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Volume 12 • Issue 8 • July 2025

ISSN 2050-4373

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DOI 10.3310/GKWP5267

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Extended Research Article

Increase in colonic propionate as a method of preventing weight gain in adults aged 20–40 years: iPREVENT, a multicentre, double-blind, randomised, parallel-group trial

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Published July 2025
DOI: 10.3310/GKWP5267

This report should be referenced as follows:

Frost G, Vasconcelos JC, Pugh JE, Anjum A, Petropoulou K, Thom G, *et al.* Increase in colonic propionate as a method of preventing weight gain in adults aged 20–40 years: iPREVENT, a multicentre, double-blind, randomised, parallel-group trial. *Efficacy Mech Eval* 2025;**12**(8). https://doi.org/10.3310/GKWP5267

Efficacy and Mechanism Evaluation

ISSN 2050-4373 (Online)

A list of Journals Library editors can be found on the NIHR Journals Library website

Efficacy and Mechanism Evaluation (EME) was launched in 2014 and is indexed by Europe PMC, DOAJ, Ulrichsweb™ (ProQuest LLC, Ann Arbor, MI, USA) and NCBI Bookshelf.

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

Editorial contact: journals.library@nihr.ac.uk

The full EME archive is freely available to view online at www.journalslibrary.nihr.ac.uk/eme.

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The EME programme is funded by the Medical Research Council (MRC) and the National Institute for Health and Care Research (NIHR), with contributions from the Chief Scientist Office (CSO) in Scotland and National Institute for Social Care and Health Research (NISCHR) in Wales and the Health and Social Care Research and Development (HSC R&D), Public Health Agency in Northern Ireland.

This article

The research reported in this issue of the journal was funded by the EME programme as award number 15/185/16. The contractual start date was in March 2018. The draft manuscript began editorial review in January 2024 and was accepted for publication in December 2024. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The EME editors and production house have tried to ensure the accuracy of the authors' manuscript and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this article.

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Abstract

Background: Overweight and obesity affect over 60% of the United Kingdom population and constitute a major risk factor for the development of comorbidities. Preventing weight gain in periods of life where there is an elevated risk of adipose tissue expansion could be beneficial in preventing associated diseases in later life. This study investigated the impact of inulin-propionate ester on the prevention of weight gain in young people who were at risk of gaining weight.

Objectives: To investigate the impact of increasing colonic production of the short-chain fatty acid propionate on preventing body weight gain, in younger adults, over 12 months. Several underpinning mechanisms were investigated in a substudy.

Design: iPREVENT was a randomised, parallel-group, placebo-controlled, double-blind trial, designed with 90% power to detect a 2 kg between-arm difference in weight gain at 12 months.

Setting: This trial was performed at the Imperial Clinical Research Facility in London and the University of Glasgow Clinical Research Facility.

Participants: Participants were 20–40 years old, with a body mass index of 24.0–27.0 kg/m^2 if of South Asian ethnicity or 25.0–30.0 kg/m^2 if non-South Asian. Participants were also required to have at least one of the following risk factors: weight gain in the past year, low physical activity, low intake of fruit and vegetables or high intake of sugar-sweetened beverages. A total of 270 participants (135 per arm) were randomised.

Intervention: Participants were randomised to either 10 g/day inulin-propionate ester or 10 g/day inulin control (fermentable oligosaccharide) consumed daily for 12 months.

Main outcome measure: The primary outcome was weight gain from baseline to 12 months.

Results: The mean (± standard deviation) body weight at baseline for inulin was 79.1 kg ± 10.6 (n = 135) and inulin-propionate ester 79.6 kg ± 10.9 (n = 135). At 12 months body weight was 78.9 kg ± 11.8 (n = 114) and 81.4 kg ± 11.9 (n = 112) for inulin and inulin-propionate ester, respectively. The baseline-adjusted mean difference in weight gain was 1.02 (95% confidence intervals −0.37 to 2.41) kg for inulin-propionate ester versus inulin control. Of the secondary outcomes, the confidence interval cautiously supports differences in fat-free mass; 1.07 kg (0.21 to 1.93) (N = 226), body water; 0.72 kg (0.1 to 1.33) (N = 226) and fasting glucose; 0.11 mmol/l (0.01 to 0.21) (N = 191). Compliance with inulin-propionate ester treatment of ≥ 50% over 12 months was 63% (n = 135). There were no unexpected adverse or serious adverse events.

Limitations: This study aimed to explore the efficacy of enhanced propionate production above background fermentation. It could be argued a negative control (a non-fermentable substrate e.g. cellulose) would have been more suitable. Metabolomic analysis of compliance could only be conducted on a subsection of the study population. There was no intention to conduct follow-up assessments on participants beyond their 12-month visit.

Conclusions: In adults below the age of 40 years, inulin-propionate ester did not affect weight gain compared with the control.

Future work: Previously we observed that inulin-propionate ester prevented weight gain in older adults, but not in this younger cohort. We aim to investigate the variation in responses between these age groups.

Trial registration: This trial is registered as ISRCTN16299902.

Funding: This award was funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme (NIHR award ref: 15/185/16) and is published in full in *Efficacy and Mechanism Evaluation*; Vol. 12, No. 8. See the NIHR Funding and Awards website for further award information.

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Report Supplementary Material 1 iPREVENT statistical analysis plan

Supplementary material can be found on the NIHR Journals Library report page (https://doi.org/10.3310/GKWP5267).

Supplementary material has been provided by the authors to support the report and any files provided at submission will have been seen by peer reviewers, but not extensively reviewed. Any supplementary material provided at a later stage in the process may not have been peer-reviewed.

List of abbreviations

(S)AE	(serious) adverse event
AUC	area under the curve
BIA	bioelectrical impedance analysis
BMI	body mass index
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	coronavirus disease 2019
CPMG	Carr–Purcell–Meiboom–Gill
CRF	Clinical Research Facility
CVD	cardiovascular disease
DMEC	Data Monitoring and Ethics Committee
DNL	de novo lipogenesis
EDC	Electronic Data Capture
ELISA	enzyme-linked immunosorbent assay
FFAR	free fatty acid receptor
FFM	fat-free mass
FM	fat mass
FMI	fat mass index
FTIR	Fourier-transform infrared spectroscopy
GC-MS	gas chromatography–mass spectrometry
GLP-1	glucagon-like peptide 1
GP	general practice/practitioner
HDL	high-density lipoprotein
iAUC	incremental areas under the curve
IPAQ	International Physical Activity Questionnaire
IPE	inulin-propionate ester
LDL	low-density lipoprotein
LME	linear mixed effects
MAR	missing at random
MCCV	Monte–Carlo cross-validation
METs	metabolic equivalents of tasks
NaN_3	sodium azide
NMR	nuclear magnetic resonance
PIS	participant information sheet
PLSDA	partial least squares discriminant analysis
PP DNL	postprandial de novo lipogenesis
PPI	patient and public involvement
PYY	peptide YY
RD	relaxation delay
REC	Research Ethics Committee
rRNA	ribosomal ribonucleic acid
SAG	Study Advisory Group
SAP	statistical analysis plan
SCFA	short-chain fatty acid
TAG	triacylglycerol
TSC	Trial Steering Committee
TSP	3-(trimethylsilyl)- [2,2,3,3, -2H4]-propionic acid
VAS	visual analogue scales

Plain language summary

Obesity is the storage of fat within the body to the degree that causes poor health outcomes. The percentage of people with overweight or obesity continues to increase in all global populations. The prevention of obesity is a major public health goal. Evidence suggests that high-fibre diets prevent weight gain. Fibre leads to increased production of short-chain fatty acids by bacteria in the colon. Short-chain fatty acids appear to have a beneficial effect on metabolic health. However, most people do not consume enough fibre and forfeit these effects. We have combined inulin (a type of dietary fibre) and propionate (a short-chain fatty acid) to form inulin-propionate ester. This novel food ingredient can deliver propionate to the colon. Inulin was chosen as the control to explore propionate independent of any effect that inulin may have on body weight. In previous studies, inulin-propionate ester has prevented further weight gain in middle-aged adults who are overweight. Here, we now investigate whether inulin-propionate ester prevents weight gain in younger adults compared with inulin. We undertook this study because evidence suggests that rapid weight gain in young adults is related to obesity and other chronic diseases in later life. We recruited 270 participants aged 20–40 years with an overweight body mass index (25–30 kg/m^2) and behaviours associated with weight gain. Participants were randomly allocated to 2 groups of 135 participants each to take 10 g inulin-propionate ester or 10 g inulin daily for 12 months. Our main interest was the change in body weight after 1 year. Other measurements of interest were changes in body fat, fasting blood biomarkers, which can indicate chronic disease risk (e.g. blood sugar and cholesterol), and blood pressure. There were no differences in body weight gain, most measures of body composition and fasting blood biomarkers, between the two groups at 12 months. In conclusion, neither inulin-propionate ester nor inulin prevented weight gain in young adults.

Scientific summary

Background

Obesity is a major global health concern and over 60% of UK adults are obese or overweight. Most research focuses on obesity treatment rather than the prevention of initial weight gain and fat deposition. Young people aged between 20 and 35 years have the fastest rates of weight gain at an average of approximately 1 kg/year. Weight gain during early adulthood increases the likelihood of poor metabolic health outcomes like elevated fasting glucose or hypertension, which contribute to chronic metabolic disease risk. Epidemiological and intervention studies have shown that dietary fibre is associated with lower body weight and better metabolic outcomes. Dietary fibre has a range of impacts on the gastrointestinal tract and gut microbiota. The fermentation of fibre by bacteria in the colon produces short-chain fatty acids (SCFAs) which may have beneficial effects on appetite and substrate metabolism. A collaboration between Scottish Universities Environmental Research Centre and Imperial College London led to the creation of inulin-propionate ester (IPE), a compound consisting of inulin (fibre) and propionate (SCFA) to specifically increase the production of propionate in the colon. This facilitates a more targeted investigation of the role of individual SCFAs on weight gain compared with general fibre supplementation. In previous work, IPE has prevented weight gain, lowered visceral fat mass (FM) and improved insulin sensitivity in middle-aged cohorts. However, the effect of increasing colonic propionate production using IPE in younger adults at risk of weight gain has not been explored. Therefore, this multicentre, randomised, placebo-controlled, double-blind trial was designed to investigate the effect of IPE on body weight in younger adults (20–40 years) with self-reported behaviours linked with phenotypic susceptibility to weight gain (e.g. low physical activity).

Main study objectives

The primary objective was to investigate whether IPE has a superior effect on preventing body weight gain, compared with inulin, in younger adults over 12 months.

The secondary objectives were to:

- Assess the effect of IPE compared to inulin on blood pressure, fasting biochemistry, and body composition.
- Determine the safety profile of IPE via adverse events (AEs), compliance and withdrawal reporting.

The substudy objectives were to assess the mechanisms by which IPE affects:

- Energy balance by measuring whole-body energy expenditure, hepatic lipid metabolism and whole-body lipid oxidation.
- Appetite by measuring peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) concentrations, subjective appetite ratings and ad libitum energy intake.
- The colonic environment via 16S ribosomal ribonucleic acid (rRNA) and metabolite analysis.
- The urinary, faecal and serum metabolite profile using nuclear magnetic resonance (NMR) spectroscopic analyses.
- Breath hydrogen as a surrogate measure of colonic bacteria fermentation.

Methods

Trial design

iPREVENT was a randomised, placebo-controlled, double-blind trial to investigate the efficacy of IPE versus inulin control upon weight gain prevention and to determine the safety profile of IPE. Participants were randomised to take 10 g/day of either IPE or inulin control daily for 12 months, with study visits at baseline, and 2, 6 and 12 months after

randomisation. A subset of participants consented, before study randomisation, to participate in further assessments of mechanistic measures in a substudy.

Study settings

The trial was performed at two UK sites: National Institute for Health and Care Research Imperial Clinical Research Facility (CRF) in London, Imperial College Healthcare NHS Trust, and University of Glasgow CRF (NHS Research Scotland).

Participants

A total of 270 participants were enrolled and randomised using Sealed Envelope software (open-source software, www.sealedenvelope.com), of whom 52 also took part in the mechanistic substudy.

The study recruited participants who were males and females aged 20–40 years with a body mass index (BMI) of 24.0–27.0 kg/m^2 if of South Asian ethnicity or 25.0–30.0 kg/m^2 if non-South Asian. Potential participants had to meet at least one of the following criteria at screening:

- a self-reported weight gain of 2 kg or more over the last 12 months
- low self-reported physical activity
- low self-reported fruit and vegetable intake (< 2 servings per day)
- high self-reported intake of sugar-sweetened beverages (> 1 serving per day).

Participants were excluded if they:

- were diagnosed with chronic disease; type 2 and type 2 diabetes, cancer, renal failure, heart disease, organic acidaemia (propionic acidaemia, methylmalonic acidaemia)
- were diagnosed with gastrointestinal conditions including coeliac disease, inflammatory bowel disease and irritable bowel syndrome
- had previous bowel reconstruction surgery
- were pregnant or lactating
- had used antibiotics at any time in the past 3 months
- had untreated vitamin B_{12} deficiency (< 160 ng/l)
- were taking part in a weight loss programme or consuming a weight loss product
- had lost 3 kg or more in the last 3 months (self-reported)
- had diarrhoea, constipation, bloating or abdominal cramping in the last 2 weeks (self-reported).

Substudy exclusion criteria included:

- anaemia or as per screening haemoglobin levels of < 130 g/l for males and < 110 g/l for females
- allergies or intolerances to any of the ingredients in the set substudy meals.

Recruitment

Study recruitment ran from July 2019 to October 2021 and was paused from March to September 2020 due to coronavirus disease 2019 (COVID-19). Trial participants were recruited from the following sources which are ranked from most to least productive:

- contacting NHS trusts
- contacting local general practices
- social media adverts
- posters
- newspaper adverts
- pop-up events.

Interventions

Participants received unlabelled, identical-looking trial interventions of IPE and inulin in 10 g pre-packed, foil-backed sachets, and they were instructed to take one sachet per day, mixed in a cold drink/water, at any time with their normal diet.

Study procedures

At the screening visit, the study rationale and protocol were explained. Participants were then asked to provide informed consent. Body weight and body composition (body water, FM, lean mass) were measured. A blood sample was taken for a full blood count to rule out the risk of B_{12} deficiency or anaemia. Participants were asked about their medical history, current medications, physical activity, alcohol intake, smoking or vaping, and recreational drug use. Blood pressure and waist and hip circumference were also measured.

Participants who were asked to attend their baseline (randomisation) study visit fasted for 12 hours and had abstained from intense physical activity and alcohol the day before. A blood sample was taken for fasting glucose, insulin, cholesterol and lipids. The participants were randomised via Sealed Envelope software and provided with IPE or inulin sachets lasting them for 2 months.

The same measurements were taken at the 2-, 6- and 12-month visits. No blood samples were taken at the 2-month visit. Compliance was measured by counting used and unused inulin/IPE sachets and the occurrence of AE or serious adverse events (SAEs) was documented.

Outcomes

Primary outcome:

Weight gain from baseline to 12 months

Secondary outcomes:

- Occurrence of AEs and SAEs over the duration of the study.
- Changes in fasting biochemistry from baseline to 6 and 12 months:
    - Glucose.
    - Insulin.
    - Triglycerides.
    - Total cholesterol.
    - Low-density lipoprotein cholesterol.
    - High-density lipoprotein cholesterol.
- Changes in blood pressure from baseline to 2, 6 and 12 months.
- Changes in body weight from baseline to 2 and 6 months.
- Changes in waist/hip/BMI/body composition measurements – FM, fat mass index (FMI), per cent body fat (fat%), fat-free mass (FFM) and FM/FFM ratio, from baseline to 2, 6 and 12 months.
- Changes in compliance (sachet count) from baseline to 2, 6 and 12 months.

Exploratory/mechanistic study outcomes

- Gut microbiota: 16S rRNA profiles from stool sample.
- Impact on neuroendocrine cell number: Proliferation in intestinal organoids using the level of SCFA and other metabolites identified from NMR spectroscopic analyses of stool.
- Appetite regulation: Measured by visual analogue scales, food diaries, ad libitum intake, and appetite-regulating gut hormones PYY, GLP-1, gastrin and cholecystokinin.
- Energy expenditure: Open-loop indirect calorimetry.
- Hepatic lipid metabolism: Stable isotope tracers of fat oxidation (^{13}C palmitate) and de novo lipogenesis.
- Total body water through dilution analysis of deuterated water.

Other data observations:

- Changes in physical activity from baseline to 2, 6 and 12 months.
- Changes in other lifestyle factors (drinking, smoking, recreational drugs) from baseline to 2, 6 and 12 months.
- Changes in diet from baseline to 2, 6 and 12 months (via food diaries).

Statistical analysis

The sample size for this study was 270 participants, based on the randomised proof-of-concept trial, the difference between arms in the change in body weight over 24 weeks was 1.4 kg [95% confidence interval (CI) −0.3 to 3.1], $p = 0.099$. A 2 kg between-arm 12-month effect size was therefore chosen. This agreed with a weight gain prevention trial over 9 months in young adults which aimed to detect a 2 kg effect and achieved 4.3 kg, with a pooled standard deviation (SD) for body weight change of 4.35 kg, and 81% retention.

The analysis of the primary end point incorporates the earlier correlated interim measurements of body weight in a linear mixed-effects (LME) model and is adjusted for baseline continuous body weight and other categorical randomisation stratifiers with further specification of the role of time point, and correlation structure, detailed in the statistical analysis plan. The implicit 'missing at random' assumption has been challenged through a set of sensitivity analyses. As these involve all randomised participants, the LME model and the sensitivity analyses taken together therefore constitute an intention-to-treat strategy.

Where possible, continuous secondary end points have been adjusted for their baseline to improve the precision of estimated intervention effects. Repeated measures have been analysed using LME models adjusting also for randomisation stratifiers. Comparisons between arms for binary outcomes are summarised as differences in proportions. Ninety-five per cent CIs have been used to make inferences from estimated effect sizes.

Results

Participants were recruited for the study from July 2019 and the target sample size was reached in October 2021 with 135 participants randomly allocated into 2 arms. Recruitment was paused from March 2020 to September 2020 during COVID-19. Participant retention was in line with estimates, with 16% (42/270) participants completely withdrawing from trial interventions. At 12 months, a compliance threshold of ≥ 50% was reached by 53% (72/135) of the inulin control arm and 63% (85/135) of the IPE arm. A high threshold of ≥ 80% was reached by 32% (43/135) of the inulin control arm and 48% (65/135) of the IPE arm, resulting in a total of 40% (108/270) of participants. Participant baseline characteristics were similar between trial arms. Primary outcome was provided for 84% (227/270) of participants.

Primary outcome

Mean (± SD) body weight at baseline was 79.1 kg ± 10.6 ($n = 135$) for inulin and 79.6 kg ± 10.9 ($n = 135$) for IPE. At 12 months, body weight was 78.9 kg ± 11.8 ($n = 114$) and 81.4 kg ± 11.9 ($n = 112$), for inulin and IPE, respectively. The baseline-adjusted difference was 1.02 (95% CI −0.37 to 2.41) kg ($p = 0.15$), between the groups.

Secondary outcomes

Lifestyle factors were comparable by study arm. There were no significant differences in measures of fasting biochemistry outcomes, except for glucose: 0.11 (0.01 to 0.21). There was a difference between arms for body water of 0.72 (0.17 to 1.28) and FFM of 1.08 (0.29 to 1.86). No changes in FM, FMI, fat%, FFM and FM/FFM ratio were detected after 12 months of IPE intake.

The AE and SAE reporting was similar between the two arms. There were a greater number of moderate-severity gastrointestinal-related AEs in the inulin control arm than in the IPE arm.

The effect of COVID-19

During the COVID-19 lockdowns, researchers pivoted to collecting participants' self-reported body weight at home. Participants were encouraged to attend the clinic for their next visit, so a clinic-reported weight could be taken.

Researchers also attempted to take clinic weights when participants had self-reported their 12-month weight but could attend the clinic within the 1-month measurement window. However, we cannot discount our results were affected by the pandemic given several studies reported increased weight gain occurred during the COVID-19 lockdowns.

Conclusions

This was the first long-term study to investigate the efficacy of increasing colonic propionate production using IPE on weight gain prevention in younger adults recruited specifically due to their risk of further weight gain. IPE did not significantly alter weight gain trajectory compared with inulin. This contrasts with previous findings demonstrating that IPE prevented weight gain, lowered body fat mass and improved insulin sensitivity in middle-aged participants who were overweight or obese. Notably, despite this population being at increased risk of weight gain, neither group exhibited significant increases in body weight nor reached the predicted 2 kg weight gain. These results are encouraging given that the study was conducted during the COVID-19 pandemic, and a recent systematic review reported that the average adult gained 1.57 kg weight from March to May 2020. The role of dietary fibre in preventing weight gain warrants further investigation.

The outcomes of this trial were not influenced by unaccounted confounding variables, such as compliance rates. High compliance at 12 months was seen in 40% of participants. The safety profile of both the intervention and control appears comparable. There were no unexpected AEs or SAEs, and complete withdrawal rates were similar for both study arms. Supplement cessation was less balanced, 42 participants and 61 participants stopped intake of IPE and inulin, respectively. The study was not confounded by high rates of gastrointestinal disturbance from IPE or inulin.

The results differ from our previous observations that IPE prevented weight gain in middle-aged people with a BMI ≥ 25 kg/m^2. At present, it is not possible to understand this difference, but we could hypothesise that young adults may be less sensitive to increased propionate production and may require a larger dose of IPE for an effect to be seen. In our previous work, 10 g was determined to be the minimally effective dose of IPE to promote weight maintenance in middle-aged participants. On the other hand, it is possible that young adults are more responsive to the effects of microbial fermentation of inulin on appetite regulation, as indicated by the lack of weight gain in the inulin control group.

Discrepancies in results between older and younger adults could partially be attributed to differences in diet and lifestyle. Younger adults have a greater tendency to eat sporadically or snack throughout the day, whereas older people tend to have more regular meal patterns and settled routines. Previous studies using stable isotopes indicate that IPE releases propionate 3–4 hours post ingestion. As propionate is a short-term signalling molecule, it may have no effect on appetite and energy intake in individuals with less consistent meal patterns. Further, evidence indicates that circulating concentrations of satiety hormones, including PYY, are lower in younger adults. These habits, powerful external cues and lower baseline concentrations of satiety hormones could overcome any satiety signal driven by IPE.

Consistent intake of IPE may have resulted in adaptations to the colonic environment and reduced sensitivity to propionate. Chronic IPE intake may have contributed to the desensitisation of free fatty acid receptors 2 and 3 located on enteroendocrine cells in the gastrointestinal tract, diminishing secretion of GLP-1 and PYY and a reducing satiety response. Further investigation is required to determine whether receptor desensitisation or alterations to certain associated proteins (e.g. those involved in gut hormone degradation) contribute to the diminished impact.

This clinical trial has some limitations. Due to the COVID-19 lockdowns and pivot to collection of self-reported weight data, the 'Principal weight' measurement for the primary outcome consists of 38% self-reported weight data. Self-reported data introduce inherent bias. Additionally, discrepancies in calibration between home weight scales and those used in the CRF may introduce further variability in the measurements. There was no plan to follow up with the participants after their final visit at the 12-month point to identify changes in their weight gain trajectory after stopping the supplement.

Future studies should aim to understand the differential effects of IPE and inulin between population groups and explore drivers of appetite regulation in younger adults.

Trial registration

This trial is registered as ISRCTN16299902.

Funding

This award was funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme (NIHR award ref: 15/185/16) and is published in full in *Efficacy and Mechanism Evaluation*; Vol. 12, No. 8. See the NIHR Funding and Awards website for further award information.

Chapter 1 Background

Overweight and obesity affect over 68.2% of men and 60.4% of women in the UK[1] and account for 160 million disability-adjusted life-years underscoring their social and economic burden.[2] Obesity drives the prevalence of several common comorbidities, including type 2 diabetes, cardiovascular disease (CVD) and cancer. Although significant advances have been made in the management of obesity, the same level of research productivity has not been seen for obesity prevention. Once an individual becomes obese, the probability of returning to a normal body weight is extremely low (1 in 210 for men and 1 in 124 for women).[3]

Body weight increases incrementally throughout adulthood, but younger adults are at the greatest risk of substantial gains in body weight. According to a National Health and Nutrition Examination Survey of adults, major weight gain over 10 years, categorised as a gain in body mass index (BMI) ≥ 5 kg/m^2, was highest in 25- to 35-year-olds.[4] Similar observations have been reported in the UK population.[5] While a modest weight gain of 1 kg over a single year would present a negligible risk to health in young adults, the accumulated weight gain over a decade or longer leads to a deterioration in cardiovascular and diabetes risk factors. For example, the 10-year Coronary Artery Risk Development in Young Adults study demonstrated that weight gain in early adulthood led to adverse changes to blood lipids, fasting insulin and blood pressure.[6]

Epidemiological studies and controlled trials report an inverse association between dietary fibre intake and body weight gain.[7,8] Although the Scientific Advisory Committee on Nutrition has recommended 30 g fibre per day since 2015, UK adults currently consume 17–20 g of fibre per day,[9] and fibre intake has not increased for the last 10 years.[10] The mechanisms by which increased dietary fibre intake affects energy balance are not fully understood. However, it is established that microbial fermentation of dietary fibre in the colon produces short-chain fatty acids (SCFAs).[11] These metabolites can stimulate the release of anorectic gastrointestinal hormones peptide YY (PYY) and glucagon-like peptide 1 (GLP 1) through binding to free fatty acid receptors (FFARs) 2 and 3 on enteroendocrine cells concentrated in the distal colon.[12-15] SCFAs can also enter the bloodstream to modulate substrate oxidation and metabolism at key metabolic sites, such as the pancreas, liver, adipose tissue and skeletal muscles.[16,17] The potential positive effects of SCFAs on energy homeostasis and metabolism have been extensively reviewed elsewhere.[16-19] However, much of this research was conducted on rodents using high-fibre intakes (5–20% total energy intake) or SCFAs in concentrations that are not physiologically translatable or realistic in human populations.

A method of delivering SCFAs to the colon and avoiding the undesirable effects of high-fibre consumption was developed by esterifying propionate to the non-digestible fructo-oligosaccharide, inulin. This novel food ingredient, inulin-propionate ester (IPE), delivers approximately 2 g of propionate to the colon and increases propionate production by sevenfold compared with inulin.[20] Propionate was selected over acetate or butyrate because it is not further converted into other SCFAs, has a high affinity for both FFAR 2 and 3 and enters the circulation exposing organs to SCFAs.[21,22]

In a proof-of-concept study, IPE stimulated the acute release of PPY and GLP-1 and lowered energy intake compared with inulin.[20,23] Further, IPE has been reported to lower neural reward responses to high-energy-density foods resulting in a lower ad libitum energy intake compared to the control.[24] IPE supplementation had no significant effect on gut hormone secretion after 6 months, although it significantly prevented weight gain compared with the inulin control in the same study.[23] IPE demonstrably improved insulin sensitivity and lowered concentrations of pro-inflammatory molecules relative to cellulose intake over 6 weeks.[25] These data suggest that the SCFAs can function via multiple mechanisms to alter energy balance and metabolic response.

Prior studies using IPE have recruited an older, more weight-stable population over a maximum study duration of 6 months. Furthermore, although the accumulated evidence suggests that IPE has a beneficial effect on energy balance and appetite, the mechanisms behind these effects are not fully understood.

This study aimed to investigate the role of IPE in weight gain prevention in younger adults who are overweight and at elevated risk of weight gain, over 12 months. The mechanistic substudy aimed to elucidate the processes by which propionate influences appetite, energy expenditure and substrate oxidation.

Chapter 2 Objectives

Primary objective

The primary objective was to investigate whether IPE has a superior effect on preventing body weight gain, compared with inulin, in younger adults over 12 months.

Secondary objectives

The secondary objectives were to determine:

1. The safety profile of IPE.
2. The effects of IPE on glucose homeostasis as a surrogate marker of type 2 diabetes risk.
3. The effects of IPE on blood lipid and cholesterol as surrogate markers of CVD risk.
4. The effects of IPE on blood pressure as a surrogate marker of CVD and stroke risk.
5. Any changes in waist and hip circumference, BMI or body composition during the 12-month intervention.
6. Compliance (sachet count) during the 12-month intervention.

Objectives for the mechanistic substudy

The objectives of the mechanistic study were to explore the effects of IPE on:

1. Colonic metabolism using metataxonomic analysis of the 16S ribosomal ribonucleic acid (rRNA) gene in stool samples to identify the relative abundance of the bacterial component of the microbiome.
2. The urinary, faecal and serum metabolite profile using nuclear magnetic resonance (NMR) spectroscopic analyses.
3. Anorectic gut hormones (GLP-1 and PYY) and subjective feelings of appetite via visual analogue scales (VAS), as measures of appetite regulation.
4. Energy expenditure and hepatic lipid metabolism [fat oxidation and de novo lipogenesis (DNL) as potential mechanisms involved in body weight maintenance].
5. Breath hydrogen as a surrogate measure of colonic bacteria fermentation.

Observational objectives

To compare changes in:

1. Physical activity during the 12-month intervention.
2. Other lifestyle factors during the 12-month intervention; smoking, drinking and recreational drugs.
3. Diet during the 12-month intervention (via food diaries).

Chapter 3 Methods

Trial design

iPREVENT was a randomised, placebo-controlled, double-blind trial to investigate the efficacy and safety of IPE versus inulin control upon weight gain prevention. Participants were randomised to either IPE or inulin control for 12 months.

Ethical approval

The trial was registered on 1 March 2018, ISRCTN16299902. Ethics approval was obtained on 29 January 2019 from the London Hampstead Research Ethics Committee (REC) (Reference 19/LO/0095). The trial protocol is available at https://f1000research.com/articles/11-1157.

Changes to protocol

Protocol amendments were approved by the sponsor and subsequently, the REC. *Table 1* details all changes to the protocol.

TABLE 1 Protocol amendments

Amendment no.	Type	Approval date by ethics/HRA	Changes
Amendment 1	Substantial	August 2019	• Revised protocol (admin changes, updated sampling details, NHS digital follow-up details, updated packaging company details) • PIS/consent form (CF) changes to reflect the above • Study templates include newspaper advertisements, participant diaries, questionnaires and instructions
Amendment 2	Substantial	January 2020	• Age in inclusion criteria extended from '20–35 years' to '20–40 years' • PIS/CF changes to reflect the above • Updated advertising details including template text message for general practices and study web page • Study templates including poster ad and updated pre-screening questionnaire
Amendment 3	Non-substantial	April 2020	• Notification of pause in recruitment due to initial COVID-19 lockdown • Guideline document created for remote follow-up visits/changes to the trial during this period
Amendment 4	Substantial	August 2020	• The title of the project was amended as requested by NIHR, following the previous change in inclusion criteria (age) • Clarification of recruitment strategies being used • Addition of Participant Identification Centres (PICs) site • Script and topic guide drafted for animation video and information videos to be created, for social media
Amendment 5	Non-substantial	November 2020	• Study clinic visits resuming at all sites, following the pause in March 2020 due to COVID-19 restrictions • Additional data collection was required as remote visits did not capture accurate primary outcome data, and blood samples (for secondary outcomes) were missed • Wider visit windows for flexibility • Additional NHS boards were added for Scotland, for a wider search of research volunteers via the SHARE database • Addition of the PIC site

continued

TABLE 1 Protocol amendments (*continued*)

Amendment no.	Type	Approval date by ethics/HRA	Changes
Amendment 6	Substantial	February 21	• Removal of ECG from screening procedures • Exclusion criteria for the main study were amended from 'vitamin B_{12} deficiency' to 'untreated vitamin B_{12} deficiency' • 'Anaemia' specified as an exclusion for substudy only (previously for main study too) • Joint screening/randomisation visit for those not taking part in the substudy and addition of remote consent as a 7-day food diary required to be completed before the joint visit • PIS/CF changes to reflect all the above
Amendment 7	Non-substantial	September 2021	• Costed extension from NIHR – new grant end date

ECG, electrocardiogram; NIHR, National Institute for Health and Care Research; PIS, participant information sheet.

Study settings

The trial was performed at two UK sites: the National Institute for Health and Care Research (NIHR) Imperial Clinical Research Facility (CRF) in London – Imperial College Healthcare NHS Trust and the University of Glasgow CRF – NHS Research Scotland.

Participants were invited to attend screening, baseline (randomisation) and all subsequent trial visits at 2, 6 and 12 months at the CRF of each participating site (*Figure 1*). Participants recruited at Imperial were also invited to participate in the mechanistic substudy, and if they agreed, additional assessments took place at baseline and 12 months at the Imperial CRF.

Participants

A total of 270 participants were randomised to the main study. Of this, 52 participants were enrolled in the mechanistic substudy.

Inclusion criteria

- Males and females aged 20–40 years (inclusive).
- BMI of 24.0–27.0 kg/m^2 if of South Asian ethnicity or 25.0–30.0 kg/m^2 if non-South Asian, and at least one of the following (at screening):
    - A self-reported weight gain of 2 kg or more over the last 12 months.
    - Low self-reported physical activity ('low' activity as per International Physical Activity Questionnaire – IPAQ).
    - Low self-reported fruit and vegetable intake (< 2 servings of fruit and vegetables per day).
    - Self-reported high intake of sugar-sweetened beverages (> 1 serving per day).
- On stable medication (if taking any) at the point of screening.
- Written informed consent.

Exclusion criteria

Participants were excluded if they:

- were diagnosed with chronic disease; type 1 and 2 diabetes, cancer, renal failure, heart disease, organic acidaemia (propionic acidaemia, methylmalonic acidaemia)
- were diagnosed with gastrointestinal conditions including coeliac disease, inflammatory bowel disease and irritable bowel syndrome
- had previous bowel reconstruction surgery
- were pregnant or lactating

- had used antibiotics at any time in the past 3 months
- had untreated vitamin B_{12} deficiency (< 160 ng/l)
- were taking part in a weight loss programme or consuming a weight loss product
- had lost 3 kg or more in the last 3 months (self-reported)
- had diarrhoea, constipation, bloating or abdominal cramping in the last 2 weeks (self-reported).

Or for any other reason according to the study investigators.

Substudy exclusion criteria

- Known anaemia or as per screening full blood count results (haemoglobin levels of < 130 g/l for males and < 110 g/l for females).
- Allergies or intolerances to any of the ingredients in the set substudy meals.

Randomisation

Randomisation was undertaken using minimisation with a random element to balance the arms by centre, sex, BMI within ethnicity (South Asians: 24.00–25.49 kg/m^2 and 25.50–27.00 kg/m^2; non-South Asians: 25.00–27.49 kg/m^2 and 27.50–30.00 kg/m^2) and whether they are taking part in the mechanistic substudy. Minimisation was conducted by researchers who enrolled/randomised participants, using a web-based randomisation and Electronic Data Capture (EDC) system, called Sealed Envelope (open source software, www.sealedenvelope.com). The unblinded statistician prepared the randomisation list which was provided to the packaging company (Melrob Nutrition, now Nordmann UK Group, Crawley, UK) by an independent Trial Manager. The packaging company added four-digit 'kit codes' to the boxes containing inulin or IPE before they were sent to the trial sites. The lists of blinded kit codes for each site were uploaded to Sealed Envelope by the Trial Manager upon delivery of IPE/inulin to the study sites, in preparation for randomisations. The researchers conducting the study did not have access to the randomisation list.

Participants whose BMI was found to have changed from screening to randomisation visits, to below the minimum or above the highest value in the BMI criteria, were still randomised. Participants who were at least half South Asian (mixed ethnicity) had the South Asian BMI category applied as they had elevated risk factors. If they were only a quarter of South Asian or less, the non-South Asian BMI category was applied.

Blinding

Participants received blinded and identical-looking trial interventions of either IPE or inulin control. IPE and inulin were white powders delivered in pre-packed plain foil-backed sachets. They had similar solubility and taste. Participants, all site researchers and the study management team were blinded. Each box of IPE or control sachets was identified with a unique kit code. These were assigned to participant trial IDs upon randomisation via Sealed Envelope. Researchers would collect boxes labelled with 'kit codes' allocated by Sealed Envelope for the participants. Neither the researcher nor the participants were aware of the treatment allocation of these kit codes. Unblinding was only considered if management of the participant would differ depending on whether they were on IPE or inulin control. The trial EDC system included an automated unblinding facility, in case unblinding of an individual participant was required. Code-break envelopes were prepared as a backup. The integrity of the trial blind was maintained, except for the Data Monitoring and Ethics Committee (DMEC) which had access to fully unblinded data and the Study Statistician who required access to fully unblinded data to prepare the DMEC reports.

Supplements

Participants were randomised to either IPE or the inulin control for 12 months. Inulin (HP inulin, Beneo-Orafti, Tienen, Belgium) was sourced commercially (Kreglinger, Chesham, UK). IPE is not yet commercially available and procurement

through an existing project partner was not an allowable cost under NIHR funding rules. Therefore, we were required to externally fund the establishment of a new IPE production route at a sufficient scale of approximately 500 kg. IPE is composed of propionate chemically bound to the inulin, allowing the delivery of propionate to the target site in the colon. Propionate by itself would be absorbed rapidly in the small intestine otherwise and is organoleptically unsuitable for direct consumption. In the early phase of the study, we sought to transfer our laboratory-scale production of IPE to a pilot-plant scale. This was established in collaboration with Moorepark Technology Limited (MTL, Fermoy, Ireland), a facility carrying out commercialisation, product and process development, training, or small-scale start-up manufacture which operates to ISO 9002 Standards and is accredited with I.S. EN 22000:2018 (Food Safety Management System/ Good Food Manufacturing Practice) and FSSC 22000 standards. After successful pilot-plant method transfer and product testing, the production of IPE proceeded in three batches predicated on the production capacity of the facility. Over 500 kg of IPE was produced successfully for the study.

To ensure IPE manufacturing met with current guidance on novel food safety and therefore was safe for human consumption, we adopted the European Food Safety Authority guidance on acceptable criteria for novel foods regarding chemical purity, heavy metal content and microbiological contamination.[26] External UKAS-accredited laboratory testing (Eurofins Food Testing UK, Wolverhampton, UK) was undertaken on each batch of IPE and the batches were only released upon satisfactory certification.

Upon release, IPE and inulin were shipped to a commercial food supplement packaging company (Melrob Nutrition, now Nordmann UK Group, Crawley, UK) where sachets were filled, boxed and shipped to trial sites according to the pre-specified supplement coding.

The IPE and the inulin control were dispensed to participants in 10 g sachets and the participants were instructed to take one sachet per day, mixed into food, cold drinks or water, at any time of day with their normal diet.

Study procedures

A summary of the study visits and procedures can be seen in the study flow chart (see *Figure 1*).

Trial assessments for the main study and mechanistic substudy are summarised in *Table 2*.

Body weight, waist and hip circumference and body composition

Body weight was measured to the nearest 0.1 kg using the Tanita BC-418MA scales (Tanita Corporation, Japan). Participants were asked to remove heavy items of clothing, shoes and accessories. They were also asked to void their bladder before the measurements were taken. Tanita scales also measure body composition via bioelectrical impedance estimating total body water, fat mass (FM), fat-free mass (FFM; FFM = body weight – FM), BMI and fat mass index (FMI). Waist and hip circumference were taken at each visit using a standard measuring tape.

Dietary intake

Participants were asked to complete 7-day food diaries at baseline, 2-, 6- and 12-month visits to monitor changes in dietary intake over the study period.

Physical activity

Participants self-reported their physical activity by completing an IPAQ at each study visit.

Lifestyle factors

Changes in any lifestyle factors such as smoking, alcohol and recreational drug use were identified through a questionnaire asked at each study visit.

Screening blood tests and fasting biochemistry

Samples were taken for full blood count at screening, to ensure participants were not anaemic or vitamin B_{12} deficient. An additional blood sample was taken for participants enrolled in the substudy to measure the natural abundance

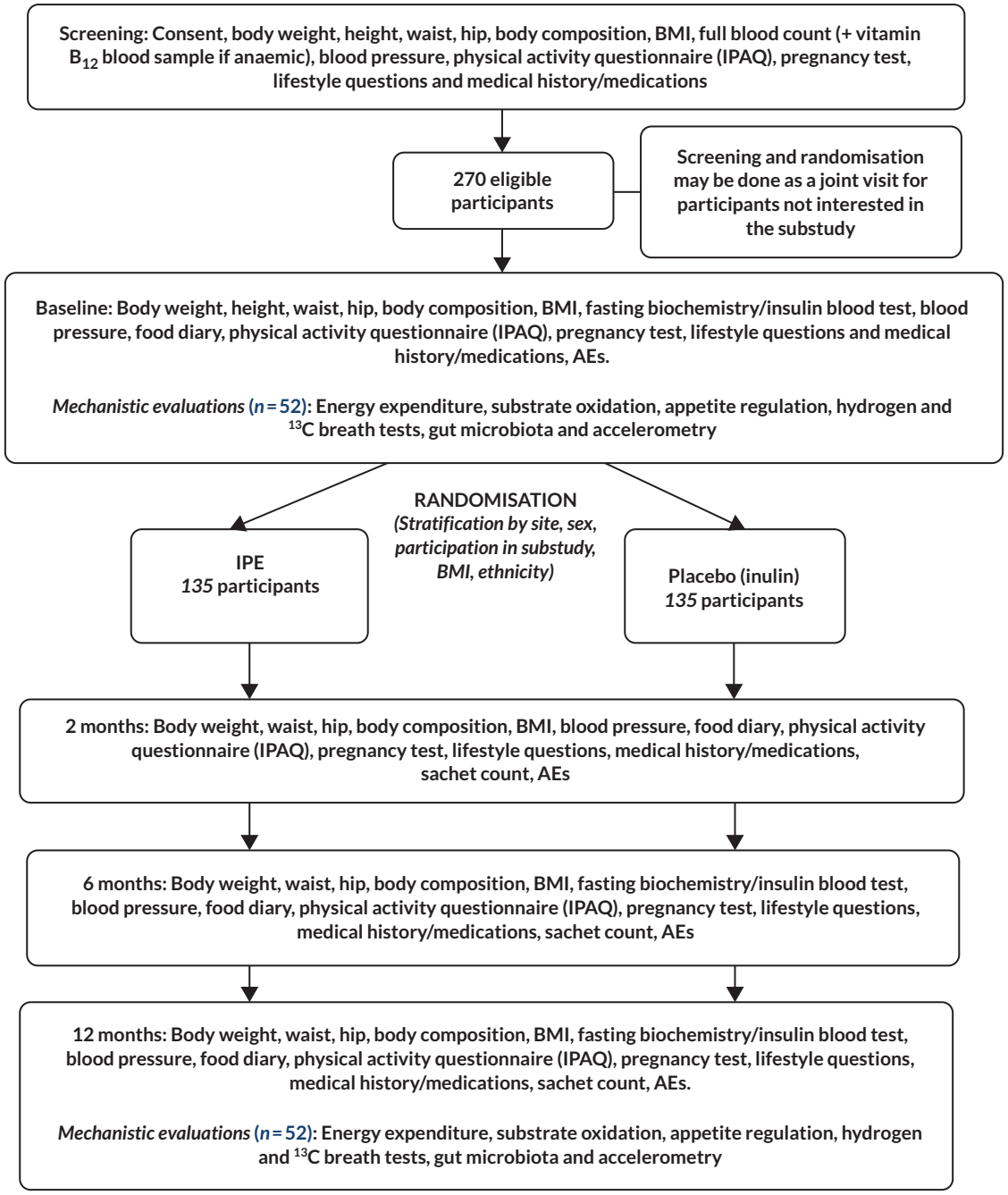

FIGURE 1 Study flow chart. AE, adverse event.

of deuterated water. Blood samples for fasting biochemistry were taken at baseline, 6- and 12-month study visits. Blood glucose, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, total cholesterol and triglyceride concentrations were analysed at the local Pathology laboratory for each site. Fasting insulin samples were analysed at Imperial College London using a commercial Human/Canine/Porcine Insulin DuoSet enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc. Bio-Techne, Minneapolis, MN, USA).

Pregnancy tests

Pregnancy tests were conducted for all female participants regardless of whether they were using contraception. Participants were also asked to use adequate contraception to prevent pregnancy or prevent pregnancy in a female partner.

TABLE 2 Study assessment schedule for the main and substudy

	Screening / Or joint screening/ randomisation	Baseline/ randomisation	2 months	6 months	12 months
Visit window		≤ 4 weeks after screening	(± 2 weeks)	(± 4 weeks)	(± 4 weeks)
Assessment					
Consent	X				
Demographics	X				
Randomisation		X			
Medical history	X	X	X	X	X
Concomitant medications	X	X	X	X	X
Pregnancy test	X	X	X	X	X
Diastolic blood pressure	X	X	X	X	X
Systolic blood pressure					
Heart rate					
Trial intervention (IPE or inulin control)		X	X	X	
Height	X	X	X	X	X
Body weight					
Waist/hip measurements					
BMI					
Body composition					
Fasting blood test (glucose, insulin, lipid profile)		X		X	X
Full blood count (and vitamin B_{12}, if required)	X				
Food diary		X	X	X	X
IPAQ	X	X	X	X	X
Lifestyle questions	X	X	X	X	X
Sachet count (compliance)			X	X	X
Adverse event tracking		X	X	X	X
Mechanistic evaluation (n = 52)					
Blood sample to measure the natural abundance of deuterated water	X				
Energy expenditure (indirect calorimetry)		X			X
Appetite regulation (VAS, ad libitum test meal, and blood tests for anorectic gut hormones)		X			X
Substrate oxidation/DNL (via stable isotope tracers in water consumption) – ^{13}C breath, urine and blood samples		X			X
Gut microbiota (stool sample and hydrogen breath test)		X			X
Accelerometry		X			X

Compliance

Compliance was monitored by asking participants to return both used and unused sachets to each study visit. Numbers of each type of sachets were recorded, and the count was verified by the Study Manager. Unused sachets from 2- to 6-month visits were re-dispensed.

Safety assessments

Participants were contacted each month to provide information on any adverse events (AEs) they had experienced since the last time they were contacted. The events were categorised based on severity and likelihood that they were related to the inulin or IPE trial intervention. These were recorded in the participant's electronic case report form and the Trial doctor was informed.

Substudy assessments

Participants who consented to the mechanistic substudy attended two 8-hour study visits, at baseline and at 12 months. Participants were asked to refrain from intense physical activity and alcohol consumption the day before the study visit. They were also asked to consume a standard evening meal, which could be consumed again at the follow-up visit and to fast overnight for 12 hours before attending the study visit. Stool, urine, blood and breath samples were collected for various analyses, as listed in *Figure 2*. A cannula was inserted into the antecubital vein to allow for repeated blood sampling. The participants were served three meals at the facility. Substudy time points and assessments are summarised in *Figure 2*.

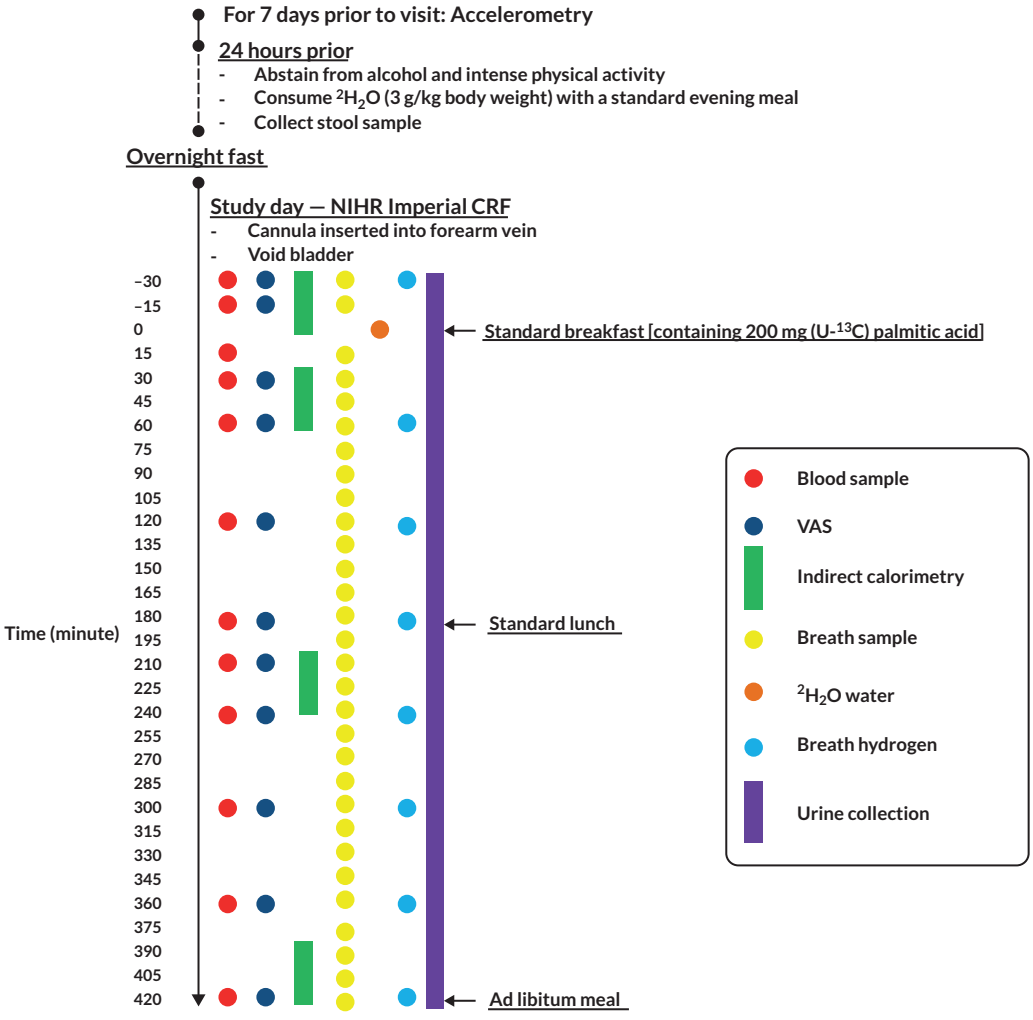

FIGURE 2 Study visit schedule for mechanistic substudy.

Appetite regulation and gut hormone concentrations

Blood samples for PYY and GLP-1 were taken at −30, −15, 15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes. Gut hormone concentrations were measured using established in-house radioimmunoassays using a previously described protocol.[27,28]

Subjective appetite measurements were taken using a standardised 100 mm appetite questionnaire VAS to assess hunger, fullness, nausea and thirst at −30, −15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes.[29]

Ad libitum energy intake was measured at 420 minutes, and the volunteers were given a pasta-based meal, served in excess. Participants were asked to eat until they felt comfortably full. The standardised meal was weighed before and after food intake to calculate the total amount eaten (g) to determine energy intake.

Energy expenditure, body composition, substrate oxidation and lipogenesis

Whole-body energy expenditure and substrate oxidation rates were measured using open-loop indirect calorimetry (Gas Exchange Monitor, GEM nutrition, Cheshire, UK). Indirect calorimetry was conducted four times throughout the study visit day, for 30 minutes at time points −30 to 0, 30 to 60, 210 to 240 and 390 to 420 minutes. Gas measurements for oxygen consumption, carbon dioxide production and a nitrogen excretion constant taken from a previous study using IPE[30] were used to calculate substrate oxidation and energy expenditure.[31,32]

Participants consumed deuterated water ($^{2}H_2O$, ~ 1.0 g/kg) the evening before and again on the day of the study visit, consumed with the mixed-meal tolerance test provided for breakfast. This was done to achieve a plasma water enrichment of 0.3% for measuring fasting and postprandial hepatic DNL as previously described.[33] To assess hepatic and whole-body fatty acid oxidation, 200 mg of (U-^{13}C) palmitic acid was added to the breakfast.

De novo lipogenesis was quantified by measurement of deuterium incorporation into palmitic acid measured in collected blood samples[34] using the modified protocol which assesses DNL from palmitic acid in the triacylglycerol (TAG) lipid fraction.[33] Briefly, basal plasma TAG (before administration of the second dose of deuterium) was processed using solid-phase extraction to isolate the TAG fraction, saponified and resultant fatty acids derivatised to their silyl esters using N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide followed by gas chromatography–mass spectrometry (GC-MS) analysis of the palmitic acid derivative.[35] Tripentadecanoin (C15:0 TAG) and heptadecanoic acid (C17:0) were added as internal process controls and $^{2}H_{31}$-palmitate (C16:0) was added as an internal quantitation standard. The isotopologues corresponding to unlabelled C16:0 (M+), $^{2}H_1$ labelled C16:0 (M + 1) and $^{2}H_{31}$ C16:0 (M + 31) were quantified by GC-MS (Agilent, Cheadle, UK) in selected ion monitoring mode. ^{2}H enrichment in body water was determined in plasma water using Fourier-transform infrared spectroscopy (FTIR). Whole-body fat oxidation from U-^{13}C palmitate was assessed from the incremental areas under the curve (iAUC) of breath $^{13}CO_2$ output by isotope ratio mass spectrometry (AP2003, Manchester, UK) of breath collected every 15 minutes from −30 to 420 minutes.

Gut microbiota

Participants collected stool samples the evening before or the morning of the study day. These were transported to the facility and immediately stored at −80 °C. Deoxyribonucleic acid was extracted from the stool samples to conduct a metataxonomic analysis of the 16S rRNA genes to identify the relative abundance of bacteria populations in the microbiome. Approximately 10,000 16S rRNA genes were sequenced per sample using an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

Metabolic phenotyping analysis

Participants voided their bladder at the beginning of the study visit and then collected their urine throughout the study day in a 24-hour urine container. The urine in the container was thoroughly mixed before it was stored at −80 °C. Participants collected stool as described above. Fasted serum samples, taken via the cannula, were aliquoted and immediately stored at −80 °C.

Nuclear magnetic resonance (^{1}H-NMR) spectroscopy was conducted on urine, serum and stool samples.

Urine samples were prepared for analysis by ^{1}H-NMR spectroscopy as follows: frozen samples (−80 °C) were thawed, vortexed and then centrifuged at 1600 *g* for 10 minutes to remove particulates and precipitated proteins. Subsequently, 540 µL of each sample was mixed with 60 µL of 1.5 M KH_2PO_4 buffer [pH 7.4, 80% deuterium oxide (D_2O)] containing 1 mM of the internal reference standard, 3-(trimethylsilyl)-[2,2,3,3,-$^{2}H_4$]-propionic acid (TSP) and 2 mM sodium azide (NaN_3), as described previously.[36]

After thawing, serum samples were centrifuged at 12,000 *g* for 5 minutes at 4 °C. Subsequently, 300 µL of serum was mixed with 300 µL of 0.075M NaH_2PO_4 buffer (pH 7.4) containing 0.8 mM of the internal reference standard TSP and 3.1 mM NaN_3, as described previously.[36,37]

Faecal water extraction was carried out by homogenising each sample with water (UHPLC grade, Fisher Chemical) at a ratio of 1 : 2 (mg of wet weight of faecal sample: µL of water). The mixture was vortexed for 5 minutes at 2000 rpm and centrifuged at 4200 *g* and 4 °C for 10 minutes; 810 µL of the supernatant was mixed with 90 µL of 1.5 M KH_2PO_4 buffer (pH 7.4, 100% of D_2O), 2 mM NaN_3 and 1% of TSP [3-trimethylsilyl-(2,2,3,3,-$^{2}H_4$)-propionic acid sodium salt].[38] The mixture was centrifuged at 18,000 *g* at 4 °C for 5 minutes. An aliquot of 600 µL of the supernatant was transferred into a 5 mm outer diameter NMR tube.[38]

^{1}H-NMR spectroscopy was performed at 300 K using a previously described protocol[36] on a 600 MHz Bruker Avance III HD spectrometer equipped with a 5 mm Double Resonance Broadband Probe (BBI) probe and fitted with the Bruker SampleJet™ robot (Bruker, Billerica, MA, USA) cooling system set to 5 °C. The following standard one-dimensional pulse sequence was used: $RDg_{z1} - 90° - t_1 - 90° - t_m - g_{z2} - 90° - ACQ$[15]. The relaxation delay (RD) was set at 4 seconds, 90° represnets the applied 90° radio frequency pulse, interpulse delay (t_1) was set to an interval of 4 µs, mixing time (t_m) was 10 ms, magnetic field gradients (g_{z1} and g_{z2}) were applied for 1 ms and the acquisition period was 2.7 seconds. Water suppression was achieved through irradiation of the water signal during RD and t_m. For the urine samples, each spectrum was acquired using 4 dummy scans followed by 32 scans while faecal spectra were acquired using 256 scans and 4 dummy scans and collected into 64 K data points. A spectral width of 12,000 Hz was used for all the samples. Prior to the Fourier transformation, the free induction decays were multiplied by an exponential function corresponding to a line broadening of 0.3 Hz. Serum samples were analysed by ^{1}H-NMR using the standard one-dimensional pulse sequence described above and Carr–Purcell–Meiboom–Gill (CPMG) one-dimensional pulse sequences. CPMG sequence was used to attenuate broad, interfering peaks from lipids and proteins present in serum. The CPMG pulse sequence had the form $RD - 90° - (t - 180° - t)n - ACQ$. The acquisition parameters were set using the same settings as the standard 1D pulse sequence, with the spin-echo delay (t) set at 0.3 ms and 128 loops (n) performed. Continuous wave irradiation was applied at the water resonance frequency during the RD.

The ^{1}H-NMR spectra underwent automated adjustments to correct for phase and baseline distortions. They were also referenced to the TSP singlet at δ 0.0 and processed using TopSpin 3.1 software (Bruker, Billerica, MA, USA). Next, the spectra were digitised into 20 K data points with a resolution of 0.0005 ppm using a custom MATLAB R2021b (The MathWorks, Inc., Natick, MA, USA) script. Sections of the spectra corresponding to the internal standard (δ −0.5 to 0.5) and water (δ 4.6–5) peaks were removed for urine and serum. To establish consistency, all the urine, stool and serum spectra were normalised using median fold-change normalisation, using the median spectrum as the reference.[39]

Breath hydrogen

Breath hydrogen, as a surrogate measure of microbial fermentation and confirming the delivery of inulin/IPE to the colon, was measured using a Gastrolyser (Bedfont Scientific Ltd., Maidstone, UK). Breath tests were taken at −30, 60, 120, 180, 240, 300, 360 and 420 minutes.

Glucose and insulin

Glucose and insulin were measured via blood samples taken from the cannula at −30, −15, 15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes. Glucose was measured using colourimetric enzymatic activity assay (GL364, Randox, UK). Insulin was measured using a commercial sandwich ELISA (DY8056-05, Biotechne, Minneapolis, MN, USA).

Physical activity

Substudy participants wore an accelerometer for 7 days before the substudy visits. The GENEActiv device (Activinsights Ltd., Kimbolton, UK) was used to determine physical activity duration and intensity, step count, time spent sedentary and sleep duration. Participants were discouraged from taking off the device at all during the 7-day measurement period to ensure adequate data collection. Data were read and processed using GENEActiv R Markdown Analysis Package (available from https://activinsights.com/support/geneactiv-support/).

Study meals

Breakfast, served after the two fasted samples (time point −30, −15 minutes), consisted of a mixed-meal tolerance test drink (Ensure Plus, Abbott, UK: 500 kcal, 67.3 g carbohydrate, 13.4 g fat, 20.8 g protein) to be consumed within 15 minutes. The nutrients from the mixed-meal tolerance test were consistent for all participants.

A standard lunch, provided as a 550, 650 or 750 kcal portion depending on the participants' basal metabolic rate, was given at T180. The lunch consisted of a white bread cheese sandwich and salted crisps (Tesco, Welwyn Garden City, UK: 10.0 g carbohydrate, 5.1 g fat, 3.1 g protein, ≤ 0.5 g fibre per 100 kcal).

The ad libitum meal consisted of an excess of durum-wheat penne pasta and ready-made tomato pasta sauce (Tesco, Welwyn Garden City, UK: 126.5 kcal, 21.4 g carbohydrate, 2.9 g fat, 3.3 g protein per 100 g) to ensure the participant could eat as much, or as little, as they desired.

Outcomes

Primary outcome

Weight gain from baseline to 12 months

Secondary outcomes

- Occurrence of AEs and serious adverse events (SAEs) over the duration of the study.
- Changes in fasting biochemistry from baseline to 6 and 12 months:
    - Glucose
    - Insulin
    - Triglycerides
    - Total cholesterol
    - LDL cholesterol
    - HDL cholesterol
- Changes in blood pressure from baseline to 2, 6 and 12 months.
- Changes in body weight from baseline to 2 and 6 months.
- Changes in waist/hip/BMI/body composition measurements FM, FMI, per cent body fat (fat%), FFM and FM/FFM ratio, from baseline to 2, 6 and 12 months.
- Changes in compliance (sachet count) from baseline to 2, 6 and 12 months.

Exploratory/mechanistic study outcomes

- Gut microbiota: 16S rRNA profiles from a stool sample.
- Impact on neuroendocrine cell number: Proliferation in intestinal organoids using the level of SCFA and other metabolites identified from NMR spectroscopic analyses of stool.
- Appetite regulation: Measured by VAS, food diaries, ad libitum intake, and appetite-regulating gut hormones PYY, GLP-1, gastrin and cholecystokinin.
- Energy expenditure: Open-loop indirect calorimetry.
- Hepatic lipid metabolism: Stable isotope tracers of fat oxidation (^{13}C palmitate) and DNL.
- Total body water through dilution analysis of deuterated water.

Other data observations:

- Changes in physical activity from baseline to 2, 6 and 12 months.
- Changes in other lifestyle factors (drinking, smoking, recreational drugs) from baseline to 2, 6 and 12 months.
- Changes in diet from baseline to 2, 6 and 12 months (via food diaries).

Sample size

In the randomised proof-of-concept trial, the difference between arms in the change in body weight over 24 weeks was 1.4 kg [95% confidence interval (CI) −0.3 to 3.1], $p = 0.099$. Using a Bayesian method recommended for preliminary trials in which evidence in the 95% CI is translated into probabilities,[40] there was a 95% posterior probability of an underlying positive between-arm difference favouring the intervention. The posterior probability of intervention-favouring differences > 1 kg, 1.5 kg and 2 kg were respectively 69%, 47% and 25% based on 24-week intervention. The difference increased in magnitude through successive 8-, 16- and 24-week time points. By 24 weeks, there were significant reductions observed in the proportion of intervention participants gaining 3% and 5% of body weight from a mean baseline of 90 kg. A 2-kg between-arm 12-month effect size was therefore chosen. This agreed with a weight gain prevention trial over 9 months in young adults,[41] which aimed to detect a 2-kg effect and achieved 4.3 kg, with a pooled standard deviation (SD) for body weight change of 4.35 kg, and 81% retention.

On this basis, a sample size of 270 randomised participants (135 per arm) was chosen to provide 90% power to detect a 2-kg difference between arms in mean body weight change over 12 months using a two-sided 5% level significance test, assuming a 4.35-kg SD and with 25% dropout allowance (68 participants).

For the mechanistic study, 34 volunteers (17 per group) would provide sufficient statistical power to detect a 15 pmol/l effect size in PYY and GLP-1 concentrations between groups, with 90% power, 5% significance level and SD 13 pmol/l. These differences were based on previously published findings that report enhanced gut hormone release following IPE supplementation.[20,23] A subsample of 52 volunteers (26 per group) was chosen to allow for a 70% retention rate.

Interim analyses and stopping guidelines

A fully independent DMEC was set up to monitor progress, participant safety and any ethical issues involved in this trial. They reviewed trial progress, recruitment rates and safety data. The stop decisions were decided upon before the study began and were not implemented during the trial.

STOP decisions:

- If the recruitment rate is below 50% of the target at 6 months (3.75 participants per centre per month), and below 50% in months 4–6, then power would be lower than 80% even with three extra sites; STOP.
- If the recruitment rate is between 50% and 66% of the target at 6 months (3.75–5 participants per centre per month) AND loss to 2-month follow-up is ≥ 25% (95% CI 12% to 38%; $n = 45$); STOP.
- 'GO' decision with changes:
    - if the recruitment rate is between 50% and 66% of the target at 6 months (3.75–5 per centre participants per month) AND retention (body weight measured) at 2 months > 75% THEN
    - add three additional sites AND
    - review power at 12 months with Funder.

GO decisions:

- If the recruitment rate is ≥ 66% of the target at 6 months (5 participants per centre per month) THEN; GO with three additional sites.

- If the recruitment rate is ≥ 75% of the target at 6 months (5.6 participants per centre per month) THEN; GO with two additional sites.
- If the recruitment rate is 85% of the target at 6 months (6.4 participants per centre per month) THEN; GO with one additional site.
- If the recruitment rate is ≥ 90% of the target at 6 months (6.75 participants per centre per month) THEN; GO, no changes are required.

The statistician analysed interim data for DMEC meetings and acted as data manager, in raising and resolving data queries with participating sites, via the Trial Manager. Closed DMEC reports included recruitment, randomisation balance and stratification effectiveness, baseline characteristics, unblinding, withdrawals, compliance, concomitant medications, efficacy, mediators and AEs. Open DMEC and Trial Steering Committee (TSC) reports were provided without outcome or arm information.[42]

Statistical methods

Primary endpoint analysis

The measurement of the body weight for the primary end-point analysis was taken to be the weight measured at the CRF; however, due to COVID-19 we collected extra self-report data, and we combined the two in the analysis model adjusting for the source of the measurement. This decision was supported by the oversight committees and recorded in the statistical analysis plan (SAP). We later called this 'principal weight' to emphasise this.

The analysis of the primary end point incorporates the earlier correlated interim measurements of body weight in a linear mixed-effects (LME) model and is adjusted for baseline continuous body weight and other categorical randomisation stratifiers. Therefore, participants who withdrew during the study without providing the 12-month measure were able to have their previously observed data from earlier time points included (i.e. 2-month and/or 6-month measures), and the statistical model accounted for these through the correlations between them and the 12-month measure. Further specification of the role of time point, and correlation structure, is detailed in the SAP (see *Report Supplementary Material 1*). The implicit 'missing at random' (MAR) assumption (that the correlation between earlier timed measures and the 12-month measure is the same for those withdrawing as was estimated in those continuing to the 12-month measure) has been challenged through a set of sensitivity analyses.[43] As these sensitivity analyses involve all randomised participants, the LME model and the sensitivity analysis taken together therefore constitute an intention-to-treat strategy.[44]

IBM SPSS Statistics (RRID: SCR_019096), version 28.0.1 (IBM Corporation, Armonk, NY, USA), was used for all statistical analyses.

Secondary end-point analysis

Where possible, continuous secondary end points have been adjusted for their baseline to improve the precision of estimated intervention effects. Repeated measures have been analysed using LME models at each outcome time point for the baseline of the measurement, age, sex, BMI within ethnicity, substudy inclusion, UK site and the missing indicator variable (unless there were no missing data for the baseline of the outcome). Comparisons between arms for binary outcomes are summarised as differences in proportions. Ninety-five per cent confidence intervals (CIs) have been used to make inferences from estimated effect sizes.

For the mechanistic substudy, iAUC were used to summarise responses at each of the baseline and 12-month follow-up visits. This iAUC approach was used for each mechanistic outcome unless indicated otherwise in the *Result* section (e.g. Oxidation) for reasons of clinical relevance or compatibility with previous research analysis methods.

As the substudy sample size was smaller, within-participant imputation was used when up to 4 post-baseline measurements were missing in measures where 10 or 11 time points were expected to be present. For measurements missing at the end of the series of measurements, the last-observation-carried-forward approach was used. For all other

missing measurements, a straight-line imputation approach was taken between observed measurements, using the values and timings of adjacent observed measurements.

For the 12-month follow-up visit, pre-baseline/baseline measurements were also imputed using the baseline measurement at the baseline visit from the same person on two occasions.

Due to non-negligible differences between groups in the baseline visit iAUC in small groups, the change from a 12-month visit to a baseline visit in the iAUC was analysed, to account for baseline differences. The number of participants present at the 12-month follow-up was lower than anticipated, so no attempt was made to further adjust for baseline using multiple regression as had been originally planned. Similarly, sensitivity analysis to imputations was not undertaken due to the small numbers with data at both time points, together with a lack of plausible pre-determined alternative assumptions.

Due to skewness in the data or small numbers present at the 12-month visit, the analysis to compare the two arms was expressed in terms of the more robust difference in medians, rather than means, and reporting the interquartile range (IQR) defined here as the first to the third quartile. The 95% CIs for the difference in medians between two independent samples were obtained using the Hodges–Lehmann estimator. An exception for this was the accelerometer outcome data, where daily data allowed more appropriately distributed summary averages to be calculated per outcome, and the non-parametric Bootstrap method was used to calculate 95% CIs.

Additional analyses

Information for all additional analyses, including sensitivity and safety, can be found in the SAP (see *Report Supplementary Material 1*).

Microbiome analysis

The data were analysed using Mothur (Dr Patrick Schloss, University of Michigan, Ann Arbor, MI, USA) and shared abundance tables and diversity indices were generated using www.microbiomeanalyst.ca/ (Xia Lab, McGill University, Montreal, Canada). A low count (four counts in < 10% of samples) and low variance (to remove 10% of samples based on IQR) filters were applied, so that samples with these characteristics are removed from data analysis. Total sum scaling was applied. Data were not rarefied or transformed.

Metabolic phenotyping multivariate data analysis

Changes in global urinary, serum and stool metabolic phenotypes were modelled using repeated measures partial least squares discriminant analysis (RM-PLSDA) in a Monte–Carlo cross-validation (MCCV) framework to potentially identify differences in the metabolic profiles between baseline versus 12 months for the IPE group and the control group, respectively. PLSDA analysis was conducted to compare differences between the control group and the IPE group and between the DELTA (12 months–baseline) control group and the IPE group. All analyses were performed using a MATLAB in-house script.[45]

Fat oxidation analysis

The iAUC was calculated as the area above the natural $^{13}CO_2$ abundance levels in breath CO_2 by the trapezoid rule and the summed area for each participant was calculated. The unit ppm ^{13}C is ppm ^{13}C above baseline ^{13}C abundance in breath. Basal ^{13}C is calculated as the average of the ^{13}C abundance in the t = −30 and −15 breath samples. Since the background (underlying) breath ^{13}C is not expected to deviate, we assume that the background ^{13}C is effectively a horizontal baseline. The added ^{13}C tracer causes an increase above the background ^{13}C natural abundance due to the oxidation of ^{13}C labelled palmitate. The areas under the curve (AUC) represents the ppm ^{13}C from the tracer that appeared in the breath as a result of whole-body palmitate oxidation.

De novo lipogenesis

Two indexes of lipogenesis were calculated: fasting DNL [fraction of newly synthesised palmitate from the first dose (previous evening) of D_2O measured at baseline] and postprandial DNL as a fraction of newly synthesised palmitate since the second D_2O dose administration. Fasting (basal) $^2H_1$ C16:0 enrichment in TAG was used to determine the fraction (F, %) of fatty acids from DNL using the measured (M + 1)/(M+) ratio in C16:0 and the precursor ^2H_2O

enrichment from FTIR as previously described.[46] Postprandial DNL was calculated using a similar methodology except the change in (M + 1)/(M+) ratio in C16:0 over the period 0–420 minutes was determined and using the average precursor pool $^{2}H_{2}O$ enrichment determined at baseline and 180 minutes measured by FTIR and a conventional fractional synthetic rate approach (%/day). Basal and postprandial DNL were analysed at baseline and 12 months and change from baseline was calculated.

Body composition by deuterium dilution

Body composition (FFM and FM) was calculated using the known gravimetrically prepared $D_{2}O$ dose administered to each participant and the measured ^{2}H enrichment in plasma with subtraction of the measured ^{2}H abundance in local tap water. FFM and FM were calculated as previously described.[47]

Chapter 4 Results

Recruitment

From July 2019 participants were recruited and screened for eligibility. Recruitment was paused between March and September 2020 due to COVID-19. The recruitment target of 270 participants was reached in October 2021. Participants were randomly allocated to receive IPE (*n* = 135) or inulin (*n* = 135). *Figure 3* shows cumulative participant recruitment per calendar month.

Recruitment strategies

Trial participants were recruited from the following sources, ranked from most to least productive:

- contacting NHS trusts
- contacting local general practices (GPs)
- social media adverts
- posters
- newspaper adverts
- pop-up events.

Screening

A total of 325 potential participants were screened. Most screening failures (*n* = 40) were due to BMI falling outside the eligible range. Other eligible individuals were not randomised due to withdrawal of consent (*n* = 6), lost to follow-up (*n* = 3) or 'other reasons', for example, job commitments or loss of interest (*n* = 3).

Participant flow

The Consolidated Standards of Reporting Trials (CONSORT) flow diagram (*Figure 4*) shows the movement of participants through the trial.

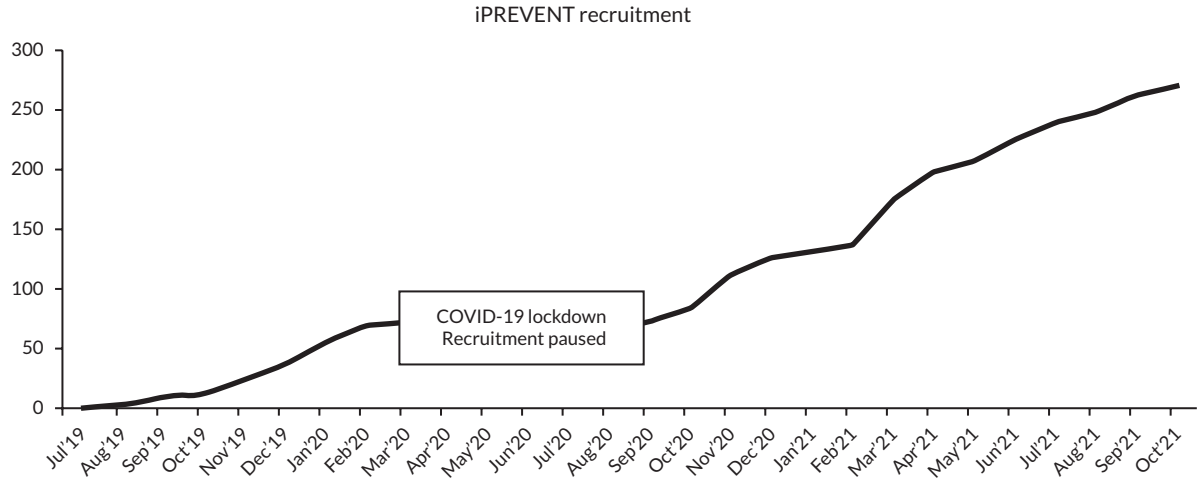

FIGURE 3 Graph demonstrating cumulative participant accrual per calendar month.

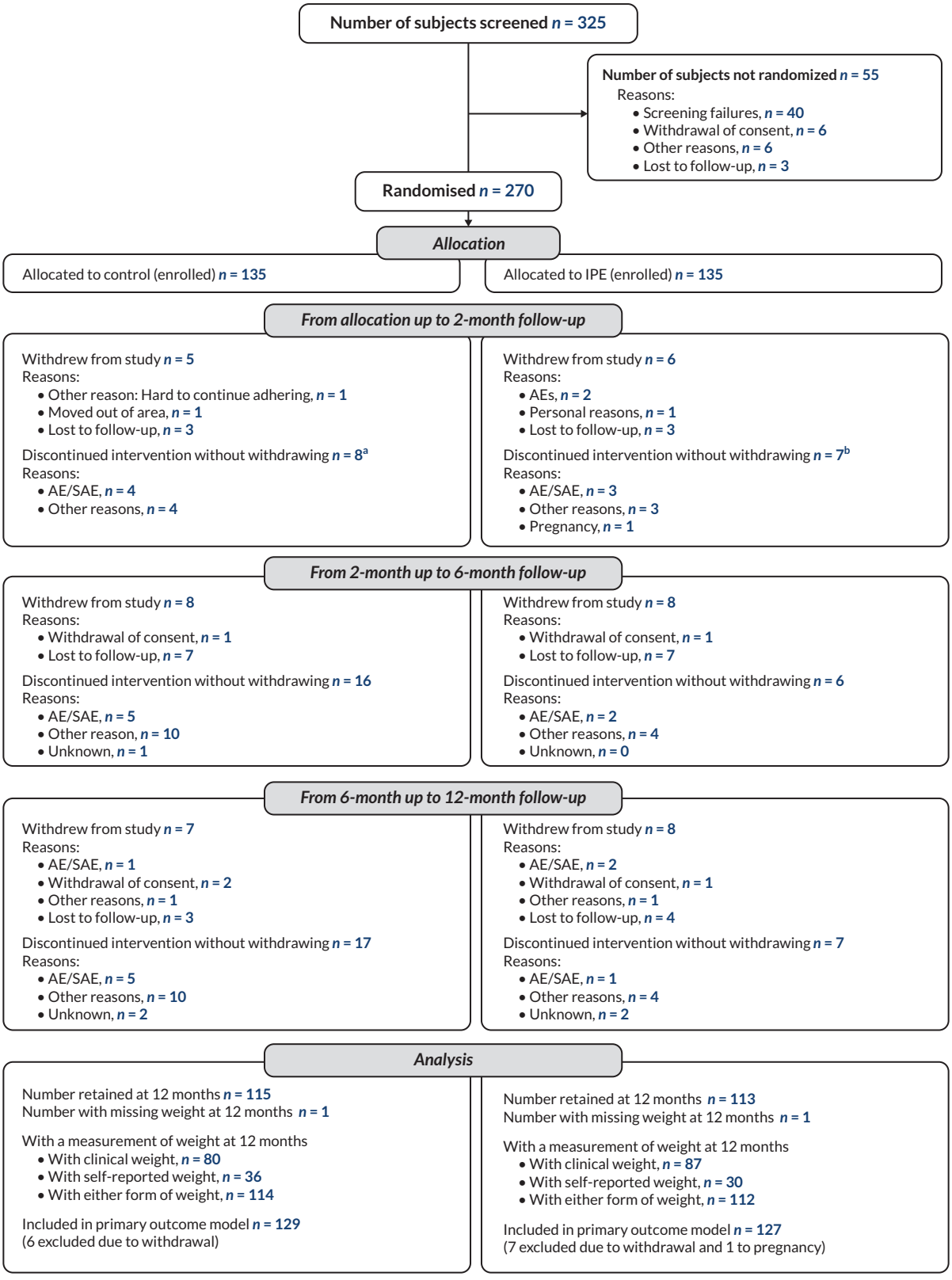

FIGURE 4 Participant CONSORT flow diagram. a, One of these participants withdrew later in the 2- to 6-month period. b, Two of these participants withdrew later in the 6- to 12-month period.

Baseline data

The baseline characteristics were well balanced between arms, as seen in *Table 3*.

TABLE 3 Sociodemographic and clinical characteristics by arm

	Con *N* = 135 *N* (% total)	IPE *N* = 135 *N* (% total)	Total *N* = 270 *N* (% total)
Age [mean (SD)]	30.1 (5.7)	30.3 (5.2)	30.2 (5.4)
(minimum–maximum)	(21–40)	(20–40)	(20–40)
Sex			
Female	86 (64%)	86 (64%)	172 (64%)
Male	49 (36%)	49 (36%)	98 (36%)
Ethnicity			
White	95 (70%)	90 (67%)	185 (68%)
Asian	20 (15%)	14 (10%)	34 (13%)
Mixed	6 (4%)	7 (5%)	13 (5%)
Black	4 (3%)	8 (6%)	12 (4%)
Any other ethnic group	10 (7%)	16 (12%)	26 (10%)
Smoking status			
Current	17 (13%)	25 (19%)	42 (16%)
Ex-smoker	28 (21%)	25 (19%)	53 (20%)
Never	90 (67%)	85 (63%)	175 (65%)
Vaping status			
Current	11 (8%)	8 (6%)	19 (7%)
Ex-vaper	3 (2%)	4 (3%)	7 (3%)
Never	121 (90%)	123 (91%)	244 (90%)
Drinking status[a]			
Current	110 (81%)	106 (79%)	216 (80%)
Ex-drinker	9 (7%)	12 (9%)	21 (8%)
Never	16 (12%)	16 (12%)	32 (12%)
Recreational drugs taken in the past year	6 (4%)	7 (5%)	13 (5%)
Weight (kg) [mean (SD)]	79.1 (10.6)	79.6 (10.9)	79.3 (10.7)
Height (cm) [mean (SD)]	169.5 (9.3)	169.6 (9.5)	169.6 (9.3)
Waist (cm) [mean (SD)]	93.2 (8.2)	94.0 (8.7)	93.6 (8.5)
BMI (kg/m²) [mean (SD)]	27.4 (1.5)	27.5 (1.6)	27.5 (1.5)

Con, inulin control.
a Missing one participant in the IPE arm.

Withdrawals

There were 42 complete study withdrawals. Rates of withdrawal were consistent until 6 months when there were 19 more withdrawals for the control group than the intervention, as seen in *Table 4*. The difference in the withdrawal rate between arms at 6 months was 0.7% (−6.4% to 7.9%) and at 12 months it was 1.5% (−10.1% to 7.2%). The difference between arms in those either withdrawing or discontinuing the supplement at 6 months was −7.4% (−17.5% to 2.7%), and at 12 months it was −14.1% (−25.5% to −2.6%). The cumulative withdrawal curve for this study can be seen in *Figure 5*. A comparison of the baseline characteristics between those who withdrew from the study and those who did not are shown in *Table 5*. One participant was found to be pregnant during the trial; the participant was withdrawn from the intervention for safety but was followed up until the end of the trial, as there was no withdrawal of consent. Follow-up data collected were only included up to the point of pregnancy.

TABLE 4 Cumulative numbers over study time points per arm who withdrew from the study and discontinued the supplement

	Con			IPE			Total
	Up to 2 months *N* = 135 *n* (%)	Up to 6 months *N* = 135 *n* (%)	Up to 12 months *N* = 135 *n* (%)	Up to 2 months *N* = 135 *n* (%)	Up to 6 months *N* = 135 *n* (%)	Up to 12 months *N* = 135 *n* (%)	Up to 12 months *N* = 270 *n* (%)
Withdrawn	5 (4)	13 (10)	20 (15)	6 (4)	14 (10)	22 (16)	42 (16)
Discontinued supplementation	8 (6)	24 (18)	41 (30)	7 (5)	13 (10)	20 (15)	61 (23)
Total	13 (10)	37 (27)	61 (45)	13 (10)	27 (20)	42 (31)	103 (38)

Con, inulin control.

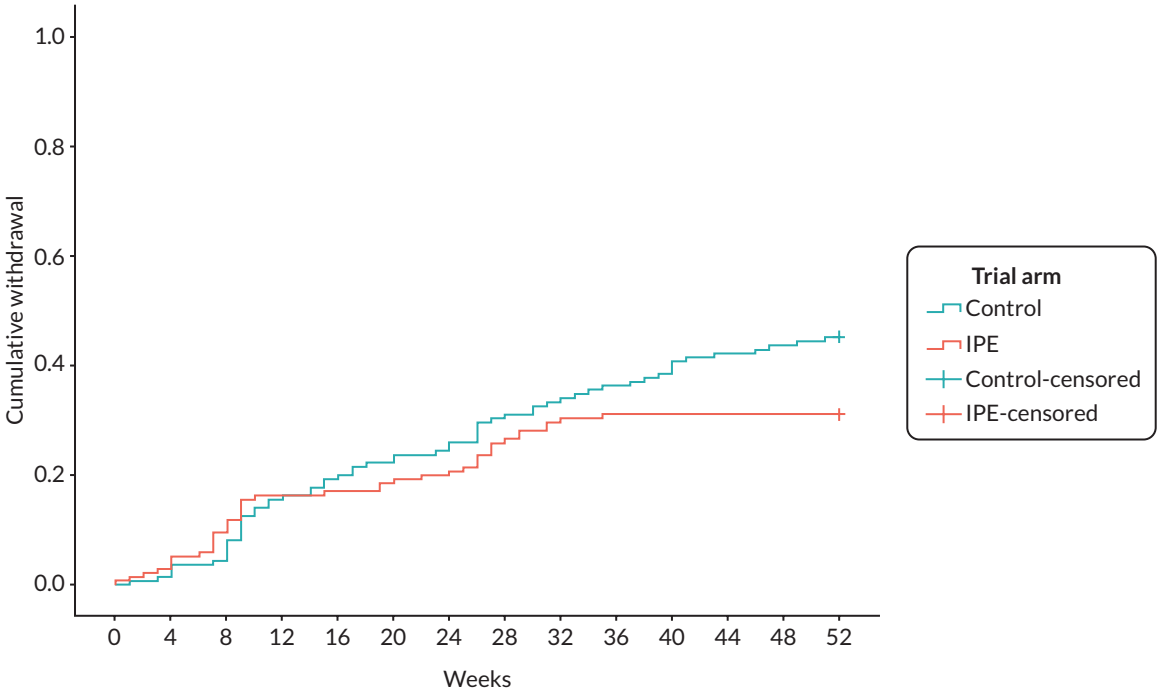

FIGURE 5 Kaplan–Meier curves showing the cumulative withdrawal in the study over time by study arm.

TABLE 5 Baseline characteristics and randomisation stratifiers of those who withdrew from the study and those who completed the study

	Withdrew from study		Completed study	
	Con *N* = 20	IPE *N* = 22	Con *N* = 115	IPE *N* = 113
Age [mean (SD)]	29.1 (4.4)	29.0 (5.6)	30.2 (5.8)	30.5 (5.1)
Sex (female) [% (*n*)]	50% (10)	73% (16)	66% (76)	62% (70)
Site (London) [% (*n*)]	60% (12)	50% (11)	63% (72)	65% (74)
In substudy [% (*n*)]	20% (4)	9% (2)	20% (23)	20% (23)
BMI within ethnicity [% (n)]				
Non-South Asian, BMI category: 25.00–27.49 kg/m^2	50% (11)	50% (10)	41% (46)	41% (47)
Non-South Asian, BMI category: 27.50–30.00 kg/m^2	41% (9)	40% (8)	46% (52)	45% (52)
South Asian, BMI category: 24.00–25.49 kg/m^2	5% (1)	0% (0)	4% (4)	4% (5)
South Asian, BMI category: 25.50–27.00 kg/m^2	5% (1)	10% (2)	10% (11)	10% (11)
Baseline weight [mean (SD)]	81.6 (10.1)	77.0 (11.6)	78.7 (10.6)	80.1 (10.7)

Con, inulin control.

Primary outcome

Numbers analysed

This resulted in 84% (226/270) of participants providing a principal weight (the weight used in the primary analysis and is the clinic-measured weight, or, where this is missing, the self-reported measured weight), for the 12-month analysis, meeting the required participant follow-up to the primary outcome of 80% in the power calculation. Of this, 62% (167/270) provided a clinic weight, and a further 22% (59/270) provided self-reported weight, reaching a total of 84%. The distribution of measured weights is comparable between arms, as shown in *Table 6*.

Unadjusted mean changes in weight from baseline

Figure 6 shows the change in weight from baseline to each study time point. This is a basic initial view of the observed data. There are reduced numbers of participants as time goes on due to dropout, and the data are unadjusted (*Table 7*). The type of weight measured (clinic vs. self-reported) also varies over time. The primary outcome analysis, shown shortly, accounts for these other factors. Ninety-five per cent CIs are added around each point in the plot to indicate the variability of each mean plotted. This error bar variability is associated with (1) the sample size and (2) the participant variability of the measurements at the time points in the relevant arm.

Adjusted weight change from baseline

The adjusted weight at each time point is shown in *Table 8*. Adjustments were made for the covariates of the source of the measured weight (clinic vs. self-reported), baseline weight, age, sex, BMI by ethnicity, whether included in the substudy and UK site in a LME model. The model was run on 256 (95% of randomised) participants (129 in the control arm and 127 in the IPE arm); 200 participants had all 3 follow-up time points, 30 had 2 and 26 had 1. There was no significant arm effect between principal weight measurement type, that is clinical or self-reported (by three-way interaction *p*-value = 0.23 at 12 months). The treatment arm effect of clinic weight measurements only at 12 months was + 0.77 (−0.78 to 2.32), giving consistent conclusions.

Sensitivity analysis for missing data

The primary outcome analysis resulted in an estimate of the treatment effect of 1.02 kg (−0.37 to + 2.41). This adjusted for baseline weight and minimisation covariates and accounted for interim measurements of weight at 2- and 6-month follow-ups in those who had a 12-month weight missing. The treatment effect therefore is estimated assuming that a

TABLE 6 Data availability for clinical and self-reported weight by visit and study arm

	Baseline			2 months			6 months			12 months		
	Con *N*	IPE *N*	Total *N*	Con *N*	IPE *N*	Total *N*	Con *N*	IPE *N*	Total *N*	Con *N*	IPE *N*	Total *N*
Clinic weight	135	135	270	89	100	189	74	78	152	80	87	167
Self-reported weight	3	5	8	35	28	63	33	30	63	36	30	66
Clinic weight only	132	130	262	86	97[a]	183	73	78[a]	151[a]	78	82	160
Self-reported weight only	0	0	0	32	25	57	32	30	62	33	24[a]	57
Both[a]	3	5	8	3	3	6	1	0	1	2	5	7
Principal weight[b] {*N* [% (*n*/*N*)]}	135 [100% (135/135)]	135 [100% (135/135)]	270 [100% (270/270)]	121 [90% (121/135)]	125 [93% (125/135)]	246 [91% (246/135)]	106 [79% (106/135)]	108 [80% (108/135)]	214 [79% (214/270)]	114 [84% (114/135)]	112[a] [83% (112/135)]	226[a] [84% (226/270)]
Clinic weight was used as principal weight	135	135	270	89	100	189	74	78	152	80	87	167
Neither	0	0	0	14	9	23	29	26	55	22	23	45

Con, inulin control.
a Self-reported weight was collected in addition to clinic weight in a small number of participants.
b Principal weight is the weight used for the analysis and is the clinic-measured weight, or, where this is missing, the self-reported measured weight.

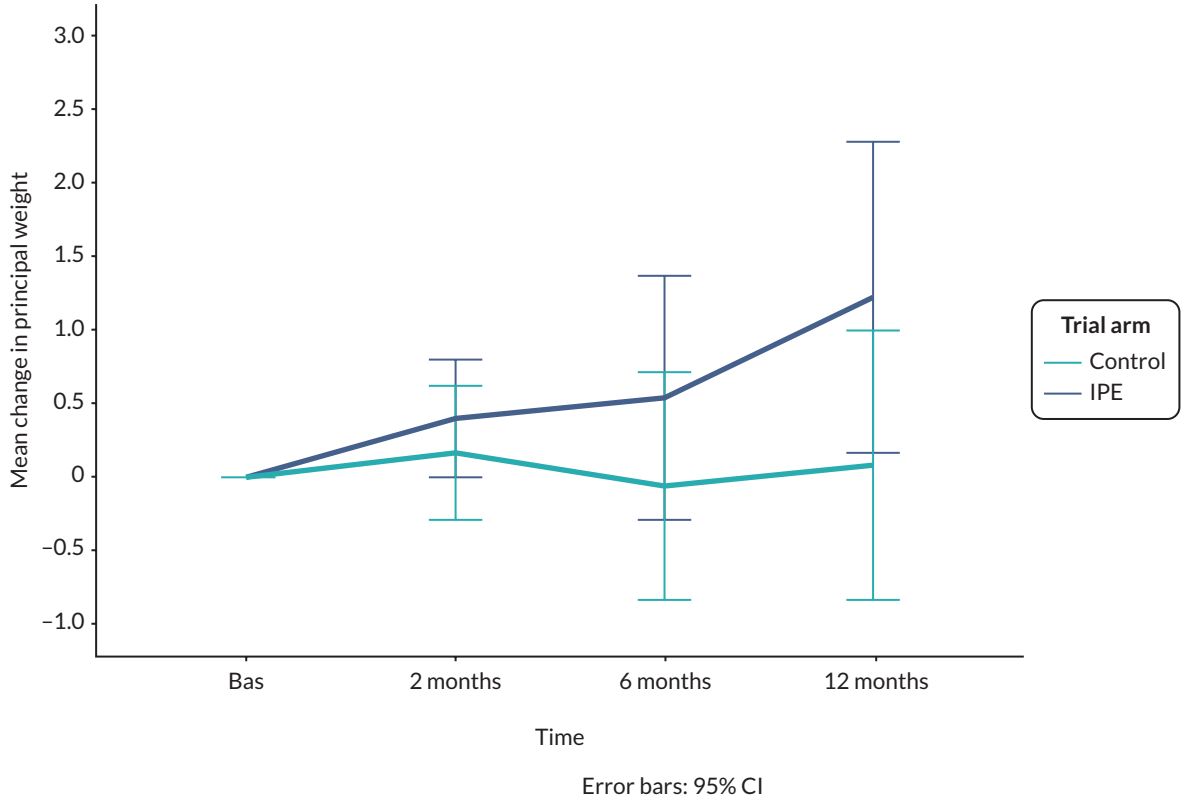

FIGURE 6 Unadjusted mean changes from baseline per arm across the study time points.

TABLE 7 Unadjusted mean changes over time within each trial arm

	Con		IPE	
Body weight (kg)	***N***	**Mean (SE)**	***N***	**Mean (SE)**
Baseline principal weight[a]	135	79.1 (0.9)	135	79.6 (0.9)
Unadjusted change from baseline to 2 months in clinical weight	89	0.24 (0.27)	100	0.32 (0.2)
Unadjusted change from baseline to 2 months in self-reported weight	35	0.03 (0.40)	28	0.69 (0.53)
Unadjusted change from baseline to 2 months in principal weight	121	0.16 (0.23)	125	0.40 (0.20)
Unadjusted change from baseline to 6 months in clinical weight	74	0.03 (0.48)	78	0.45 (0.46)
Unadjusted change from baseline to 6 months in self-reported weight	33	−0.39 (0.67)	30	0.75 (0.95)
Unadjusted change from baseline to 6 months in principal weight	106	−0.07 (0.39)	108	0.53 (0.42)
Unadjusted change from baseline to 12 months in clinical weight	80	0.30 (0.57)	87	1.03 (0.58)
Unadjusted change from baseline to 12 months in self-reported weight	36	−0.70 (0.80)	30	1.24 (1.23)
Unadjusted change from baseline to 12 months in principal weight	114	0.07 (0.47)	112	1.22 (0.53)

Con, inulin control; SE, standard error.
a Principal weight is the weight used for the analysis and is the clinic-measured weight, or, where this is missing, the self-reported measured weight.

TABLE 8 Adjusted difference in weight between arms

Body weight (kg)	Con		IPE		Adjusted difference in means[a] (IPE – Con) (95% CI)
	N	Mean (SD)	*N*	Mean (SD)	
At baseline					
Clinical weight	135	79.1 (10.6)	135	79.6 (10.9)	N/A
Self-reported weight	3	83.3 (3.1)	5	80.0 (12.7)	
Principal weight	135	79.1 (10.6)	135	79.6 (10.9)	
At 2 months					
Clinical weight	89	80.3 (11.5)	100	80.7 (11)	
Self-reported weight	35	77.6 (10.0)	28	79.2 (9.9)	
Principal weight	121	79.5 (11.2)	125	80.5 (10.8)	0.20 (−0.41 to 0.81)
At 6 months					
Clinical weight	74	80.3 (11.1)	78	80.1 (10.6)	
Self-reported weight	33	75.9 (9.9)	30	83.1 (11.3)	
Principal weight	106	78.9 (10.9)	108	80.9 (10.9)	0.19 (−0.90 to 1.28)
At 12 months					
Clinical weight	80	79.8 (10.5)	87	80.8 (11.1)	
Self-reported weight	36	76.4 (13.9)	30	81.4 (14.6)	
Principal[b] weight	114	78.9 (11.8)	112	81.4 (11.9)	1.02 (−0.37 to 2.41) $p = 0.15$

Con, inulin control; NA, not applicable.
a This was adjusted at each time point for the covariates of the source of the measured weight (clinic vs. self-reported), baseline weight, age, sex, BMI by ethnicity, whether included in substudy and UK site.
b Principal weight is the weight used for the analysis and is the clinic-measured weight, or, where this is missing, the self-reported measured weight.

person with unobserved 12-month weight would have no (or zero) difference, on average, from a similar person with the same covariates and interim weight measurements.

Sensitivity analysis to investigate the potential impact on the treatment effect under this 'missing at random' assumption involves instead assuming that for those with unobserved 12-month weight, a quantity δ, other than zero, is added to the 1.02 kg treatment effect that was estimated under the MAR assumption.

Under the first scenario, in IPE participants only, the quantity δ is assumed to range, broadly, from −5 kg (more weight lost compared to those reporting weight) up to a value of +5 kg (more weight gained), whereas the control participants with unobserved weight are not varied relative to their counterparts with weight observed. This causes departures from the 1.02 (−0.37, 2.41) estimate. These are limited by the 17% of IPE participants with missing 12-month weight data. *Table 9* shows that if the IPE participants with missing weight had 5 kg lower weight than expected, then the treatment effect would be estimated to be 0.18 kg, whereas 5 kg higher than expected would increase the effect size to 1.89 kg.

The second scenario considers variation in the control arm. If those with missing weights in the control arm had 5 kg lower weight than expected from their counterparts with reported weight, the treatment effect would rise to 1.81 kg. It would fall to 0.26 kg on the assumption of 5 kg higher weight in control arm participants with weight unobserved. The resulting treatment effects are similar in range to those in the first scenario, as the dropout in the control arm of 16% is similar to that of the IPE arm (see *Table 9*).

The third scenario considers the same effect operating in both arms, and the treatment effect is estimated to range between 0.96 kg and 1.11 kg, largely unchanged from the estimated 1.02 kg treatment effect (see *Table 9*).

In summary, as the estimated treatment effects in the table are all positive-valued, these broad-range departures from the assumptions do not alter the direction of the estimated treatment effect. *Figure 7* gives a comprehensive view of the impact of the treatment effect under the three scenarios and the three coloured scenario lines lie above zero, indicating that a treatment effect estimate favouring IPE is unlikely to arise from alternative assumptions about the missing data.

TABLE 9 Linear mixed effects treatment estimates for the three sensitivity analysis scenarios

	$\delta = -5$		$\delta = +5$	
	Estimated treatment effect	**95% CI**	**Estimated treatment effect**	**95% CI**
First scenario: Depart from MAR in the IPE arm only	0.17	−1.22 to 1.56	1.87	0.48 to 3.26
Second scenario: Depart from MAR in the control arm only	1.80	0.41 to 3.19	0.24	−1.15 to 1.63
Third scenario: Depart from MAR in both arms	0.95	−0.45 to 2.34	1.09	−0.30 to 2.49

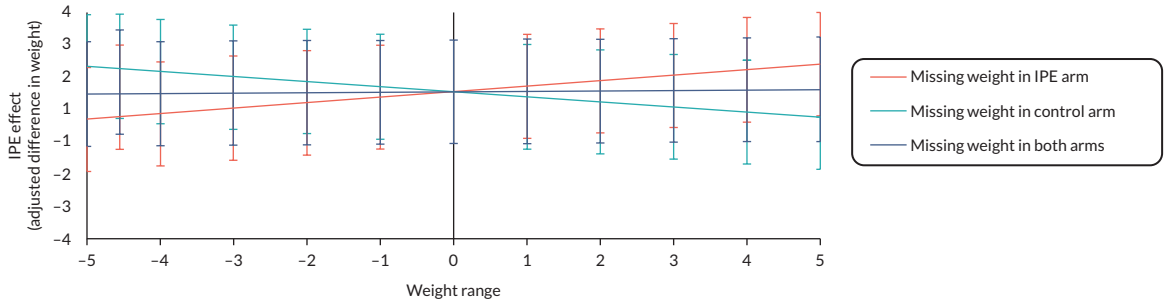

FIGURE 7 Estimates of the LME treatment effect under three sensitivity analysis scenarios.

The fraction of missing data is quite small. Those with unobserved data compared to those with observed data would have to have a δ of at least −6 kg in the first scenario (IPE arm only) to have a negative estimate, or at least −15 kg for a negative and statistically significant estimate. On the other hand, for scenario two (control arm only), δ would have to be at least + 7 kg for a negative estimate or + 16 kg to have a negative and significant estimate.

Sensitivity analysis for adverse events potentially affecting weight

In the sensitivity analysis for those participants who reported an AE directly or indirectly affecting their weight, there was one control-arm participant who reported having COVID-19 during their 6-month visit. No effect on treatment estimate was detected after removing this participant's weight at this time point.

Secondary outcomes

Body mass index, waist and waist–hip ratio

There was no significant difference in measures of BMI, waist circumference and waist-to-hip ratio, after 12 months of IPE intake (*Table 10*).

TABLE 10 Adjusted difference in means in BMI, waist, and waist–hip ratio between arms

	Con		IPE		
	N	Mean (SD)	*N*	Mean (SD)	Adjusted difference in means (IPE – Con) (95% CI)
BMI (kg/m²)					
Baseline	135	27.4 (1.5)	135	27.5 (1.6)	
2 months	89	27.6 (1.7)	100	27.7 (1.6)	0.08 (−0.13 to 0.28)
6 months	74	27.6 (1.8)	78	27.6 (1.9)	0.11 (−0.26 to 0.48)
12 months	80	27.6 (2.2)	87	27.9 (2.4)	0.39 (−0.10 to 0.87) (*N* = 227)[a]
Waist (cm)					
Baseline	135	93.1 (8.1)	135	93.8 (8.7)	
2 months	88	92.2 (8.5)	101	93.7 (8.5)	0.15 (−0.95 to 1.26)
6 months	74	92.5 (8.6)	78	92.0 (8.0)	−0.77 (−2.32 to 0.78)
12 months	78	92.3 (8.8)	85	92.9 (8.1)	0.01 (−1.58 to 1.60) (*N* = 226)[a]
Waist/hip ratio					
Baseline	135	0.86 (0.07)	135	0.87 (0.08)	
2 months	88	0.85 (0.07)	101	0.86 (0.07)	0.00 (−0.01 to 0.02)
6 months	74	0.85 (0.07)	78	0.85 (0.07)	−0.01 (−0.02 to 0.01)
12 months	78	0.86 (0.07)	85	0.90 (0.10)	−0.01 (−0.02 to 0.01) (*N* = 226)[a]

Con, inulin control.
a *N* = total number of participants including those whose 2- or 6-month visit measurements were carried over for participants who did not have 12-month measurements.

Body composition measurements

Body composition measurement results can be found in *Table 11*. No differences were seen for body fat (kg and %) and FM to FFM ratio. There were significant differences for body water of 0.72 (0.10 to 1.33), which remained significant at 0.72 (0.17 to 1.28) after removal in sensitivity analysis of two outliers in the 12-month data (> 4 SD residuals). Similarly, the estimate of 1.07 (0.21 to 1.23) for FFM remained significant 1.08 (0.29 to 1.86) after removing two outliers.

Pulse and blood pressure measurements

There were no significant differences in pulse and blood pressure measurements after the 12-month intervention. These data can be found in *Table 12*.

Fasting biochemistry measurements

No significant differences were found for the fasting biochemistry outcomes, except glucose which had a treatment effect of 0.11 (0.01 to 0.21). Natural log transformation was conducted prior to analysis for fasting triglycerides and insulin. The result was not sensitive to the single outlier (a participant in the IPE arm with HDL values of 1.9, 1.0 and 0.8 at baseline, 6 and 12 months). The treatment effect excluding this outlier was 0.00 (−0.06 to 0.07). The results of the fasting biochemistry analyses are seen in *Table 13*.

TABLE 11 Adjusted difference in means in body composition measures between arms

	Con		IPE		Adjusted difference in means (IPE – Con) (95% CI)
	N	Mean (SD)	*N*	Mean (SD)	
Body fat (kg)					
Baseline	135	24.2 (6.2)	134	24.8 (5.7)	
2 months	88	23.7 (6.9)	100	24.6 (5.6)	−0.15 (−0.73 to 0.43)
6 months	74	24.5 (6.9)	79	25.0 (6.4)	0.36 (−0.64 to 1.35)
12 months	78	24.4 (7.0)	86	24.9 (6.6)	0.07 (−1.07 to 1.21) (*N* = 226)[a]
Body fat (%)					
Baseline	135	30.8 (7.6)	134	31.5 (7.2)	
2 months	88	29.7 (8.1)	99	30.8 (7.1)	−0.1 (−0.8 to 0.5)
6 months	74	30.6 (8.6)	85	31.3 (8.0)	0.2 (−0.1 to 1.5)
12 months	68	31.1 (8.4)	76	31.3 (7.2)	0.2 (−1.0 to 1.3) (*N* = 217)[a]
Body water (kg)					
Baseline	135	39.7 (7.8)	134	39.5 (7.9)	
2 months	88	41.0 (8.3)	100	40.4 (8.0)	0.30 (−0.14 to 0.74)
6 months	74	40.4 (8.1)	79	39.8 (7.3)	0.07 (−0.56 to 0.71)
12 months	78	40.0 (7.5)	86	40.5 (7.6)	0.72 (0.10 to 1.33) (*N* = 226)[a]
FFM (kg)					
Baseline	135	55.0 (10.9)	134	54.8 (11.1)	
2 months	88	56.7 (11.4)	100	56.2 (11.5)	0.53 (−0.10 to 1.17)
6 months	74	55.8 (11.1)	79	55.2 (10.3)	0.04 (−0.82 to 0.91)
12 months	77	55.2 (10.5)	86	56.1 (10.7)	1.07 (0.21 to 1.93) (*N* = 226)[a]
FM/FFM ratio					
Baseline	135	46.2 (15.9)	134	47.5 (15.7)	
2 months	88	44.1 (16.7)	100	46.0 (14.6)	−0.3 (−1.7 to 0.1)
6 months	58	45.6 (17.6)	68	46.8 (15.8)	0.1 (−2.3 to 2.4)
12 months	58	45.6 (16.9)	68	46.2 (14.6)	0.0 (−1.9 to 1.9) (*N* = 198)[a]
FM index (kg/m²)					
Baseline	135	8.5 (2.3)	134	8.7 (2.1)	
2 months	88	8.2 (2.5)	100	8.5 (2.1)	−0.04 (−0.24 to 0.16)
6 months	74	8.5 (2.5)	79	8.7 (2.3)	0.14 (−0.20 to 0.48)
12 months	78	8.5 (2.6)	86	8.7 (2.4)	0.02 (−0.38 to 0.42) (*N* = 226)[a]

Con, inulin control.

a *N* = total number of participants including those whose 2- or 6-month visit measurements were carried over for participants who did not have 12-month measurements.

TABLE 12 Adjusted difference in means in pulse, systolic and distal blood pressure between trial arms

	Con		IPE		Adjusted difference in means (IPE – Con) (95% CI)
	N	Mean (SD)	*N*	Mean (SD)	
Pulse (bpm)					
Baseline	135	68.9 (10.2)	135	71.4 (11.0)	
2 months	88	71.6 (9.6)	101	72.5 (11.2)	−0.69 (−3.01 to 1.62)
6 months	74	69.5 (10.2)	79	70.3 (10.6)	0.53 (−1.76 to 2.82)
12 months	79	71.3 (11.1)	86	71.7 (10.5)	0.18 (−2.59 to 2.95) (*N* = 226)[a]
DBP (mmHg)					
Baseline	135	70.3 (9.7)	135	72.1 (9.8)	
2 months	88	72.6 (9.2)	101	70.9 (8.6)	−1.63 (−3.33 to 0.06)
6 months	74	71.9 (9.0)	79	70.5 (9.6)	−1.44 (−3.42 to 0.54)
12 months	79	71.4 (8.9)	86	71.2 (9.7)	−0.64 (−2.71 to 1.44) (*N* = 226)[a]
SBP (mmHg)					
Baseline	135	113.2 (10.5)	135	116.2 (11.3)	
2 months	88	116.1 (9.6)	101	115.7 (10.9)	−1.25 (−3.62 to 1.11)
6 months	74	115.4 (10.8)	79	114.3 (10.6)	−2.19 (−4.90 to 0.52)
12 months	79	115.8 (10.6)	86	115.7 (11.6)	−1.30 (−3.84 to 1.24) (*N* = 226)[a]

Con, inulin control; DBP, distal blood pressure; SBP, systolic blood pressure.
a *N* = total number of participants including those whose 2- or 6-month visit measurements were carried over for participants who did not have 12-month measurements.

TABLE 13 Adjusted difference in means in fasting biochemistry measurements and glucose between trial arms

	Con		IPE		Adjusted difference in means (IPE – Con) (95% CI)
	N	Mean (SD)	*N*	Mean (SD)	
Ln triglycerides (mmol/l)					
Baseline	133	−0.07 (0.52)	132	−0.10 (0.46)	
6 months	71	−0.06 (0.46)	77	−0.07 (0.47)	0.04 (−0.07 to 0.15)[a]
12 months	80	−0.07 (0.58)	86	−0.11 (0.46)	0.01 (−0.11 to 0.13)[a] (*N* = 191)[b]
Total cholesterol (mmol/l)					
Baseline	133	4.58 (0.74)	134	4.70 (0.94)	
6 months	71	4.68 (0.84)	77	4.71 (0.91)	−0.10 (−0.30 to 0.09)
12 months	80	4.69 (0.93)	86	4.66 (0.85)	−0.12 (−0.32 to 0.09) (*N* = 191)[b]
LDL cholesterol (mmol/l)					
Baseline	132	2.72 (0.67)	131	2.90 (0.83)	
6 months	71	2.75 (0.79)	77	2.82 (0.79)	−0.08 (−0.26 to 0.09)
12 months	79	2.78 (0.79)	86	2.82 (0.73)	−0.12 (−0.30 to 0.05) (*N* = 191)[b]

TABLE 13 Adjusted difference in means in fasting biochemistry measurements and glucose between trial arms (*continued*)

	Con		IPE		Adjusted difference in means (IPE – Con) (95% CI)
	N	Mean (SD)	*N*	Mean (SD)	
HDL cholesterol (mmol/l)					
Baseline	133	1.37 (0.36)	133	1.35 (0.31)	
6 months	71	1.44 (0.37)	77	1.42 (0.35)	−0.04 (−0.11 to 0.02)
12 months	80	1.40 (0.38)	86	1.37 (0.30)	0.00 (−0.07 to 0.07) (*N* = 191)[b]
Glucose (mmol/l)					
Baseline	125	4.65 (0.42)	127	4.59 (0.40)	
6 months	71	4.74 (0.42)	78	4.68 (0.47)	0.01 (−0.11 to 0.14)
12 months	80	4.66 (0.39)	85	4.74 (0.39)	0.11 (0.01 to 0.21) (*N* = 191)[b]
Ln insulin (pmol/l)					
Baseline	131	4.52 (0.88)	130	4.50 (0.97)	
6 months	68	4.42 (0.98)	75	4.45 (0.89)	0.10 (−0.10 to 0.30)[c]
12 months	75	4.60 (0.80)	84	4.47 (0.86)	0.03 (−0.13 to 0.20)[c] (*N* = 187)[b]

Con, inulin control; Ln, natural log.
a Before natural log transformation, the means (SD) of triglycerides at baseline, 6 and 12 months in the control arm, respectively, were, in mmol/l, 1.08 (0.06), 1.05 (0.07), 1.14 (0.10) and for IPE were 1.01 (0.05), 1.04 (0.06), 1.00 (0.06). The adjusted difference in means (with CI) is presented on the natural log scale, and on taking the anti-log is equivalent to a more interpretable ratio of the geometric means of 1.04 mmol/l 95% CI (0.93 to 1.16) at 6 months and 1.01 95% CI (0.90 to 1.14) at 12 months.
b *N* = total number of participants including those whose 2- or 6-month visit measurements were carried over for participants who did not have 12-month measurements.
c Before natural log transformation, the means (SD) of insulin at baseline, 6 and 12 months in the control arm, respectively, were, in mmol/l, 126.6 (100.5), 121.5 (100.7) and 133.7 (109.3), and for IPE were 138.7 (159.1), 122.6 (112.2) and 125.0 (123.4). The adjusted difference in means (with CI) is presented on the natural log scale, and on taking the anti-log is equivalent to a more interpretable ratio of the geometric means of 1.10 mmol/l with 95% CI (0.90 to 1.34) at 6 months and 1.03 with 95% CI (0.88 to 1.22) at 12 months.

Lifestyle factors

Physical activity, as measured by IPAQ, was similar between the arms. Over time in each arm, there was a slight shift towards higher physical activity as seen in *Table 14*.

Similar numbers in smoking, vaping, drinking and recreational drug status are found between arms and over time, as shown in *Table 15*. For participants who did not report lifestyle changes but had a visit date, it was assumed that the smoking, vaping, drinking and drugs status had not changed from the previous visit.

TABLE 14 International Physical Activity Questionnaire summary statistics over time and by study arm

	Baseline		2 months		6 months		12 months	
Physical activity level	Con % (*n*)	IPE % (*n*)	Con % (*n*)	IPE % (*n*)	Con % (*n*)	IPE % (*n*)	Con % (*n*)	IPE % (*n*)
High	17 (23)	18 (24)	27 (32)	17 (21)	24 (25)	28 (31)	24 (23)	24 (24)
Moderate	40 (54)	38 (51)	31 (36)	36 (44)	38 (39)	42 (46)	38 (36)	41 (41)
Low	43 (58)	44 (60)	42 (49)	46 (56)	38 (39)	29 (32)	39 (37)	34 (34)
Total *n*	135	135	117	121	103	109	96	99
No. missing	0	0	13 (18)	10 (14)	24 (32)	19 (26)	29 (39)	27 (36)

Con, inulin control.

TABLE 15 Smoking, vaping, drinking and recreational drug status over time and by study arm

	Baseline		2 months		6 months		12 months	
	Con *N* = 135 % (*n*)	IPE *N* = 135 % (*n*)	Con *N* = 135 % (*n*)	IPE *N* = 135 % (*n*)	Con *N* = 135 % (*n*)	IPE *N* = 135 % (*n*)	Con *N* = 135 % (*n*)	IPE *N* = 135 % (*n*)
Smoking								
Current	14 (19)	19 (25)	17 (20)	17 (21)	13 (14)	15 (16)	11 (13)	14 (16)
Ex-smoker	19 (26)	19 (25)	18 (22)	21 (26)	21 (22)	20 (22)	22 (25)	22 (25)
Never	67 (90)	63 (85)	65 (79)	63 (79)	66 (69)	65 (72)	67 (77)	63 (71)
No. missing	0	0	17 (14)	7 (9)	22 (30)	19 (25)	15 (20)	17 (23)
Vaping								
Current	9 (12)	6 (8)	8 (10)	6 (8)	9 (9)	7 (8)	9 (10)	7 (8)
Ex-vaper	2 (3)	3 (4)	4 (5)	3 (4)	4 (4)	3 (3)	5 (6)	4 (4)
Never	89 (120)	91 (123)	88 (106)	90 (114)	88 (92)	90 (99)	86 (99)	89 (100)
No. missing	0	0	10 (14)	7 (9)	22 (30)	19 (25)	15 (20)	17 (23)
Drinking								
Current	81 (110)	79 (106)	82 (99)	78 (98)	82 (86)	80 (87)	82 (94)	79 (88)
Ex-drinker	7 (9)	9 (12)	5 (6)	10 (12)	5 (5)	9 (10)	6 (7)	10 (11)
Never	12 (16)	12 (16)	13 (16)	12 (15)	13 (14)	11 (12)	12 (14)	11 (12)
No. missing	0	1 (1)	10 (14)	7 (10)	22 (30)	19 (26)	15 (20)	18 (24)
Recreational drugs								
No	95 (128)	96 (129)	93 (112)	95 (121)	92 (99)	94 (104)	92 (107)	94 (107)
Yes	5 (7)	4 (6)	7 (9)	5 (6)	8 (9)	6 (7)	8 (9)	6 (7)
No. missing	0	0	10 (14)	6 (8)	20 (27)	18 (24)	14 (19)	16 (21)

Con, inulin control.

Safety outcomes

The safety population included in this report relates to all randomised participants. This differs from the SAP, where the safety population was envisaged to be defined to be all participants who took at least one sachet. However, there is no certainty that participants took zero sachets between baseline and the date they provided for supplement cessation, to be able to assume that safety events were not associated with having taken treatment. Additionally, other participants were lost to follow-up before returning any used or unused sachets. There were 339 AEs in a total of 151 (56%) participants with 160 AEs in 76 (56%) participants in the IPE arm, and 180 in 76 (56%) participants in the control arm. There were four SAEs, two in the control arm and two in the IPE arm. Therefore, reporting of AEs and SAEs did not differ between the two arms. The Wilson score-based method with no continuity correction for 95% CIs was used to provide an uncertainty range when there were fewer than five events in either of the arms.[48] *Tables 16–18* summarise AEs and SAEs.

There were a greater number of moderate-severity gastrointestinal-related AEs in the control arm in comparison to the IPE arm as seen in *Figure 8*.

TABLE 16 Summary of AEs by arm

	IPE *N* = 135 *N* (%)	Con *N* = 135 *N* (%)	Difference in proportions (95% CI) (IPE – control)
Participants reporting ≥ 1 AE	76 (56)	76 (56)	0.0% (−11.7 to 11.7%)
Participants reporting a SAES	2 (1.5)	2 (1.5)	0.0% (−3.9% to 3.9%)
Death	0 (0)	0 (0)	0.0% (−2.8% to 2.8%)
AEs of special interest			
Participants reporting at least one AE in gastrointestinal disorders SOC	33 (24)	45 (33)	−8.9% (−19.4% to 1.9%)
Participants reporting at least one AE in infection and manifestations of SOC	29 (21)	28 (21)	0.7% (−9.0% to 10.5%)
Participants reporting at least one AE in food supplement taste/texture SOC[a]	10 (7)	6 (4)	3.0% (−3.0% to 9.17%)
Number of participants discontinued from study due to AE[a]	4 (3)[b]	1 (1)	2.2% (−1.6% to 6.7%)

Con, inulin control; SOC, system organ class.
a There were three participants who mentioned the reason for stopping the supplement as 'Did not like taking supplement'.
b One of these was the ectopic pregnancy at randomisation.

TABLE 17 Serious adverse events

	Arm	Duration (days)	Severity	Study month	Relationship to study treatment	Outcome
Stab wound	IPE	30	Severe	10	Not related	Resolved
Hospitalisation	IPE	61	Severe	8	Not related	Resolved
Renal stone removal	Con	1	Moderate	4	Not related	Resolved
Meningitis bacterial	Con	17	Severe	6	Not related	Resolved

Con, inulin control.

Legend of the system organ class:

Blood and lymph syst	Blood and lymphatic system disorders
Cardiac	Cardiac disorders
Diarrhoea	Diarrhoea
Food sup taste/texture	Food supplement taste/texture
Gastro	Gastrointestinal disorders
General	General disorders and administration site conditions
Immune syst	Immune system disorders
Infect and infestations	Infections and infestations
Injury, poisoning and proc compl	Injury, poisoning and procedural complications
Investigations	Investigations

Metabolism and nutrition	Metabolism and nutrition disorders
Musc and connective tissue	Musculoskeletal and connective tissue disorders
Neoplasms	Neoplasms benign, malignant and unspecified (including cysts and polyps)
Nervous syst	Nervous system disorders
Pregn, puerp and perinatal	Pregnancy, puerperium and perinatal conditions
Psychiatric disorders	Psychiatric disorders
Reproductive syst	Reproductive system and breast disorders
Respiratory	Respiratory, thoracic and mediastinal disorders
Skin	Skin and subcutaneous tissue disorders
Social circumstances	Social circumstances
Surg and med proc	Surgical and medical procedures
Therapeutic proc	Therapeutic procedures and supportive care not elsewhere classified

Compliance

Compliance after the study withdrawal date was taken to be zero. Within-participant pro-rata imputation was otherwise used where compliance data were missing. In total, 61 participants discontinued the study treatment but remained in the study (41 controls and 20 in the IPE arm). A post hoc conservative sensitivity analysis assuming zero compliance instead of pro-rata compliance did not alter the percentages markedly (at most 6% difference). Percentage compliance for a subject was defined as the number of used sachets divided by the total number of sachets (expected to have been taken over the 12-month study period) multiplied by 100.

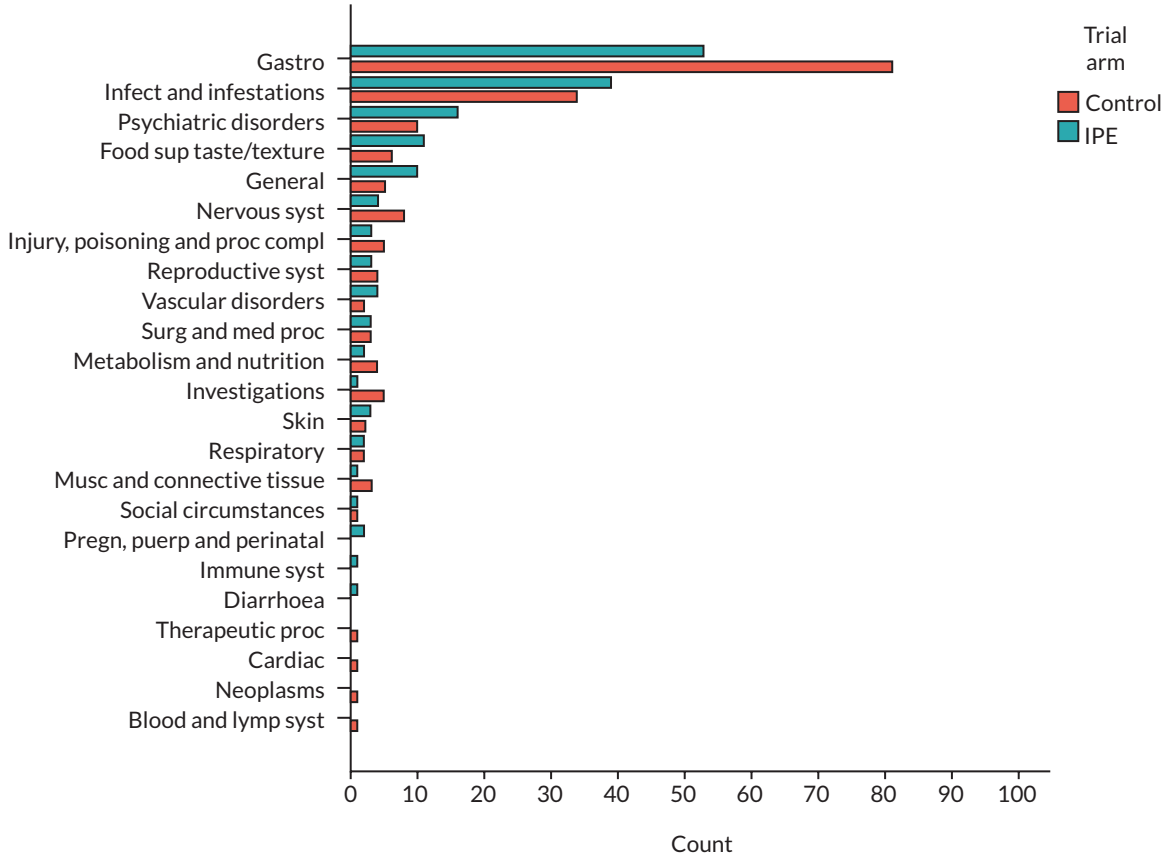

FIGURE 8 Number of AEs by system organ class and study arm.

TABLE 18 Number of AEs by system organ class, intensity and relationship to study intervention

		Mild			Moderate			Severe			Total		
System organ class[a]	Arm	NR	R	Total	NR	R	Total	NR	R	Total	NR	R	Total
Blood and lymphatic system	IPE	0	0	0	0	0	0	0	0	0	0	0	0
	Con	0	0	0	1	0	1	0	0	0	1	0	1
Cardiac	IPE	0	0	0	0	0	0	0	0	0	0	0	0
	Con	0	0	0	1	0	1	0	0	0	1	0	1
Diarrhoea	IPE	0	0	0	0	1	1	0	0	0	0	1	1
	Con	0	0	0	0	0	0	0	0	0	0	0	0
Food supplement taste/texture	IPE	0	5	5	0	2	2	0	0	0	0	7	7
	Con	0	2	2	0	0	0	0	0	0	0	2	2
Gastrointestinal disorders	IPE	4	21	25	11	16	27	0	1	1	15	38	53
	Con	2	23	25	9	46	55	0	2	2	11	69	80
General	IPE	1	0	1	8	1	9	0	0	0	9	1	10
	Con	1	0	1	4	0	4	0	0	0	5	0	5
Immune system	IPE	1	0	1	0	0	0	0	0	0	1	0	1
	Con	0	0	0	0	0	0	0	0	0	0	0	0
Infections and infestations	IPE	13	0	13	24	0	24	0	0	0	37	0	37
	Con	8	0	8	22	0	22	1	0	1	31	0	31
Injury, poisoning and procedural complications	IPE	0	0	0	2	0	2	1	0	1	3	0	3
	Con	2	0	2	3	0	3	0	0	0	5	0	5
Investigations	IPE	1	0	1	0	0	0	0	0	0	1	0	1
	Con	2	0	2	3	0	3	0	0	0	5	0	5
Metabolism and nutrition	IPE	0	1	1	1	0	1	0	0	0	1	1	2
	Con	2	0	2	2	0	2	0	0	0	4	0	4
Musculoskeletal and connective tissue	IPE	0	0	0	1	0	1	0	0	0	1	0	1
	Con	0	0	0	2	0	2	1	0	1	3	0	3

continued

TABLE 18 Number of AEs by system organ class, intensity and relationship to study intervention (*continued*)

		Mild			Moderate			Severe			Total		
System organ class[a]	Arm	NR	R	Total	NR	R	Total	NR	R	Total	NR	R	Total
Neoplasms	IPE	0	0	0	0	0	0	0	0	0	0	0	0
	Con	0	0	0	1	0	1	0	0	0	1	0	1
Nervous system	IPE	1	0	1	2	1	3	0	0	0	3	1	4
	Con	2	1	3	4	1	5	0	0	0	6	2	8
Pregnancy, puerperium and perinatal	IPE	1	0	1	1	0	1	0	0	0	2	0	2
	Con	0	0	0	0	0	0	0	0	0	0	0	0
Psychiatric disorders	IPE	5	0	5	7	2	9	0	0	0	12	2	14
	Con	1	0	1	8	0	8	0	0	0	9	0	9
Reproductive system	IPE	0	0	0	3	0	3	0	0	0	3	0	3
	Con	3	0	3	1	0	1	0	0	0	4	0	4
Respiratory	IPE	1	0	1	1	0	1	0	0	0	2	0	2
	Con	1	0	1	0	0	0	1	0	1	2	0	2
Skin	IPE	0	0	0	0	1	1	1	0	1	1	1	2
	Con	1	1	2	0	0	0	0	0	0	1	1	2
Social circumstances	IPE	0	0	0	1	0	1	0	0	0	1	0	1
	Con	0	0	0	1	0	1	0	0	0	1	0	1
Surgical and medical procedures	IPE	1	0	1	0	0	0	2	0	2	3	0	3
	Con	1	0	1	2	0	2	0	0	0	3	0	3
Therapeutic procedures	IPE	0	0	0	0	0	0	0	0	0	0	0	0
	Con	0	0	0	1	0	1	0	0	0	1	0	1
Vascular disorders	IPE	3	0	3	1	0	1	0	0	0	4	0	4
	Con	2	0	2	0	0	0	0	0	0	2	0	2
TOTAL	IPE	58	54	112	128	71	199	7	3	10	193	126	319
	Con	32	27	59	63	24	87	4	1	5	99	52	151

Con, inulin control; NR, not related or unlikely; R, possibly, probably, or definitely related.
a Legend in the footnote of the figure above.

TABLE 19 Percentage compliance up to 2 months of follow-up by treatment arm

Percentage compliance levels by 2 months	Con % (*N*) *N* = 135	IPE % (*N*) *N* = 135	Total % (*N*) *N* = 270
> 80%	61% (82)	64% (86)	62% (168)
50% to < 80%	10% (13)	16% (22)	13% (35)
< 50%[a]	30% (40)	20% (27)	25% (67)
≥ 50%	70% (95)	80% (108)	75% (203)

Con, inulin control.
a This included 11 (5 in control and 6 in IPE) participants who dropped out from the study at or between baseline and 2 months' follow-up.

TABLE 20 Percentage compliance up to 6 months of follow-up by treatment arm

Percentage compliance levels by 6 months	Con % (*N*) *N* = 135	IPE % (*N*) *N* = 135	Total % (*N*) *N* = 270
> 80%	45% (61)	56% (75)	50% (136)
50% to < 80%	24% (32)	14% (19)	19% (51)
< 50%[a]	31% (42)	30% (41)	31% (83)
≥ 50%	69% (93)	70% (94)	69% (187)

Con, inulin control.
a This included 11 (5 in control and 6 in IPE) participants who dropped out from the study at or between baseline and 2 months' follow-up plus 16 (8 in each arm) who dropped out from 2- and 6-month follow-up.

Inulin-propionate ester 50%-sachet compliance is estimated to have been met for 80% (108/135), 70% (94/135) and 63% (85/135) of participants up to 2, 6 and 12 months, respectively. The corresponding statistics for 80%-sachet compliance are 64% (86/135), 56% (75/135) and 48% (65/135). Compliance after a participant's study withdrawal date was taken to be zero. Compliance at 2 and 6 months can be seen in *Tables 19* and *20* for the IPE and Control arms.

Due to the COVID-19 pandemic, sachets were returned by some participants at the end of the study rather than at the 6-month visit, so it is not possible to have a clear view of the compliance between 2 and 12 months. Forty-eight per cent of the IPE achieved 80% sachet-day compliance and a further 15% achieved 50–80%, giving a total of 63% (85/135) achieving 50% compliance (*Figure 9*). The comparable level of compliance was observed to be 53% (72/135), 10% lower, in the control arm. *Table 21* relies on the pro-rata distribution of compliance data within this period, up to the study end or stated discontinuation/withdrawal date.

Protocol deviations

Protocol deviations were recorded throughout the study and have been summarised in *Table 22*.

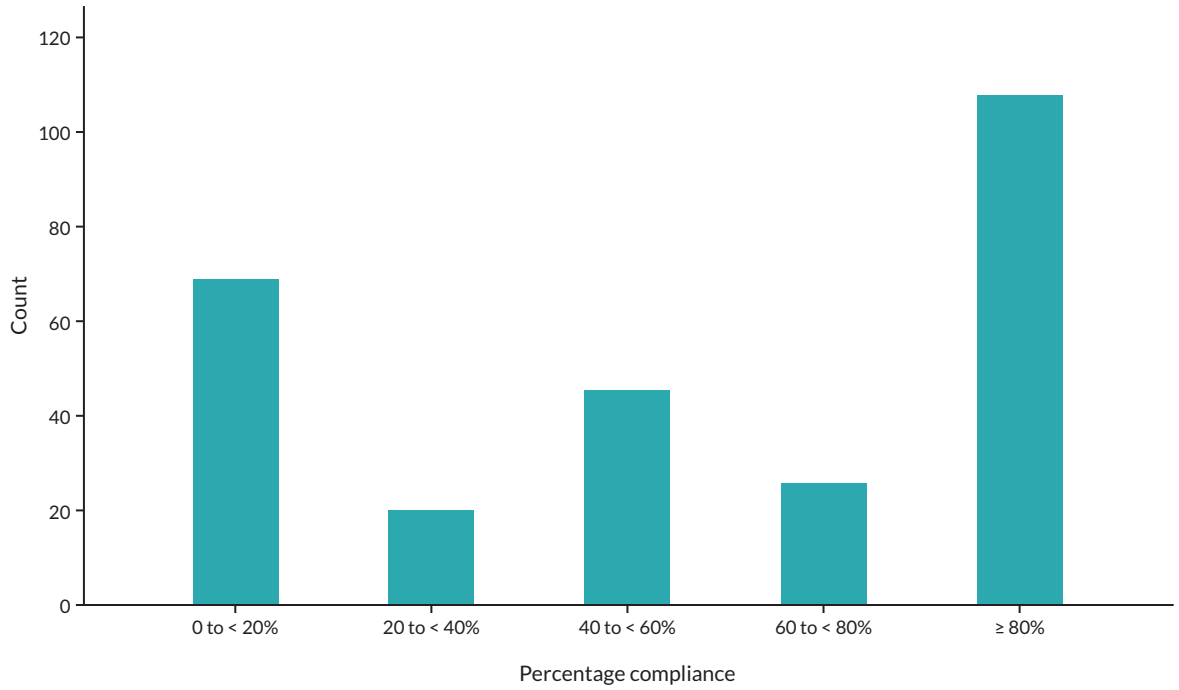

FIGURE 9 Distribution of participants' compliance measured by the percentage of used sachets returned over the 12 months in the study (both arms combined).

TABLE 21 Percentage compliance over the 12 months by treatment arm

Percentage compliance by 12 months	Con % (*N*) *N* = 135	IPE % (*N*) *N* = 135	Total % (*N*) *N* = 270	The difference in proportions between arms (IPE – control) (95% CI)
> 80%	32% (43)	48% (65)	40% (108)	16.3% (4.8% to 11.5%)
50% to < 80%[a]	22% (29)	15% (20)	18% (49)	
< 50%[b]	47% (63)	37% (50)	42% (113)	
≥ 50%	53% (72)	63% (85)	58% (157)	9.6% (−2.1% to 21.3%)

Con, inulin control.
a This included two participants (one in each arm) who dropped out from the study between the 6- and the 12-month follow-up.
b This included the 40 (19 in control and 21 in IPE) participants who dropped out from the study.

TABLE 22 Protocol deviations listed by type

Protocol deviation type	Number of reports
Visit deviations	
Remote visit	112
Self-reported weight	13
Lost to follow-up	5
Missed study visit	4
Withdrawn from substudy	5
Sachets not returned for compliance count	47
Study visits outside visit window	4
Deviation from substudy protocol	7

TABLE 22 Protocol deviations listed by type (*continued*)

Protocol deviation type	Number of reports
Study treatment missed	
Illness	13
Difficulty with supplement	3
COVID-19	5
Travel	19
Forgetfulness	9
Side effects	2
Other	13
Reason not given	35
Non-compliance	
Intentional weight loss	5
Double-dosing IPE/inulin	1
Changed diet	2
Changed physical activity	2
Change in medication	1
Sachets not returned for compliance count	47
Researcher error	
Extra sachets allocated	5
Incorrect randomisation allocation	9
Incorrect participant information sheet or consent form	14
Missed study measurement	
Blood sample	17
Body composition	1
Blood pressure	1
Missed food diary	3
Weight	1

Mechanistic outcomes

A total of 52 participants were enrolled in the mechanistic substudy, drawn from the main study population. All substudy assessments were conducted at the Imperial College London site. Higher rates of withdrawal were detected for the mechanistic substudy compared with the main study. This is attributable to lifestyle changes after the COVID-19 lockdowns, such as relocation and returning to work. Consequently, some participants did not completely withdraw from the trial but were unable or chose not to participate in the 12-month follow-up visit for the study. Of the initial 52 participants, 23 returned for the follow-up mechanistic study at 12 months. For this reason, the baseline results of the whole substudy cohort are described separately from those with measurements at both baseline and 12-month follow-up visits.

TABLE 23 Medians (IQR) of the iAUC of GLP-1 and PYY at baseline visit

	IPE Median (IQR) *N* = 22	Con Median (IQR) *N* = 25
GLP-1	64,793 (22,262–97,977)	56,646 (29,754–85,509)
PYY	3293 (1258–6160)	2308 (719–6771)

Con, inulin control.

TABLE 24 Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for GLP-1 and PYY

	Baseline		12-month follow-up		Change from baseline		
	IPE Median (IQR) *N* = 15[a]	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 15	Con Median (IQR) *N* = 8	Difference between arms (95% CI)
GLP-1	67,019 (15,985–116,443)	47,547 (20,464–56,880)	58,295 (20,278–94,274)	31,665 (8653–49,388)	−7214 (−36,780 to 25,174)	−8486 (−34,067 to 15,281)	1272 (−33,734 to 38,013)
PYY	3980 (822–8494)	1893 (916–5651)	6124 (1008–9842)	1304 (108–2734)	437 (−5068 to 5078)	−150 (−4251 to 769)	586 (−5174 to 6629)

Con, inulin control.
a One participant with a follow-up measurement was excluded from this baseline summary due to having only two measurements at baseline.

TABLE 25 Mean change in weight of ad libitum meal for IPE and inulin arms at baseline visit

IPE Mean (SD) *N* = 24	Con Mean (SD) *N* = 24
−556 (265)	−470 (273)

Con, inulin control.

TABLE 26 Weight (g) of ad libitum meal after eaten minus before meal was eaten

	Baseline		12-month follow-up		Changes from baseline		
	IPE Mean (SD) *N* = 15	Con Mean (SD) *N* = 8	IPE Mean (SD) *N* = 15	Con Mean (SD) *N* = 8	IPE Mean (SD) *N* = 15	Con Mean (SD) *N* = 8	Difference in means (95% CIs)
Weight of meal after minus weight of meal before eating (g)	−619 (257)	−679 (143)	−640 (329)	−531 (250)	−20 (161)	148 (146)	−169 (−311 to −26)

Con, inulin control.

Primary outcome for mechanistic substudy: glucagon-like peptide 1 and peptide YY

There were no significant differences in gut hormone iAUC at the baseline visit (*Table 23*) and no differences in iAUC within or between treatment arms at 12 months (*Table 24*).

TABLE 27 Baseline medians (IQR) of the iAUC of subjective appetite ratings

	IPE Median (IQR) *N* = 24	Con Median (IQR) *N* = 25
Desire to eat	25 (0–605)	384 (11–2498)
Fullness	13,458 (6255–17,614)	7260 (2505–9385)
Hunger	147 (1–1862)	325 (0–2314)
Nausea	879 (7–2116)	407 (41–1823)

Con, inulin control.

TABLE 28 Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for subjective appetite ratings

	Baseline		12-month follow-up		Difference (95% CI) between arms at FU	Change from baseline		Difference (95% CI) between arms of change
	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8		IPE *N* = 16	Con *N* = 8	
Desire to eat	4 (0–382)	194 (27–4094)	128 (0–773)	76 (0–3890)	52 (−2804 to 264)	11 (−193 to 467)	−118 (−648 to 113)	129 (−240 to 1074)
Fullness	13,693 (7815–20,252)	7382 (2290–8186)	10,174 (5482–17,559)	6247 (1878–12,821)	3927 (−1928 to 9978)	−998 (−7770 to 3166)	−268 (−5200 to 4277)	−730 (−7062 to 3717)
Hunger	106 (1–1135)	253 (0–4276)	174 (0–425)	0 (0–1527)	174 (−1079 to 206)	−23 (−489 to 411)	0 (−301 to 596)	−23 (−681 to 1044)
Nausea	879 (13–1744)	279 (73–2444)	26 (0–1118)	55 (0–4284)	−28 (−1428 to 533)	−297 (−1448 to 1106)	−41 (−144 to 2214)	−256 (−2648 to 886)

Con, inulin control; FU, follow-up.

Ad libitum energy intake

The weights of the ad libitum meal consumed at baseline were similar between the two study arms (*Table 25*). There was a statistically significant difference in mean change in ad libitum meal intake between the study arms of −169 (−311 to −26) (*Table 26*).

Subjective appetite ratings

There are differences in the baseline median subjective appetite rating iAUC between the IPE and inulin arms, reinforcing the decision to analyse the change from the baseline (*Table 27*). There were no significant differences in iAUC for self-reported subjective appetite ratings from appetite VAS between treatment arms at 12 months (*Table 28*).

Calorimetry data

There were no marked differences in the sample in distributions of the iAUC at baseline between IPE and inulin for iAUC in energy expenditure, respiratory exchange ratio, carbohydrate, or fat oxidation (see *Appendix 1*, *Table 29*) nor were there any significant differences between the study arms for median iAUC for these outcomes at the 12-month visit (see *Appendix 1*, *Table 30*).

Fat oxidation

There were no significant differences in baseline iAUC for fat oxidation (see *Appendix 1*, *Table 31*). There were no significant differences between IPE and inulin in changes in fat oxidation iAUC at 12-month follow-up (see *Appendix 1*, *Table 32*).

De novo lipogenesis

Two measurements of DNL were possible using the double-dosing protocol with D_2O as the tracer. Fasting DNL is the contribution of newly synthesised palmitate to the plasma TAG pool in the period since the administration of the first dose of D_2O (on the previous evening). Owing to the rapid isotopic equilibration of D_2O in body water (~ 1 hour) and the extended elimination half-life of D_2O (~ 10 days), it can be assumed that ^2H enrichment is effectively at plateau (steady state) in the body water precursor pool. Thus, the ^2H enrichment of palmitate in the basal plasma (before the second D_2O dose) represents the fraction of newly synthesised palmitate [fasting DNL (%)]. The second DNL measure used a classical fractional synthesis rate approach to measure the rate of synthesis in the postprandial phase [PP DNL (%/day)].

At baseline, there were no marked differences between groups in measured fasting DNL or postprandial DNL (see *Appendix 1*, *Table 33*). In participants who completed both baseline and 12-month DNL measurements (n = 6 in the control and n = 15 in the IPE group), there were no marked changes either from baseline to follow-up or between groups at the 12-month follow-up period (see *Appendix 1*, *Table 34*).

Insulin

The baseline iAUC for insulin was not different between study arms (see *Appendix 1*, *Table 35*). There were no differences in change in median iAUC between study arms at 12 months (see *Appendix 1*, *Table 36*).

Glucose

There were no marked differences between IPE and inulin for glucose iAUC at baseline (see *Appendix 1*, *Table 37*) nor any appreciable or significant changes in glucose iAUC at 12-month follow-up (see *Appendix 1*, *Table 38*).

Breath hydrogen

Breath hydrogen was used as a surrogate measure of bacteria fermentation in the colon, and no differences were found in baseline or change at 12-month iAUC between the inulin and IPE study arms (see *Appendix 1*, *Tables 39* and *40*).

Physical activity data

At the baseline visit, there were 21 participants with all 7 days of data for all outcomes and 3 had fewer than 3 days of data. At follow-up, 14 participants had 7 days of data and 9 had 3 or fewer days of data.

There was acceptable duration on the body with a median (IQR) of 1439 (1292–1440) minutes per day in the control group (n = 24) and 1439 (1413–1440) minutes per day in the IPE group (n = 24) at baseline. At 12 months' follow-up, it was similarly high.

Baseline physical activity measurements are shown in *Appendix 1*, *Table 41*. Sleep duration appears to be statistically significant between arms with a change from baseline −74 (−124 to −29) minutes; no other measurements of physical activity were different between the two study arms (see *Appendix 1*, *Table 42*). However, this result was based on 16 and 7 participants in the 2 arms and the result needs to be interpreted cautiously. In the sensitivity analysis, this remained significant when comparing the two medians. The 95% bootstrap CIs were based on approximately 10,000 samples.

Metabolomics

There were no significant differences within or between IPE or inulin between baseline and 12 months for urinary or plasma metabolites (see *Appendix 2*, *Figures 10* and *11*). There were no significant changes in the faecal metabolome between baseline and 12 months in the IPE study arm (see *Appendix 2*, *Figure 12*). There were four faecal samples available for analysis at 12 months in the inulin study arm which was an insufficient number for comparative analysis.

Gut microbiota

There were no significant differences in alpha- or beta-diversity within or between the IPE or inulin study arms from baseline and 12 months (see *Appendix 3*, *Figures 13* and *14*).

There were some significant differences in abundance of bacteria species between the inulin and IPE study arms at 12 months (see *Appendix 2*, *Table 43*). Comparative to the inulin arm, the species *Bifidobacterium bifidum*, *Escherichia coli*, *Dialister invisus*, *Lachnospiraceae bacterium*, *Desulfovibrio piger* and *Roseburia intestinalis* were in higher abundance, while the species *Bifidobacterium pseudocatenulatum*, *Alistipes onderdonkii*, *Alistipes inops*, *Bacteroides ovatus*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Parabacteroides merdae*, *Coprobacter secundus* and *Alistipes obesi* were in greater abundance in the participants within the inulin control arm.

Body composition by deuterium dilution

An additional output accessible from D_2O labelling (primarily undertaken for DNL measurements) is body composition analysis. There is an alternative and primary reference method for body composition and was used to compare with body composition from bioelectrical impedance analysis (BIA). At baseline, there was no meaningful difference in either FFM (kg) or FM (kg) between groups (see *Appendix 4*, *Table 44*).

Comparing the concordance of FFM measured by ^2H_2O and BIA, as expected, a strong correlation was observed and a high degree of concordance in the Bland–Altman analysis except for perhaps the very highest levels of FFM (see *Appendix 4*, *Figure 15*). Two participants were excluded from the concordance analysis because of implausibly high ^2H enrichment in their plasma measurements which would only have been possible with the consumption of more than a single ^2H_2O dose prior to blood collection. In those participants who completed both baseline and 12-month follow-up visits, there was no meaningful difference in FFM or FM (see *Appendix 4*, *Table 45*).

Outstanding analyses

There are four outcomes from the original protocol which are yet to be analysed. These include 7-day food diaries, the use of a human organoid model to determine how these specific changes in the colonic environment influence L-cell differentiation, ^{13}C beta-hydroxybutyrate and targeted SCFA analysis.

Chapter 5 Discussion

The incidence and prevalence of obesity continue to increase globally. Research to date has focused on the treatment and management of obesity, whereas few studies investigate weight gain prevention. Young adulthood can be a time of rapid and substantial weight gain, which can set the trajectory for life and is linked to an increased risk of metabolic disease later.[5] Evidence suggests SCFAs play a role in energy homeostasis and appetite.[17,19,49] Previous work by our group and others suggests that SCFAs influence appetite by stimulating the release of appetite-regulating hormones from the gut enteroendocrine system through the two G protein-coupled receptors FFAR 2/3. Of the main SCFAs produced in the colon, propionate has the highest affinity across the two receptors. We therefore developed IPE as a method to increase propionate in the human colon. IPE releases approximately 2.0 g of propionate to the colon per dose.

This was the first study to investigate the efficacy of IPE on weight gain prevention in younger adults via a year-long randomised control trial. This study shows IPE had no differential effect on weight gain over 1 year when compared to inulin, although neither the IPE nor inulin group experienced the predicted weight gain of 2 kg. This is encouraging considering that this cohort consisted of participants with a BMI of ≥ 25 kg/m^2 who were selected on the basis that they had: high intakes of sugar-sweetened beverages, low intakes of fruit and vegetables, low physical activity or were at risk of further weight gain (they had gained > 1 kg in the last year). This population was phenotypically susceptible to weight gain and therefore ideal for investigating the strength of the effect of IPE on weight gain prevention. One of the few other studies examining weight gain prevention reported an average weight increase of 2 kg in a high-risk population similar to our study cohort.[41] Furthermore, considering the study was conducted during COVID-19, and a recent review indicated that the average adult gained 1.57 kg between March and May 2020,[50] weight gain was even more likely. It is also worth noting that participants were not given dietary advice, behavioural support, or a physical activity intervention to induce a negative energy balance during the study.

We believe that the observations made above were not influenced by other confounding factors over the 12-month follow-up period. Withdrawal rates were similar for both study arms, with 15% of inulin control and 16% of IPE participants withdrawing from all study procedures. Supplement discontinuation was higher in the inulin group. Inulin may have been less well tolerated by participants compared with the IPE intervention as indicated by higher gastrointestinal AEs in the inulin group. This was as expected due to the larger dose of fibre provided by the control and could have contributed to the lower compliance detected in the inulin group. High compliance, measured by ≥ 80% IPE or inulin sachet intake, was detected in 60% of participants after 2 months of the intervention. By 12 months, high compliance had reduced to 48% and 32% of participants in IPE and inulin groups, respectively. Lower rates of compliance did not result in greater body weight gain in the control group, whereas poorer compliers within the IPE group experienced the greatest weight gain indicated in the complier-average causal effect analysis. No unexpected, related AEs or SAEs were detected during the trial, confirming that IPE is safe for consumption.

We have previously demonstrated that increasing the production of propionate in the colon of middle-aged adults leads to reduced weight gain over 6 months. IPE attenuated substantial weight gain (≥ 3% body weight) in 96% of middle-aged participants (mean age: 54.4) with a BMI ≥ 25 kg/m^2, significantly more than the 75% of participants in the inulin control arm who did not experience substantial weight gain.[23] In the present study, the data diverged in the opposite direction, that is those consuming IPE trended towards weight gain, it could be suggested that younger adults are less sensitive to propionate. Conversely, as the weight within the inulin-consuming group was stable throughout the study, younger adults could be more responsive to enhanced satiety signalling from the products of inulin fermentation.

Younger adults (aged 20–40 years) are at greatest risk of weight gain.[51] Adults of this age tend to engage in less physical activity and have highly sedentary lifestyles, resulting in a positive energy balance and weight gain of up to 1 kg/year.[6,52,53] Young adults tend to eat outside of the home, follow inconsistent meal patterns and snack throughout the day.[54–57] These eating habits are associated with higher energy intake.[58,59] Hyperpalatable foods and other external cues driving appetite and food intake may have overpowered any satiety signal by propionate in the colon. Previous studies using ^{13}C-labelling indicate that IPE reaches the colon and releases propionate 3–4 hours post ingestion.[20] As propionate is a short-term signalling molecule, if the timing of the main meal was inconsistent or fell outside of this

window, there may not have been any discernible effect on fullness and energy intake. As there is no evidence of a difference in gut response (e.g. gut transit or fermentation time) between these population groups, the release and sensing of propionate should be similar in all healthy adults and between these study cohorts.[60]

The 10 g/day IPE dose was chosen as it was identified as the minimally effective dose in middle-aged adults and no differences were seen when the dosage was increased to 20 g/day.[23,25] However, this dose may have been insufficient in younger adults and could indicate an IPE dose for age effect. Ageing adults are reported to have 75% concentrations of SCFAs compared with adults below 50 years old and could therefore be more sensitive to changes in SCFA production in the colon.[61] This suggests that higher concentrations of SCFA may be required to over-ride drivers of energy intake in younger adults.

Differences in production and circulating concentrations of satiety hormones may also have contributed to the discrepancy in the results. A recent systematic review found that younger adults exhibit lower levels of fasting and postprandial cholecystokinin, leptin and insulin, along with lower post-meal PYY secretion.[62] Additionally, young people reported higher subjective hunger ratings and increased energy intake compared to older adults.[62] Previous work has repeatedly shown that acute IPE intake enhanced GLP-1 and PYY secretion or lowered subjective appetite and ad libitum food intake in middle-aged adults.[20,23,24] The effects of chronic IPE intake on gut hormone secretion appear less consistent. No changes in GLP-1 or PYY secretion were reported after 1- and 6-month-long supplementation period in the middle-aged cohorts.[23,63] Consistent intake of IPE may result in adaptations to the colonic environment and increased tolerance to enhanced propionate. It is also possible that there could be differences in the duration and extent to which younger and older populations adapt to increased SCFA concentrations in the colon or enhanced satiety hormone secretion. The desensitisation of SCFA receptors, FFAR 2 and 3, located on enteroendocrine cells could mean that GLP-1 or PYY secretion returns to basal levels. Alternatively, changes to other proteins like an increase in dipeptidyl peptidase-4 activity, a protein responsible for enzymatic degradation of GLP-1,[15] could have shortened the length of satiety signalling. Further investigation is required to understand the nature and duration of these adaptations.

Consistently, studies using IPE have reported its positive effect on body composition. In the present study, a rise in FFM was observed without alterations to FM. Previous studies have reported lowered visceral adipose tissue mass in middle-aged adults.[25] Similarly, reductions in body weight and FM were observed following a 1-month intervention combining IPE with moderate exercise, while no changes were seen in the inulin group.[63] This is consistent with evidence from rodent studies which suggest that increased fat oxidation may prevent further adipose deposition.[64] Therefore, replacing a small percentage of adipose tissue with lean tissue may benefit health over time. Though not definitive, this increase in lean tissue could increase energy expenditure and contribute to improved overall health.

There was a significant increase in fasting glucose in the IPE group and no other changes in the fasting biochemistry. This aligns with the absence of significant changes to FM or body weight in either group. Further analysis is required to determine whether elevated fasting glucose is linked to the trend towards an increase in body weight in the IPE group. In previous studies, improvements in markers of glycaemic control have been reported such as improvements in insulin sensitivity,[23,25] enhanced insulin secretion,[65] reductions in cholesterol[23] and lowered markers of inflammation.[25]

Given that the findings of this study diverge from both our hypothesis and previous evidence, it seems necessary to verify the existence of propionate in the intervention product. Serendipitously, there was a suggestion that there could have been a supplement mix-up at packaging, during the trial. This meant that additional blinded assays were conducted on the intervention and control batch. These assays and certificates of authenticity confirmed that propionate was present within the intervention batch and in the original analysis of the IPE product at manufacture.

Compliance with IPE and inulin could have differentially affected weight gain outcomes according to the complier-average causal effect analysis. High compliance with IPE (≥ 80% sachet intake) over the study period resulted in lower weight gain, whereas compliance with inulin did not affect weight outcomes. Higher compliance was identified in male participants, those aged over 30 years and males with a lower starting body weight (< 89 kg). These participants demonstrated less weight gain compared with females, adults between 20 and 30 years and those with a higher starting body weight. However, the overall treatment effect was statistically non-significant and still trended towards weight gain.

Post hoc analyses detected a larger, albeit non-significant, treatment effect in the primary outcome of the London cohort compared to those from the Glasgow site. There were also some site-dependent differences in compliance and withdrawals, whereby 60% of total withdrawals occurred at the London site and 47% and 50% of London and Glasgow participants, respectively, met the 80% compliance threshold.

Results from the mechanistic substudy

Although the study met the 52-participant recruitment target, there was a severe decrease in the volunteers returning for their follow-up visit at 12 months, with withdrawal rates at around 50%. The substudy appears to have been influenced by lifestyle changes due to the pandemic and other uncontrollable factors to a greater extent than the main study. As participants stopped working from home or moved out of London, they could not or did not wish to attend the 8-hour study visit for the mechanistic investigations. In some cases, they only attended the main study visit. The limited follow-up numbers at 12 months and the multiple tests conducted on mechanistic outcomes mean that caution should be taken when interpreting these study results.

The primary outcome of the mechanistic study was a change in appetite hormone excursion from baseline to the follow-up visit at 12 months. Analyses indicate that there was no significant difference between GLP-1 and PYY iAUC between IPE and inulin at follow-up. This outcome aligns with our original findings in middle-aged adults which reported a significant difference in acute gut hormone secretion but no effect after 6 months of IPE or inulin intervention.[23]

The difference in mean ad libitum energy intake was significant between baseline and 12-month visits for inulin and IPE, demonstrating increased energy intake in participants who took IPE for 12 months. Delayed satiety signalling contributing to later cessation of food intake may have contributed towards the trend of increased body weight detected in the IPE arm of the main study. These results differed from previous data that demonstrated an anorectic pattern through the VAS and a reduction in food intake during the ad libitum buffet meal.[20,23,30]

Propionate is a potential gluconeogenic substrate; however, evidence on its overall role in glycaemic control is contradictory.[66] There was no significant difference in the postprandial insulin and glucose AUC between the IPE and the inulin group. However, previous analyses have identified a reduction in postprandial glucose AUC[23] and an improvement in insulin secretion and beta cell function following IPE intake.[65] In another study, no differences were seen between the effects of inulin and IPE.[25] However, improved insulin sensitivity was detected by multiple indices in both groups compared to the non-fermentable control, cellulose.[25] These results obstruct our ability to disentangle the differences in the effect of IPE versus inulin alone and there remains an important question on the hepatic handling of propionate in humans.

Overall, the 16S rRNA microbial analysis revealed a trend towards a lower bacterial abundance in both study arms, which is consistent with previous studies using IPE and inulin. Notably, enrichment of the potentially beneficial bacteria, *Bifidobacteriaceae*, occurred in both study arms, associated with increased delivery of inulin to the colon.[67] In the IPE group, higher rates of species belonging to the *Lachnospiracae* family were detected, a family of saccharolytic bacteria associated with improved gut eubiosis.[68] Higher populations of *E. coli* could be associated with an increase in the metabolite 2-deoxy-D-ribose as it can be utilised by some strains of this species.[69] However, the organisms producing this metabolite and its source are unknown. During the microbial analysis of the final IPE batch, higher aerobic cell counts were detected compared with previous batches, but the nonspecific assay does not speciate the bacteria responsible for the higher count. In food/ingredient manufacture, this measure is used as a general hygiene marker facilitating additional control measures in the production process where required. The cell counts however remained far below the stringent safety threshold for this type of food product but may have contributed to higher populations of *E. coli*.

Interestingly, several members of the *Bacteroidetes* phylum were reduced in the IPE arm, possibly indicating that increased colonic propionate creates an environment less favourable for *Bacteroidetes*. However, this is counter-intuitive as one major propionate-producing group in the microbiota is *Prevotella*, which are part of the *Bacteroidetes* phylum

and may suggest that the environmental drivers of bacterial composition are more nuanced and fine-tuned than major phylogenetic group-level effects.

In the inulin group, alongside elevations in species belonging to the *Bifidobacteriaceae*, several species within the *Rikenellaceae* family exhibited increased abundance identified, which has been associated with reduced visceral adipose tissue deposition.[70] Moreover, a higher abundance of bacteria associated with propionate, specifically *Bacteroidaceae* and *Tannerellaceae*, was noted in the inulin group.[71] *Coriobacteriaceae*, identified in our analysis, has previously been associated with positive changes in type 2 diabetes outcomes following gastric bypass procedures in rodents.[72]

No significant differences in breath hydrogen (H_2) AUC were identified. However, the inulin control arm trended towards elevated breath hydrogen iAUC at baseline and 12 months. In both arms, we would expect to see an increase in breath hydrogen at approximately 240 minutes, as an indicator that the inulin or IPE has reached the site of fermentation in the colon. The reasons for the difference, albeit non-significant, in AUC between the IPE and inulin study arms are currently unknown. We would expect a slightly lower H_2 production in the IPE group as approximately 20% of the molecule is composed of propionate as opposed to inulin, a fermentable fibre. Increased inulin may have promoted acetate and butyrate production, which is also associated with H_2 production, particularly butyrate.[73] Conversely, increasing propionate in the colon using IPE may have shifted the microbiome towards a more propiogenic profile from 'normal' fermentation. This is associated with a shift in H_2 sequestration into propionate as opposed to methane as a means of H_2 disposal in the colon, as evidenced in animal and in vitro data, meaning that less H_2 would be detected.[74] Evidence suggests approximately 20% of the population are low H_2 producers.[75,76] There may have been more individuals who failed to produce H_2 in the IPE study arm. However, it is likely that low H_2 producers should have been distributed equally into the groups at randomisation. The low numbers at follow-up may have resulted in an imbalance in low to normal H_2 producers.

The NMR metabolomic enquiry of stool, blood and urine showed no significant difference between the groups at baseline or 12 months suggesting that neither inulin nor IPE altered metabolomic profiles after 12 months of intake. Similarly, analyses of plasma metabolomic profiles revealed no differences following the consumption of IPE and inulin. Our previous work indicated an elevation in metabolites linked to insulin resistance following the ingestion of cellulose but not IPE or inulin.[25]

We have reported previously that propionate significantly increases energy expenditure and fat oxidation.[30,63,77] This may contribute to weight gain prevention and higher lean mass percentage, independent of food intake.[30] However, no significant differences in energy expenditure and fat oxidation were observed in this study. The lack of effect observed in the current study may have contributed to the absence of an impact on weight gain. There was also no meaningful difference in the measurements of DNL which is unsurprising given the lack of difference in FM observed between study arms. This may indicate that either propionate has no effect on DNL or that it is activated by a more generic mechanism via a combination of SCFAs (principally acetate and propionate which escape colonic metabolism), given the lack of difference between study arms. The concordance between different measurements of body composition was as expected.

Accelerometry data were collected to control for the confounding variable of physical activity when investigating the effects of inulin and IPE on body weight. There was no change in physical activity in metabolic equivalents of tasks (METs) or duration between the IPE and the inulin group. However, there was a significant difference observed in sleep, although based on 16 and 7 participants in the 2 arms. The IPE group slept 74 minutes less than the inulin group, expressed as a change from baseline. It is unclear at present how this is linked to the IPE supplementation. Our previous findings using functional magnetic resonance imaging indicated that IPE is associated with lower blood-oxygen-level-dependent signalling in the caudate and nucleus accumbens. This brain activity is associated with a reduced desire for high-energy food. Recent reports have also suggested that the nucleus accumbens has a role in sleep–wake behaviour and mediates arousal and that lesions to this area increase wakefulness.[78]

Ongoing analysis

At present, we are still processing the 7-day diet diaries completed at each time point (baseline, 2, 6 and 12 months). This delay is attributed to various technical issues surrounding the nutrition coding software that were outside of our control. Without this data, it is difficult to ascertain whether these younger adults had irregular meal patterns and a tendency to eat outside the home to distinguish them from previous older-aged cohorts. Additionally, without these data, we cannot confirm whether the daily supplementation of IPE or inulin had an impact on overall energy intake or whether any observed outcomes were influenced by differential changes in energy intake between the study groups.

Impact of availability of inulin-propionate ester and resourcing inulin-propionate ester production on implementation

In preliminary work leading up to the application for funding, we identified a production partner and production costs associated with the production of sufficient IPE for the study. IPE is not commercially available, so dedicated production facilities were required. The decision by NIHR to withhold funding for the procurement of IPE led to some challenges. We were unable to proceed as planned and were required to find alternative funding and facilities to produce IPE at the scale required. This led to significant delays in commencing the study as the translation of our laboratory-scale production and testing of pilot plant procedures and product quality took considerable time after finding a new production partner. However, the work undertaken in this study has demonstrated that IPE can be manufactured at scale and of a quality that meets novel food safety standards and is organoleptically acceptable (and indistinguishable) and comparable with inulin.

Impact of COVID-19 on study implementation

During COVID-19, researchers pivoted to self-reported methods of data collection, including weight so that visits could continue during the lockdowns. Participants were encouraged to attend the clinic for their next visit as soon as restrictions were lifted, so a clinic-reported weight could be taken. Clinic weights were also taken when participants had self-reported 12-month weight but were still within the 1-month measurement window. There is no evidence that COVID lockdowns incurred differential compliance, however, it may have contributed to the lower compliance or higher withdrawal rates seen at the end of the intervention period.

The primary outcome of weight gain may have been affected by the change in lifestyle over the COVID period. It has been reported that there was an increase in weight gain. The alternative is that neither group gained significant weight, and it could be argued that both IPE and inulin were protective.

The strengths of this trial are the intervention duration, cohort size and participant adherence rates at 12 months (63% of participants had 50% or greater treatment adherence). However, this trial has limitations. Firstly, the dose of IPE may not have been suitable for this younger study population when dosage studies were conducted on older adults with naturally lower SCFA concentrations. Additionally, inulin was used as the control to determine the effect of propionate on weight gain, independent of the inulin backbone. However, as inulin is a fermentable fibre, it may itself reduce appetite. To disentangle these effects, a third study arm with a negative control such as cellulose could have been used. The implications of COVID-19 on dietary intake and weight gain are still unclear. While we still intend to analyse participant food diaries, preliminary data suggest an energy intake misreporting rate of 40%, calling into question data validity. COVID-19 interrupted the study, and the protocol needed to be changed to allow home measurement of weight within a new primary outcome. This was discussed and agreed with the trial oversight committees as seen by the signed SAP. ‘Principal weight’ was therefore used to assess the primary outcome of body weight change. This means that for 38% of participants, this was self-reported, which introduces bias as for any self-reported measure. It is also likely that differences in scale calibration exist between the scales used at home by participants and those in the research facility. Lastly, the protocol did not include plans for follow-up after the 12-month study visit. It may have been valuable to assess changes in weight trajectory after stopping the study treatment.

Chapter 6 Patient and public involvement

Public involvement in this trial involved the formation of a Study Advisory Group (SAG), consisting of four members of the public with similar characteristics to the trial population, joining public engagement events, study pop-ups and public focus groups. The role of the SAG was to liaise with community groups and advise on recruitment, retention, engagement and dissemination strategies for the trial. The Ethics application and participant materials were prepared in collaboration with the SAG.

Separate from the public involvement was the TSC which consisted of an independent Chair, an independent clinician, the chief investigator, a public representative (a member of the SAG) and members of the Imperial Clinical Trials Unit. The role of the TSC was to provide overall supervision of trial conduct and progress. The TSC met every 6 months throughout the trial.

These events and groups were implemented to steer the design of study protocol and materials for participants, as well as assist with recruitment and participant engagement during the trial. The methods used to engage these groups and members of the public, as well as the findings from the meetings and events, are described below.

Research design

Aims and methods

Three meetings were held with the SAG before the study opened for recruitment. The first meeting was to discuss the role of the SAG, introduce the study and study team and gain feedback on the study logo. During the second meeting, the SAG met to review the participant information sheet (PIS) and consent forms. The third meeting was to gain insight into how best to recruit participants, gain public interest and interact with participants during the study. We also participated in an Imperial Fringe event, attended by over 1200 people. At this event, we presented a summary of the trial, and the following questions were asked:

- How can we encourage study participation of young adults who are overweight?
- What are the best forms of advertising/methods to reach young adults who are overweight?
- What would make someone want to/not want to participate and maintain interest for the entire 12 months of the study?
- How much do young adults care about weight gain prevention?
- Do young adults know about the health benefits of fibre?

Outcomes and reflections

The SAG thought the 'I' in the iPREVENT logo suggested the study had to do with technology and should not be included; however, the study could not be called Prevent as that is the name of another research study. The SAG provided detailed feedback on the PIS to ensure the language and phrasing were accessible and unambiguous and the structure was easy to follow. Many amendments to the PIS were made with the assistance of the SAG surrounding clarity of descriptions of study procedures and expectations of participants, as well as making suggestions to improve the readability and accessibility (e.g. addition of images) of the PIS. During this meeting, the SAG also highlighted how certain aspects of the trial protocol (e.g. the need to keep used sachets until the next study visit) were impractical. This helped us to identify potential challenges with participant compliance with the study protocol; however, unfortunately, in the case of measuring compliance, an alternative practical and realistic method could not be identified. In the third SAG meeting, members suggested allowing participants to choose how often they were contacted by the study team so that the participant is given the appropriate level of support. The SAG members brainstormed many recruitment ideas (e.g. use of social media, poster locations, pop-up stalls), all of which were implemented by one or both sites during the study. From these meetings and the event, we were able to gain an insight into the queries, concerns and levels of understanding of our study population providing an understanding of how best to communicate concepts and procedures involved in the study. Additionally, this insight prepared the team for what to expect during the recruitment and screening process and helped to identify how we could best engage, encourage and reassure participants throughout the trial process.

Research management and implementation

Aims and methods

The aim of the public involvement during the study implementation phase was to understand how to effectively recruit, retain and engage participants and to highlight any novel concerns arising during this stage in the research cycle. Two SAG groups were held to discuss participant retention and participant concerns regarding COVID-19. Further, two public focus groups were held on Zoom video software (Zoom Video Communications, San Jose, CA, USA), to discuss recruitment and any queries or concerns the focus group had as representatives of the public.

Public engagement and involvement were also conducted through the People's Research Café at The Imperial Great Exhibition Road Festival 2022, where members of the public of all ages and backgrounds were asked:

1. How concerned are you about weight gain as you get older? Are you more concerned about preventing weight gain or about losing weight if you were to become overweight?
2. Which things do you personally feel are important when it comes to managing weight?
3. If you were to take a food supplement for a long time, which things would matter to you the most?

Outcomes and reflections

Both the SAG and the focus groups suggested many useful and practical methods for recruitment, including short videos or animations for social media, texts from GP surgeries, engaging ethnic minority communities via community centres, places of worship and asking employers to advertise the study via their staff mailing lists. These strategies were utilised when recruitment resumed after the COVID-19 restrictions were lifted. Some ideas such as posting study leaflets through letterboxes and pop-up stands in supermarkets could not be implemented due to time and staffing constraints.

The SAG and public focus groups suggested some strategies for us to implement to maintain contact with the participants and encourage compliance with the study. They suggested buddy systems with other participants or with one of the researchers. However, this was not feasible, as it would have required sharing participants' data with others. Per the focus groups' suggestion, a newsletter was designed and sent out to participants; unfortunately, there was not much engagement or response. Regular/monthly contact with the study team was implemented from March 2020 to October 2020 to ensure participants were coping with the study during the most isolating part of the COVID-19 restrictions. The SAG also advised it may be wise to provide advice on how to manage potential side effects before they arise, to reduce the risk of withdrawal, and this advice was implemented at both sites. Some other practical suggestions for participant retention were merchandise for each visit attended, celebratory online socials when milestones were reached and circulation of learning resources relevant to the trial. However, the study team did not think these were within the study budget or necessarily appropriate for participants.

The SAG also provided ideas about how we could encourage participants to attend the research facility for visits after COVID-19 restrictions were lifted. As the study was resumed early, in September 2020, participants were understandably hesitant to make the journey or attend the research facility. The following SAG suggestions were implemented: e-mail or call participants beforehand to reassure them that the necessary safety precautions will be taken at the study visit and offer reimbursement for private transport (taxis) to the research facility. It was also suggested that we incentivise attendance by offering COVID-19 tests and that we reimburse gloves and mask purchases for study visits; however, participants did not feel this was necessary.

Novel perspectives from Study Advisory Group, focus groups and the People's Research Café

Challenges identified by the SAG:

- Public understanding of BMI, weight gain prevention and fibre is lacking.
- IPE seems unnatural.
- Weight-related supplements have negative connotations.

- Participants may believe there is no benefit from the placebo.
- 'Fat phobia' may play a role in individuals not wanting to or being able to identify themselves as overweight.
- Barriers to participation that were unique to ethnic minority communities (e.g. differences in diet and presentations of chronic disease).
- Clinical research has a negative stigma.

Actions in response to challenges highlighted:

- More detailed explanation of current findings for IPE during the recruitment and screening process.
- Highlight that few side effects had been identified in previous trials.
- Calling the trial 'a nutrition study' when describing the trial to members of the public.

People's Research Café responses:

- No concern about current weight. This was because the interviewees were young, and believed they were a healthy weight or would lose weight once they had gained it.
- An overall healthy lifestyle was perceived to be of greater importance than weight and other metrics such as breathlessness when walking upstairs would be a preferred indicator of poorer health.
- Perceived factors of importance relating to weight management were diet, nutrition, energy, ability to cook, affordability of healthy food, education, exercise, stress, work, sleep, mental health, mindful eating and government policy.
- The need to understand the systemic problems behind weight gain was highlighted.
- The fact that it only had to be taken once a day with no time restriction was convenient and most had taken a supplement in the past idea in the past.

Concerns from People's Research Café:

- Public concerns regarding appetite suppressant supplements were side effects, and ramifications of stopping or long-term effects on health.
- IPE could be exploited by those with eating disorders, or it may lead to over-reliance in the future.
- Encouraging people to continue with poor dietary habits.

The feedback from the first two questions clarified some of the barriers and issues within the iPREVENT study and helped us to explore the perceived importance of weight gain prevention. The feedback also facilitated our understanding of why people had stopped taking the supplement or why sometimes it was challenging to recruit participants.

Research dissemination

Advice on dissemination is being sought from the SAG, the patient and public involvement (PPI) coordinator at the Imperial Clinical Trials Unit and the Imperial College/University of Glasgow press or media teams for methods of dissemination. The aim of dissemination is to raise awareness of the results and highlight potential future work and mechanisms of IPE. Dissemination channels will include posters or leaflets, posts on the Imperial College/University of Glasgow websites, press releases, social media posts, video clips from researchers and engagement at future science festivals. The PPI sections and lay summaries of this report and other research outputs have been or will be reviewed by SAG members in future.

Chapter 7 Equality, diversity and inclusion

In this study, we included participants of all ethnicities with an overweight BMI (25–30 kg/m^2) and the population recruited met the current proportions of global majority populations in the UK. This was facilitated by the geographically diverse locations of the study sites. It is worth noting that the BMI range for inclusion of South Asian volunteers differed from other ethnicities, as there is a tendency for an increased risk of chronic disease in this population group (BMI for South Asians: 24–27 kg/m^2).

Our recruitment methods included the use of different strategies such as GP surgery recruitment texts, websites and posters. We believe that these methods reached populations that are often under-represented in research such as those on lower incomes or ethnically diverse backgrounds. A barrier to study participation was that participants had to attend the research facility during working hours. If they were working in a job that had no flexibility, they would not be able to participate. This tends to affect those in less-secure work, indicative of lower income or lower educational attainment which are also socioeconomic risk factors for the development of obesity. Although we were able to conduct some study visits outside of working hours, a better way to combat this would have been to conduct visits during the weekend. However, this introduces other biases as it is dependent on research and medical staff availability.

A gap in research that was addressed in this project is the effect of IPE specifically in younger adults. This population group tends to be under-represented in research as individuals of this age tend to have many commitments that prevent them from participating. To alleviate some of the burden on participants, the screening visit was combined with the randomisation visit, as advised by the TMG. Furthermore, phone calls were suggested as an alternative way of continued participation if the participant was no longer willing to travel to the research facility for study visits, either due to COVID, work constraints or if they had withdrawn from the intervention.

To ensure that the study population was representative during recruitment and to identify any differences in results after study completion, study data were disaggregated based on ethnic group, age and gender of participants.

The study team and the wider trial management group consist of individuals with a wide range of experience and expertise. Unique perspectives on public opinion and concerns regarding research in general and specific to the iPREVENT study protocol were offered by members of the public/PPI members who shared similar characteristics as the study population.

Chapter 8 Impact and learning

This clinical trial indicated that IPE had no significantly different effect on weight gain compared to inulin. As neither group gained the predicted 2 kg body weight, these results underscore the importance of fibre in weight gain prevention and highlight the need to continue to promote high-fibre intakes for overall population health and weight maintenance.

Chapter 9 Implications for decision-makers

This evidence supports previous findings that emphasise the challenge of weight maintenance in younger adults. Numerous observational studies indicate that this is a period of substantial weight gain contributing to the onset of obesity. This evidence also highlights the dichotomy between the results seen in older adults and within this cohort raises questions about the differences in appetite regulation between these population groups. Understanding of appetite regulation in young people is lacking, and there is still an urgent need for research to understand what drives appetite and weight gain during this crucial life stage. There was no significant weight gain in either of the study arms. This highlights the importance of exploring the potential effect of fermentable dietary fibre on weight gain prevention and energy balance.

Chapter 10 Research recommendations

Evidence points to weight gain in early adulthood being a major driver of future obesity. Prevention of weight gain remains an under-researched topic. Our recommendations for further research are:

1. To investigate the drivers of appetite regulation and food intake in young adults, to discern factors contributing to the incremental weight gain during this period.
2. To understand the relationship between eating behaviour and IPE/inulin intake in younger people. Younger people tend to have a less traditional eating pattern of three meals a day. It is critical to understand this eating pattern to target the rise in propionate in the colon to be coincidental with major nutrient intake.
3. To investigate the optimum effective dose of IPE across different phenotypes, such as age, sex, or rate of adaptation to increases in colonic SCFA.
4. Given the observation made in this study what both inulin and IPE appear to prevent weight gain, understanding how fermentable fibre regulates appetite regulation and energy balance could yield new avenues for prevention of weight gain. Such studies should include negative controls for better understanding of underpinning mechanisms.

Chapter 11 Conclusions

To conclude, IPE did not exert a significantly greater effect on weight gain compared to inulin as demonstrated by an absence of significant differences in weight gain at 12 months. IPE was well tolerated as indicated by fewer withdrawals from treatment within this group, and no unexpected, related AEs were reported. Robust analysis leads us to conclude that compliance or withdrawals did not influence the result. No significant difference was observed between the two groups at 12 months and neither group surpassed the estimated 2 kg weight gain. This raises the possibility that fermentable carbohydrates may play a protective role in maintaining energy balance and preventing weight gain. In line with previous analyses, in this study, IPE appears to affect body composition, with significant elevations in FFM detected.

The mechanistic substudy was inadequately powered due to high rates of participant withdrawals, linked to life changes such as relocation and employment after the COVID-19 lockdowns compromising the statistical robustness of the findings. However, there was a significantly greater ad libitum meal intake in the IPE arm at 12 months. Further, IPE participants had significantly lower sleep durations according to physical activity data. These results deviate from previous evidence from IPE studies. In middle-aged cohorts, significant reductions in FM and greater weight gain prevention have been reported after IPE intake and a cohort of younger women experienced lower body fat and body weight after IPE intake combined with a moderate exercise intervention. Consistently, IPE intake has resulted in lower ad libitum energy intake, indicative of enhanced satiety signalling. Future investigations should aim to elucidate differences in behavioural and psychosocial drivers of appetite between younger and older adults. Furthermore, research is warranted to explore the role of fermentable fibre on energy balance and appetite.

Additional information

CRediT contribution statement

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Acknowledgements

All the clinical trials were conducted at the NIHR Imperial Clinical Research Facility and University of Glasgow Clinical Research Facilities; we thank all the staff and volunteers who took part in the study. We thank Professor Julian R Marchesi, Dr Marko Storch, Despoina Chrysostomou and Monica Hill for their assistance with the 16S rRNA analysis. We thank Dr Isabel Garcia Perez and Dr Jose Ivan Serrano Contreras for the urinary, plasma and faecal metabolomic analysis. We thank Eleanor McKay and Gaby Morillo Santander for the stable isotope analysis. We also thank Professor Kevin Murphy for his assistance in the GLP-1 and PYY radioimmunoassays. The views expressed in this report are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

Patient data statement

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that they are stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation. All results will be shared with the volunteers of the study once the code has been broken. Our intention is to invite all volunteers to a dissemination event to share the results of the study. Volunteers will be offered copies of the study and their own results.

Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to anonymised data may be granted following review.

Ethics statement

Ethics approval was obtained on 29 January 2019 from the London Hampstead Research Ethics Committee (REC) (Reference 19/LO/0095).

Information governance statement

For the purposes of the iPREVENT study, Imperial College London (Sponsor) acted as both the data controller and data processor. All data were collected, processed and managed in strict accordance with the General Data Protection Regulation (GDPR) (EU 2016/679) and the UK Data Protection Act (2018). A GDPR Application was submitted to the Imperial College London Data Protection Information Officer and was accepted with minor corrections (Application Reference Number: 1417 – iPREVENT). We ensured that all personal data were handled with the highest standards of confidentiality and security. Participants were fully informed about the nature of the study, the types of data collected, the purposes for which their data were used, and their rights under data protection laws, including the right to withdraw consent at any time without their care being affected. Appropriate technical and organisational measures were implemented to protect personal data against unauthorised access, loss or misuse. Access to data was restricted to authorised personnel only, and data were stored securely in compliance with our institutional data protection policies.

Data were anonymised or pseudonymised where applicable to further protect participant identities. Any transfer of data followed strict protocols to ensure the security and integrity of the information. The handling of data throughout the study was continuously monitored to maintain compliance with GDPR and the Data Protection Act, ensuring that participants' privacy rights were respected at all stages of the research. You can find out more about how we handle personal data, including how to exercise your individual rights and the contact details for our Data Protection Officer here www.imperial.ac.uk/clinical-trials-unit/dataprotection/.

Disclosure of interests

Full disclosure of interests: Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page at https://doi.org/10.3310/GKWP5267.

Primary conflicts of interest: Gary Frost, Tom Preston and Douglas Morrison are named inventors on the patent Compounds and their effects on appetite control and insulin sensitivity WO2014020344A1 and are founding directors of a Spinout company aimed at commercialising IPE production. A Toby Prevost was a member of the NIHR Public Health Research funding committee (2014–20) and a member of the NIHR COVID-19 Recovery and Learning funding committee (2020).

Publications

Protocol

Pugh JE, Anjum A, Petropoulou K, Thom G, McCombie L, Tashkova M, *et al.* Increase in colonic PRopionate as a method of prEVENTing weight gain in adults aged 20–40 years (iPREVENT): protocol of a multi-centre, double-blind, randomised, parallel-group trial to investigate the efficacy of inulin-propionate ester versus inulin (control) in the prevention of weight gain over 12 months [Internet]. *F1000Research* 2022. URL: https://f1000research.com/articles/11-1157 (accessed 24 June 2025).

Main study outcomes

Pugh JE, Petropoulou K, Vasconcelos JC, Anjum A, Thom G, McCombie L, *et al.* Increase in colonic PRopionate as a method of prEVENTing weight gain over 12 months in adults aged 20–40 years (iPREVENT): a multi-centre, double-blind, randomised, parallel-group trial. *eClinicalMedicine [Internet]* 2024 [cited 2024 Sep 26];**76**. URL: www.thelancet.com/journals/eclinm/article/PIIS2589-5370(24)00423-1/fulltext (accessed 14 April 2025).

Conference presentations

Dietary Manipulations for Health and in the Prevention and Management of Disease (Manchester Metropolitan University, UK). *Proc Physiol Soc* 2024;**56**:C08. Oral Communications: iPREVENT: Increasing colonic propionate as a method of preventing weight gain in adults aged 20–40 years, a 12-month randomised controlled trial.

International Congress on Obesity (Sao Paulo, Brazil) (2024). Oral Presentation: iPREVENT: Increasing colonic propionate as a method of preventing weight gain in adults aged 20–40 years, a 12-month randomised controlled trial.

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Appendix 1 Secondary outcomes of the iPREVENT mechanistic study

Indirect calorimetry

TABLE 29 Medians (IQR) of the iAUC at baseline for calorimetry outcomes

	IPE Median (IQR) *N* = 23	Con Median (IQR) *N* = 22
Energy expenditure	85,349 (42,632–117,819)	55,458 (32,367–110,121)
Respiratory exchange ratio	1 (0–8)	8 (1–18)
Carbohydrate oxidation	4 (0–14)	12 (6–17)
Fat oxidation	6 (2–12)	2 (0–5)

Con, inulin control.

TABLE 30 Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for calorimetry outcomes

	Baseline		12-month FU			Change from baseline		
	IPE Median (IQR) *N* = 13	Con Median (IQR) *N* = 6	IPE Median (IQR) *N* = 14	Con Median (IQR) *N* = 7	Difference (95% CI) between arms at FU	IPE *N* = 13	Con *N* = 6	Difference (95% CI) between arms of change
Energy expenditure	102,219 (60,333–161,471)	34,461 (24,195–70,383)	76,246 (28,429–129,345)	99,198 (9816–137,038)	−22,952 (−65,063 to 69,397)	−49,237 (−97,538 to 43,923)	32,035 (−31,915 to 109,039)	−81,272 (−165,218 to 34,569)
Respiratory exchange ratio	1 (0–14)	9 (2–27)	2 (1–10)	1 (0–16)	0 (−4 to 13)	2 (−2 to 6)	−4 (−18 to 9)	6 (−9 to 19)
Carbohydrate oxidation	6 (1–18)	10 (6–22)	4 (2–14)	4 (1–12)	1 (−9 to 8)	0 (−4 to 6)	−6 (−16 to 5)	5 (−4 to 17)
Fat oxidation	6 (2–11)	0 (0–4)	4 (2–6)	8 (0–9)	−4 (−4 to 7)	−3 (−7 to 2)	3 (−2 to 10)	−6 (−13 to 2)

Con, inulin control; FU, follow-up.

Fat oxidation

TABLE 31 Medians (IQR) of the AUC at baseline for fat oxidation

IPE Median (IQR) *N* = 25	Con Median (IQR) *N* = 24
12,062 (11,857–12,446)	12,058 (11,832–12,593)

Con, inulin control.

TABLE 32 Medians (IQR) of the AUC at baseline, follow-up and change from baseline for fat oxidation

Baseline		12-month follow-up		Change from baseline		
IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	IPE *N* = 16	Con *N* = 8	Difference (95% CI)
12,062 (11,857–12,449)	12,344 (12,003–13,083)	12,051 (11,926–12,216)	12,260 (12,020–12,405)	−31 (−345 to 200)	5 (−916 to 377)	−35 (−480 to 692)

Con, inulin control.

De novo lipogenesis

TABLE 33 Medians (IQR) of fasting and postprandial DNL at baseline

	IPE	Con
	Median (IQR)	Median (IQR)
	N = 16	*N* = 10
Fasting DNL (%)	10.84 (2.45–15.79)	2.94 (1.19–9.48)
PP DNL (%/day)	1.52 (−0.88 to 4.84)	0.55 (−2.07 to 2.24)

Con, inulin control.

TABLE 34 Medians (IQR) of fasting and PP DNL at baseline and 12-month follow-up with comparison of differences between study arms

	Baseline		12-month FU			Change from baseline		
	IPE	Con	IPE	Con				
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Difference (95% CI) between arms at FU	IPE	Con	Difference (95% CI) between arms of change
	N = 15	*N* = 6	*N* = 15	*N* = 6		*N* = 15	*N* = 6	
Fasting DNL (%)	11.64 (3.36–15.81)	3.55 (1.19–9.48)	6.85 (0.63–14.72)	0.22 (−2.48 to 6.60)	6.64 (−4.58 to 17.85)	−1.00 (−7.16 to 3.88)	−1.82 (−7.49 to 2.04)	0.82 (−10.31 to 11.95)
PP DNL (%/day)	1.29 (−1.06 to 4.72)	0.55 (−1.52 to 2.04)	4.53 (0.41–5.47)	4.09 (2.45–5.84)	0.45 (−3.71 to 4.60)	1.39 (−0.87 to 4.75)	3.92 (2.54–5.42)	2.52 (−7.90 to 2.85)

Con, inulin control; FU, follow-up.

Insulin

TABLE 35 Medians (IQR) of the iAUC at baseline for insulin

IPE Median (IQR) *N* = 22	Con Median (IQR) *N* = 25
48,271 (33,605–117,414)	68,755 (34,127–116,232)

Con, inulin control.

TABLE 36 Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for insulin

Baseline		12-month FU			Change from baseline		
IPE Median (IQR) *N* = 15	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	Difference (95% CI) between arms at FU	IPE *N* = 15	Con *N* = 8	Difference (95% CI) between arms of change
43,839 (29,268–84,992)	66,735 (38,990–10,6919)	42,961 (18,056–79,609)	42,591 (28,537–101,735)	370 (−40,628 to 22,879)	−4885 (−31,520 to 19,557)	−3914 (−37,425 to 16,011)	−971 (−29,868 to 24,549)

Con, inulin control; FU, follow-up.

Glucose

TABLE 37 Medians (IQR) of the iAUC at baseline for glucose

IPE Median (IQR) *N* = 21	Con Median (IQR) *N* = 25
189 (64–277)	228 (118–347)

Con, inulin control.

TABLE 38 Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for glucose

Baseline		12-month FU			Change from baseline		
IPE Median (IQR) *N* = 15	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	Difference (95% CI) between arms at FU	IPE *N* = 15	Con *N* = 8	Difference (95% CI) between arms of change
189 (71–293)	204 (122–281)	231 (136–291)	142 (75–263)	89 (−56 to 162)	2 (−45 to 66)	−28 (−112 to 67)	30 (−81 to 122)

Con, inulin control; FU, follow-up.

Breath hydrogen

TABLE 39 Medians (IQR) of the iAUC at baseline for breath hydrogen

IPE Median (IQR) *N* = 23	Con Median (IQR) *N* = 25
214 (0–720)	3168 (690–5141)

Con, inulin control.

TABLE 40 Medians (IQR) of the iAUC at baseline, follow-up and change from baseline for breath hydrogen

Baseline		12-month FU			Change from baseline		
IPE Median (IQR) *N* = 15	Con Median (IQR) *N* = 8	IPE Median (IQR) *N* = 16	Con Median (IQR) *N* = 8	Difference (95% CI) between arms at FU	IPE *N* = 15	Con *N* = 8	Difference (95% CI) between arms of change
214 (20–719)	3573 (1804–4840)	0 (0–993)	4061 (220–11,438)	−4061 (−9859 to 181)	−50 (−324 to 0)	1464 (−1456 to 5124)	−1514 (−5171 to 1480)

Con, inulin control; FU, follow-up.

Physical activity

TABLE 41 Means (SD) of the different physical activity measurements at baseline

	IPE Mean (SD) *N* = 24	Con Mean (SD) *N* = 24
Sleep duration (minutes)	437 (86)	411 (77)
Step count	9021 (4622)	8521 (4224)
Moderate + vigorous (minutes)	132 (71)	124 (66)
METs	1245 (379)	1142 (510)
PA duration[a]	344 (102)	316 (145)

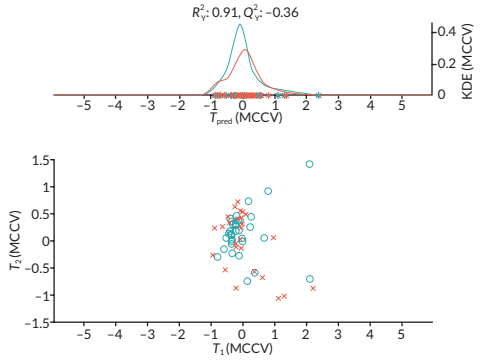
Con, inulin control; PA, physical activity.
a Defined as the sum of light + moderate + vigorous.

TABLE 42 Means (SD) of the different physical activity measurements

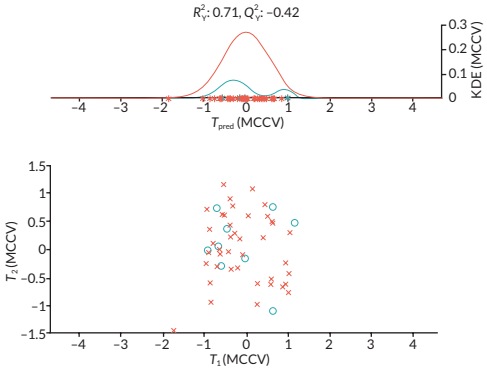
	Baseline		12-month follow-up		Change from baseline		
	IPE Mean (SD) *N* = 16	Con Mean (SD) *N* = 7	IPE Mean (SD) *N* = 16	Con Mean (SD) *N* = 7	IPE Mean (SD) *N* = 16	Con Mean (SD) *N* = 7	Difference (95% CI) between arms of the change from baseline
Sleep duration (minutes)	432 (66)	379 (50)	374 (60)	394 (60)	−58 (76)	15 (42)	−74 (−124 to −29)[a]
Step count	9406 (5130)	8473 (2988)	10,655 (5227)	9992 (2188)	1250 (3371)	1519 (1992)	−269 (−2485 to 1913)
Moderate + vigorous (minutes)	134 (66)	121 (48)	151 (84)	132 (51)	17 (60)	11 (22)	5.8 (−26.5 to 39.4)
METs	1278 (367)	1277 (395)	1461 (391)	1423 (457)	183 (306)	146 (253)	36 (−198 to 269)
PA duration (minutes)[b]	352 (99)	358 (116)	405 (104)	401 (136)	53 (86)	43 (75)	10 (−59 to 77)

Con, inulin control; PA, physical activity.
a Statistically significant.
b Defined as the sum of light + moderate + vigorous.

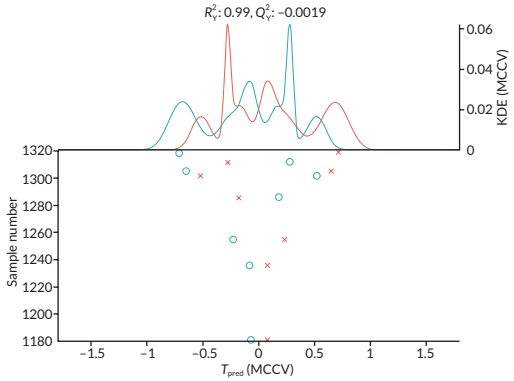
Appendix 2 Monte–Carlo cross-validation – partial least squares discriminant metabolomics analysis



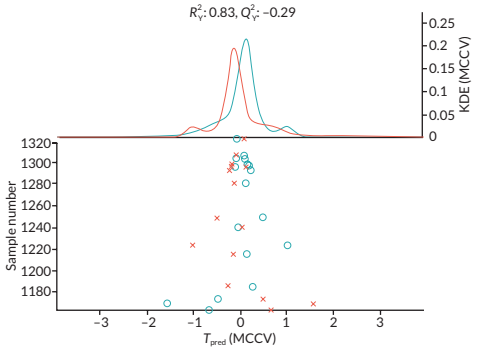
MCCV-PLSDA for baseline control and IPE to identify changes in urinary metabolites



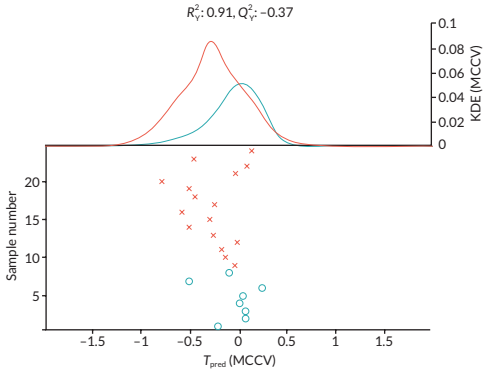
MCCV-PLSDA for 12 months control and IPE to identify changes in urinary metabolites



MCCV-PLSDA for baseline and 12 months control to identify changes in urinary metabolites

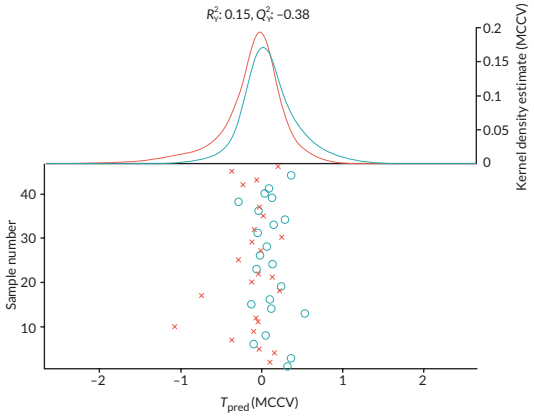


MCCV-PLSDA for baseline and 12 months IPE to identify changes in urinary metabolites

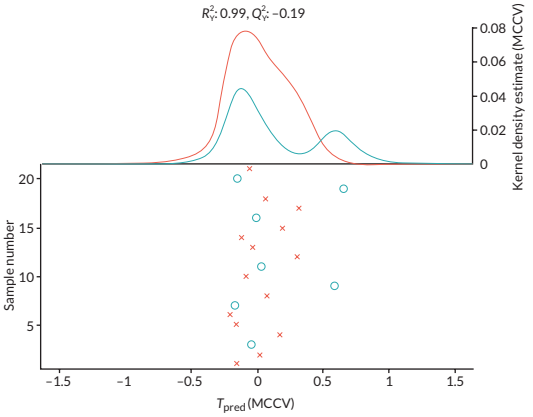


MCCV-PLSDA for Δ-change from baseline to 12 months for control vs. IPE to identify changes in urinary metabolites

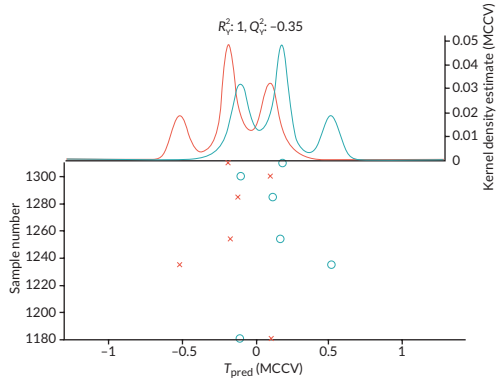
FIGURE 10 Urinary metabolites.



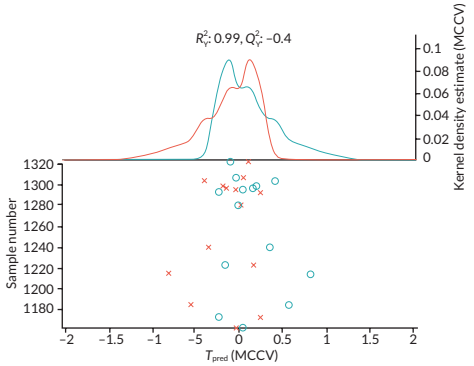
MCCV-PLSDA for baseline control and IPE to identify changes in serum metabolites



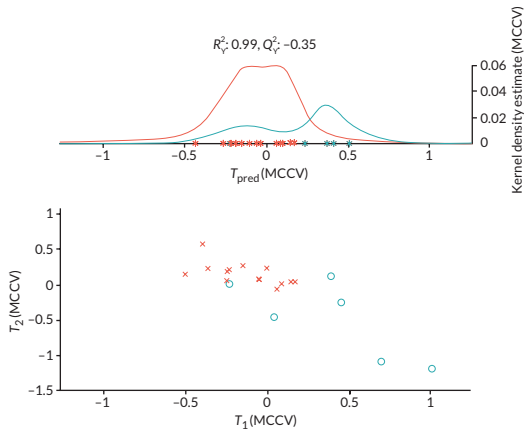
MCCV-PLSDA for 12 months control and IPE to identify changes in serum metabolites



MCCV-PLSDA for baseline and 12 months control to identify changes in serum metabolites



MCCV-PLSDA for baseline and 12 months IPE to identify changes in serum metabolites



MCCV-PLSDA for Δ−change from baseline to 12 months for control vs. IPE to identify changes in serum metabolites

FIGURE 11 Serum metabolites.

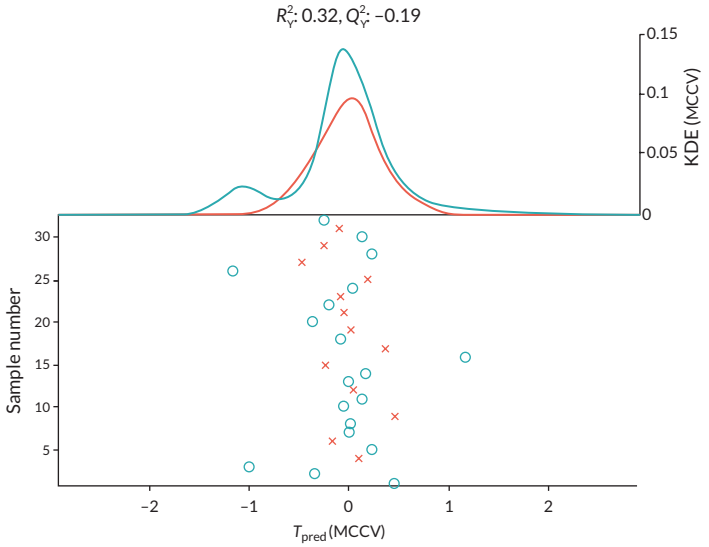


FIGURE 12 Faecal metabolomics.

Appendix 3 Changes to gut microbiome

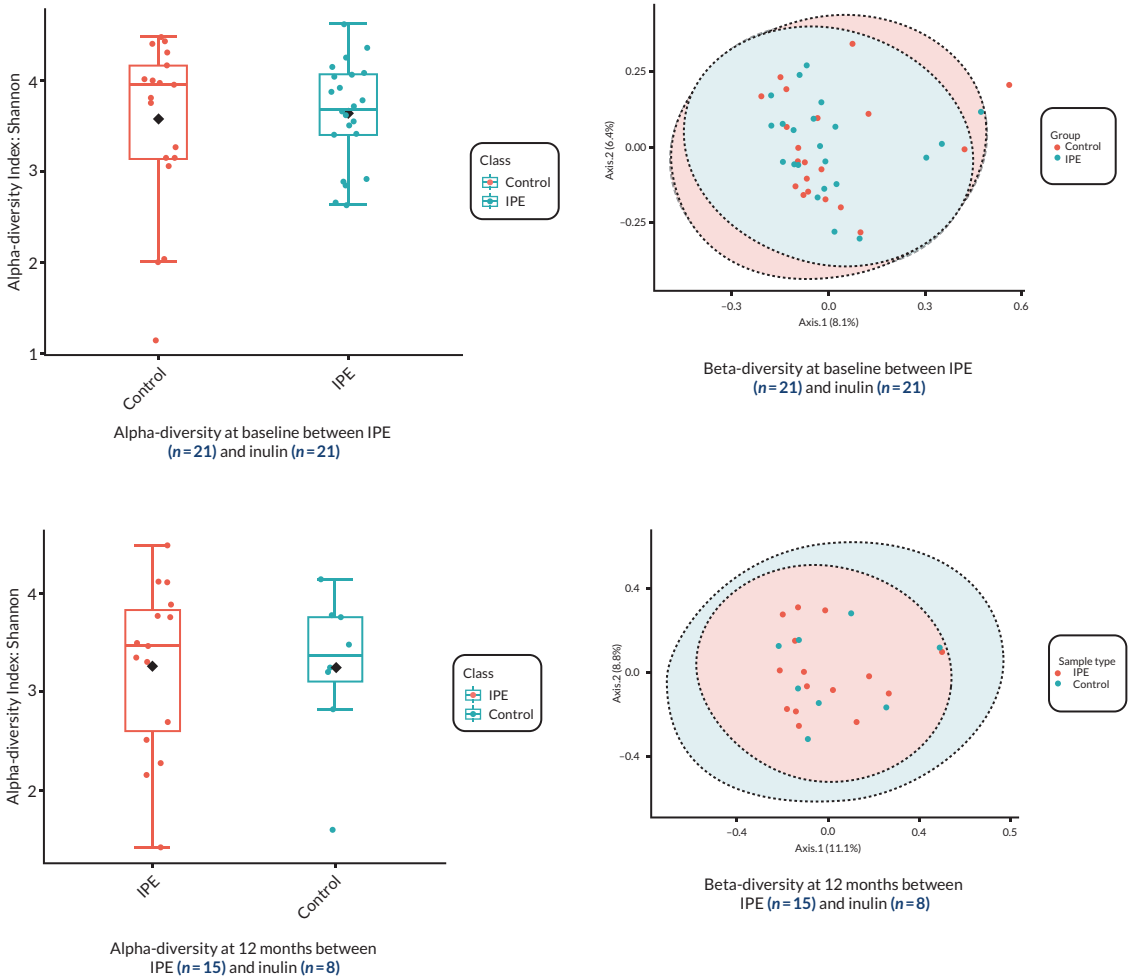


FIGURE 13 Comparing alpha- and beta-diversity between IPE and inulin study arms.

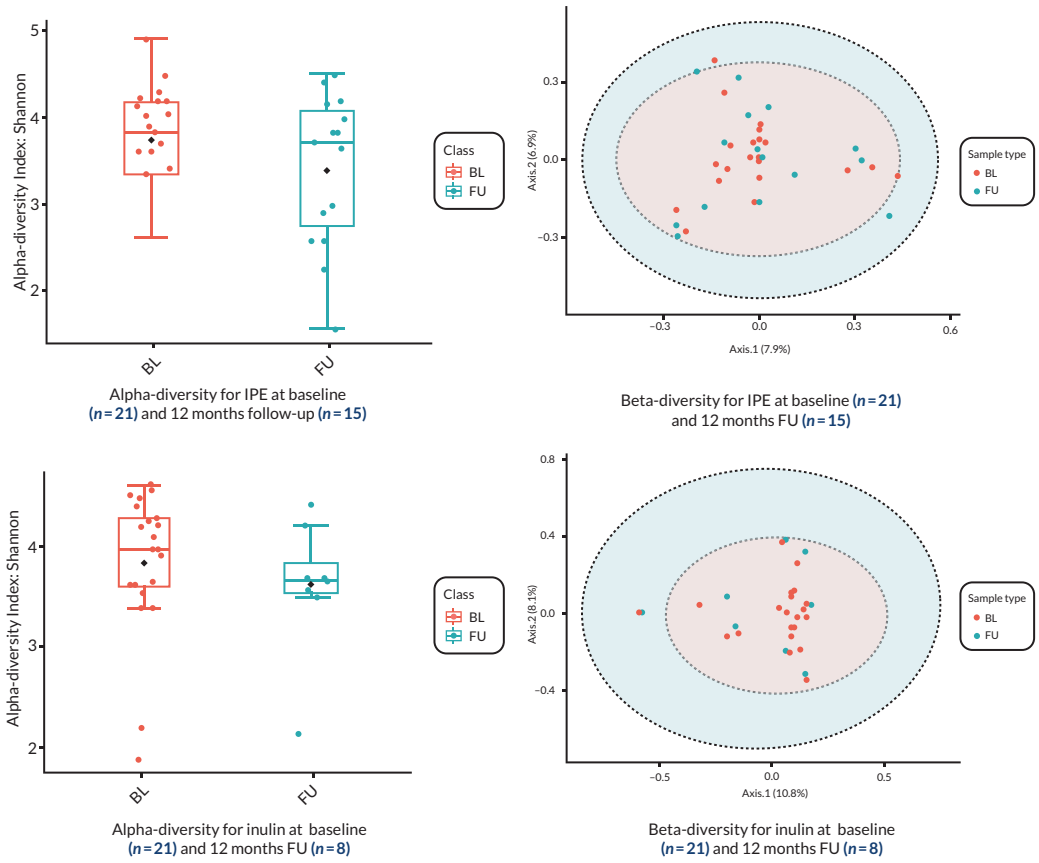


FIGURE 14 Comparing alpha- and beta-diversity baseline and 12 months gut microbiota in the IPE and inulin study arms. BL, baseline; FU, follow-up.

TABLE 43 Differences in 12-month bacteria species diversity between inulin and IPE

Bacteria species	Log2FC	LogCPM	*p*-value
Bifidobacterium bifidum	3.93	12.28	0.002
Escherichia coli	2.64	11.88	0.006
Dialister invisus	2.89	11.47	0.007
Lachnospiraceae bacterium	2.15	12.48	0.012
Desulfovibrio piger	2.28	11.07	0.016
Roseburia intestinalis	2.01	11.63	0.048
Bifidobacterium pseudocatenulatum	−6.61	14.12	0.000
Alistipes onderdonkii	−4.30	13.95	0.000
Alistipes inops	−3.11	11.24	0.000
Bacteroides ovatus	−3.99	15.35	0.000
Collinsella aerofaciens	−3.20	14.93	0.001
Bifidobacterium adolescentis	−3.13	14.93	0.001
Parabacteroides merdae	−2.84	14.55	0.004
Coprobacter secundus	−1.23	10.69	0.038
Alistipes obesi	−1.55	12.43	0.047

Note
Log2FC, log twofold change (+ve Log2FC indicates higher abundance in IPE group, −ve Log2FC indicates lower abundance in IPE group compared with inulin control); logCPM, log counts per million.

Appendix 4 Body composition by deuterium dilution

TABLE 44 Median (IQR), FFM (kg) and FM (kg) measured by deuterium dilution at baseline

	IPE	Con
	Median (IQR)	Median (IQR)
	N = 22	*N* = 22
FFM (kg)	51.3 (44.6–60.1)	46.6 (42.8–52.4)
FM (kg)	26.7 (22.8–29.1)	25.6 (20.9–31.7)

Con, inulin control.

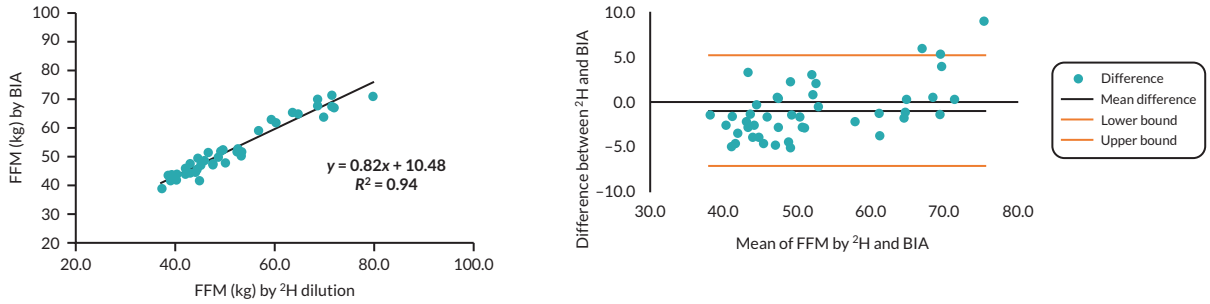


FIGURE 15 Analysis of correlation and concordance between FFM assessed by bioelectrical impedance and deuterated water dilution.

TABLE 45 Medians (IQR) for FFM and FM by ^2H_2O at baseline and 12-month follow-up with comparison of differences between study arms

	Baseline		12-month follow-up			Change from baseline		
	IPE	Con	IPE	Con		IPE	Con	
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Difference (95% CI) between arms at FU	Median (IQR)	Median (IQR)	Difference (95% CI) between arms of change
	N = 13	*N* = 6	*N* = 13	*N* = 6		*N* = 13	*N* = 6	
FFM (kg)	56.8 (45.0–64.1)	47.1 (43.8–51.9)	53.8 (49.0–66.2)	49.1 (47.2–52.6)	4.7 (−8.51 to 17.90)	1.2 (−0.1 to 5.5)	2.3 (−0.2 to 7.8)	1.1 (−3.30 to 5.58)
FM (kg)	25.4 (23.4–27.2)	23.1 (20.3–31.7)	22.2 (17.3–24.3)	23.1 (14.5–26.3)	0.9 (−6.43 to 8.32)	−2.0 (−9.2 to −0.6)	−4.6 (−7.9 to 0.0)	2.6 (−2.51 to 7.66)

Con, inulin control; FU, follow-up.

EME
HSDR
HTA
PGfAR
PHR

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