCFA to a sufficient concentration to stimulate gut hormone release.⁴⁰ Recent evidence suggests the colonic microbiota adapt rapidly to a change in substrate availability.⁴¹ Data from batch culture experiments demonstrated that inulin-control and inulin-propionate ester stimulate changes to the gut microbiota, although only inulin-control had a selective effect on *Bifidobacterium*. This would suggest that the observed short-term effects on appetite regulation were independent of alterations to gut microbial composition.

Longitudinal studies demonstrate that adults gain weight gradually through middle age, with an average yearly weight gain of 0.3–0.8 kg.^{6–9} This accumulation of weight would result from a small daily positive energy balance of around 50–100 kcal.¹⁰ The 14% (162 kcal) reduction in food intake observed following acute administration of 10 g/day inulin-propionate ester would have a significant impact on weight gain and health if sustained over the long term.⁴² We therefore investigated if long-term elevation of colonic propionate would prevent weight gain by conducting a 24 week randomised controlled trial of inulin-propionate ester supplementation in overweight middle-aged adults. We demonstrated lower weight gain in the inulin-propionate ester group, with significantly fewer volunteers gaining $\geq 3\%$ or $\geq 5\%$ body weight. This was coupled with a reduced gain in intra-abdominal adipose tissue compared with the inulin-control group and prevention of the deterioration of postprandial glucose response. Furthermore, long-term elevations in colonic propionate production reduced IHCL content in subjects meeting the diagnostic criteria for NAFLD. A reduction in IHCL is a reproducible finding in rodents fed a high level of fermentable dietary fibre,^{16 43} however the mechanism behind this is not well understood. Intra-abdominal adipose tissue and NAFLD are regarded as major risk factors in the development of insulin resistance and type 2 diabetes.⁴⁴

Interestingly, in this long-term study we were unable to detect any change in PYY or GLP-1 release following ingestion of the inulin-propionate ester compared with inulin-control, in contrast to our acute administration studies. This suggests that there may be a desensitisation of the FFAR2/3 receptor response over time and that the beneficial effects of long-term inulin-propionate ester supplementation may not be mediated by PYY and GLP-1. However, subjective ratings of postprandial appetite were significantly reduced within the inulin-propionate ester group and we observed a trend towards a significant decrease in food intake of 8.7% (73 kcal), suggesting that propionate may influence appetite and energy intake via mechanisms unrelated to PYY or GLP-1 release. It has been demonstrated that propionate can stimulate leptin release through activation of FFAR2 on adipocytes,⁴⁵ although we did not observe any changes in circulating leptin concentrations following acute or long-term supplementation with inulin-propionate ester. Recent reports suggest that propionate could also have a positive effect on energy balance and body weight independent of energy intake. An investigation observed weight loss in germ-free mice transplanted with microbiota from animals which had undergone gastric bypass surgery. The reduced body weight was associated with increased microbial production of propionate, but no differences in energy intake

were observed.⁵ In addition, when the intestines of germ-free mice are transplanted with microbiota from an obese or lean human twin it was found that animals receiving the transplant from the lean twin donor developed decreased lower body mass and adiposity level compared with those receiving the transplant from the obese twin, despite comparable energy intake.⁴ The inhibition of adipose tissue accumulation observed in the lean twin transplanted mice was associated with greater amounts of propionate produced by the gut microbiota. The outcome of these investigations could be attributed to the observation that propionate promotes sympathetic activity via FFAR3, resulting in elevated energy expenditure.⁴⁶ Furthermore, SCFA activation of FFAR2 has been shown to reduce the sensitivity of murine adipocytes to insulin, leading to reduced lipid clearance by adipocytes and to increased energy expenditure, with preferential oxidation of lipid.⁴⁷ Propionate has also been shown to stimulate a gut-brain circuit via FFAR3 in the portal vein wall, leading to the induction of intestinal gluconeogenesis (IGN) gene expression.⁴⁸ The authors suggest that the glucose released by IGN is detected by a portal vein glucose sensor that transmits its signal to the brain by the peripheral nervous system to promote beneficial effects on energy homeostasis. Using a rodent-model it was found that upregulation of IGN by propionate reduced body weight gain and adiposity independent of food intake. These reports indicate that propionate can contribute to energy homeostasis through effects on numerous cellular metabolic pathways and receptor-mediated mechanisms and provide a potential explanation for the differences in body weight gain and adiposity observed between supplementation groups in the long-term study. Additional investigations are therefore warranted to clarify the effects of long-term supplementation with the inulin-propionate ester on energy expenditure and the metabolic and neural pathways that regulate substrate oxidation. Given that acutely elevating colonic propionate increases plasma PYY and GLP-1 levels and inhibits energy intake in healthy subjects, and that this effect on gut hormone release appears to be lost following long-term supplementation while a reduction in body weight gain is maintained, the short-term and long-term effects of colonic propionate may have divergent underlying biological mechanisms.

A possible limitation of our study design would be the choice of inulin as a control for the inulin-propionate ester. Inulin was used as a control to specifically account for any effects that may derive from colonic fermentation of inulin itself, rather than the release of the esterified propionate. As our *in vitro* faecal fermentation data demonstrates, the levels of propionate produced by the inulin-control are relatively small compared with those produced by the inulin-propionate ester, but the production of acetate and butyrate are comparable. Previous studies have shown that much larger doses of inulin-type fructans (>30 g/day) are required to modulate gut hormone release and appetite regulation than used as a control in the present design.^{40–49} It is therefore unlikely that the 10 g/day dose of inulin used as a control is masking any effects of the inulin-propionate ester on our primary and secondary outcome measures. Nevertheless, the SCFA production from 10 g/day inulin may be sufficient to explain some of the significant long-term changes observed within the inulin-control group, particularly the reductions in fasting cholesterol.⁵⁰

In summary, these studies provide the first direct evidence that colonic propionate can acutely reduce energy intake and prevent long-term weight gain in humans. The present results support a role specifically for colonic propionate in weight management and may provide a molecular explanation of recent

data that have observed changes in the gut microbiome and associated SCFA production profiles in weight loss. In humans, the beneficial actions of propionate appear to be mediated by different mechanisms in the short term compared with the long term, which warrants further study. Optimum delivery of propionate to the colon through selection of propiogenic components of the diet may represent a novel route to improve weight management at the population level.

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Contributors All authors contributed to the design of the study. ESC, AV, AP and SEKZ-V conducted the human studies. DJM, CT and TP developed and produced the inulin-propionate ester. All authors contributed to writing the manuscript.

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Boosting chemical by-product of dietary fibre fermentation in gut slims and trims

Propionate-friendly fibre intake may offer new weight management option, say researchers

[Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults Online First: 11 Dec 2014. doi 10.1136/gutjnl-2014-307913]

Boosting levels of a naturally produced by-product of dietary fibre fermentation in the bowel can help trim the waistline and stave off weight gain, reveals a small study published online in the journal **Gut**.

This approach may offer a new weight management option, suggest the researchers.

Animal studies have shown that the natural fermentation of dietary fibre by gut bacteria produces short chain fatty acids, one of which is propionate.

These fatty acids stimulate the release of the gut hormones PYY and GLP-1, which in turn suppress appetite. And propionate seems to be the most effective at stimulating PYY and GLP-1 release.

To find out if increasing levels of propionate in the bowel could reduce food intake and stave off weight gain in people, the researchers developed a propionate supplement primed to target propionate release in the bowel.

First, 20 volunteers were given either the propionate supplement or just inulin, a predominantly fructose-containing plant fibre, and allowed to eat as much as they liked from a buffet. When given the propionate supplement, participants ate 14% less, on average, and had higher levels of PYY and GLP-1 in their blood.

Next, 60 overweight adults between the ages of 40 and 65 received either a daily 10 g dose of the propionate supplement or 10 g of inulin alone over a period of 24 weeks. They were asked to follow their normal dietary and exercise routines throughout.

Body weight was assessed at the beginning and end of the study period, as was the distribution of fat around the body.

A fasting blood sample was also taken to check on risk factors for cardiovascular disease and diabetes, including blood fats, liver enzymes, and markers of inflammation.

Forty nine of the original 60 participants completed the trial.

Among the 25 people taking the propionate supplement, just one put on more than 3% of their baseline weight, compared with six of the 24 treated with inulin alone.

Furthermore, the propionate supplement altered the distribution of body fat, significantly trimming abdominal fat tissue compared with inulin alone, and lowering the total amount of fat in the liver.

Both the propionate supplement and inulin cut the risk factors for cardiovascular disease and diabetes, although only the propionate supplement significantly reduced 'bad' low density cholesterol and the enzyme aspartate transaminase, high levels of which are associated with tissue damage, particularly of the heart and liver.

The evidence suggests that adults gain around half to 1 kilo in weight every year throughout middle age by just consuming 50-100 extra calories a day. And the findings provide the first direct evidence that raising propionate levels in the bowel can cut energy intake and stave off longer term weight gain, say the researchers.

“The present results support a role specifically for colonic propionate in weight management and may provide a molecular explanation of recent data that have observed changes in the gut [range of bacteria] and associated [short chain fatty acid production] profiles in weight loss,” they conclude.

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SUPPLEMENTARY MATERIAL

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SUPPLEMENTARY METHODS

Isolation of human colonic cells

Colonic biopsies were obtained from patients undergoing diagnostic colonoscopy at Hammersmith Hospital. Biopsies were usually obtained from the right side of the colon. All subjects provided informed, written consent prior to the study, approval for which was granted by the Hammersmith and Queen Charlotte's Research Ethics Committee (Registration No: 2000/5795).

The colonic tissue was prepared as described previously^{1 2}. Briefly, the tissue was digested with 0.4mg/ml collagenase XI (Sigma, UK) in Dulbecco's Modified Eagle Medium (DMEM) at 37°C. The resulting cell suspensions were centrifuged for 5 minutes at $500 \times g$ and the pellets re-suspended in DMEM (supplemented with 10% foetal calf serum (FCS) and 1% penicillin/streptomycin). The digestion process was repeated three times and the combined cell suspensions plated onto 24-well, 1% Matrigel-coated plates and incubated at 37°C in an atmosphere of 5% CO₂.

In vitro gut hormone secretion experiments

Secretion experiments were carried out 18-24 hours after the colonic cells were plated. Cells were washed twice with secretion buffer (4.5mM KCl, 138mM NaCl, 4.2mM NaHCO₃, 1.2mM NaH₂PO₄, 2.6mM CaCl₂, 1.2mM MgCl₂ and 10mM HEPES, adjusted to pH 7.4 with NaOH) containing 0.1% bovine serum albumin (BSA)¹ and incubated with sodium propionate (Sigma, UK) for 2 hours at 37°C in an atmosphere of 5% CO₂. Concentrations of 200 and 400 mmol/L were used as estimates suggest that the concentration of SCFA in the human colon is approximately 150 mmol/L and modelling data from *in vitro* systems demonstrate that these levels will more than double with a high fermentable carbohydrate diet^{3 4}. In addition, these concentrations are in line with those previously shown to stimulate PYY release from the rodent colon (500 mmol/L)⁵. Following incubation, cell supernatants were centrifuged and the plates treated with lysis buffer and freeze-thawed. Percentage gut hormone release was calculated for each well and normalised to basal secretion¹. Cell health was confirmed using a CytoScanTM lactate dehydrogenase assay (G-Biosciences, USA). PYY and GLP-1 data was obtained from separate cell cultures.

Production of inulin-propionate ester

The inulin-propionate ester was synthesized by reacting inulin with propionic anhydride (0.8 L/Kg inulin) in water whilst maintaining pH between 8 - 8.5 and the reaction temperature $< 20^{\circ}\text{C}$. Excess unreacted propionate was removed by filtration twice through activated carbon columns whilst the mixture was maintained at pH 2. Finally, the product was recovered as a fine amorphous crystalline product through spray drying. A ^{13}C stable isotope labelled variant of the propionate ester was synthesised in an identical fashion to produce ^{13}C -propionate ester with (1- $^{13}\text{C}_1$)-propionate bound to inulin.

To determine the amount of free propionate in the final product, 100 mg of ester product was dissolved in 2 ml water containing 10 mM butyric acid as the internal standard (IS). To quantify free propionate, 200 μl of the solution was treated with 100 μl of concentrated orthophosphoric acid followed immediately by ether extraction (1 ml). To quantify total propionate (free + bound), another 200 μl of the solution was treated with 100 μl of concentrated orthophosphoric acid and heated at 80°C for 1 hour before being extracted with 1 ml ether. Propionate and butyrate in the ether extracts were quantified by gas chromatography with flame ionisation detection ⁶. Propionate yield was calculated relative to the IS and the amount of free propionate calculated by the ratio $(\text{free}/\text{total}) \times 100 (\%)$. The degree of esterification (d_e) was also calculated using this analysis by using the yield of bound propionate ($\text{total} - \text{free}$) per gram of ester to compute moles of propionate yielded per mole of propionate ester.

An inulin-propionate ester was produced with a degree of esterification of 0.74 ± 0.02 , meaning on average that $24.6 \pm 0.67\%$ (0.74 out of maximum of 3 per monosaccharide unit) of all hydroxyl groups were replaced by an ester group. The level of free propionate was $2.57 \pm 0.26 \%$ of the total propionate available from the molecule, demonstrating that more than 97% of propionate was chemically bound to the inulin polymer. Less than 1% of the bound propionate is released when the inulin-propionate ester is solubilised in acid (pH 1-2), a similar low pH to the environment of the stomach. Heating to $>80^{\circ}\text{C}$ is required in order to release the propionate from the ester in this preparation.

***In vitro* fermentation profiles of inulin-propionate ester and inulin-control**

Faecal samples were collected from three healthy volunteers (who had no history of gastrointestinal complaints and were antibiotic free for >6 months prior to faecal collection) and prepared separately in triplicate for each substrate. Faecal fermentation systems consisted of the fermentation medium (2.25g tryptone in 450 ml of distilled water), 112.5 µl of micromineral solution (13.2 g of CaCl_2 , 10.0 g of MnCl_2 , 1.0 g CoCl_2 , FeCl_3 made up to 100ml with distilled water), 225 ml of macromineral solution (2.85 g Na_2HPO_4 , 3.1 g KH_2PO_4 , 0.3 g MgSO_4 made up to 500 ml with distilled water), 225 ml of buffer solution (2 g NH_4HCO_3 , 17.5 g NaHCO_3 made up to 500 ml with distilled water) and 1 ml of 0.1% (w/v) resazurin solution (a redox indicator). This medium was adjusted to pH 7 using 6M HCl, after which it was sterilized by boiling for 5 min. Reducing solution prepared on the day of fermentation (312.5 mg cysteine hydrochloride, 2 ml 1M NaOH, 312.5 mg sodium sulfide, and 47.5 ml distilled water) was added at 0.5 ml per 10 ml of medium after the solution was cooled to 37°C under oxygen-free nitrogen (OFN) until anaerobic conditions were achieved as indicated by a colour change from pale indigo to colourless. A 32% faecal slurry was prepared for each subject in 66.6 mM phosphate buffer (pH=7), homogenized in a household blender for 2 min and strained through a nylon stocking. The final *in vitro* system containing 0.5 ml of the slurry was added to 4.2 ml of the pre-reduced fermentation medium in 10 ml autoclaved fermentation bottles. Each batch culture consisted of 4.9 ml of the above slurry mixture to which 100 mg of inulin-propionate ester or inulin-control was added. At 0 and 24 h, 800 µL of the aqueous volume was removed from each vial. For SCFA extraction, 100 µL of internal standard (IS; 2-ethylbutyrate, 73.8 mM) and 25 µL concentrated orthophosphoric acid was added to 225 µL of sample and thoroughly mixed and extracted with 3 x 1 mL of ether. The ether aliquots were pooled and a sub-sample transferred to a clean vial for analysis. Samples were analysed by GC-FID (Trace GC, ThermoFisher, UK) using a ZB-WAX column (15 m × 0.53 mm × 0.25 µm; Phenomenex, Cheshire UK). The GC operating parameters were nitrogen carrier gas (1.89 ml/min) and GC oven parameters starting at an initial temp of 80°C, ramp temp (10°C /min) to 210°C with splitless injection. The concentration of acetate, propionate and butyrate calculated using the area ratio to the IS and the calibrated response factor of each SCFA to the IS as determined by a gravimetrically prepared external standard. The concentration (production) of SCFAs and molar ratios were calculated.

Acute intervention with inulin-propionate ester

Nine healthy subjects (8 males and 1 female) were recruited for the colonic delivery investigation. The mean (\pm SEM) age, weight and body mass index (BMI) were 32 ± 4 years, 75.0 ± 4.0 kg and 25.0 ± 1.3 kg/m², respectively. Twenty healthy subjects (15 males and 5 females) were recruited for the acute food intake investigation. The mean (\pm SEM) age, weight and BMI were 31 ± 2 years, 75.0 ± 3.0 kg and 25.4 ± 0.8 kg/m², respectively. Fourteen healthy subjects (8 males and 6 females) were recruited for the gastric emptying investigation. The mean (\pm SEM) age, weight and BMI were 32 ± 4 years, 69.4 ± 3.5 kg and 24.0 ± 0.9 kg/m², respectively. The inclusion criteria for these investigations were a BMI of 20 to 35 kg/m² and 21 to 65 years of age. The exclusion criteria were smoking, substance abuse, pregnancy, use of medications (except for oral contraceptives), a change in body weight >3 kg in the previous 2 months, medical or psychiatric illness, and any abnormalities detected on physical examination, electrocardiography, or screening blood tests (measurement of complete blood count, electrolytes, fasting glucose, thyroid function and liver function).

Colonic delivery investigation

This investigation compared the appearance of ¹³C in breath CO₂ with the appearance of breath H₂, a methodology previously used to investigate gut transit times ⁷. Subjects arrived at Hammersmith Hospital at 08:30 and were served a standardized breakfast (533 kcal; 78 g carbohydrate (CHO), 17 g fat, 18 g protein) containing 10g of propionate ester and 100mg ¹³C labelled inulin propionate ester (containing ~30 mg of bound (1-¹³C₁)-propionate). Breath H₂ was collected and measured in real-time using a handheld H₂ monitor (Bedfont Scientific Ltd, Kent UK). Breath CO₂ was collected serially over 24 h (excluding the sleep period) by exhaling alveolar breath through a straw into Exetainers (Labco, Buckinghamshire, UK). ¹³CO₂ enrichment was determined by isotope ratio mass spectrometry (IRMS). Breath H₂ was expressed as parts per million (ppm) and ¹³CO₂ as ppm xs, defined as ppm ¹³C enrichment above baseline samples collected before isotope ingestion. Cumulative ¹³CO₂ excretion was also calculated. Plasma was collected at -15, 0 and 360 min, and urine over 24 h for analysis of ¹³C propionate enrichment by GC-combustion-IRMS (GC-C-IRMS) ⁶. Acetate ¹³C enrichment was also measured in the same analysis as a control for dietary influence on SCFA ¹³C enrichment and for evidence of inter-conversion between propionate and acetate. Although our experimental methodology was not designed to explore gluconeogenesis, ¹³C enrichment was measured in plasma glucose and plasma alanine by LC-IRMS ⁸ and GC-C-IRMS ⁹, respectively. Enrichment in plasma ¹³C glucose would occur through gluconeogenesis from propionate directly but alanine would indicate the enrichment in the triose pool, with which it

comes rapidly into isotopic equilibrium ¹⁰. Data were expressed as $\delta^{13}\text{C}$ (per mil or ‰) which represents the change in the measured ratio in parts per thousand from the internationally accepted standard carbon Vienna Pee Dee Belemnite (VPDB). Isotopic enrichment was expressed as $\delta^{13}\text{C}$ (‰). SCFA concentrations ($\mu\text{mol/L}$) were measured relative to an internal standard (3-methyl valerate).

Energy intake and gut hormone investigation

The study was performed in a randomized, double-blind, crossover manner, with each subject studied on two occasions > 7 days apart. Subjects refrained from alcohol and strenuous exercise for the 24 hours prior to each study day and consumed an identical meal between 19:00 and 20:00 the evening before. Subjects then fasted overnight and arrived at Hammersmith Hospital at 08:30 on each study day. A cannula was inserted into a forearm vein and baseline blood samples were collected at -10 and 0 min. Following the 0 min sample, subjects were served a standardized breakfast (398 kcal; 71 g CHO, 8 g fat, 10 g protein) containing either 10 g of inulin-propionate ester, or 10 g inulin control. At 180 min a standardized lunch (356 kcal; 34 g CHO, 12 g fat, 28 g protein) was provided, and at 420 min subjects were offered a buffet dinner with food served in excess and asked to eat until they felt comfortably full. The amount of food was quantified and energy intake calculated. Postprandial blood samples were taken at 15, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min and collected into heparin-coated tubes containing 0.2 ml of aprotinin (Bayer, UK). Plasma was separated immediately by centrifugation at 4°C and then stored at -70°C until analyzed. Subjective hunger, satiety, and nausea were monitored with the use of 100 mm visual analog scales (VAS) ¹¹. Subjects were asked to complete the VAS before each blood sample.

Gastric emptying investigation

The study was performed in a randomized, double-blind, crossover manner, with each subject studied on two occasions > 7 days apart. Subjects refrained from alcohol and strenuous exercise for the 24 hours prior to each study day and asked to consume a standard meal between 19:00 and 20:00 the evening before. On the study days, subjects attended having fasted overnight. Following baseline blood samples, subjects were given 10 g inulin-propionate ester or 10 g inulin control in a standardized breakfast (641 kcal; 113 g CHO, 15 g fat, 16 g protein). At 300 min volunteers were served a standard lunch (354 kcal; 47 g CHO, 12 g fat, 12 g protein) together with 100 mg ¹³C-octanoic acid ¹². Breath CO₂ was collected serially for 480 min after the standard lunch by exhaling alveolar breath through a straw into Exetainers (Labco, Buckinghamshire, UK). ¹³CO₂ enrichment

was determined by isotope ratio mass spectrometry (IRMS). The time to 50% AUC excretion of ^{13}C in breath ($T_{1/2}$) was calculated as a proxy for gastric emptying rate.

Long-term supplementation with inulin-propionate ester

Randomisation

Four strata were defined according to sex (male, female) and BMI ($<30.0 \text{ kg/m}^2$, $\geq 30.0 \text{ kg/m}^2$) and randomisation sequences for each stratum were generated using the random number generator using Stata (version 11.0). The two supplementation groups were labelled 'A' and 'B' by DJM who held the key to the allocations for the duration of the trial. Participants were randomised to either 'A' or 'B' by the trial statistician (SMI) according to the randomisation sequence and assigned a randomisation number. Throughout the trial, none of the participants or investigators involved in the trial had access to complete information on the randomisation allocations.

Self-reported assessment of energy intake and physical activity

Participants were asked to record energy intake by 3-day food diary at baseline and during the last week of the 24 week supplementation period. Food diaries were analysed using Dietplan6 (Forestfield Software, West Sussex, UK). Physical activity was measured at baseline and at week 24 using the short self-administered format of the International Physical Activity Questionnaire (IPAQ)¹³. IPAQ measures the frequency and duration of any walking and other moderate-to-vigorous intensity physical activity undertaken for more than 10-continuous minutes across all contexts (e.g. work, home and leisure) over a 7-day period. IPAQ calculates metabolic equivalents (MET-h/week), derived by assigning standardised MET values for walking, moderate-intensity and vigorous-intensity of 3.3, 4.0 and 8.0, respectively.

Fluorescent in situ hybridisation to assess gut microbial changes in response to inulin-propionate ester and control

The effects of inulin-propionate ester on gut microbial populations were studied using an *in vitro* culture system. Faecal samples were obtained from three healthy human volunteers (two males; age 30 – 50 years; BMI 25-31 kg/m^2). Volunteers were excluded if they suffered from any gastrointestinal disorder (e.g. ulcerative colitis, Crohn's disease, irritable bowel syndrome, peptic

ulcers and cancer) and/or had taken antibiotics in the six months preceding sample donation. Samples were kept under anaerobic conditions (10% H₂, 10% CO₂ and 80% N₂) and used within a maximum of 30 min after voiding. Faecal samples were diluted 1/10 w/w in anaerobic phosphate buffered saline (0.1 mol/l phosphate buffer solution, pH 7.4) and homogenised in a stomacher (Stomacher 400, Seward, West Sussex, UK) for 2 min at normal speed.

Batch culture fermentations

Sterile anaerobic batch culture fermenters (150ml working volume) were set up in parallel and aseptically filled with 135ml pre-reduced, sterile basal culture medium (peptone water 2 g/l (Oxoid), yeast extract 2 g/l (Oxoid, Basingstoke, UK), NaCl 0.1 g/l, K₂HPO₄ 0.04 g/l, KH₂PO₄ 0.04 g/l, MgSO₄·7H₂O 0.01 g/l, CaCl₂·6H₂O 0.01, NaHCO₃ 2 g/l, Tween 80 2 ml (BDH, Poole, UK), haemin 0.05 g/l, vitamin K₁ 10 µl, cysteine.HCl 0.5 g/l, bile salts 0.5 g/l). The fermenters were gassed overnight with O₂ free N₂ at a rate of 15mL/min. Inulin-propionate ester and inulin control substrates (1/10 w/v) were added to their respective fermenters just prior to the addition of the faecal slurry. A substrate-free vessel was set up for each volunteer as negative controls. Cultures were continuously stirred and kept at 37°C by means of a circulating water bath. Culture pH was kept between 9.7 and 6.9 using automated pH controllers (Fermac 260, Electrolab, Tewkesbury, UK). Each vessel was inoculated with 15 ml fresh fecal slurry (1/10 w/w). Batch fermentations were ran for 48 h and 6 ml samples were obtained from each vessel at 0, 10, 24, 34 and 48 h for microbial enumeration by fluorescent *in situ* hybridisation (FISH) analysis. Three replicate fermentations were set up, each inoculated with one of three different human fecal samples.

Bacterial enumeration

Fluorescent in situ hybridization targeting *Bifidobacterium* spp. (Bif164), *Bacteroides/Prevotella* (Bac303), *Lactobacillus/Enterococcus* (Erec482), *Clostridium histolyticum* (Chis150), *Atopobium* cluster (Ato291) and *Eubacterium rectale/Clostridium coccoides* (Erec482), was used as described by Sarbini et al (2011). Cells were visualized by fluorescent microscopy (Eclipse 400, Nikon, Surrey, UK) using the Fluor 100 lens. For each sample, 15 different fields of view were enumerated.

Statistical analysis

One-way ANOVA was performed to determine differences in *in vitro* secreted gut hormone levels. Differences in energy intake and area under the curve (AUC) for plasma hormone levels and gastric emptying between trials were assessed using Paired Student's *t* test. Two-way (trial×time) repeated measures ANOVA was performed to determine differences in plasma hormone levels, VAS, breath hydrogen, and bacterial group populations. Significant effects were followed up by Tukey's *post hoc* comparisons. Data are presented as means \pm SEM or \pm 95% Confidence Interval [CI]. P values < 0.05 were considered statistically significant.

SUPPLEMENTARY RESULTS

Colonic delivery investigation

¹³C enrichment in plasma glucose and plasma alanine

Enrichments in plasma $\delta^{13}\text{C}$ glucose and $\delta^{13}\text{C}$ alanine were 1.5 ± 1.7 per mil ($p=0.27$) and 0.9 ± 0.8 per mil ($p=0.11$) at 360 min compared with baseline, respectively.

Estimation of daily colonic short chain fatty acid production from inulin-propionate ester

Data from sudden death victims suggests that the average propionate pool size in the proximal large intestine is 4.5 mmol and 10 g of inulin-propionate ester releases an additional 15.0 mmol and 36.2 mmol from inulin fermentation and bound propionate respectively³. Using stoichiometric equations for hexose fermentation by intestinal bacteria¹⁴, we calculate that a daily dietary intake of 15 g non-starch polysaccharide yields a daily production of 94.1, 34.5 and 28.2 mmol for acetate, propionate and butyrate, respectively. Addition of 10 g inulin-control leads to a 2.0-, 1.6- and 1.4-fold change for daily acetate, propionate and butyrate production, respectively, whereas addition of 10 g inulin-propionate ester leads to a 1.7-, 2.5- and 1.3-fold change for daily acetate, propionate and butyrate production respectively.

Gastric emptying investigation

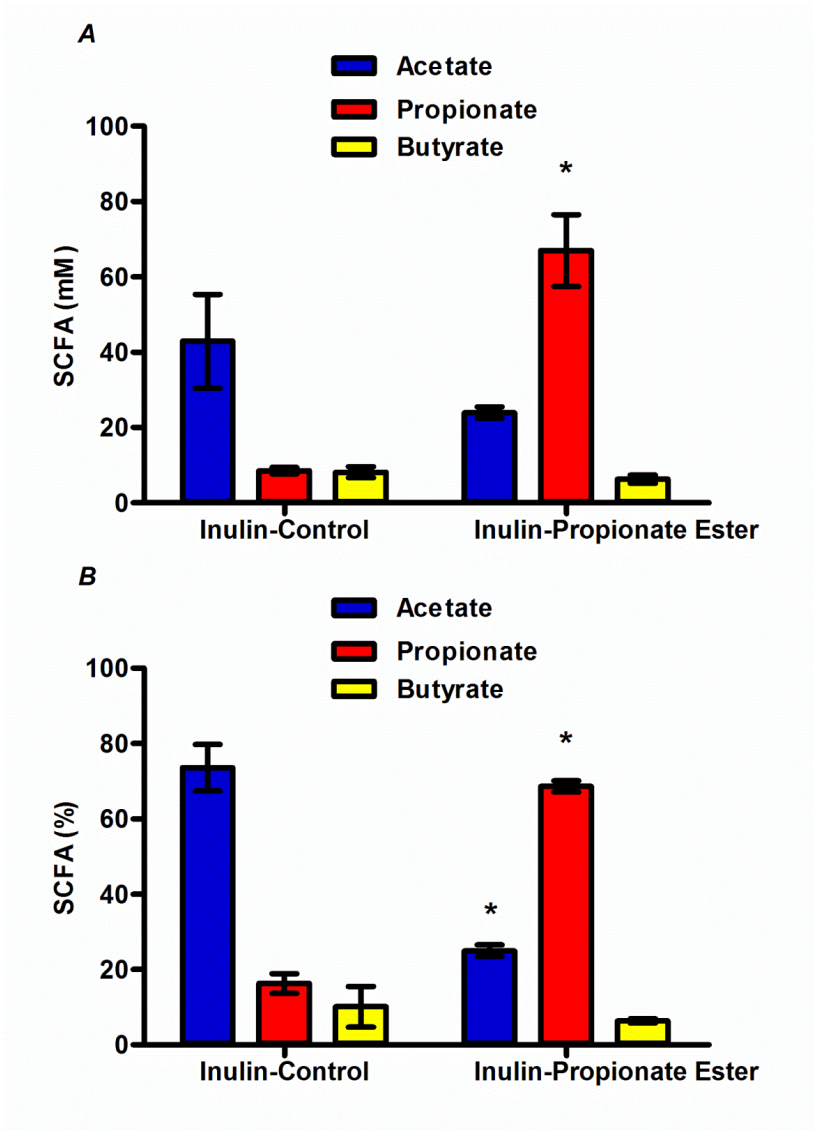
Rates of gastric emptying were not significantly different between acute propionate ester and control treatments (Siegel $T_{1/2}$ 185 min [95% CI, 168 to 204] inulin-propionate ester vs. 180 min [95% CI, 163 to 198] control, $P=0.506$).

Gut microbial changes to inulin-propionate ester and inulin-control

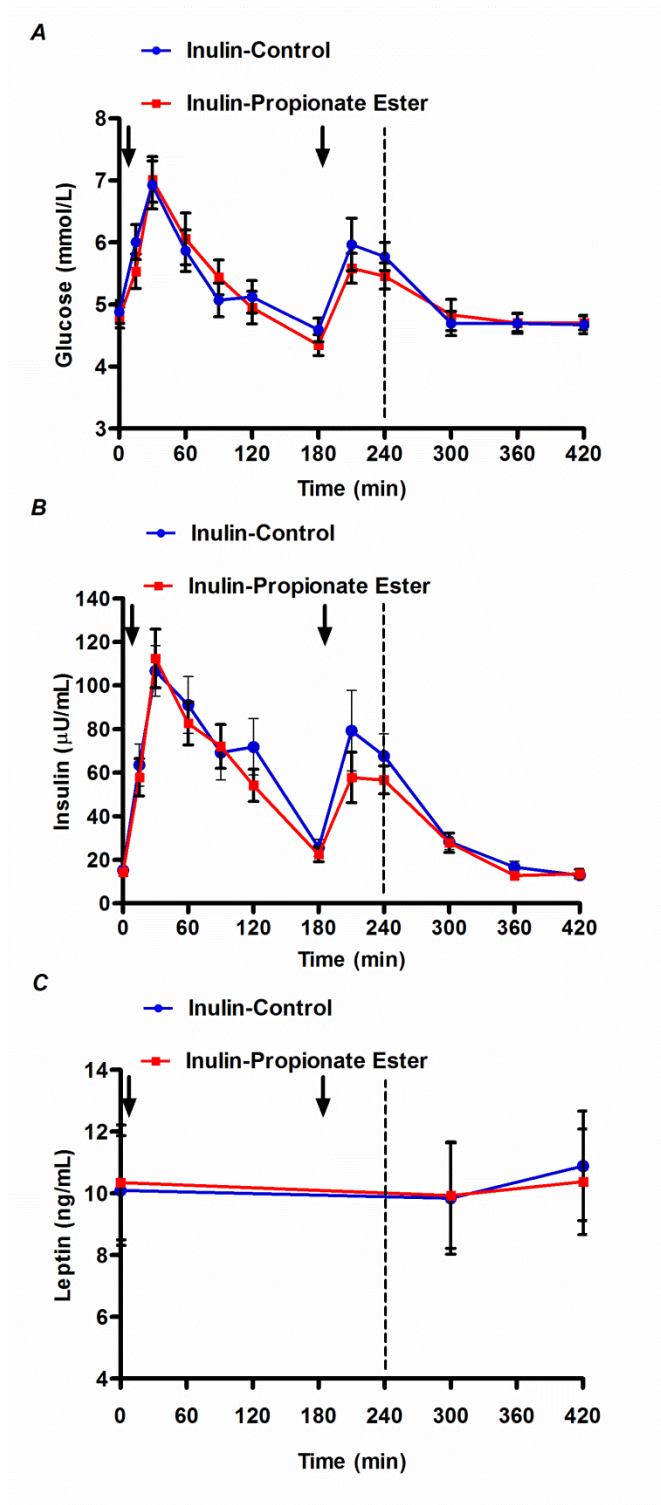
Inulin control significantly increased Bif164 labelled cells at all time points compared to baseline. Bif164 levels with control were significantly higher compared to the propionate ester cultures throughout fermentation. Both propionate ester and inulin control significantly increased Bac303 labelled cells at all sampling points compared to baseline levels. Ato291 labelled cells increased

significantly with both test substrates between 10 and 34 h compared with baseline concentrations. No other significant changes were seen in any of the groups targeted or total bacteria levels. It appears that propionate ester was fermentable by *Bacteroides* and *Atopobium* but not by *Bifidobacterium* spp.

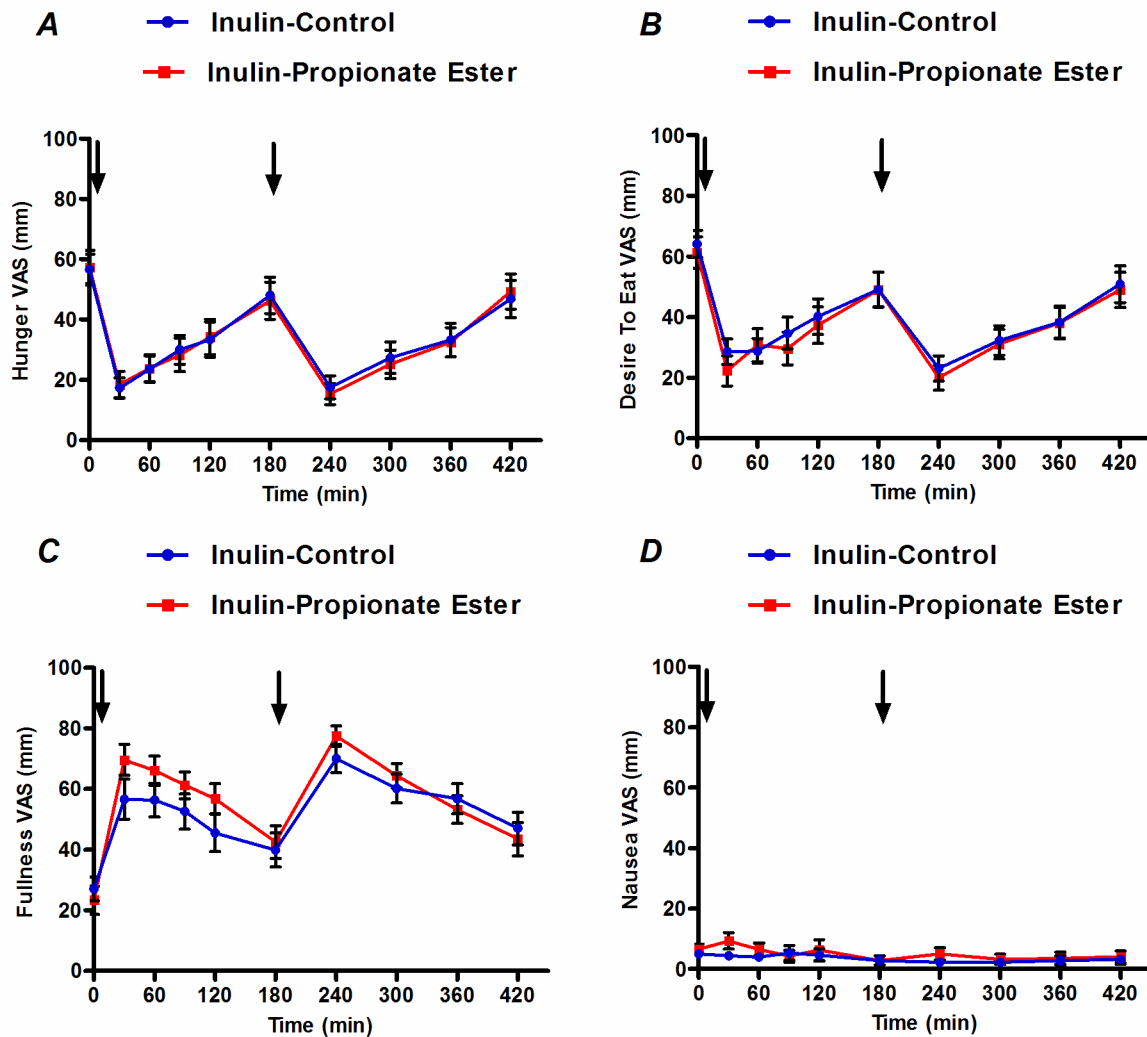
SUPPLEMENTARY FIGURES



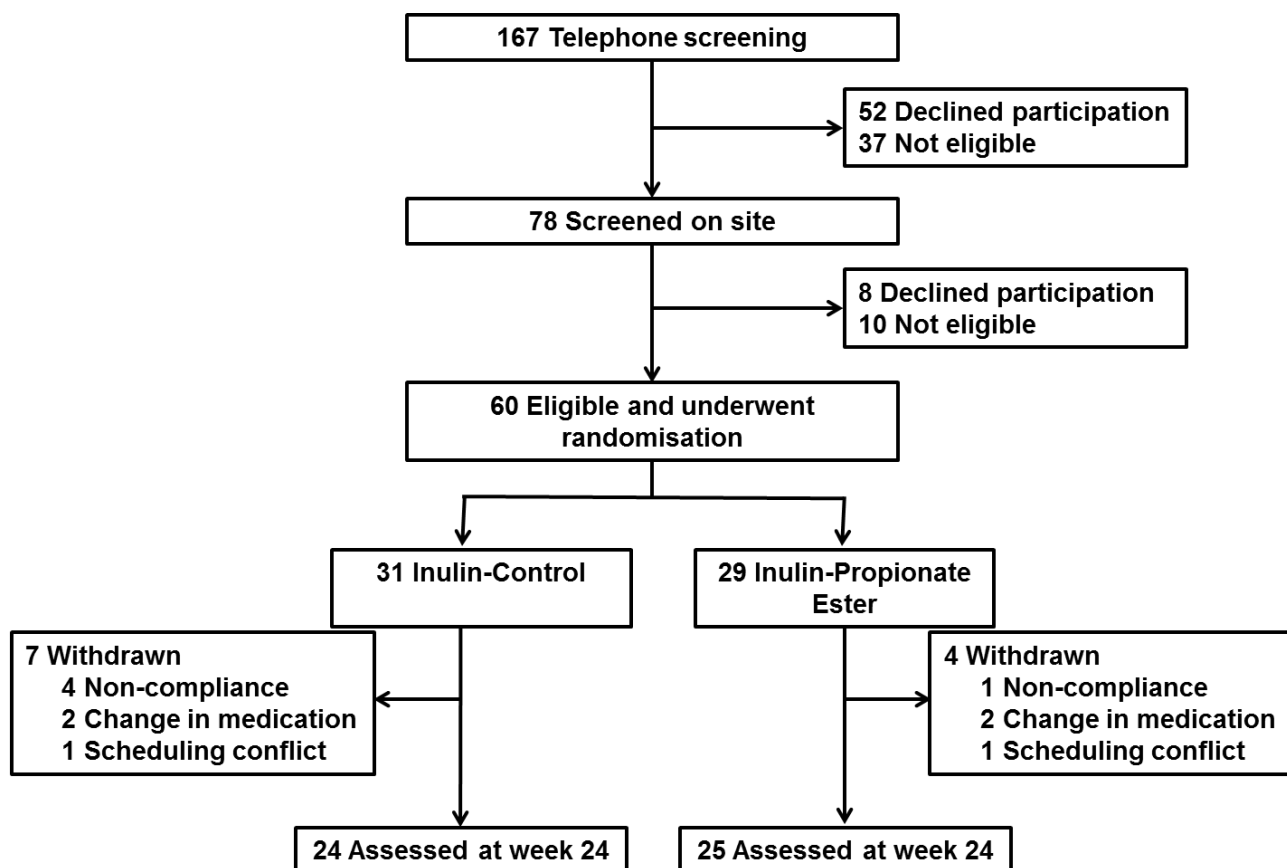
Suppl. Figure 1. *A.* Short chain fatty acid production and *B.* molar ratios of inulin-control and inulin-propionate ester in fecal fermentations. Mean \pm SEM, * $P < 0.05$ ($n = 3$).



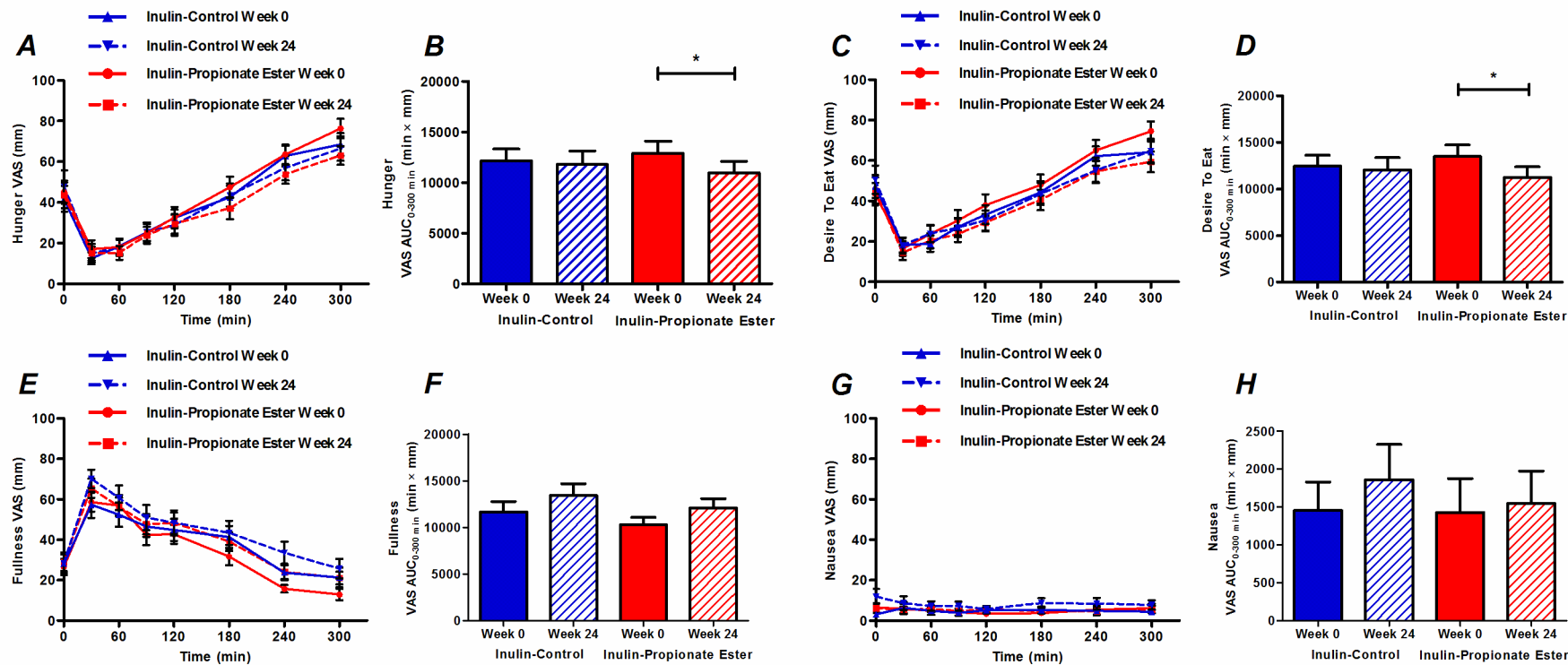
Suppl. Figure 2. The effect of acute inulin-propionate ester supplementation on postprandial A. glucose, B. insulin or C. leptin response. Arrows indicate timings of standardized meals. 10 g inulin-control or 10 g inulin-propionate ester were provided with breakfast at 0 min. Dotted lines signify the time point after which >80% inulin-propionate ester enters the colon as determined by the enrichment of ^{13}C in expired air and breath H_2 methodology (Figure 1C). Data are presented as means \pm SEM (n=20).



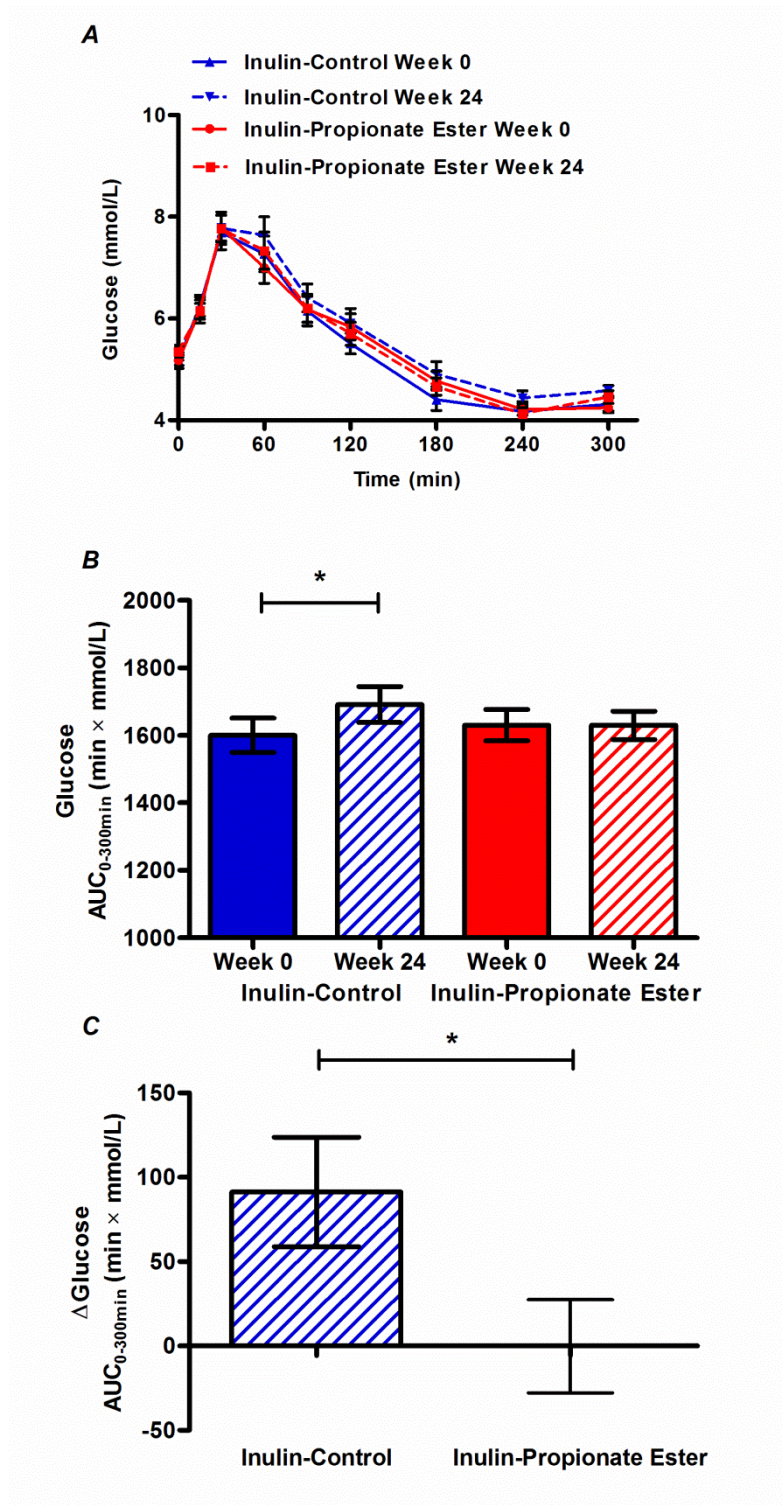
Suppl. Figure 3. An acute increase in colonic propionate content does not affect postprandial ratings of appetite or nausea. A. Hunger, B. Desire To Eat, C. Fullness, and D. Nausea. Ratings were made using 100 mm visual analogue scales (VAS), with extreme statements anchored at each end of the rating scale (e.g. 0 mm *Not at all hungry*, 100 mm *Extremely hungry*). Arrows indicate standardized meals. 10 g inulin-control or 10 g inulin-propionate ester were provided with breakfast at 0 min. Data are presented as means \pm SEM (n=20).



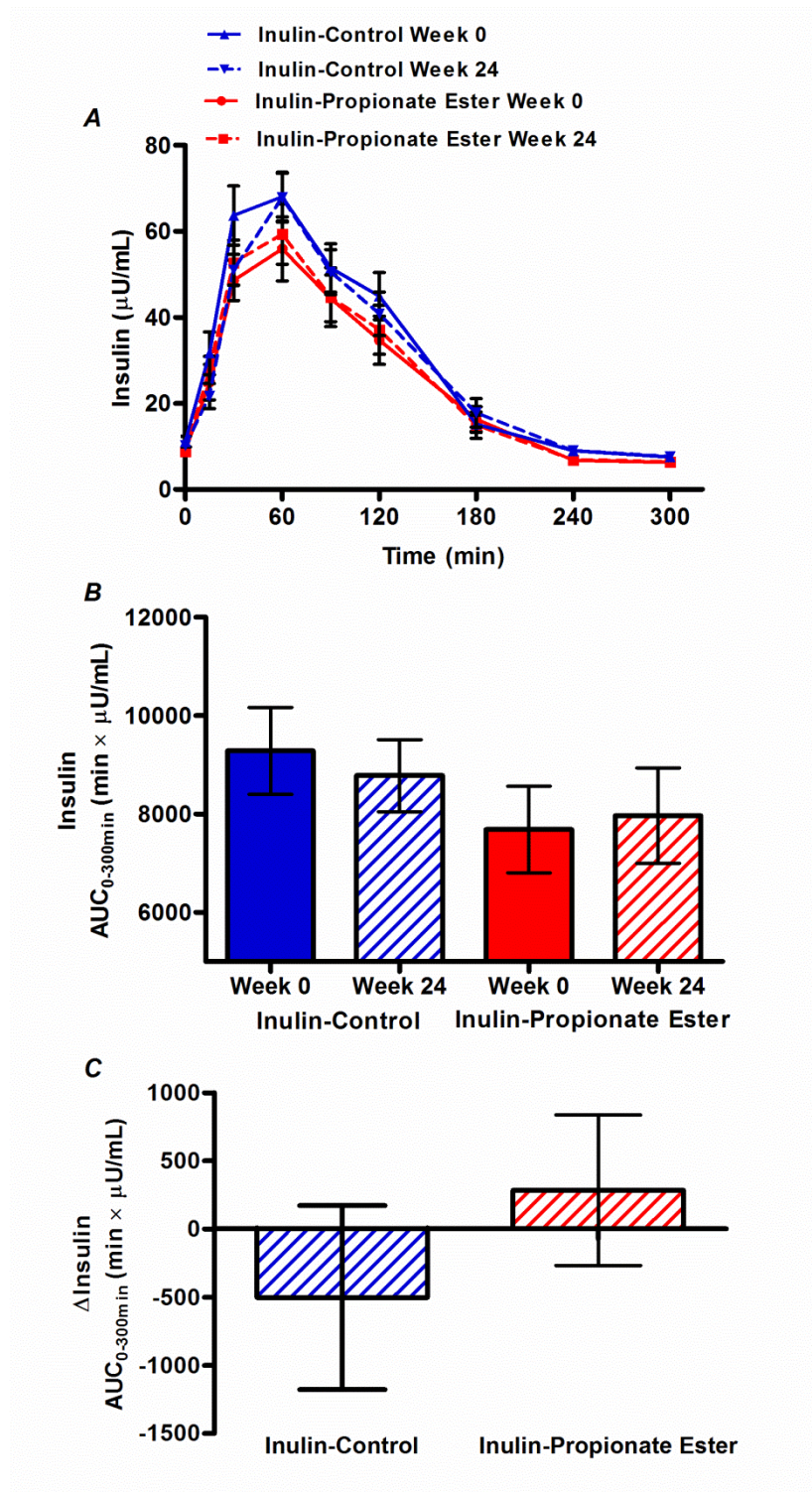
Suppl. Figure 4. Flow chart showing recruitment and retention in the 24 week supplementation study.



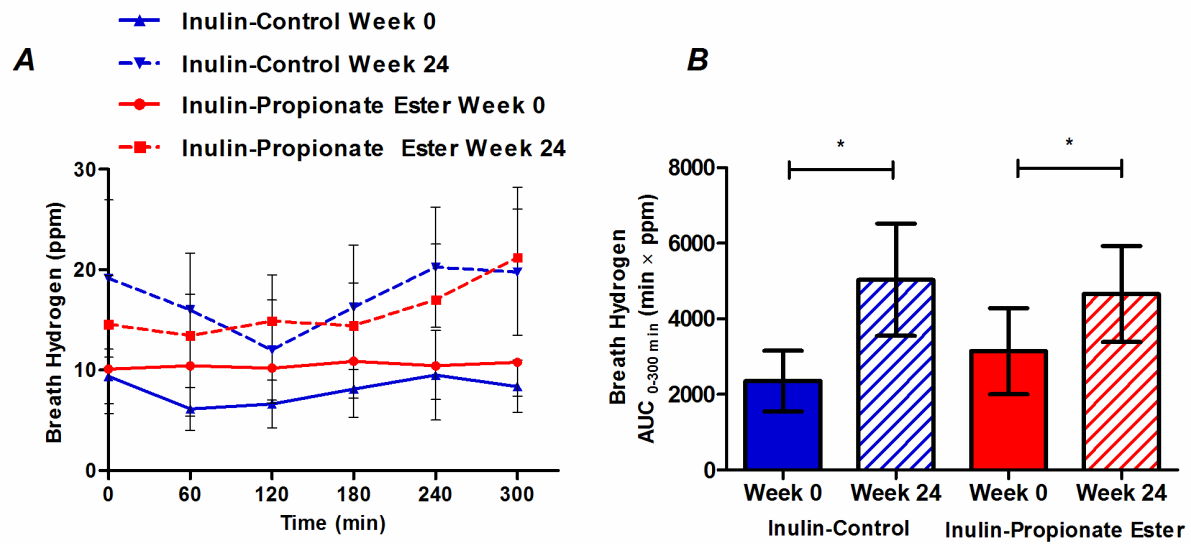
Suppl. Figure 5. Postprandial ratings of appetite and nausea at baseline and following 24 weeks supplementation with inulin-control and inulin-propionate ester. A. and B. *Hunger*, C. and D. *Desire To Eat*, E. and F. *Fullness*, and G. and H. *Nausea*. Ratings were made using 100 mm visual analogue scales (VAS), with extreme statements anchored at each end of the rating scale (e.g. 0 mm *Not at all hungry*, 100 mm *Extremely hungry*). * $P < 0.05$, Mean \pm SEM (inulin-control, $n = 24$; inulin-propionate ester, $n = 25$).



Suppl. Figure 6. Effect of 24 weeks supplementation with inulin-control and inulin-propionate ester on glucose response. **A.** Postprandial glucose response at baseline and following 24 weeks of supplementation with inulin-control and inulin-propionate ester. **B.** Glucose AUC_{0-300min} at week 0 and week 24. **C.** Change from baseline (week 0) glucose AUC_{0-300min} at week 24. * $P < 0.05$. Mean \pm SEM (inulin-control, $n = 24$; inulin-propionate ester, $n = 25$).



Suppl. Figure 7. Effect of 24 weeks supplementation with inulin-control and inulin-propionate ester on insulin response. A. Postprandial insulin response at baseline and following 24 weeks of supplementation with inulin-control and inulin-propionate ester. **B.** Insulin $\text{AUC}_{0-300\text{min}}$ at week 0 and week 24. **C.** Change from baseline (week 0) insulin $\text{AUC}_{0-300\text{min}}$ at week 24. Mean \pm SEM (inulin-control, $n = 24$; inulin-propionate ester, $n = 25$).



Suppl. Figure 8. Effect of 24 weeks supplementation with inulin-control and inulin-propionate ester on breath hydrogen. **A.** Fasting and postprandial breath hydrogen at baseline and following 24 weeks of supplementation with inulin-control and inulin-propionate ester and **B.** Breath hydrogen AUC_{0-300min} at week 0 and week 24. *P<0.05. Mean \pm SEM (Propionate Ester, n = 9; Control, n = 8).

SUPPLEMENTARY TABLES

Suppl. Table 1. Gastrointestinal adverse events reported during the 24 week supplementation period.

	Inulin-Control	Inulin-Propionate Ester	Inulin-Propionate Ester - Inulin-Control	
	(N = 24)	(N = 25)		
Side Effect	Mean ± SD	Mean ± SD	Difference (95% CI)	P Value
Stomach Discomfort	31.0 ± 26.6	10.5 ± 13.0	-11.1 (-29.0, 6.7)	0.221
Nausea	7.5 ± 10.6	4.6 ± 5.8	1.5 (-7.4, 10.4)	0.736
Bloating	34.0 ± 27.4	19.7 ± 22.7	-6.8 (-24.4, 10.8)	0.448
Flatulence	56.0 ± 28.4	25.2 ± 22.6	-24.9 (-41.9, -8.0)	0.004
Heartburn	17.8 ± 19.5	9.9 ± 14.4	-1.9 (-18.0, 14.2)	0.820
Belching	15.1 ± 14.3	10.0 ± 14.0	-0.0 (-11.8, 11.8)	1.000

Ratings were made at weeks 8, 16 and 24 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*).

Suppl. Table 2. Self-reported food intake and physical activity of subjects at baseline and following 24 weeks of inulin-control and inulin-propionate ester supplementation. Means \pm SEM.

	Inulin-Control		Inulin-Propionate Ester	
	Week 0	Week 24	Week 0	Week 24
Energy (kcal)	2215 \pm 247	2508 \pm 317	1957 \pm 249	1753 \pm 359
Energy (kJ)	9306 \pm 1034	10544 \pm 1316	8213 \pm 1043	7385 \pm 1522
Protein (g)	95 \pm 15	95 \pm 11	75 \pm 9	70 \pm 11
Fat (g)	84 \pm 12	94 \pm 18	82 \pm 13	73 \pm 12
Carbohydrate (g)	256 \pm 19	294 \pm 43	237 \pm 29	213 \pm 68
Fibre (g)	20 \pm 4	21 \pm 5	17 \pm 2	12 \pm 5
Total physical activity (MET-h/week)	33.3 \pm 8.0	31.3 \pm 6.8	25.7 \pm 4.3	26.8 \pm 4.0
Vigorous-intensity (MET-h/week)	3.5 \pm 3.3	4.9 \pm 3.4	4.3 \pm 1.8	4.4 \pm 1.9
Moderate-intensity (MET-h/week)	5.9 \pm 2.5	4.9 \pm 2.5	3.6 \pm 1.1	4.1 \pm 0.9
Walking (MET-h/week)	23.9 \pm 5.0	21.5 \pm 3.8	17.8 \pm 3.7	18.3 \pm 3.6

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)¹³. IPAQ calculates metabolic equivalents (MET-h/week), derived by assigning standardised MET values for walking, moderate-intensity and vigorous-intensity of 3.3, 4.0 and 8.0, respectively. No significant differences were found in any measure either within- or between-groups ($P > 0.05$).

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