PROTOCOL

The Acute Effect of Propionate on Energy Homeostasis

Propionate and Energy Homeostasis

Main Sponsor	Imperial College London

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Long title:

The Acute Effect of Propionate on Energy Homeostasis

Short title:

Propionate and Energy Homeostasis

Study Management Group

Chief Investigator	Professor Gary Frost
Co-investigators	Dr. Edward Chambers
Statistician	Dr. Douglas Morrison
Study Management	Dr Edward Chambers

Sponsor

Imperial College is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager at:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 215, Level 2
Medical School Building
Norfolk Place
London, W2 1PG

www.imperial.ac.uk/clinicalresearchoffice

PROBLEMS RELATED TO THIS TRIAL SHOULD BE REFERRED TO PROFESSOR GARY FROST
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1. INTRODUCTION

Dietary fibres have long been recognized for their important role in a healthy diet due to their negative association with, and even management of, chronic diseases such as obesity, diabetes, metabolic syndrome, cardiovascular disease and inflammatory-bowel disease among others (1–3).Emerging evidence has suggested that these benefits could largely be attributed to short chain fatty acids (SCFA) (acetate, propionate and butyrate), the main by-products of fibre fermentation in the gut (1,4). Our research group has demonstrated that a long-term elevation in the SCFA propionate significantly reduced body weight gain in overweight adults and reduced liver fat storage (5).

The current project will examine potential mechanisms for the positive effect of propionate on energy homeostasis and metabolic profile. We will examine the effects of propionate on circulating glucose, insulin and lipid levels at rest, following moderate-intensity exercise and mixed meal tolerance test.

To acutely increase propionate absorption from the gut the present project will use a simple nutritional supplement: sodium propionate in a hydroxypropylmethyl cellulose (HPMC) capsule. This capsule is coated with an enteric film which prevents gastric digestion until the capsule reaches the intestine. This nutritional supplement has been used in human volunteers in a previously approved ethics application (12/LO/1769: Oral propionate and glucose homeostasis). A 5g acute dose of sodium propionate had previously been tested and reported no adverse effects (6) . The MHRA have confirmed that encapsulated sodium propionate is not classed as an investigative medicinal product (correspondence attached with application).

STUDY: The acute effects of short chain fatty acids on energy homeostasis

Obesity is a major global health concern where it has more than doubled since 1980 inflicting around 13% of the population world-wide(7). In England alone, more than half of the population is either overweight or obese (8). Causes of obesity are mainly environmental where excess dietary intake and physical inactivity play a major role (9). Obesity increases the risk of various chronic diseases such as type 2 diabetes, hypertension, dyslipidaemia and cardiovascular disease which place a significant economic burden on health care systems (9,10). And these diseases often develop as a consequence of the metabolic changes that occur with obesity such as disturbances in glucose homeostasis, insulin sensitivity and lipid profile (11,12). Thus, efforts have been directed to address these metabolic abnormalities and environmental causes to help alleviate obesity and its adverse outcomes.

Short chain fatty acids, acetate, propionate and butyrate, have shown to exert multiple physiological changes that are beneficial in obese states. Propionate, in particular, has shown to exert multiple physiological benefits. For instance, it seems to regulate hepatic lipid metabolism through a reduction in intrahepatic triglyceride concentration (5). It can also enhance the lipid buffering capacity of adipose tissue through an increase in lipoprotein lipase activity and consequently, can decrease serum free fatty acids (13). Moreover, it can increase energy expenditure via diet-induced-thermogenesis through activation of the sympathetic nervous system (14), and can reduce energy intake through its effect on appetite hormones and decrease long-term weight gain and fat accretion in overweight adults (5). As for glucose metabolism, propionate serves as a gluconeogenic

substrate (15–17) and can thus spare/preserve the use of amino acids for gluconeogenesis (18,19). Moreover, it seems to hold promising results in enhancing hepatic and peripheral insulin sensitivity(20) and overall glucose homeostasis (20,21).

An intervention which elevates propionate release from the gut may therefore protect against obesity and its associated metabolic disruptions through enhanced glucose tolerance, lipid profile, and increased energy expenditure. Thus, the aim of this project is to investigate the acute effects of gut-derived propionate on the regulation of energy expenditure, substrate oxidation and overall metabolic profile in humans throughout different physiological states (fasting, postprandial and during physical activity). This study is an extrapolation based on a previously ethically approved research study (15/WA/0415) which demonstrated an increase in energy expenditure, lipid oxidation rates and circulating levels of propionate following an acute dose of 5 g sodium propionate supplemented over a 180 min time period. As can be seen from the below graphs, circulating levels of propionate increased over time to reach a peak at 180 min and thus for this study we would want to determine the trend in propionate levels over a longer period (300 minutes) while also examining energy expenditure and lipid oxidation with a properly matched energy control.

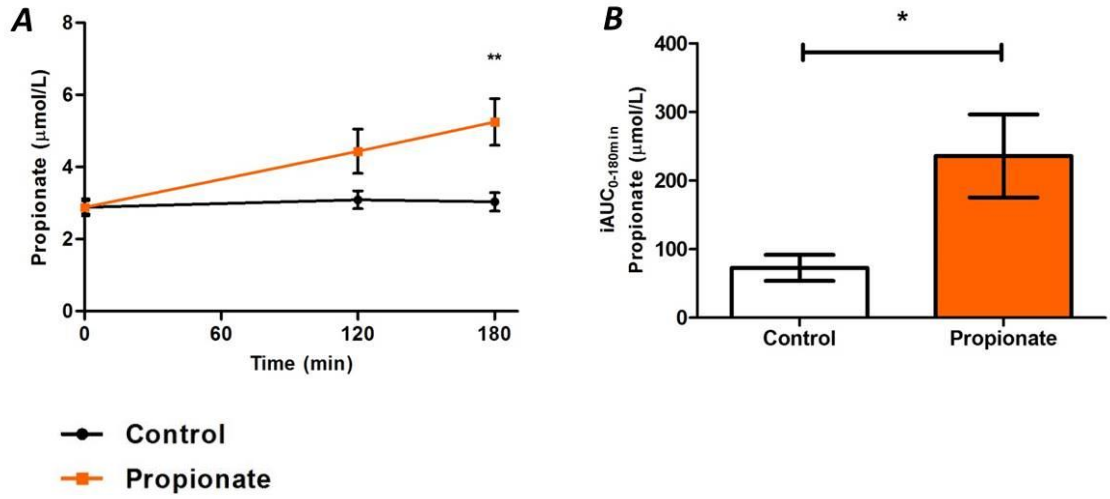


Figure 1. The effect of oral propionate supplementation on short chain fatty acid levels in peripheral blood. A. Propionate (Time×Trial: P= 0.043) and **B.** propionate iAUC (P=0.021).

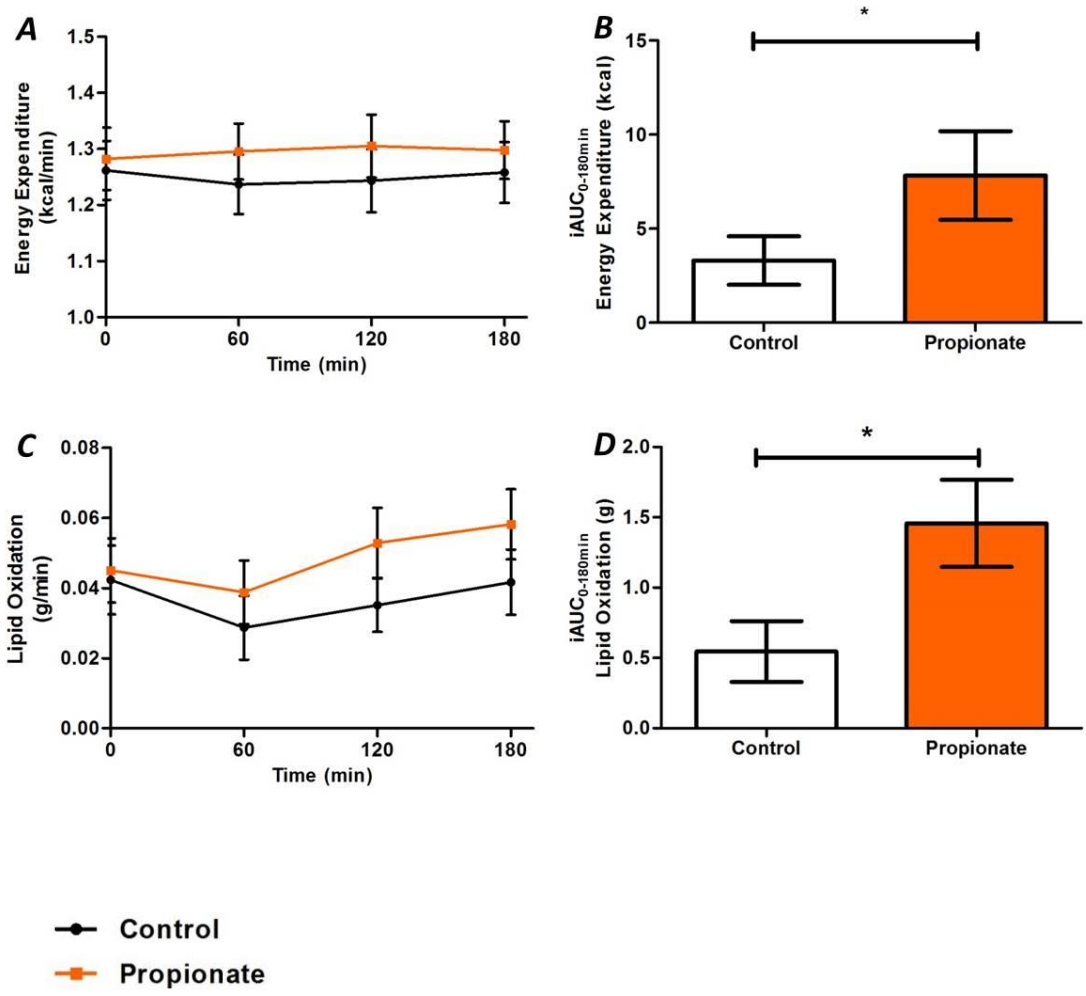


Figure 2. The effect of oral propionate supplementation on resting energy expenditure and substrate oxidation. A. Resting energy expenditure (Time×Trial: P= 0.388) and **B.** resting energy expenditure iAUC (P=0.036). **C.** Lipid oxidation (Time×Trial: P= 0.075) and **D.** lipid oxidation iAUC (P=0.019).

2. Study 1: The acute effects of propionate on energy metabolism during fasting.

Study methodology: A randomized, placebo controlled, double blind, cross-over study.

Participants: 40 healthy male and female volunteers aged between 18 and 65 years with body mass index (BMI) of 18-35 kg/m^2.

Support of number of volunteers: A power calculation confirmed that 34 participants would be sufficient to detect a 5% difference in the primary outcome, resting energy expenditure, with a standard deviation (SD) of 10 %(α=0.05, power=0.80). 40 participants were recruited to allow a dropout rate of 15%.

Recruitment

Volunteers will be recruited via email using a healthy volunteer database of the NIHR/Wellcome Trust Imperial Clinical Research Facility (CRF) or via a study advertisement. Interested volunteers will be asked to fill in a pre-screening questionnaire and if deemed eligible will be invited for a screening visit at the CRF in Hammersmith Hospital.

Visit 1- Health Screening

Participants will attend the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital where their eligibility will be assessed via an interview which discusses medical history, family history of medical illness and drug history. They will have a blood test (Full blood count liver function test, urea & electrolytes and lipids) and height and weight measurements will also be taken. They will also have an electrocardiogram (ECG) and blood pressure will be recorded. All women of child bearing age will have a pregnancy test. These results will be examined and approved by a qualified research doctor before the volunteers will be formally enrolled in the study.

Visits 2,3,4– Study Visits

Subjects will be asked to complete three study visits that will last approximately 6 hours each. The three study visits will need to be separated by a period of at least 3 days. 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.

In a randomized order, volunteers will complete the following three study visits:

1. Sodium Chloride (Sodium Control)
2. Palmitic Acid (Fatty Acid Control)
3. Sodium Propionate

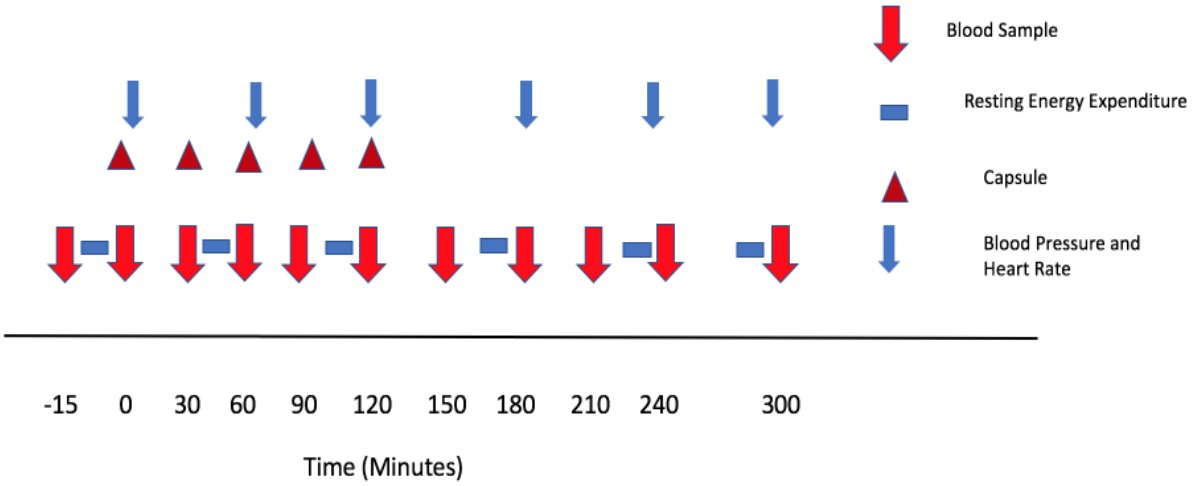
The day prior to each study visit, participants will be requested to refrain from strenuous exercise, caffeine and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water) and will come to the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital the following morning. Fasting blood samples will be taken through an intravenous peripheral cannula at -15 and 0 min.

Between the fasting blood samples, resting energy expenditure will be measured with a standard indirect calorimeter. The calorimeter captures expired air from a large, transparent canopy, worn over the head and thorax, and is typically very well tolerated. Each participant will be asked to lie in a semi-recumbent position under the canopy. Measurements will be taken for 20 min in order to assess the participants' resting metabolic rate (RMR) and respiratory quotient (RQ). Additional measures of RMR and RQ will be recorded at 40-60 min, 100-120 min,-160-180 220-240 min and 280-300.

Following the 0 min sample volunteers will ingest a capsule containing either 1g sodium propionate (Propionate) or 1 g sodium chloride (Sodium Control) or 1g palmitic acid (fatty acid control) at 30, 60, 90 and 120 (5g in total)

Blood samples will be taken at 30, 60, 90, 120, 150, 180, 210, 240 and 300 min to measure hormones and metabolites. 110 ml (11 × 10 ml) will be collected throughout each study visit. Blood pressure and heart rate will be measured at baseline and every hour at 60,120,180,240 and 300 min. Urine will be collected throughout the study visit to measure urea concentrations for calculation of protein oxidation rates. Subjects will empty their bladder before baseline blood samples and collect all urine thereafter for a period of 300 min using appropriate measurement containers.

Figure 1: Study visit protocol for study 1.



3. Study 2: The acute effects of propionate on energy metabolism during exercise.

Study methodology: A randomized, placebo controlled, double blind, cross-over study.

Participants: 40 healthy male and female volunteers aged between 18 and 65 years with body mass index (BMI) of 18-35 kg/m^2.

Support of number of volunteers: A power calculation confirmed that 34 participants would be sufficient to detect a 5% difference in the primary outcome, resting energy expenditure, with a standard deviation (SD) of 10 %(α=0.05, power=0.80). 40 participants were recruited to allow a dropout rate of 15%.

Recruitment

Volunteers will be recruited via email using a healthy volunteer database of the NIHR/Wellcome Trust Imperial Clinical Research Facility (CRF) or via a study advertisement. Interested volunteers will be asked to fill in a pre-screening questionnaire and if deemed eligible will be invited for a screening visit at the CRF in Hammersmith Hospital.

Visit 1- Health Screening

Participants will attend the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital where their eligibility will be assessed via an interview which discusses medical history, family history of medical illness and drug history. They will have a blood test (Full blood count liver function test, urea & electrolytes and lipids) and height and weight measurements will also be taken. They will also have an electrocardiogram (ECG) and blood pressure will be recorded. All women of child bearing age will have a pregnancy test. These results will be examined and approved by a qualified research doctor before the volunteers will be formally enrolled in the study.

Visit 2 – Maximal Exercise Test

Subjects will come to the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital and perform an incremental exercise test to volitional exhaustion on a cycle ergometer. Volunteers will start cycling at 50 Watts (W) and the workload will increase in 25 W increments every 3 min until voluntary exhaustion. This will determine maximal oxygen uptake (VO_{2max}), maximal aerobic power output (W_{max}) and maximal heart rate (HR_{max}). Non-invasive measures of respiratory exchange and heart rate will be recorded throughout the test. The maximal exercise test is a common measurement in exercise physiology research and presents no greater risk than the exercise activities normally undertaken by the recruited volunteers. The visit would last approximately 1 hour.

Visits 3,4,5 – Study Visits

Subjects will be asked to complete three study visits that will last approximately 6 hours each. The three study visits will need to be separated by a period of at least 3 days. 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.

In a randomized order, volunteers will complete the following three study visits:

1. Sodium Chloride (Sodium Control)
2. Palmitic Acid (Fatty Acid Control)
3. Sodium Propionate

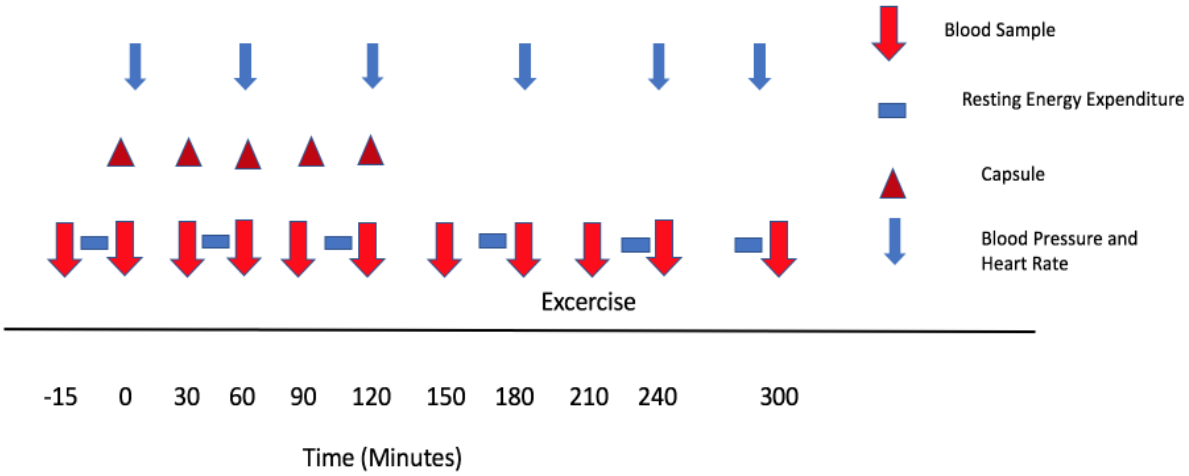
The day prior to each study visit, participants will be requested to refrain from strenuous exercise, caffeine and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water) and will come to the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital the following morning. Fasting blood samples will be taken through an intravenous peripheral cannula at -15 and 0 min.

Between the fasting blood samples, resting energy expenditure will be measured with a standard indirect calorimeter. The calorimeter captures expired air from a large, transparent canopy, worn over the head and thorax, and is typically very well tolerated. Each participant will be asked to lie in a semi-recumbent position under the canopy. Measurements will be taken for 20 min in order to assess the participants' resting metabolic rate (RMR) and respiratory quotient (RQ). Additional measures of RMR and RQ will be recorded at 40-60 min, 100-120 min,-160-180 220-240 min and 280-300.

Following the 0 min sample volunteers will ingest a capsule containing either 1g sodium propionate (Propionate) or 1 g sodium chloride (Sodium Control) or 1g palmitic acid (fatty acid control) at 30, 60, 90 and 120 (5g in total)

At 180 min, volunteers will have their heart rate recorded while they complete 60 min of constant-load cycling at a workload corresponding to 40% Wmax, determined from Visit 2 whilst breathing through a mouthpiece connected to an indirect calorimeter to determine energy expenditure and substrate oxidation rates during exercise. Blood samples will be taken at 30, 60, 90, 120, 150, 180, 210, 240 and 300 min to measure hormones and metabolites. 110 ml (11 × 10 ml) will be collected throughout each study visit. Blood pressure and heart rate will be measured at baseline and every hour at 60,120,180,240 and 300 min. Urine will be collected throughout the study visit to measure urea concentrations for calculation of protein oxidation rates. Subjects will empty their bladder before baseline blood samples and collect all urine thereafter for a period of 300 min using appropriate measurement containers.

Figure 2: Study visit protocol for study 2.



4. Study 3: The acute effects of propionate on postprandial metabolism:

Study methodology: A randomized, placebo controlled, double blind, cross-over study.

Participants: 40 healthy male and female volunteers aged between 18 and 65 years with body mass index (BMI) of 18-35 kg/m^2.

Support of number of volunteers: A power calculation confirmed that 34 participants would be sufficient to detect a 5% difference in the primary outcome, resting energy expenditure, with a standard deviation (SD) of 10 %(α=0.05, power=0.80). 40 participants were recruited to allow a dropout rate of 15%..

Recruitment

Volunteers will be recruited via email using a healthy volunteer database of the NIHR/Wellcome Trust Imperial Clinical Research Facility (CRF) or via a study advertisement. Interested volunteers will be asked to fill in a pre-screening questionnaire and if deemed eligible will be invited for a screening visit at the CRF in Hammersmith Hospital.

Visit 1- Health Screening

Participants will attend the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital where their eligibility will be assessed via an interview which discusses medical history, family history of medical illness and drug history. They will have a blood test (Full blood count liver function test, urea & electrolytes and lipids) and height and weight measurements will also be taken. They will also have an electrocardiogram (ECG) and blood pressure will be recorded. All women of child bearing age will have a pregnancy test. These results will be examined and approved by a qualified research doctor before the volunteers will be formally enrolled in the study.

Visits 2,3,4 – Study Visits

Subjects will be asked to complete four study visits that will last approximately 6 hours each. The three study visits will need to be separated by a period of at least 3 days. 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.

In a randomized order, volunteers will complete the following four study visits:

1. Sodium Chloride (Sodium Control)
2. Palmitic Acid (Fatty Acid Control)
3. Sodium Propionate

The day prior to each study visit, participants will be requested to refrain from strenuous exercise, caffeine and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water) and will come to the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital the following morning. Fasting blood samples will be taken through an intravenous peripheral cannula at -15 and 0 min.

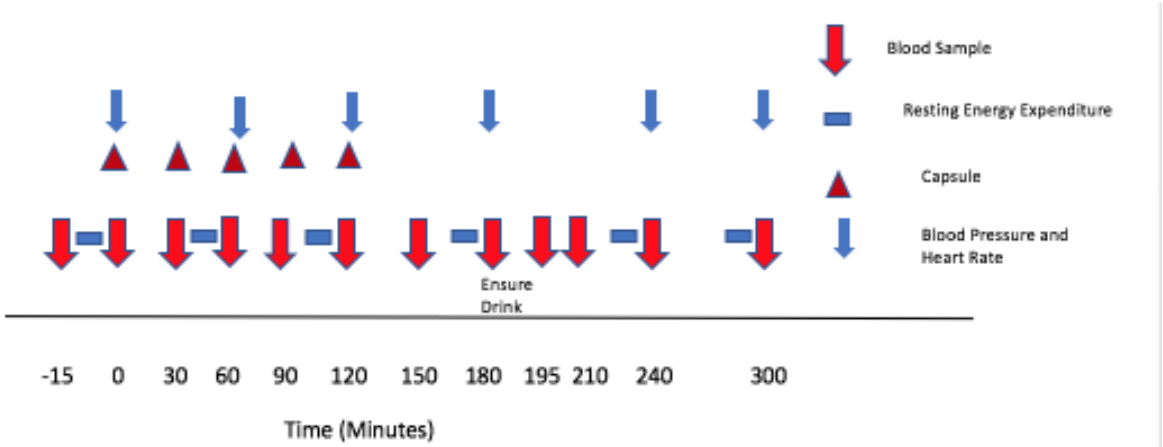
Between the fasting blood samples, resting energy expenditure will be measured with a standard indirect calorimeter. The calorimeter captures expired air from a large, transparent canopy, worn over the head and thorax, and is typically very well tolerated. Each participant will be asked to lie in a semi-recumbent position under the canopy. Measurements will be taken for 15 min in order to assess the participants' resting metabolic rate (RMR) and respiratory quotient (RQ). Additional measures of RMR and RQ will be recorded at 40-60 min, 100-120 min,-160-180 220-240 min and 280-300.

Following the 0 min sample volunteers will ingest a capsule containing either 1g sodium propionate (Propionate) or 1 g sodium chloride (Sodium Control) or 1g palmitic acid (fatty acid control) at 30, 60, 90 and 120 (5g in total)

At 180 min participants will have mixed meal tolerance test in the form of an ensure drink (500 kcal). Blood samples will be taken at 30, 60, 90, 120, 150, 180, 195, 210, 240 and 300 min to measure hormones and metabolites. 120 ml (12 × 10 ml) will be collected throughout each study visit. Blood pressure and heart rate will be measured at baseline and every hour at 60, 120, 180, 240 and 300 min.

Urine will be collected throughout the study visit to measure urea concentrations for calculation of protein oxidation rates. Subjects will empty their bladder before baseline blood samples and collect all urine thereafter for a period of 300 min using appropriate measurement containers.

Figure 3: Study visit protocol for study 3.



5. PARTICIPANT ENTRY

INCLUSION CRITERIA

Study 1:

- Healthy volunteers (body mass index (BMI) of 18-35 kg/m^2)
- Age between 18-65 years (inclusive)

Study 2:

- Healthy volunteers (body mass index (BMI) of 18-35 kg/m^2)
- Age between 18-65 years (inclusive)

Study 3:

- Healthy volunteers (body mass index (BMI) of 18-35 kg/m^2)
- Age between 18-65 years (inclusive)

EXCLUSION CRITERIA

Study 1, 2, 3:

- Weight change of ≥ 3kg in the preceding 2 months
- Current smokers

- Substance abuse
- Excess alcohol intake
- Pregnancy
- Diabetes
- Cardiovascular disease
- Cancer
- Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome
- Kidney disease
- Liver disease
- Pancreatitis
- Started new medication within the last 3 months likely to interfere with energy metabolism, appetite regulation and hormonal balance, including: anti-inflammatory drugs or steroids, antibiotics, androgens, phenytoin, erythromycin or thyroid hormones.
- Involved in current research or have recently been involved in any research prior to recruitment in the past 12 weeks.

WITHDRAWAL CRITERIA

The safety of the study participants takes priority. Any significant adverse event (as assessed by the researchers) will halt the study and the ethics committee and sponsor will be informed as per standard protocol. All adverse events will be recorded and investigators will review each adverse event as it arises. In addition, participants will be free to withdraw at any time and are not required to give a reason.

5. ADVERSE EVENTS

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

Serious Adverse Event (SAE): Any untoward and unexpected medical occurrence 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 was more severe.
- requires hospitalisation, or prolongation of existing inpatients' hospitalisation.
- results in persistent or significant disability or incapacity
- is a congenital abnormality 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.

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.

Non-serious AEs

All such events, whether expected or not, should be recorded.

Serious AEs (SEAs)

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

All SAEs should be reported to the xxxx Research Ethics Committee where in the opinion of the Chief Investigator the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. 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.

Local investigators should report any SAEs to the sponsor and their Local Research Ethics Committee and/ or Research and Development Office.

Contact details for reporting SAEs
Fax 020 838 33142, attention Professor Gary Frost
Please send SAE forms to Professor Gary Frost
Tel: 020 838 33242 (Mon to Fri 09.00- 17.00)

6. REGULATORY ISSUES

ETHICS APPROVAL

This study is awaiting ethical approval from the Wales REC 6 Ethics Committee. 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. Amendments to the protocol should be approved by the sponsor before being sent to ethics.

CONSENT

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 such cases, the participants remain within the study for the purposes of follow-up and data analyses. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

CONFIDENTIALITY
The Chief Investigator will preserve the confidentiality of participants in the study and is registered under the Data Protection Act.

DATA AND SAMPLE COLLECTION AND ANALYSIS
All data and samples will be collected at the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital. All data and samples will be stored and analysed at the laboratories of the Department of Investigative Medicine, Hammersmith Hospital. They data and samples will be available only to the study researchers. All analysis will be carried out on anonymised samples. The code linking samples to the donor will only be broken once all analysis has been conducted.

INDEMNITY
Imperial College holds negligent harm and non-negligent harm insurance policies, which apply to this study.

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.

REIMBURSMENT
Participants will be reimbursed for their time. £75will be awarded for completion of Study 1 (£25 for completing Visits 2 –4). £100 will be awarded for completion of Study 2 (£25 for completing Visits 2 - 5). £75will be awarded for completion of Study 3 (£25 for completing Visits 2 –4)

AUDITS AND INSPECTIONS
The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

7. 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.

8. References:

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