Imperial College London

Imperial College Healthcare NHS Trust

# Propionate and bone health

**Increasing gut-derived propionate to improve bone health in postmenopausal women**

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MAIN SPONSOR: Imperial College London
FUNDERS: Imperial College London
STUDY COORDINATION CENTRE: Imperial College London

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**Protocol authorised by:**

| **Name & Role** | **Date** | **Signature** |
|---|---|---|

## Study Management Group

Chief Investigator: Dr Edward Chambers

Co-investigators:

Statistician: Dr Edward Chambers

Study Management: Dr Edward Chambers

## Clinical Queries

Clinical queries should be directed to Dr Edward Chambers who will direct the query to the appropriate person

## Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Research Governance and Integrity Team
Imperial College London and Imperial College Healthcare NHS Trust
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Imperial College - Research Governance and Integrity Team (RGIT) Website

## Funder

Imperial College London

This protocol describes the "Propionate and bone health" study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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## GLOSSARY OF ABBREVIATIONS

| | |
|---|---|
| IPE | Inulin-propionate ester |
| BMD | Bone mineral density |
| SCFA | Short chain fatty acid |
| BMI | Body mass index |
| CTX-I | Carboxy-terminal telopeptide of type I collagen |
| NTX-I | N-telopeptide of type I collagen |
| PINP | N-terminal propeptide of type I procollagen |
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**KEYWORDS**
Propionate
Bone
Fibre
Short chain fatty acids
Menopause

## STUDY SUMMARY

**TITLE** Increasing gut-derived propionate to improve bone health in postmenopausal women

**DESIGN** A randomised, placebo controlled, double blind, parallel design.

**AIMS** 1) Control (control) 2) Inulin-propionate ester (IPE; intervention)

**OUTCOME MEASURES** Bone turnover markers, short chain fatty acids, HbA1c, glucose, insulin, c-reactive protein, gut bacterial composition

**POPULATION** 28 post-menopausal females aged 50-75 years

**ELIGIBILITY**

**INCLUSION CRITERIA**

- Post-menopausal females (>5 years post-menopause)
- Healthy non-obese volunteers (body mass index (BMI) of 20-30 kg/m$^2$)
- Age between 50-75 years (inclusive)
- Non-diabetic (HbA1c $\leq$48 mmol/mol)

**EXCLUSION CRITERIA**

- Weight change of ≥ 3kg in the preceding 2 months
- Current smokers
- Substance abuse
- Excess alcohol intake
- Cardiovascular disease
- Cancer
- Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome
- Kidney disease
- Pancreatitis
- Use of medications likely to interfere with energy metabolism, appetite regulation and hormonal balance, including: anti-inflammatory drugs or steroids, antibiotics, androgens, phenytoin, erythromycin or thyroid hormones.

**DURATION** 1 year. Each participant will complete 4 study visits over an 8 week period.

# 1. INTRODUCTION

## 1.1. BACKGROUND

There are >500,000 osteoporotic fractures per year in the UK, which causes considerable individual suffering and costs the NHS £4.4bn[1]. Older women (>50 years) have a 2.5-fold increased risk of osteoporotic fractures compared with older men, owing to the profound impact of the menopause on bone turnover[1]. Oestrogen deficiency is characterised by uncoupled activities of bone resorbing osteoclasts and bone forming osteoblasts, resulting in accelerated bone loss[2].Pharmacological interventions to improve bone turnover and prevent osteoporotic fractures are limited by the cost and adverse side effects of approved drugs. Consequently, there is an urgent need for effective, safe, inexpensive, and widely applicable interventions to prevent osteoporotic fractures in postmenopausal women.

Higher intakes of dietary fibre in postmenopausal women improve bone mineral density (BMD)[3], an important risk determinant for osteoporotic fractures[4]. Investigations highlight that the short chain fatty acid (SCFA) propionate, generated from gut bacterial fermentation of dietary fibre, improves bone turnover and mass[5]. Studies also report that, despite similar dietary fibre intake, gut-derived propionate is ~25% lower in older (≥50 years) compared to younger (<50 years) adults[6]. Accordingly, interventions that can augment gut-derived propionate in postmenopausal women may be an effective strategy at improving bone turnover and preventing osteoporotic fractures.

To selectively raise gut-derived propionate we have developed an inulin-propionate ester (IPE)[7]. We estimate that the addition of 10 g IPE to the diet of a typical UK adult leads to a 2.5-fold increase in daily propionate production[7]. The IPE is a food supplement produced by Dr Douglas Morrison at The University of Glasgow. The IPE can therefore be incorporated into common food products, which is a major competitive advantage when targeting improvements in bone health at the population level.

## 1.2. RATIONALE FOR CURRENT STUDY

The primary objective of this project is to develop *in vivo* proof-of-concept for IPE as a novel therapeutic to improve bone turnover markers in postmenopausal women. The secondary objective is to explore the associations between gut-derived propionate, bone turnover markers and changes in glucose homeostasis and systemic inflammation. Our previous work found that IPE supplementation improved insulin sensitivity and systemic inflammation[8], which modulate bone turnover.

# 2. STUDY OBJECTIVES

**Primary objective:**

- To assess the utility of IPE to improve bone turnover markers

**Secondary objectives:**

- To assess the impact of IPE on production and systemic availability of SCFAs from stool and plasma samples.

- To assess the impact of IPE on glucose homeostasis and systemic inflammation.

## 3. STUDY DESIGN

**Visit 1- Health Screening**

Participants will be recruited from the healthy volunteer database at the NIHR Imperial Clinical Research Facility. Potential participants will be identified based on a database search using the inclusion/exclusion criteria of the study. Identified individuals will be sent a letter that briefly describes the study. A contact number and email on the letter will enable potential participants to contact the research team at Imperial College. Participants will also be recruited from poster adverts placed around Imperial College London and Imperial NHS Trust sites. A contact number and email on the poster will enable potential participants to contact the research team at Imperial College.

These individuals will be invited to attend the NIHR Imperial Clinical Research Facility at Hammersmith Hospital where their eligibility for the study will be assessed. Informed consent will be obtained. Individuals will have a blood test (HbA1c) and height and weight measurements will also be taken.

**Visit 2 – Baseline Visit**

The day prior to the study visit, the participants will be requested to refrain from strenuous exercise and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water).

At approximately 09:00 participants will attend the NIHR Imperial Clinical Research Facility at Hammersmith Hospital where an intravenous cannula will be inserted for blood sampling throughout the study day. At approximately 09:30 fasting blood samples will be taken through the intravenous peripheral cannula to measure bone resorption CTX-I, N-telopeptide of type I collagen (NTX-I)) and bone formation (N-terminal propeptide of type I procollagen (PINP), Osteocalcin) markers. NTX will also be measured in a urine sample. HbA1c and C-reactive protein will also be measured in fasting blood samples.

Participants will then receive a standardised breakfast (0 min) containing 660 kcal; 89 g carbohydrate, 22 g fat, 28 g protein. Postprandial blood samples will be taken at 30, 60, 120, 180, 240, 300 min to measure SCFAs, glucose and insulin. 100 ml of blood will be taken during the study visit.

Participants will be asked to provide a stool sample to assess gut microbial composition and SCFAs. Participants will be provided with detailed instructions of how to do this and will be given appropriate containers.

**Supplementation Period**

Participants will be randomised to take 10 g/day of one the following dietary supplement for 8-weeks:

1) Cellulose (Control)

2) IPE (Intervention)

Cellulose will be used as a control due to its negligible impact on saccharolytic gut bacteria and production of SCFAs[11].

Supplements will be given to participants in 10 g ready-to-use sachets and instructed to mix the contents into their normal diet twice a day. The sachets will be prepared by Dr Douglas Morrison at the University of Glasgow. An independent researcher (i.e. not linked to the study) will be given the task of randomisation, which will be by sealed envelopes. During the supplementation period, participants will have weekly telephone contact with researchers to review compliance and monitor any adverse events. Participants will be told not to start any other new diets or intensive exercise regimes during the study period as this may give us conflicting results.

At the end of the 8-week supplementation period, participants will attend the NIHR Imperial Clinical Research Facility at Hammersmith Hospital to determine outcome measures.

**Visit 3 - Week 1 Follow-up Visit**

The day prior to the study visit, the participants will be requested to refrain from strenuous exercise and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water).

At approximately 09:00 participants will attend the NIHR Imperial Clinical Research Facility at Hammersmith Hospital where a venepuncture will measure bone turnover markers described in Visit 2. NTX will also be measured in a urine sample.

**Visit 4 – Week 8 Follow-Up Visit**

This will be identical to Visit 2. The standardised breakfast would also contain 10 g cellulose or 10 g IPE depending on supplementation group.

All participants will receive a weekly telephone call during the 8-week supplementation period to monitor compliance and any side-effects. Participants will complete a 7-day food diary and physical activity questionnaire at baseline and during the final weeks of the supplementation period.

### 3.1. STUDY OUTCOME MEASURES

Primary: Bone turnover markers (CTX-I, NTX-I, PINP, Osteocalcin)
Secondary: Short chain fatty acids, HbA1c, glucose, insulin, c-reactive protein, gut bacterial composition

# 4. PARTICIPANT ENTRY

## 4.1. PRE-REGISTRATION EVALUATIONS

A pre-evaluation (screening visit) will be completed at the NIHR Imperial Clinical Research Facility at Hammersmith Hospital Informed consent will be obtained. Individuals will have a blood test (HbA1c) and height and weight measurements will also be taken.

## 4.2. INCLUSION CRITERIA

- Post-menopausal females (>5 years post-menopause)
- Healthy non-obese volunteers (body mass index (BMI) of 20-30 kg/m$^2$)
- Age between 50-75 years (inclusive)
- Non-diabetic (HbA1c $\leq$48 mmol/mol)

## 4.3. EXCLUSION CRITERIA

- Weight change of ≥ 3kg in the preceding 2 months
- Current smokers
- Substance abuse
- Excess alcohol intake
- Cardiovascular disease
- Cancer
- Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome
- Kidney disease
- Pancreatitis
- Use of medications likely to interfere with energy metabolism, appetite regulation and hormonal balance, including: anti-inflammatory drugs or steroids, antibiotics, androgens, phenytoin, erythromycin or thyroid hormones.

Any participants with the above conditions would already have an altered pattern of hormones and inflammatory molecules because of their disease process and would therefore give us confounding or misleading results.

## 4.4. WITHDRAWAL CRITERIA

Participants will be withdrawn if their ability to give informed consent is impaired or they no longer meet eligibility criteria.

# 5. ADVERSE EVENTS

## 5.1. DEFINITIONS

**Adverse Event (AE):** any untoward medical occurrence in a patient or clinical study subject.

**Serious Adverse Event (SAE):** any untoward medical occurrence or effect that:

- **Results in death**

- **Is life-threatening** – *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*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
  - **Results in persistent or significant disability or incapacity**
  - **Is a congenital anomaly or birth defect**

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

### 5.2. REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

#### 5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded- it should be specified if only some non-serious AEs will be recorded, any reporting should be consistent with the purpose of the trial end points.

#### 5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours. However, relapse and death due to <condition>, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the <name of REC> where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

**Contact details for reporting SAEs**
**RGIT@imperial.ac.uk**
**CI email (and contact details below)**
**Fax 020 838 33142, attention Dr Edward Chambers**
**Please send SAE forms to: Dr Edward Chambers**
**Tel: 07786361040 (Mon to Fri 09.00 – 17.00)**
**Email: e.chambers@imperial.ac.uk**

## 6. ASSESSMENT AND FOLLOW-UP

There will be no follow-up to the study. The participant will have finished the study in its entirety following the completion of study visit 3.

End of the study will be the last visit of the last participant in the study.

Any incidental findings from blood tests (high blood glucose and/or c-reactive protein) would be reported to the participants GP to arrange follow-up care.

## 7. STATISTICS AND DATA ANALYSIS

A previous dietary study demonstrated a 20% reduction in the bone resorption marker carboxy-terminal telopeptide of type I collagen (CTX) after 8-weeks[9]. Assuming CTX values of 0.23±0.045 ng/mL and 0.18±0.045ng/mL in control and IPE groups post-supplementation, 11 participants per group would provide statistical power (power=0.8, α=0.05) to detect this difference (effect size=1.11). Fourteen participants per group will be recruited to allow a drop-out rate of ~25%[7].

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

## 8. REGULATORY ISSUES

### 8.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the xxx Research Ethics Committee (REC) and Health Research Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

### 8.2. CONSENT

(If study does not involve consent, this section is not relevant)

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

### 8.3. CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. Data will be pseudonymised.

### 8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study

### 8.5. SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

### 8.6. FUNDING

Imperial College London are funding this study. Participants will be reimbursed for their time. £150 will be awarded for completion of the study, or £50 per study visit (excluding screening visit).

### 8.7. AUDITS

The study may be subject to audit by Imperial College London/ Imperial College Healthcare NHS Trust under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

## 9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated by Dr Edward Chambers.

## 10. PUBLICATION POLICY

The findings of the research will be published in an open-access, peer-reviewed journal. In addition, we will be collaborating with patient groups and professional groups to disseminate the findings via multiple media channels such as patient association publications, print and broadcast media. All data will be anonymised prior to publication.

## 11. REFERENCES

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[2]J Bone Miner Res. (1996) 11(3):337-49.
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[5]Nat Commun. (2018) Jan 4;9(1):55.
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[9]Bone. (2010) 46(3):759-67.