CLINICAL STUDY PROTOCOL

Full Study Title: Increase in colonic PRopionate as a method of prEVENTing weight gain in adults aged 20-40 years

Acronym: iPREVENT

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ABBREVIATIONS

AE	Adverse Event
A&E	Accident & Emergency
BDAU	Big Data Analytics Unit
BMI	Body Mass Index
BP	Blood Pressure
^{13}C	Carbon-13
CARDIA	Coronary Artery Risk Development In Young Adults (study)
CCK	Cholecystokinin
CF	Consent Form
CI	Chief Investigator
CIn	Confidence Interval
CO_2	Carbon Dioxide
CRF	Clinical Research Facility
CRN	Clinical Research Network
CSR	Clinical Study Report
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DMEC	Data Monitoring and Ethics Committee
DNA	Deoxyribonucleic Acid
DNL	De Novo Lipogenesis
DSP	Digital Signal Processor
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EME	Efficacy and Mechanism Evaluation (Programme)
FBC	Full Blood Count
FFA2/FFA3	Free Fatty Acid Receptor 2/3
FM	Fat Mass
FMI	Fat Mass Index
FFM	Fat Free Mass
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation

GEM	Gas Exchange Monitor
GI	Gastrointestinal
GLP-1	Glucagon-like Peptide 1
GP	General Practitioner
H_2O	Water
HDL	High Density Lipoprotein
HR	Heart Rate
HRA	Health Research Authority
ICH GCP	International Conference on Harmonisation for Good Clinical Practice
ICMJE	International Committee of Medical Journal Editors
ICTU	Imperial Clinical Trials Unit
ID	Identification
IPAQ	International Physical Activity Questionnaire
IPE	Inulin-Propionate Ester
ISRCTN	International Standard Randomised Controlled Trials Number
ISF	Investigator Site File
LDL	Low Density Lipoprotein
MET	Metabolic equivalent of task
MRI	Magnetic Resonance Imaging
NHANES	National Health and Nutrition Examination Survey
NIHR	National Institute of Health Research
NHS	National Health Service
NMR	Nuclear Magnetic Resonance
PAL	Physical activity level
PCA	Principal Component Analysis
PD	Protocol Deviation
PDG	Protocol Development Group
PI	Principal Investigator
PV	Protocol Violation
PIS	Patient Information Sheet
PYY	Peptide YY

QA	Quality Assurance
QC	Quality Control
REC	Research Ethics Committee
RER	Respiratory Exchange Ratio
rRNA	Ribosomal Ribonucleic Acid
SAE	Serious Adverse Event
SAG	Study Advisory Group
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SCFA	Short Chain Fatty Acid
SD	Standard Deviation
SOP	Standard Operating Procedure
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UK	United Kingdom
UKCRC	United Kingdom Clinical Research Collaboration
VAS	Visual Analogue Scales
$^{2}H_2O$	Deuterated water
3D	3-dimensional
4-CMTB	Free fatty acid receptor FFA2 agonist

Table of Contents

1. TRIAL SUMMARY

TITLE

Increase in colonic PROpionate as a method of prEVENTing weight gain in adults aged 20-40 years.

AIMS

To investigate the effects of increasing colonic propionate production on preventing weight gain, in a population of adults who are at the greatest risk of substantial increases in body weight.

DESIGN

Double-blind, randomised, placebo-controlled trial, taking place at two United Kingdom (UK) sites; Imperial College London and University of Glasgow.

SAMPLE SIZE

270 randomised participants (135 per arm), to allow a dropout rate of 25% and to provide 90% power to detect a 2kg difference between arms in mean body weight change over 12 months.

Mechanistic sub-study; 52 participants (26 per group), to allow a dropout rate of 30% and to provide 90% power to detect a 15 pmol/L effect size in Peptide YY (PYY) and Glucagon-like Peptide 1 (GLP-1) concentrations between arms.

INCLUSION/EXCLUSION CRITERIA

Main/Sub-study Inclusion

1. Males and Females aged 20-40 years*
2. Body Mass Index (BMI) of 24.00-27.00kg/m^2 if of South Asian ethnicity or 25.00-30.00kg/m^2 if non-South Asian, and at least one of the following (at screening)**:
    - A self-reported weight gain of 2kg 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)
3. On stable medication (if taking any) at point of screening
4. Written informed consent

*Notes: *20 years + 0 months to <41 years at screening*

***If BMI changes by randomisation visit and becomes outside of these ranges, participant will still be randomised*

South Asian ethnicity: This includes participants who are at least half South Asian (mixed ethnicity) as they have elevated risk factors. If only a quarter South Asian or less, participants will be considered non South Asian, for BMI categorisation purposes.

Main/Sub-study Exclusion

1. Diagnosed chronic disease; Type I and II diabetes, cancer, renal failure, heart disease, organic acidaemia (propionic acidaemia, methyl malonic acidaemia)
2. Diagnosed gastrointestinal condition including coeliac disease, inflammatory bowel disease and irritable bowel syndrome
3. Previous bowel reconstruction surgery

4. Pregnancy or lactation
5. Use of antibiotics at any time in the past 3 months
6. Untreated Vitamin B12 deficiency (<160 ng/L)*
7. Taking part in a weight loss program or consuming a weight loss product
8. Have lost 3kg or more in the last 3 months
9. Any other gastrointestinal upset (such as diarrhoea/constipation in the last 2 weeks, abdominal cramping etc.)
10. Any other reason in the opinion of the investigator

*Note: *participants with Vitamin B12 deficiency may be reconsidered for the trial, once on stable treatment for 3 months*

Sub-study Exclusion only

1. Known anaemia or as per screening FBC results (Haemoglobin levels of <130g/L for males and <110g/L for females)
2. Allergies or intolerances to any of the ingredients in the set sub-study meals

INTERVENTION / MAIN TRIAL PROCEDURES

Participants will be randomised to either Inulin Propionate Ester (IPE) or Inulin control for 12 months. Participants will attend screening, baseline (randomisation), 2, 6 and 12 month study visits at the Clinical Research Facility (CRF) of each participating site.

Participants recruited to the mechanistic sub-study will have additional assessments at baseline and 12 month study visits.

PRIMARY ENDPOINT

Weight gain from baseline to 12 months.

SECONDARY ENDPOINT(S)

- Occurrence of Adverse Events and Serious Adverse Events 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 (LDL) cholesterol
  - High Density Lipoprotein (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 (Fat mass (FM), Fat Mass Index (FMI). Percent body fat (Fat%), fat free body 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

2. BACKGROUND

2.1 Overweight and obesity are growing health concerns

Overweight and obesity affects over 60% of the UK population and drives the prevalence of a number of common co-morbidities, including type 2 diabetes, cardiovascular disease and cancer. It is estimated that the cost of obesity to the UK economy was £27million in 2015 and this expenditure is set to rise, as 700,000 new cancer diagnoses directly caused by being overweight or obese are predicted by 2035, whilst the number of people living with diabetes in the UK has topped 4 million for the first time (1). Although there has been a great focus on treatments for obesity, there has been comparatively very little on prevention. A greater emphasis on the prevention of obesity at the population level is urgently required, as recent findings indicate that current non-surgical obesity treatments are ineffective, as 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) (2).

Young adults gain weight the fastest

Weight gain occurs commonly throughout adulthood and is related to many negative health outcomes. Young adults are at the greatest risk of substantial gains in body weight, as according to a National Health and Examination Survey (NHANES) of adults aged 25–74, major weight gain over 10 years, categorised as a gain in Body Mass Index (BMI) ≥5 kg/m^2, was highest in those aged 25–35 years (3). The longitudinal data highlighted that young adults gained an average of 10 kg over a 10 year period and were hence more likely to become obese. Whilst a relatively modest weight gain of 1 kg over a single year would present a very low risk to health in young adults, the accumulated weight gain over a decade or longer leads to a clear deterioration of cardiovascular and diabetes risk factors. For example, the 10 year Coronary Artery Risk Development in Young Adults (CARDIA) study demonstrated that weight gain during early adulthood produced measurable adverse changes in blood lipids, fasting insulin, and blood pressure irrespective of race or gender (4). Furthermore, a recent study identified a clear association between an excessive BMI increase during early-life and increased risk of cardiovascular mortality in later-life (5).

Although the evidence is limited, a systematic review of weight gain prevention in young adults concludes that early lifestyle intervention can prevent onset of chronic disease biomarkers associated with weight gain (6). Consequently, any treatment or strategy that can prevent the incremental upward trajectory in body weight currently observed in young adults will have significant benefits to long-term health in this population.

Dietary fibre and short chain fatty acids

Epidemiological and experimental studies have consistently highlighted an inverse association between dietary fibre intake and body weight gain (7, 8). Current Western diets contain energy-dense foods that are generally low in fibre (10–20 g/d) and high in sugars and fats (9) .This is in marked contrast to the Palaeolithic diet, which contained >100 g/d dietary fibre, to which the human gastrointestinal (GI) system has evolved over several millennia (10). Dietary fibre passes through the small intestine unaffected by digestive enzymes, and upon reaching the colon, anaerobic bacteria are able to degrade some of these dietary fibres via a fermentation process that yields energy for the resident micro-organisms. The main end-products of microbial fermentation are short chain fatty acids (SCFA), classified as carboxylic acids that contain less than six carbon atoms. The most abundant (about 95%) SCFA present in the human colon lumen are acetate (C2), propionate (C3) and butyrate (C4), in the approximate molar ratio 60: 20: 20 (11). It is estimated that fermentation of dietary fibre in humans can yield 400–600 mM SCFA/d, and it has been consistently demonstrated that the amount of dietary fibre consumed has a considerable impact on the concentrations of SCFA produced in the large bowel (12, 13).

A significant body of evidence suggests that increasing colonic SCFA production has a beneficial role in energy homeostasis and body weight (14). However, the majority of this evidence is derived from rodent studies, which have provided animals with large amounts of dietary fibre (5–20% total energy intake) that are not feasible or translatable in human trials. Consequently, the utility of population wide supplementation with high doses of dietary fibre for body weight management is limited, as the large amounts of fermentable fibre needed to substantially increase SCFA in man results in adverse GI side effects.

2.2 Inulin-propionate ester for colonic delivery of SCFA in humans

A novel system has been developed to induce large changes in SCFA production in the human large bowel, whilst avoiding the unwanted GI side effects of high fibre diets. It was decided to increase the levels of propionate levels in the colon, as several recent studies have demonstrated that mice receiving a faecal transplant from a donor with a gut microbiota composition that elevates propionate production have reduced weight gain and adiposity (15, 16). An Inulin-Propionate Ester (IPE) was produced, whereby propionate is conjugated by an ester linkage to an inulin carrier molecule. Inulin is a naturally occurring non-digestible carbohydrate that is readily fermented by the gut microbiota in the colon. Following oral administration of IPE, the majority of the bound propionate is released only when the inulin 'carrier molecule' is fermented in the colon, providing targeted delivery of propionate to its natural site of production. Initial investigations confirmed that the majority of the conjugated propionate was released through fermentation in the colon (17, 18). 10 g IPE delivers 2.4 g propionate to the colon, which it is estimated would lead to a 2.5-fold increase in daily colonic propionate production. To achieve this through traditional dietary fibre supplementation would require ~60 g/day fibre intake. The effects of dietary supplementation with 10 g/day IPE for 24 weeks in overweight middle-aged (40-65 years) adults has been investigated. Compared with the control group, long-term supplementation with IPE significantly reduced body weight gain, which was associated with a reduced expansion in visceral abdominal adipose tissue (19) and enhanced β-cell function (20).

Based on the observed improvements in body weight management in middle-aged adults, it is hypothesised that long-term supplementation of IPE will reduce weight gain in a younger population (20-35 years) who are at a greater risk of substantial increases in body weight.

It is hypothesised that IPE will prevent weight gain by a number of complimentary mechanisms that will improve long-term energy balance:

1. **Changes in the colonic environment that promote enteroendocrine L-cell differentiation and anorectic hormone release**

   SCFA act as signalling molecules at the recently de-orphaned G-protein coupled receptors, free fatty acid receptor 2 (FFA2) and FFA3 (21, 22). Both receptors are expressed in various peripheral tissues, including the gastrointestinal (GI) tract, pancreas and adipose tissue. Within the GI tract, FFA2 and 3 have been localised to enteroendocrine L cells and these specialised gut cells secrete the appetite-suppressing hormones PYY and GLP-1 in response to luminal nutrients (23). The highest density of L cells is found in the colon where SCFA are most concentrated. Consequently, SCFA have been shown to stimulate PYY and GLP-1 release from L-cells *in vitro* (24) , whilst *in vivo* elevation of SCFA in the colonic lumen enhance release of these anorectic hormones (25). Furthermore, these effects are attenuated by deletion of FFA2 (25). Recent work in rodents also suggests that SCFA drive an expansion of the L-cell population within the colon via FFA2, leading to increased GLP-1 and PYY release, thus potentiating anorectic signalling by the gut (26).
   The profile of SCFA and concentration in the colon is determined by colonic microbiota and the nutrients available to them, particularly fermentable carbohydrate.

The IPE specifically increases the concentration of propionate in the colon, whilst the inulin backbone of the molecule produces additional SCFA when fermented by the microbiota (18). Inulin has been used as the control molecule in experiments. In the first in human studies, fluorescence in situ hybridisation was used to determine the effect of IPE on targeted phylum within the microbiota and did not detect difference in numbers or phylum between the IPE or inulin control (17).

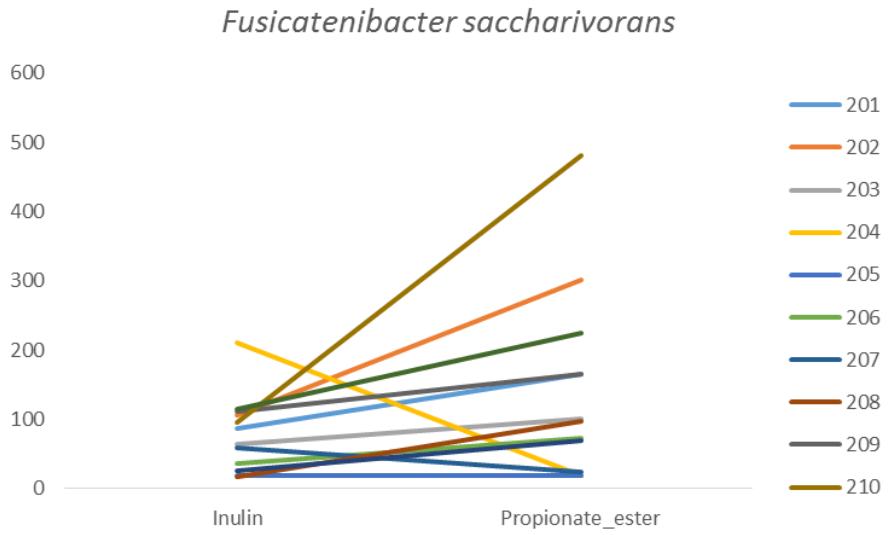


Figure 1. Comparison of the abundance of *Fusicatenibacter saccharivorans* in individuals consuming inulin or inulin propionate ester

More recent analysis using 16S metagenomics has revealed differences between the inulin and IPE. The species *Fusicatenibacter saccharivorans* was significantly increased after 6 weeks of daily IPE intake compared with the inulin control (Fig 1). This species may metabolise propionate to succinate and the production of succinate will enhance the propionate signal.

To understand the impact of IPE on the metabolite profile Nuclear Magnetic Resonance (NMR) will be used to investigate the faecal metabolome. This will provide insight into the specific metabolic changes that occur within the colon with IPE feeding. In addition, the impact of these metabolic changes on enteroendocrine L-cell differentiation using a human colonic organoid cell model will be determined, a method established in previous studies demonstrating they respond to SCFA by releasing PYY (Figure 2).

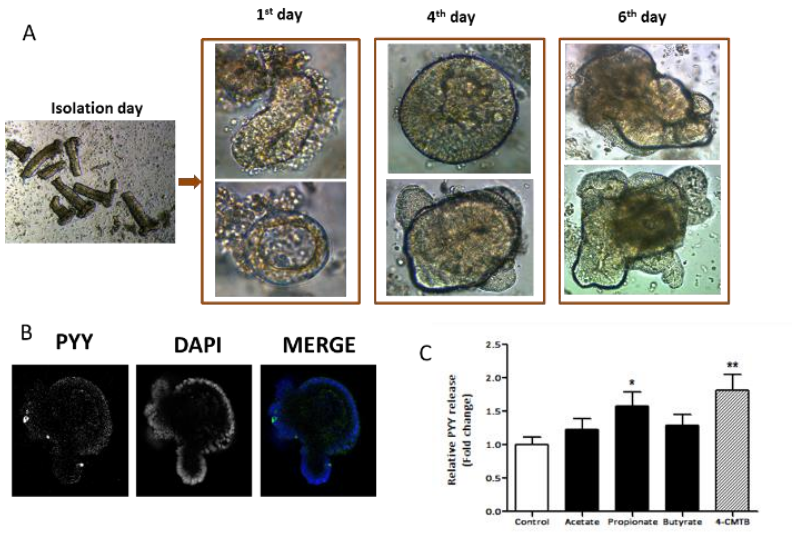


Figure 2: A) showing the development of colonic organoids from crypts isolated from colonic biopsy; B) PYY staining of a colonic organoid; C) Relative release of PYY in response to SCFA and Free fatty acid receptor FFA2 agonist **(4-CMTB)**

This is a 3-dimensional (3D) model of the human colon derived for stem cells taken from humans at colonic biopsy. Human colonic organoids will be exposed to the metabolic profile induced by IPE supplementation and it is hypothesised that this will promote L-cell differentiation and the release of anorectic gut hormones. The impact the IPE metabolic profile has on transcription factors that drive L-cell differentiation will also be investigated. It has recently been demonstrated in mice that the PYY containing L-cell differentiation is associated with an increase in expression of transcription factor Pax4 (26). This enquiry will give a fundamental understanding of the impact of the metabolic profile induced by IPE has on L-cell physiology in a human organoid model.

2. Increased anorectic signalling from the gut will improve appetite regulation

It has previously been demonstrated that acute ingestion of IPE significantly increases circulating levels of PYY and GLP-1 and reduces ad libitum food intake (17, 27). Furthermore, the effects of IPE on central nervous activity have been observed, as measured by functional Magnetic Resonance Imaging (MRI), showing that IPE lowered the responses to pictures of high-energy food in reward-related areas of the brain (27). The impact of long-term IPE supplementation on appetite regulation will therefore be monitored. Previous work has shown that supplementation of propionate leads to a specific effect on satiation. No effect on subjective appetite has been shown, as assessed by visual analogue scales between meals, but the intake at a meal is decreased (17, 27). Recent evidence suggests that circulating SCFA increase the release of gastrin (28), which will stimulate the release of (Cholecystokinin) CCK, both of which promote satiation (29). This aligns with the increased satiation and early termination of eating that we have reported following IPE supplementation.

3. Increased energy expenditure through enhanced hepatic lipid oxidation

Preliminary data and research from other groups suggests that there may be an impact of colonic-derived propionate on energy expenditure and lipid oxidation (30, 31). Pilot data demonstrates that supplementation with IPE for one week significantly increased both fasting and postprandial energy expenditure by 7%. It is hypothesised that this is driven through the stimulation of vagal afferents in the gut and the metabolism of propionate in the liver. SCFA that are not metabolised by colonocytes are released from the gut via the hepatic and portal vein system and measurements in human subjects have found that the molar fractions of acetate, propionate and butyrate change from approximately 70:20:10 in the portal vein to 90:5:5 in the peripheral circulation, demonstrating that the liver extracts a considerable amount of propionate from the circulation (11). Indeed, it has been found that that about 90% of propionate in the portal vein is extracted by the liver (32). It is hypothesised that the IPE will modulate hepatic lipid metabolism to augment lipid oxidation and supress De Novo Lipogenesis (DNL), as demonstrated in rodent models (30). Changes in resting energy expenditure will therefore be monitored using indirect calorimetry (fasting and postprandial) and accelerometry to assess active energy expenditure. To quantify changes in lipid metabolism palmitate(^{13}C) and deuterated water ($^{2}H_2O$) will be used, consumed before and during a test meal to assess the specific impact of the IPE on hepatic DNL and lipid oxidation. The appearance of ^{13}C in plasma 3-hydroxybutyrate and breath carbon dioxide (CO_2) will assess hepatic and whole body fatty acid oxidation, respectively (33). Fasting and postprandial DNL will be assessed through incorporation of ^{2}H into hepatic very low-density lipoprotein palmitate (33).

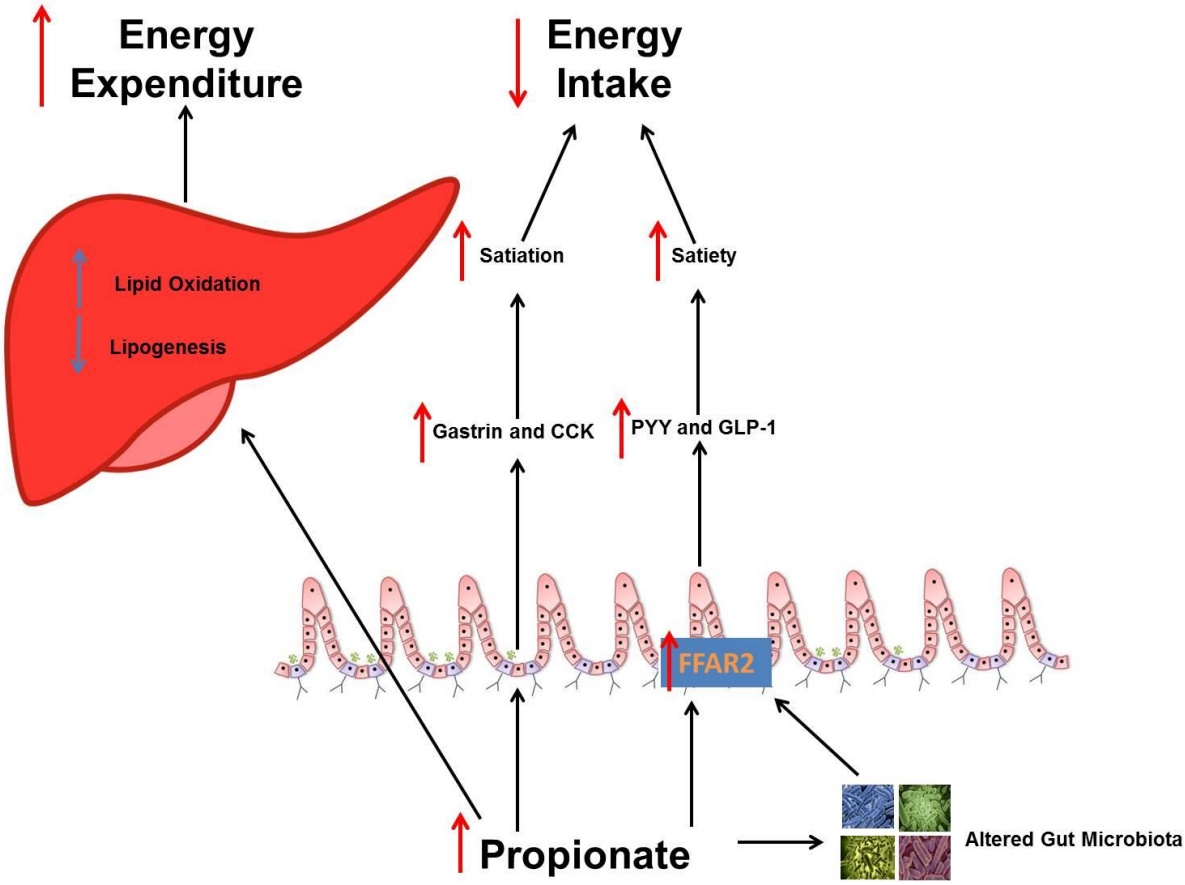


Figure 3. A schematic of the physiological mechanisms that underlie improvements in energy balance with increased colonic propionate production.

2.3 Risk / Benefit Assessment

Based on the previous 24 week study with overweight and obese adults (17), incidence of nausea and gastrointestinal side effects were extremely uncommon and not different between the IPE and inulin-control supplementation groups. The dietary intervention therefore presents a very low risk to participants, particularly as those with any previous or current gastrointestinal conditions are excluded from participating in the trial. It is predicted that long-term IPE supplementation will prevent weight-gain in young adults at the greatest risk of substantial gains in body weight. Consequently, the prevention of weight gain in this population will have benefits to long-term health in this population.

2.4 Rationale for the study

The rationale for the proposed study is to confirm the beneficial effects of increasing colonic propionate production on preventing weight gain in a population of adults who are at the greatest risk of substantial increases in body weight. This trial will test the primary hypothesis that IPE has a superior effect on preventing body weight gain, compared with inulin, over 12 months. The trial will also investigate a number of complementary mechanisms that may explain prevention of weight gain and improved long-term energy balance from consuming IPE (Fig 3). These mechanisms include monitoring changes in the colonic environment that will promote L-cell differentiation and anorectic hormone release to reduce energy intake, and increase energy expenditure via enhanced hepatic lipid oxidation.

3. OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

To measure weight gain following a 12 month intervention of IPE versus inulin control.

3.2 Secondary Objectives

1. To determine the safety profile of IPE
2. To determine effects on glucose homeostasis as a surrogate marker of type-2 diabetes risk
3. To determine effects on blood lipid and cholesterol as surrogate markers of cardiovascular disease (CVD) risk
4. To determine effects on blood pressure as a surrogate marker of CVD and stroke risk
5. To compare changes in body weight/waist/BMI/body composition during the 12 month intervention
6. To determine compliance (sachet count) during the 12 month intervention

3.3 Exploratory / Mechanistic Sub-study Objectives

a. To explore the effects of IPE on 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.
b. To explore the effects of IPE on the metabolite profile using nuclear magnetic resonance spectroscopic analyses. These data will be used to determine how these specific changes in the colonic environment influence L-cell differentiation using a human organoid model.
c. To explore the effects of IPE on anorectic gut hormones (GLP-1, PYY, gastrin and CCK) and subjective feelings of appetite via visual analogue scales (VAS), as measures of appetite regulation.
d. To explore the effects of IPE on energy expenditure and hepatic lipid metabolism as potential mechanisms involved in body weight maintenance.

3.4 Other data observations

- To compare changes in physical activity during the 12 month intervention
- To compare changes in other lifestyle factors during the 12 month intervention; smoking, drinking and recreational drugs
- To compare changes in diet during the 12 month intervention (via food diaries)

3.5 Primary Endpoint

Weight gain from baseline to 12 months

3.6 Secondary Endpoints

- Occurrence of Adverse Events and Serious Adverse Events 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 (LDL) cholesterol
    - High Density Lipoprotein (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 - Fat mass (FM), Fat Mass Index (FMI), percent body fat (Fat%), fat free body 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

3.7 Exploratory / Mechanistic sub-study endpoints

- 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 nuclear magnetic resonance spectroscopic analyses of stool
- Appetite regulation: Measured by visual analogue scales (VAS), food diaries, ad libitum intake, and appetite regulating gut hormones PYY, GLP-1, Gastrin and CCK
- 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 $^{2}H_2O$ as already applied.

3.8 Lifestyle factors

- 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)

3.9 Summary Table of Objectives and Endpoints

Objectives	Procedure / measurement	Endpoints	Timepoint (s) of evaluation of endpoint
Primary Objective:			
To measure weight gain following a 12 month intervention of IPE versus inulin control	Body weight	Body weight gain at 12 months	12 months (change from baseline)
Secondary Objectives:			
1. To determine the safety profile of IPE	Adverse events and serious adverse events reporting	Adverse events and serious adverse events rates, causality etc.	Duration of study
2. To determine effects on glucose homeostasis as a surrogate marker of type-2 diabetes risk	Fasting blood test	Fasting biochemistry: - Glucose - Insulin	6 and 12 months (changes from baseline)

3. To determine effects on blood lipid and cholesterol as surrogate markers of CVD risk	Fasting blood test	Fasting biochemistry: - Triglycerides - Total cholesterol - LDL cholesterol - HDL cholesterol	6 and 12 months (changes from baseline)
4. To determine effects on blood pressure as a surrogate marker of CVD and stroke risk	Blood pressure (BP) (Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP))	Blood pressure (SBP and DBP)	2, 6 and 12 months (changes from baseline)
5. To compare changes in body weight/ waist/ BMI/body composition during the 12 months intervention	Body weight Waist/hip BMI Body composition -Fat mass (FM), Fat Mass Index (FMI). Percent body fat (Fat%), fat free body mass) (FFM) and FM/FFM ratio	Body weight Waist/hip BMI Body composition (((Fat mass (FM), Fat Mass Index (FMI). Percent body fat (Fat%), fat free body mass) (FFM) and FM/FFM ratio))	2 and 6 months (changes from baseline) 2, 6 and 12 months (changes from baseline)
6. To determine compliance during the 12 month intervention	Accountability of returned unused sachets	Compliance	2, 6 and 12 months (changes from baseline)
Exploratory / mechanistic sub-study objectives:			
a. To compare changes in colonic metabolism to identify the relative abundance of the bacterial component of the microbiome	Stool sample - metataxonomic analysis of the 16S rRNA gene Hydrogen breath test	Gut microbiota abundance	12 months (changes from baseline)
b. (i) To analyse the metabolite profile (ii) To determine how these specific changes in the colonic environment influence L-cell differentiation	(i) I Stool sample - nuclear magnetic resonance spectroscopic analyses (ii) Human organoid model	(i) Level of SCFA and metabolites profile (ii) Proliferation of L-cells in intestinal organoid	12 months (changes from baseline)

c. Appetite regulation: (i) To compare subjective feelings of appetite (ii) To compare anorectic gut hormones	(i) Visual Analogue Scales (VAS) (ii) Ad libitum test meal (meal in excess) followed by series of blood tests	(i) Visual Analogue Scales (VAS) scores of appetite (ii) Gut hormones: - PYY - GLP-1 - Gastrin - CCK and Insulin/glucose	12 months (changes from baseline)
d. Mechanisms involved in weight maintenance: (i) To compare energy expenditure (ii) To compare hepatic lipid metabolism (iii) To compare Total Body Water within the mechanistic sub-study	(i) Open loop indirect calorimetry (ii) Stable isotope tracers of fat oxidation ($^{13}CO_2$ + ^{13}C-beta-hydroxybutyrate) and De Novo Lipogenesis (^2H_2O) (iii) ^2H_2O in body water (blood)	(i) Energy expenditure (carbohydrate oxidation, fat oxidation, energy expenditure, RER – respiratory exchange ratio) (ii) Fat oxidation (breath and blood samples) and De Novo Lipogenesis (blood + urine samples) (iii) Total Body Water	12 months (changes from baseline)

Lifestyle factors			
A. To compare changes in physical activity during the 12 month intervention	IPAQ Accelerometer - 7 days	IPAQ overall score (for all participants) Accelerometer data (for sub study participants only): - total energy expenditure - active energy expenditure - average metabolic equivalent of tasks (METs) - physical activity duration - step count - duration on body - lying down - sleep duration	2, 6 and 12 months (changes from baseline for all participants)
B. To compare changes in other lifestyle factors during the 12 month intervention	Questions regarding smoking, drinking, drug-taking	Smoking, drinking, drug-taking prevalence	2, 6 and 12 months (changes from baseline, for all participants)
C. To compare changes in diet during the 12 month intervention	Food diaries	-Energy (in kcal or kJ) -Protein (g) -Fat (g) -Carbohydrate (g) -Fibre (g)	2, 6 and 12 months (changes from baseline, for all participants)

4. STUDY DESIGN

4.1 Design

This trial will be performed at two UK sites; Imperial Clinical Research Facility (CRF) in London - Imperial College Healthcare NHS Trust and Glasgow CRF - NHS Research Scotland. This is a randomised, placebo-controlled, double-blind trial to investigate the impact of inulin propionate ester (IPE) on weight gain prevention. Participants will be randomised to either IPE or inulin control for 12 months.

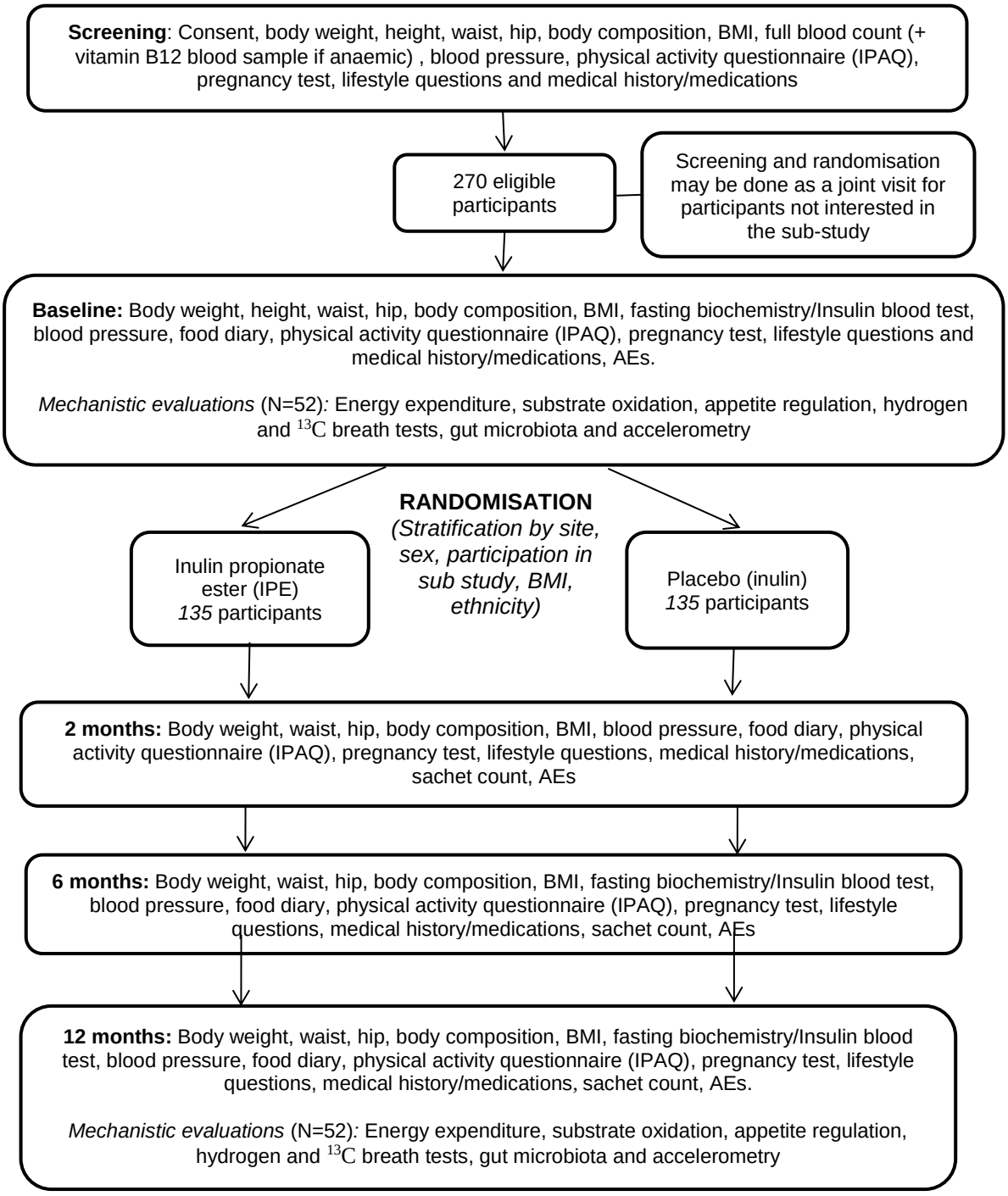


Figure 4. Trial flow chart

5. PARTICIPANT ENTRY

5.1 Trial setting and population

Adults aged 20-40 years, who are overweight (South Asians BMI 24-27kg/m^2 / non-South Asians BMI 25-30kg/m^2) but not obese and at a high risk of weight gain.

(i) Inclusion criteria

1. Males and Females aged 20-40 years*
2. Body Mass Index (BMI) of 24.00-27.00kg/m^2 if of South Asian ethnicity or 25.00-30.00kg/m^2 if non-South Asian, and at least one of the following (at screening)**:

    a. A self-reported weight gain of 2kg or more over the last 12 months

    b. Low self-reported physical activity ('low' as per International Physical Activity Questionnaire - IPAQ)

    c. Low self-reported fruit and vegetable intake (<2 servings of fruit and vegetables per day)

    d. Self-reported high intake of sugar sweetened beverages (>1 serving per day)

3. On stable medication (if taking any) at point of screening
4. Written informed consent

*Notes: *20 years + 0 months to <41 years at screening*
***If BMI changes by randomisation visit and becomes outside of these ranges, participant will still be randomised*

South Asian ethnicity: This includes participants who are at least half South Asian (mixed ethnicity) as they have elevated risk factors. If only a quarter South Asian or less, participants will be considered non South Asian, for BMI categorisation purposes.

(ii) Exclusion criteria

1. Diagnosed chronic disease; Type I and II diabetes, cancer, renal failure, heart disease, organic acidaemia (propionic acidaemia, methyl malonic acidaemia)

2. Diagnosed gastrointestinal condition including coeliac disease, inflammatory bowel disease and irritable bowel syndrome.

3. Previous bowel reconstruction surgery

4. Pregnancy or lactation

5. Use of antibiotics at any time in the past 3 months

6. Untreated Vitamin B12 deficiency (<160 ng/L)*

7. Taking part in a weight loss program or consuming a weight loss product

8. Have lost 3kg or more in the last 3 months

9. Any other gastrointestinal upset (such as diarrhoea/constipation in the last 2 weeks, abdominal cramping etc.)

10. Any other reason in the opinion of the investigator

*Note: *participants with Vitamin B12 deficiency may be reconsidered for the trial, once on stable treatment for 3 months*

Sub-study Exclusion only

1. Known anaemia or as per screening FBC results (Haemoglobin levels of <130g/L for males and <110g/L for females)
2. Allergies or intolerances to any of the ingredients in the set sub-study meals

6. PROCEDURES AND MEASUREMENTS

6.1 Identification and recruitment of patients

Participants will be recruited via a range of methods. The trial will be advertised in General Practitioner (GP) clinics, CRFs, universities and other public places using posters, via local newspapers e.g. Metro and Evening Standard, via social media and radio, videos, pop up events, research recruitment websites, research recruitment phone apps and University/NHS staff and departmental mailing lists.

GP mailshots may be coordinated using docmail via GP clinics as well as GP texting services. The Discover database (London) and SHARE database (Glasgow) which are databases of volunteers who have consented to be contacted for research, will also be a source of potential participants.

Participant Identification Centres (PICs) may be added.

Eligibility of potential participants will be identified by a pre-screening questionnaire, following received interest/response to advertising or contact through the above database organisations. This will likely be done via telephone or by email, and verbal/email consent obtained for this pre-screening.

The trial will be registered on the National Institute of Health Research (NIHR) Clinical Research Network (CRN) Portfolio, enabling eligibility for National Health Service (NHS) service support costs and interaction with the UK Clinical Research Facilities and the Study Support Service.

6.2 Screening and pre-randomisation evaluations

Written informed consent will be obtained before the participant undergoes any trial procedures including blood pressure measurements, physical activity questionnaire (IPAQ), blood sample collection, height/weight/waist/hip/body composition measurements and pregnancy test (females only). Data about other lifestyle factors and medical history and concomitant medications will also be obtained. Participants meeting the trial eligibility criteria will be asked to consider taking part in the trial., The above will be repeated at screening and baseline/randomisation.

For participants that are not interested in the sub-study, screening and randomisation may be done as a joint visit, in which case measurements and blood samples will only be taken once. FBC results are not required to be known prior to confirming eligibility for the main study.

For participants that are interested in doing the sub-study, FBC results must be awaited to confirm that participants are not anaemic, as a larger blood volume is being drawn for this aspect of the trial. Therefore, participants will be required to come back for a separate baseline/randomisation visit.

If a potential participant is known to have Vitamin B12 deficiency which is untreated, they will be excluded from both the main and sub study. They may choose to return to be reconsidered for the study, following 3 months on stable treatment.

If anaemia is known or confirmed as per FBC results, and the cause is not due to Vitamin B12 deficiency, the participant may proceed to take part in the main study only or the participant may choose to be treated for anaemia and return after 3 months of stable treatment, to be re-considered for both the main and sub-study.

6.3 Randomisation and Blinding

Randomisation and unblinding will be carried out using a web-based randomisation and Electronic Data Capture (EDC) system, called Sealed Envelope.

The trial data will be collected on a separate Electronic Data Capture (EDC) system called InForm.

If at the randomisation visit a participant's BMI has changed since screening, and is now below the minimum or above the highest value in the BMI criteria that applies to their ethnicity (non-South Asians 25.00-30.00kg/m^2 ; South Asians 24.00-27.00kg/m^2) , they will still be randomised.

Participants who are at least half South Asian (mixed ethnicity) will have the South Asian BMI category applied as they have elevated risk factors. If only a quarter South Asian or less, participants will have the non-South Asian BMI category applied.

Randomisation will be undertaken using minimisation with a random element in order to balance the arms by centre, sex, BMI and 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 volunteer to take part in the mechanistic sub study. This will ensure that equal and representative numbers are assigned to each intervention group.

Participants will receive blinded, identical-looking and identically packaged trial intervention of either active IPE or inulin control.

Participants will be identified with a unique trial identifier and each IPE or control sachet will be identified with a unique treatment code linked to the allocation and trial identification (ID).

6.4 Code-breaking / Unblinding

6.4.1 Emergency unblinding

Each participant will be assigned a unique trial ID and each sachet dispensed will be identified with a unique treatment code which is linked to the treatment allocation. The treatment code must not be broken except in medical emergencies when the appropriate management of the participant necessitates knowledge of the treatment, or in the event that expedited reporting to the Research Ethics Committee (REC) of an unexpected and related Serious Adverse Event (SAE) is required.

The trial EDC system will include an automated unblinding facility, in case unblinding is required. In the event that emergency unblinding of an individual participant is required, authorised staff (as documented on the delegation log) will follow trial procedures to unblind the participant in question and proceed with expedited reporting if required.

Unblinding should only be considered if management of the participant would differ depending on whether they are on IPE or Inulin control.

6.4.2 Unblinding for statistical analysis

When performing safety and interim analyses, the integrity of trial blind should be maintained, with the exception of the Data Monitoring and Ethics Committee (DMEC) who will have access to fully unblinded data.

The Study Statistician preparing the DMEC report will also require access to fully unblinded data in order to prepare the report. Unblinding in these cases should take place in accordance with Imperial Clinical Trials Unit (ICTU) Standard Operating Procedures (SOP) and documented accordingly.

6.5 Visit Schedule

The schedule of visits for the trial is summarised in the Table below.

Assessment	Screening	Baseline/Randomisation (up to 4 weeks after screening)	2 months (+/- 2 weeks)	6 months (+/- 4 weeks)	12 months (+/- 4 weeks)
	Or joint screening/randomisation				
Consent	X				
Demographics	X				
Randomisation		X			
Medical History	X	X	X	X	X
Concomitant medications	X	X	X	X	X
Pregnancy test - females only	X	X	X	X	X
Vital signs: Diastolic Blood Pressure(DBP) Systolic Blood Pressure (SBP) Heart rate (HR)	X	X	X	X	X
Trial intervention (IPE or inulin control)		X	X	X	
Height, body weight, waist/hip measurements, BMI, body composition	X	X	X	X	X
Fasting blood test (glucose, insulin, lipid profile)		X		X	X
Full Blood Count (and Vitamin B12, if required)	X				
Food diary		X	X	X	X
International Physical Activity Questionnaire (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 (52 ppts; Imperial CRF only)					
Blood sample to measure natural abundance of deuterated water	X				
Energy expenditure (indirect calorimetry)		X			X
Appetite regulation (VAS, food diary, ad libitum test meal, and blood tests for anorectic gut hormones)		X			X
Substrate oxidation/DNL (via stable isotype tracers in water consumption) – ^{13}C breath, urine and blood samples		X			X
Gut microbiota (stool sample and hydrogen breath test)		X			X
Neuroendocrine cell number (stool sample)		X			X
Accelerometry		X			X

6.6 Follow-up

Participants will be invited to attend screening, baseline/randomisation and all subsequent trial visits at 2, 6 and 12 months at the Clinical Research Facility (CRF) of each participating site.

Participants recruited at Imperial will also be invited to participate in the mechanistic sub-study and if they agree these assessments will take place at baseline and 12 months at the Imperial CRF.

There must be a maximum 4 week gap between screening and randomisation (if these are not a joint visit), to avoid weight changes that may occur over time. A window of +/- 2 weeks will be allowed for the 2 month visit, whilst a window of +/- 4 weeks (28 days) will be allowed for 6 and 12 months visits, for convenience and availability of participants.

6.7 Trial assessments

6.7.1 Body weight and height

Body weight will be measured to the nearest 0.1 kg with standard electronic weighing scales (Tanita BC-418MA). Participants will be asked to take heavy/outer layers of clothing off, as well as accessories and shoes, and to void their bladder before measurements are taken to improve accuracy of the recorded body weight. Height will be measured once at screening and used throughout the study along with weight to calculate BMI.

6.7.2 Waist-hip ratio

Waist and hip measurements will be taken at every study visit and waist-hip ratio calculated. This is an indicator of fat around the abdomen.

6.7.3 Body composition

Body composition will be measured using bioelectrical impedance. This estimates total body water which is used to estimate fat-free body mass and, by difference with body weight, body fat.

6.7.4 Food diary

Participants will be asked to complete 7-day food diaries at baseline, 2, 6 and 12 months, to monitor changes in food intake in the home environment.

6.7.5 Physical activity

All participants will complete the International Physical Activity Questionnaire (IPAQ) at each study visit.

Sub study participants will additionally wear an accelerometer for 7 days prior to the trial visits, to assess changes in total energy expenditure, active energy expenditure, average metabolic equivalent of tasks (METs), physical activity level (PAL), physical activity duration, step count, total distance, lying down, sleep duration and sleep efficiency. For a day's data to be valid, the accelerometer must have been worn and data collected for at least a total of 20 hours in the day.
The accelerometer does not need to be taken off whilst showering, swimming etc. and should be continuously worn, including when sleeping.

6.7.6 Other lifestyle

Questions relating to smoking, alcohol and recreational drugs will be asked at each study visit, to monitor any changes from screening over the duration of the study.

6.8 Laboratory Evaluations

Blood samples will be obtained from participants for Full Blood Count (FBC) and Vitamin B12 at screening. For participants intending to take part in the sub study, an additional blood sample will be collected to measure their natural abundance of deuterated water. For participants already part of the sub study, this sample will be collected at the 6 month visit instead, when no consumption of deuterated water is required for the study.

Blood samples for fasting biochemistry will be taken at baseline, 6 month and 12 month study visits, along with a pregnancy test at every study visit. The total amount of blood to be taken from each subject during the study is approximately 39-44ml (3-8ml at screening, 12ml at three study visits).

The total amount of blood to be taken from each subject for the sub-study is an additional 120ml per baseline and 12m sub-study visits (10ml taken at 12 time points). A urine sample, stool sample and breath samples will also be taken at each sub study visit.

Sample handling / processing details will be described in a separate laboratory manual.

6.8.1 Fasting biochemistry

Glucose, insulin, triglycerides, total cholesterol, LDL cholesterol, HDL-cholesterol will be measured at baseline, 6 and 12 months. Glucose and lipid profile will be analysed by local NHS laboratories in accordance with standard practice. Insulin will be measured by the research labs at the Imperial College London. These analyses will give an indicator of secondary markers of health benefit.

Vitamin B12 will only be measured at screening for potential participants who are known to be anaemic but the cause is unknown or for potential participants who are shown to be anaemic when the full blood count is done at screening. These participants may have an additional visit at screening for a further blood sample to be collected, to measure Vitamin B12.

6.8.2 Pregnancy Test

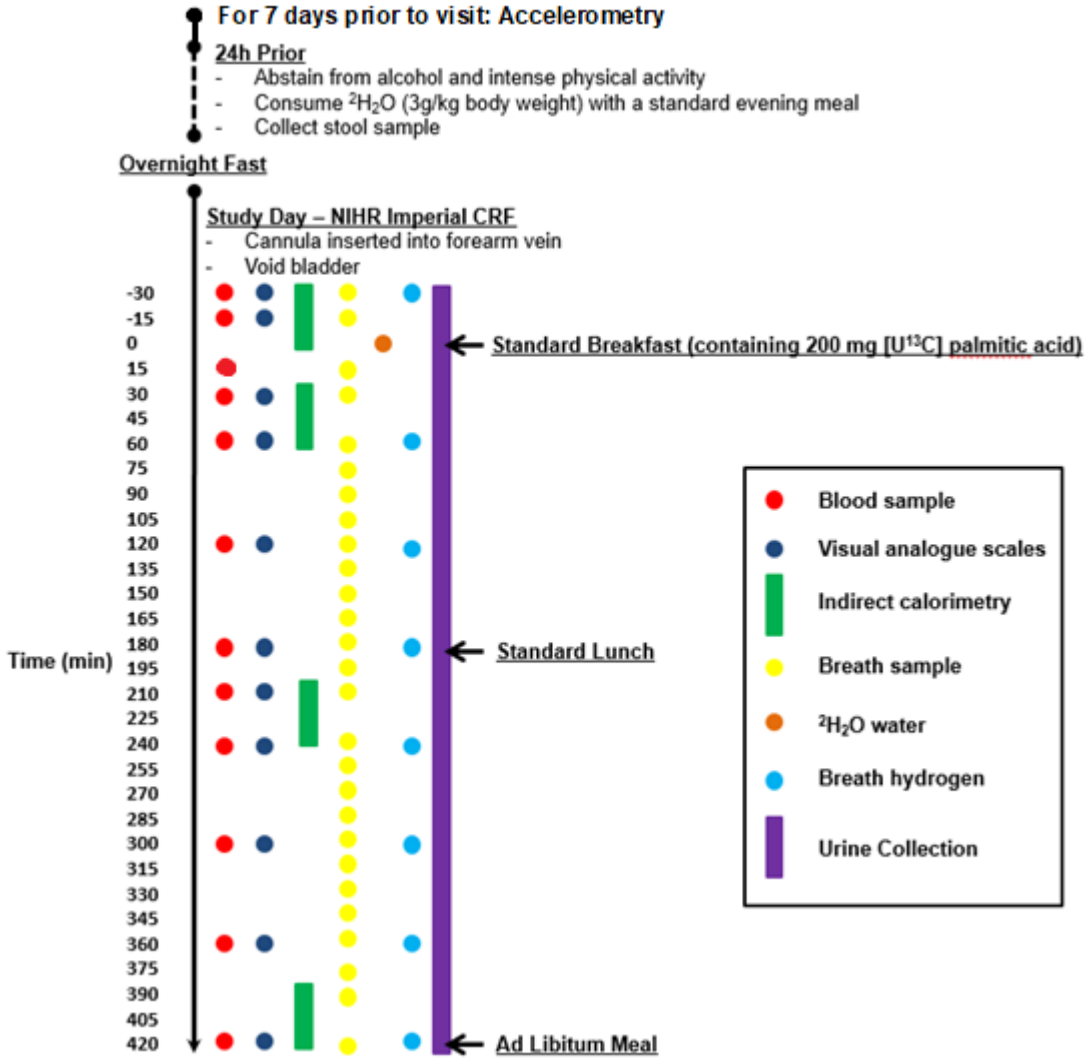
A pregnancy test will be performed at all trial visits for all female participants regardless of whether they are considered to be using adequate contraception. Participants will be asked that they and/or their partners use adequate contraception whilst taking part in the trial to prevent pregnancy or for male participants to prevent pregnancy in a female partner.

If a participant is found to be pregnant at any point during the trial, the intervention will be withdrawn for safety, but follow up will continue until the end of trial, unless there is withdrawal of consent. Follow up data collected after intervention withdrawal will be excluded from the analysis populations.

The event of pregnancy in a participant will be collected on the trial database on a specific pregnancy notification eCRF. Pregnant partners of participants will not be followed up for outcome of pregnancy, although they are welcome to call the CRF/study team should they have any study related concerns at any point during or after the study.

6.8.3 Exploratory mechanistic sub-study samples

The schedule of exploratory samples and investigations is summarised in the chart below.



(i) Gut bacteria microbiome and metabolite analysis

Participants consenting to the mechanistic sub study will provide one stool sample at baseline and one after 12 months of dietary intervention. Deoxyribonucleic Acid (DNA) extracted from stool samples will be subjected to a metataxonomic analysis of the 16S rRNA gene to identify the relative abundance of the bacterial component of the microbiome. Approximately 10,000 16S rRNA genes will be sequenced per sample using an Illumina MiSeq platform and data analysed using Mothur to generate shared abundance tables and diversity indices. Nuclear magnetic resonance spectroscopic data will be acquired using well-established sample preparation and spectral acquisition protocols (34).

Multivariate pattern recognition analyses will be carried out on global spectral profiles using similarly well-established and validated techniques such as Principal Component Analysis (PCA), Orthogonal Partial Least Squares Discriminant Analysis and non-linear methods such as Bayesian probabilistic analyses.

Impact on neuroendocrine cell number and proliferation in intestinal organoids will be analysed by levels of SCFA and other metabolites identified from stool analysis.

(ii) Appetite regulation and gut hormone levels

The role of anorectic hormones released from the gastrointestinal tract will be investigated along with subjective feelings of appetite and ad libitum food intake. Participants will arrive at the CRF following an overnight fast and a cannula will be inserted into a forearm vein for repeated blood sampling. Blood samples for analysis of Insulin, glucose, PYY, GLP-1, gastrin and CCK will be collected at -30, -15, 15, 30, 60, 120, 180, 210, 240, 300, 360 and 420 minutes. Subjective hunger, satiety and nausea will be monitored with the use of 100 mm Visual Analogue Scales (VAS) at these time points too, before each blood sample.

At 0 minutes a standard breakfast will be provided with a sachet of food supplement and at 180 minutes a standard lunch will be served. At 420 minutes, volunteers will be offered a buffet meal with food served in excess, and asked to eat until they feel comfortably full. The amount of food will be quantified and energy intake calculated.

(iii) Energy expenditure and substrate oxidation (lipid metabolism)

Whole body energy expenditure and substrate oxidation rates will be quantified throughout the test meals using indirect calorimetry (Gas Exchange Monitor; GEM nutrition). Each assessment will last approximately 30 minutes and measurements will be recorded following the overnight fast between -30 to 0 minutes, 30-60 minutes, 210-240 minutes, and 390-420 minutes of the test meal protocol, to assess postprandial changes. We have considerable experience with indirect calorimetry in our laboratory (35, 36).

The evening prior to the trial visit, participants will be asked to consume deuterated water (^2H_2O) (approximately 2-3 g/kg body water) and then again on the day of the study visit with the breakfast, in order to achieve and maintain a plasma water enrichment of 0.3% for the measurement of fasting and postprandial hepatic De Novo Lipogenesis (DNL). 200mg of [U-^{13}C] palmitic acid will be added to the standard breakfast at 0 minutes.

DNL will be quantified through the incorporation of heavy Hydrogen (^2H) into very low-density lipoprotein palmitate measured in the collected blood samples (33). The appearance of ^{13}C in plasma 3-hydroxybutyrate and in breath CO_2 in breath tests done every 15 minutes (from -30 minutes to 420 minutes, except at 0, 45, 225 and 405 minutes), will assess hepatic and whole body fatty acid oxidation, respectively (33). Hydrogen breath tests will also be done at -30 , 60, 120, 180, 240, 300, 360 and 420 minutes.

6.8.4 Sample storage and analysis

Screening full blood count (FBC), fasting biochemistry (except for Insulin and pregnancy test samples) will be transferred to local laboratories immediately for analysis. Blood samples for Insulin will be stored locally

and sent to the Imperial College London research labs for analysis, by the end of the study. Any remainders of samples will be discarded. Urine samples collected from females for a pregnancy test will be analysed on the spot for a positive or negative result and then discarded of.

Mechanistic sub study blood, stool, urine and breath samples will be stored at participating sites.

Some of these samples will later be transferred by appropriate courier to central laboratories at the University of Glasgow for storage and analysis, after which they will be destroyed. Remaining samples will be analysed at the research labs at Imperial College London, by the trial investigators and then transferred to the Imperial College Healthcare Tissue Bank.

Further details will be defined in study specific guidelines for sample collection and handling.

Samples will be kept beyond the end of the trial and stored in accordance with the Human Tissue Act. These will be banked in a HTA licensed Imperial College Healthcare Tissue Bank for use in future ethically approved research, except for samples whose' participants did not consent to this on their consent form – these will be safely disposed of.

6.9 Remote visits, additional study visits and measurements

6.9.1 Remote visits

Participants may have had their 2-month, 6-month or 12-month visit done remotely via telephone (due to covid-19 or other reasons), and a self-reported weight may have been collected where possible. For 6-month and 12-month remote visits, the blood sample, blood pressure and weight-related body measurements would have been missed.

Further supply of the study food supplement would have been posted to those who completed their 2 or 6-month remote visit. Those who had their 12-month visit would require no further supply as this is the final visit.

A note will be added on the InForm database and/or a log kept, to specify when visits have been done remotely.

6.9.2 Additional visits

For those who had a remote 6-month visit, these participants will be asked to come in for a study clinic visit as soon as possible before 11 months, to obtain an accurate clinic weight (calibrated study scales) and body measurements, blood pressure and a blood sample for biochemistry results. If they have reached 11 months of being in the study, this should not be done as this would fall within the 12-month visit window.

For those who had a remote 12-month visit, these participants will be asked to come in for a study clinic visit as soon as possible up to one month after the remote visit was done, to obtain an accurate clinic weight (calibrated study scales) and body measurements, blood pressure and a blood sample for biochemistry results. If it has been more than one month since their 12-month (final) remote visit, this should not be done as any physiological changes will no longer be present after stopping the food supplement, following completion of the study.

The above clinic visit data will be recorded under an additional visit tab on the InForm database.

The SAP will be updated to specify how the above data will be managed.

6.9.1 Additional measurements

For participants that had a self-reported weight collected for a remote visit, a pre-clinic self-reported weight will be collected again at the time of their next clinic visit, in addition to the clinic weight being taken. This is so that the accuracy of home scales (or equivalent) can be assessed/compared to the accuracy of the study

clinic scales. This will help with interpreting/analysing the self-reported weight data captured during remote visits.

A 'pre-clinic' self-reported weight field will be added to study visits on the InForm database.

The SAP will be updated to specify how the above data will be managed.

7. INTERVENTION

The IPE and the inulin control will be given to participants in 10g sachets and they will be instructed to take one sachet per day, at any time with their normal diet for 12 months.

The trial IPE supply will be manufactured by Moorepark in Ireland and both the IPE and Inulin control will be packaged in the UK. The packaging company will distribute packaged and labelled sachets to the trial sites for dispensing to participants.

Participants will be given an initial trial supply at the baseline visit and subsequent supply will be dispensed at 2 and 6 months. Participants will be asked to bring used and unused sachets back to each visit so that these can be counted by the researcher and an assessment of adherence made.

7.1 Permanent Discontinuation of Trial Intervention and Withdrawal from the Trial

(i) Permanent discontinuation of trial intervention

Participants may discontinue trial intervention for the following reasons:

- At the request of the participant
- Due to an Adverse Event / Serious Adverse Event
- If the investigator considers that a participant's health will be compromised due to adverse events or concomitant illness that develop after entering the trial.

(ii) Withdrawal from the trial

Withdrawal from the trial refers to discontinuation of trial intervention and procedures and can occur for the following reasons:

- Participant decision (withdrawal of consent)
- Loss to follow-up

(iii) Procedures for permanent discontinuation of trial intervention or withdrawal from the trial

If a participant permanently discontinues the trial intervention, they will be invited to continue to attend trial visits if possible to allow for collection of key outcome and safety data.

If a participant withdraws from trial procedures, an attempt will be made to obtain self-reported body weight (primary endpoint) at the point of withdrawal and at the final study visit time point.
The decision to withdraw from further trial procedures will be documented on the electronic case report form (eCRF) and in the medical notes.

If the participant does not agree for data and samples collected to be retained, the samples must be destroyed and data excluded from the analyses.

If the participant withdraws consent to further be contacted at all for the study purposes, this will be documented on the electronic case report form (eCRF) and in the medical notes. No attempts of further contact to obtain a self-reported body weight for the primary endpoint, will be made.

Participants who have discontinued the trial intervention and/or have withdrawn from the trial will not be replaced, as the sample size for both the main study and sub study allow for a 25% and 30% drop-out rate, respectively.

8. SAFETY REPORTING

8.1 Definition of Adverse Event (AE)

An AE is any untoward medical occurrence in a clinical trial subject. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, whether or not considered related to the trial protocol or intervention.

8.2 Definition of Serious Adverse Event (SAE)

An SAE is defined as any event that:

- Results in death;
- Is life-threatening*;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**;
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;
- Is a medically important event

* "Life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
** "Hospitalisation" means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to (Accident & Emergency) A&E (without admission).

Medical judgement should be exercised in deciding whether an adverse event is serious in other situations. Important adverse events that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a subject, or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

8.3 Severity of Adverse Events / Serious Adverse Events

Definitions for assessment of severity:

Mild: Awareness of event but easily tolerated
Moderate: Discomfort enough to cause some interference with usual activity
Severe: Inability to carry out usual activity

8.4 Causality of Adverse Events / Serious Adverse Events

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions listed below. If any doubt about the causality exists, the local investigator should inform the trial coordination centre who will notify the Chief Investigator (CI).

Unrelated: No evidence of any causal relationship
Unlikely: There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial intervention). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possibly: There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial intervention). However, the influence of other factors may have contributed

to the event (e.g. the patient's clinical condition, other concomitant treatments).

Probably: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Definitely: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

8.5 Expectedness of AEs/SAEs

Serious adverse events will be marked as either expected, or unexpected. An unexpected event would be a type of event that is not listed in the trial protocol or document associated with the intervention, as an expected occurrence.

Expected adverse events for this trial are: Gastrointestinal effects.

8.6 Reporting procedures

All adverse events, non-serious and serious, occurring after consent has been obtained will be reported for this trial, except for elective medical procedures.

(i) Non serious AEs

All such AEs, whether expected or not, should be recorded in the adverse event section of the relevant case record form within one month of the form being due.
The trial physician will decide what the best course of action is i.e. referral to GP, hospital, clinic or other. AEs will be followed up according to local practice until stabilised, resolved, diagnosed/treated or the last trial follow-up visit, whichever is sooner.

(ii) SAEs

Participating sites must report all SAEs to the trial coordination centre within 24 hours of being notified, as detailed in the study specific safety reporting instructions.
Reporting of SAEs will be via the trial data collection system (InForm eCRF). The SAE form asks for the nature of the event, date of onset, severity, corrective therapies given, outcome, expectedness and causality.

All SAEs will be reviewed by both the local Investigator and CI or a designated medically qualified representative to confirm 'expectedness' and 'causality', within a reasonable timeframe as described in the study specific safety reporting instructions.

If the investigator becomes aware of safety information that appears to be related to the trial, involving a participant who took part in the trial, even after an individual subject has completed the trial, this should be reported to the trial co-ordination centre and Sponsor.

(iii) Abnormal Laboratory Test Results

All clinically important abnormal laboratory test results occurring during the trial will be recorded as adverse events. The trial physician will decide what the best course of action is i.e. referral to GP, hospital, clinic or other. Again, the event will be followed up according to local practice until stabilised, resolved, diagnosed/treated, or the last trial follow-up visit, whichever is sooner.

(iv) Reporting of SAEs that are related and unexpected

SAEs that are thought to be possibly, probably or definitely related to the trial protocol/intervention and are unexpected should be notified to the relevant REC and the Sponsor within 15 days.

Follow up of patients who have experienced a related and unexpected SAE should continue until recovery is complete or the condition has stabilised. Reports for related and unexpected SAEs should be unblinded prior to submission.

(v) Reporting urgent safety measures

If any urgent safety measures are taken, the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC of the measures taken and the circumstances giving rise to those measures.

9. STATISTICAL ANALYSES

9.1 Sample Size and power considerations

In the randomised proof of concept trial, the difference between arms in the change in body weight over 24 weeks was 1.4kg (95% CIn: -0.3 to 3.1), p=0.099. Using a Bayesian method recommended for preliminary trials in which evidence in the 95% CIn is translated into probabilities (37), there was a 95% posterior probability of an underlying positive between-arm difference favouring the intervention. The posterior probability of intervention-favouring differences greater than 1kg, than 1.5kg, and 2kg were respectively 69%, 47% and 25% based on 24-week intervention. The difference increased in magnitude through successive 8-week, 16-week, and 24-week time points. By 24 weeks there were significant reductions in the proportion of intervention participants gaining 3%, and 5% of body weight from a mean baseline of 90kg. We therefore chose a 2kg between-arm 12-month effect size. This agreed with a weight gain prevention trial over 9 months in young adults (38) which aimed to detect a 2kg effect and achieved 4.3kg, with a pooled standard deviation (SD) for body weight change of 4.35kg, 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 2kg difference between arms in mean body weight change over 12 months using a two-sided 5% significance test, assuming a 4.35kg SD and with 25% dropout allowance (67.5 participants).

9.2 Power calculation for Mechanistic outcomes

For the mechanistic studies, 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 value, SD 13 pmol/L. These differences are based on our previous published findings that report enhanced gut hormone release following IPE supplementation (17, 18). We will therefore recruit a subsample of 52 volunteers (26 per group) to allow a 70% retention rate.

9.3 Data Analysis

This trial has both a pragmatic element, to answer the question of whether the policy of prescribing and uptake of inulin propionate ester as specified in the trial will reduce further weight gain compared with control, and an explanatory element to understand the mechanisms on the causal pathway of such body weight change and any limitations from compliance. Therefore analyses will be primarily on an 'Intention to Treat' basis. Secondarily, analyses will incorporate mechanistic sub study data, and use this to understand the 'Intention to Treat' effect estimated in the trial.

Statistical methods for analysis of the study endpoints are described below. A separate detailed Statistical Analysis Plan (SAP) will be prepared and finalised prior to database lock. This will contain the rationale for the methods chosen and the assessment of their assumptions. It will include pre specifying the handling of covariates and missing data, per protocol and compliance analyses. Changes to the plan will be re-approved by trial oversight committees.

Any deviations from the SAP will be documented and signed off by the statisticians and CI, and filed in the statistics section of the Trial Master File (TMF) which will be merged with the main TMF at the end of the study.

(i) Primary Endpoint Analysis

The analysis of the primary endpoint will incorporate the earlier correlated interim measurements of body weight in a linear mixed effects model and will adjust for baseline continuous body weight and other categorical randomisation stratifiers. The implicit 'missing at random' assumption will be challenged through a set of sensitivity analyses (39). As these involve all randomised participants, this is therefore an Intention to Treat Strategy (40).

(ii) Secondary Endpoints Analysis

Where possible, continuous secondary endpoints will be adjusted for their baseline to improve the precision of estimated intervention effects. Repeated measures will be analysed using linear mixed effects models adjusting also for randomisation stratifiers. Comparisons between arms for binary outcomes will be summarised as differences in proportions. 95% confidence intervals will be used to make inferences from estimated effect sizes of secondary endpoints.

(iii) Adjusted analysis

In the case of the baseline of a continuous endpoint having missing data, the principal analysis of the endpoint would involve adjustment for a missing indicator (41). No further adjusted between-arm trial comparisons are planned beyond those already described. If a need arose, such as through arm imbalance, then any such identified additional sensitivity analysis would require approval within the statistical analysis plan.

(iv) Safety Analysis

Safety outcomes will be reported as unadjusted patient proportions and rates within and between arms with 95% confidence intervals using exact methods where appropriate.

(v) Statistical analysis of mechanistic enquiry

The mechanism of the randomised effect of the intervention on body weight will be assessed through using hypothesised intermediate variables, including energy intake, drivers of appetite and energy expenditure. Randomised effects on these hypothesised mediators will be reported, but, more formally, the participant level data relationships between randomisation status, these mediators and outcome will be explored in addition. Regression analyses within the mechanistic subsample will involve between-arm comparison of energy expenditure, appetite regulation, breath hydrogen, lipid metabolism/substrate oxidation, gut microbiota, and will account for baseline where applicable.

10. REGULATORY, ETHICAL AND LEGAL ISSUES

10.1 Declaration of Helsinki

The investigator(s) will ensure that this trial is conducted in full conformity with the *7th* revision of the 1964 Declaration of Helsinki.

10.2 Good Clinical Practice

The trial will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines and Addendum ICH GCP E6 (R2)).

10.3 Independent Research Ethics Committee (REC) Approval

(i) Initial Approval

Prior to the enrolment of participants, the REC must provide written approval of the conduct of the trial at named sites, the protocol and any amendments, the Participant Information Sheet (PIS) and Consent Form (CF), any other written information that will be provided to the subjects, any advertisements that will be used and details of any participant compensation.

(ii) Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the REC for approval as instructed by the Sponsor. Amendments requiring REC approval may be implemented only after a copy of the REC's approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or REC approval. However, in this case, approval must be obtained as soon as possible after implementation.

The trial team, in collaboration with the Sponsor will assess whether a proposed amendment is substantial or non-substantial. For each proposed amendment, a revised version of the protocol will be prepared using tracked changes, a new version number assigned and the revised document will be reviewed and approved by Protocol Development Group and Sponsor prior to submission to the REC and Health Research Authority (HRA). The amended protocol will be sent to participating sites for local approval to be granted and the approved version will be shared with all staff involved in the trial.

(iii) Annual Progress Reports

The REC will be sent annual progress reports on the anniversary of each REC approval until the end of trial, in order to facilitate their continuing review of the study.

(iv) End of Trial Notification

The REC will be informed of the End of Trial, as defined in section 10.12 of this trial protocol, within the required timelines.

10.4 Health Research Authority (HRA) approval

Health Research Authority (HRA) approval will be obtained prior to starting the trial. Each participating site will then confirm capacity and capability prior to commencing.

The HRA and all participating sites also need to be notified of all protocol amendments to categorise the amendment and assess whether the amendment affects the institutional approval for each site.

10.5 Non-Compliance and Serious Breaches

All protocol deviations (PD) and protocol violations (PV) will be reported via the eCRF/ and reviewed by the Principal and Chief Investigators (PI/CI), and reported to the ICTU Quality Assurance (QA) manager on a monthly basis. PVs will also be reported to the Sponsor.

An assessment of whether the PD/PV constitutes a serious breach will be made.

A serious breach is defined as:

A breach of the conditions and principles of Good Clinical Practice (GCP) in connection with a trial or the trial protocol, which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the UK trial subjects; or
- The overall scientific value of the trial

The Sponsor will be notified within 24 hours of identifying a likely Serious Breach. If a decision is made that the incident constitutes a Serious Breach, this will be reported to the REC within 7 days of becoming aware of the serious breach.

10.6 Sponsor Insurance and Indemnity

Imperial College London, the Sponsor of the trial, has civil liability insurance which covers this study in the UK. Imperial College London also holds negligent harm and non-negligent harm insurance policies which apply to this study.

10.7 Trial Registration

The trial will be registered on the International Standard Randomised Controlled Trials Number (ISRCTN) database in accordance with requirements of the International Committee of Medical Journal Editors (ICMJE) regulations.

10.8 Informed Consent

Informed consent will be obtained from all participants using REC approved Participant Information Sheet(s) (PIS) and Consent Form(s) (CF) during the screening visit.

The participant will be informed about the trial by the responsible clinician or a member of the research team and given a copy of the PIS. Informed subjects will be given an adequate amount of time to consider their participation in the trial. If the subject decides to participate in the trial they will be asked to sign the CF which will then be countersigned by the responsible clinician / researcher. The patient will retain one copy of the signed CF. Another copy will be placed in the participant's medical records whilst the original will be retained in the participant's research record, at site.

Participants having a joint screening/randomisation visit (if they are only taking part in the main study) will be required to complete a 7-day food diary which they should bring with them to their visit. Therefore, prior to this, they will be asked to complete electronic consent. This will be setup by the trial management team, using the ReDcap secure, web-based interface, designed for non-commercial research use.

Study teams at participating sites will be asked to print all completed e-consent forms, with a copy placed in participant medical records and a copy retained in research records.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol intervention without giving reasons and without prejudicing further treatment.

10.9 Contact with General Practitioner

It is the participating site investigator's responsibility to inform the participant's General Practitioner (GP) by letter that the participant is taking part in the trial provided the subject consents to this, and information to this effect is included in the PIS and CF. A copy of the letter to the GP should be filed in the Investigator Site File (ISF). Participants are not obligated to consent to this in order to take part in the trial.

10.10 Data Protection and Participant Confidentiality

The participating site investigator must ensure that the subject's confidentiality is maintained. On the eCRF or other documents submitted to the Sponsor, subjects will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g. signed informed consent form) should be kept in a strictly confidential file by the site investigator.

The site investigator will preserve the confidentiality of all participants taking part in the trial, which will be conducted in accordance with the General Data Protection Regulation (GDPR).

Imperial College London is the Sponsor for this study based in the United Kingdom. The Sponsor will be using information from participants and their medical records in order to undertake this study and will act as the data controller for this study. This means that the Sponsor is responsible for looking after participant information and using it properly. Imperial College London will keep identifiable information about participants for 10 years after the study has finished.

Participants' rights to access, change or move their information are limited, as information needs to be managed in specific ways in order for the research to be reliable and accurate. If participants withdraw from the study, the information about them already obtained will be kept. To safeguard participant rights, the minimum personally-identifiable information possible will be used.

When participants agree to take part in a research study, the information about their health and care may be provided to researchers running other research studies in the organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Their information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

With permission of participants (optional), participating sites may collect and provide participant identifiers to NHS Digital (England)/NHS in Scotland in order to link to their health records data for future follow-up and receive pseudonymised data regarding their health status. Participant identifiers (full name, DOB, NHS number, address/post code) would be collected and stored securely on the NHS computer network, on a password encrypted spreadsheet. Pseudonymised data received from NHS Digital/NHS Scotland would be identified only by participant trial ID numbers and stored securely in a Digital Signal Processor (DSP) toolkit environment on an Imperial College London secure server, managed by the Big Data Analytics Unit (BDAU). The toolkit environment is certified with ISO 27001:2013 (international standard recognised globally for managing risks to the security of information for an information security management system) and fully compliant with NHS Information Governance Toolkit Level 3 (EE133887).

The reason for obtaining information by linking to health records after the end of the study is to see how the health of participants may have changed since then and up to 10 years afterwards (weight, metabolism and related health changes or conditions etc.). This would be a 'follow-up' study under a new Ethics approval. The data may be transferred between Imperial College London and University of Glasgow by secure and approved means, as these are where the collaborating research teams are based and where the data would be analysed.

10.11 Monitoring, audits and inspections

The participating site investigator shall permit direct access to subjects' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor and RECs.

10.12 End of Trial

The end of the trial will occur when the final participant has completed the final follow up visit and all trial data have been captured on the trial database.

11. DATA MANAGEMENT

11.1 Source Data

Source documents include original documents related to the trial, to medical treatment and to the history of the participant, and adequate source documentation must be maintained at participating sites to allow reliable verification and validation of the trial data. What constitutes source data for this trial will be outlined in the trial Source Identifier Document.

11.2 Language

eCRFs will be in English. Generic names for concomitant medications should be recorded in the eCRF wherever possible. All written material to be used by subjects must use vocabulary that is clearly understood, and be in the language appropriate for the participating site. Translations of any study documents will be made if necessary.

11.3 Database

Trial data will be collected on an electronic case report form (eCRF). The principal means of data collection from participant visits will be Electronic Data Capture (EDC) via the internet using the InForm database. Data is entered into the EDC system by trained site personnel. All data recorded in the eCRF will be signed off by the Investigator or his/her appropriate designee. All changes made following initial submission of data will have an electronic audit trail with a date. Specific instructions and further details will be outlined in the study specific eCRF manual.

Randomisation and unblinding will be carried out using a different Electronic Data Capture (EDC) system, called Sealed Envelope, as the InForm database does not support the minimisation randomisation strategy that the trial requires.

Participants having a joint screening/randomisation visit will be required to complete electronic consent. This will be setup by the trial management team, using the ReDcap secure, web-based interface, designed for non-commercial research use.

Imperial College London is registered as a ReDcap partner with the ReDcap consortium. Under the terms of this licence, a central instance of the software has been installed on ICT run Imperial College infrastructure and provides a secure hosting environment for the ReDcap software, suitable for non-CTIMP studies.

11.4 Data Collection

Data from all trial visits will be collected and entered on the trial eCRF built in the InForm system. Data will include demographics, vital signs, diet and physical activity information, blood test results, questionnaire data and data arising from the mechanistic evaluations.

Details of procedures for eCRF completion will be provided in a study specific manual.

11.5 Data Storage and Archiving

The participating site investigator must retain essential documents until notified by the Sponsor, and for at least 10 years following the end of trial. Participant files and other source data (including copies of protocols, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be retained. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the participating site investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

12. STUDY MANAGEMENT STRUCTURE

12.1 Trial coordination

The trial will be managed by the United Kingdom Clinical Research Collaboration (UKCRC) registered Imperial Clinical Trials Unit (ICTU).

12.2 Trial Steering Committee (TSC)

A Trial Steering Committee (TSC) will be convened including as a minimum an independent Chair, independent clinician, the CI, a public representative and members of ICTU. The role of the TSC is to provide overall supervision of trial conduct and progress. The TSC will meet approximately 6-monthly throughout the duration of the trial. Full details of membership, responsibilities and frequency of meetings will be defined in a separate Charter.

12.3 Trial Management Group (TMG)

A Trial Management Group (TMG) will be convened including the CI, Co-Investigators and key collaborators, at least one public representative, the Trial Statistician, Senior Statistician, Operations Manager and Trial Manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Details of membership, responsibilities and frequency of meetings will be defined in separate Terms of Reference.

12.4 Data Monitoring and Ethics Committee (DMEC)

A fully independent Data Monitoring and Ethics Committee (DMEC) will be set up to monitor progress, participant safety and any ethical issues involved in this trial. They will review trial progress, recruitment rates and safety data. A separate DMEC Charter will be drawn up defining their responsibilities, frequency of meetings and reporting to the TSC. Meetings will be approximately 6-monthly.

The statistician will analyse interim data for DMEC meetings and act as data manager, in raising and resolving data queries with participating sites, via the Trial Manager. Closed DMEC reports will include recruitment, randomisation balance and stratification effectiveness, baseline characteristics, unblinding, withdrawals, compliance, concomitant medications, efficacy, mediators, and adverse events. Open DMEC and TSC reports will be provided without outcome or arm information.

12.5 Study Advisory Group (SAG)

A Study Advisory Group (SAG) consisting of approximately four members of the public with similar characteristics to the trial population, will be convened. The role of this group will be to liaise with community groups and advise on recruitment, retention, engagement and dissemination strategies for the trial. The Ethics application and participant materials will be prepared in collaboration with the SAG.

12.6 Early Discontinuation of the Study

There are no statistical criteria for termination of the trial but the funder National Institute of Health Research; Efficacy and Mechanism Evaluation programme (NIHR/EME) has stipulated the following Stop / Go decisions which will be reviewed regularly by the Trial Management Group and Trial Steering Committee.

STOP' decisions:

- If the recruitment rate is below 50% of target at 6 months (3.75 participants per centre per month), and also below 50% in months 4-6, then power would be lower than 80% even with 3 extra sites; STOP
- If the recruitment rate is between 50% and 66% of target at 6 months (3.75 - 5 participants per centre per month) AND loss to 2-month follow-up is ≥ 25% (95% CIn: 12%-38% ;n=45); STOP

'GO' decision with changes:

- If the recruitment rate is between 50% and 66% of target at 6 months (3.75 - 5 per centre participants per month) AND retention (body weight measured) at 2-months > 75% THEN;
    - Add 3 additional sites AND
    - Review power at 12 months with Funder

- If the recruitment rate is ≥ 66% of target at 6 months (5 participants per centre per month) THEN; GO with 3 additional sites
- If the recruitment rate is ≥ 75% of target at 6 months (5.6 participants per centre per month) THEN; GO with 2 additional sites
- If the recruitment rate is = 85% of target at 6 months (6.4 participants per centre per month) THEN; GO with 1 additional site
- If the recruitment rate is ≥ 90% of target at 6 months (6.75 participants per centre per month) THEN; GO no changes required

12.7 Risk Assessment

A study-specific risk assessment will be performed prior to the start of the trial to assign a risk category of 'low', 'medium' or 'high' to the trial. Risk assessment will be carried out by the ICTU QA Manager in collaboration with the Trial Manager and the result will be used to guide the Monitoring Plan. The risk assessment will consider all aspects of the trial and will be updated as required during the course of the trial.

12.8 Monitoring

The trial will be monitored periodically by the trial Monitor to assess the progress of the trial, verify adherence to the trial protocol, ICH GCP E6 (and R2) guidelines and other national requirements and to review the completeness, accuracy and consistency of the data.

Monitoring procedures and requirements will be documented in a Monitoring Plan, in accordance with the risk assessment.

12.9 Quality Control (QC) and Quality Assurance (QA)

Quality Control (QC) will be performed according to ICTU internal procedures. The trial may be audited by a Quality Assurance (QA) representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

The trial may be subject to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

12.10 Peer review

The trial has undergone independent peer review via the NIHR-EME funding programme. The trial has also been reviewed by senior members of ICTU.

12.11 Participant and Public Involvement

Members of the public have been involved in this trial through a number of avenues and will be involved throughout the trial:

- *Design of the research:* a summary of the trial was presented at an Imperial Fringe event attended by over 1,200 people where young adults were asked their opinions. Feedback was taken into account and adjustments to the trial protocol made on the basis of this.
- *Management and undertaking of the research:* public representatives will be included on the TSC and TMG to provide guidance on all aspects of the trial from the public perspective. A Study Advisory

Group (SAG) consisting of approximately 4 public members will also be set up to advise on the trial as mentioned in section 11.5

- *Analysis of results and dissemination of findings:* Trial findings will be shared with the TSC and SAG and advice sought on suitable dissemination strategies.

12.12 Publication and Dissemination policy

Information concerning the trial, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The trial investigator(s) may use this information for the purposes of the trial only.

It is understood by the investigator(s) that the Sponsor will use information developed in this clinical trial and, therefore, may disclose it as required to other clinical investigators. In order to allow the use of the information derived from this clinical trial, the investigator(s) understand that he/she has an obligation to provide complete test results and all data developed during this trial to the Sponsor.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor. Therefore all information obtained as a result of the trial will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed.

Permission from the Writing Committee is necessary prior to disclosing any information relative to this study outside of the Trial Steering Committee. Any request by participating site investigators or other collaborators to access the trial dataset must be formally reviewed by the TSC.

The results may be published or presented by the trial investigator(s), but the Funder will be given the opportunity to review and comment on any such results for up to 1 month before any presentations or publications are produced.

A Clinical Study Report (CSR) summarising the trial results will be prepared and submitted to the REC within a year of the end of trial.

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SIGNATURE PAGE 1 (CHIEF INVESTIGATOR - CI)

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults: iPREVENT

Protocol Number: 18HH4903

Signed: _____

Print Name and Title: _____

Date: _____

SIGNATURE PAGE 2 (SPONSOR)

The signatures below constitute approval of this protocol by the signatory.

Study Title: Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults: iPREVENT

Protocol Number: 18HH4903

Signed: _____

Print Name and Title: _____

Date: _____

SIGNATURE PAGE 3 (STATISTICIAN)

The signatures below constitute approval of this protocol by the signatory.

Study Title: Increase in colonic PROpionate as a method of prEVENTing weight gain in young adults: iPREVENT

Protocol Number: 18HH4903

Signed: _____

Print Name and Title: _____

Date: _____

SIGNATURE PAGE 4 (PRINCIPAL INVESTIGATOR - PI)

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Increase in colonic PRopionate as a method of prEVENTing weight gain in young adults: iPREVENT

Protocol Number: 18HH4903

Address of Institution: _____

_____

_____

Signed: _____

Print Name and Title: _____

Date: _____