Imperial College London

MRC Clinical Sciences Centre

# STUDY PROTOCOL

## The Acute Effects of Food Structure on Appetite Regulation

**< Version 3, 18/11/2015>**

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**Funder:** Royal Embassy of Saudi Arabia-Cultural Bureau in London (King Saud bin Abdul-Aziz University for Health Sciences) will be funding this study.

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This protocol refers to the study **'The acute effects of food structure on appetite regulation'** and presents information about procedures for recruiting human participants. **Problems related to this trial should be referred to Professor Gary Frost: g.frost@imperial.ac.uk**

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

**Protocol authorized by:**

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## GLOSSARY OF ABBREVIATIONS

| | |
|---|---|
| **CCK** | **Cholecystokinin** |
| **GLP-1** | **Glucagon-Like Peptide 1** |
| **PYY** | **Peptide YY** |
| **VAS** | **Visual Analogue Scale** |
| **NIDDM** | **Non-insulin-dependent diabetes mellitus** |

**CONTENTS**

## 8. STUDY STRUCTURE

- ✓ Solid meal group.
- ✓ Milled (intact-cell) meal group.
- ✓ Milled (destroyed-cell) meal group.
- ✓ Assessments of gastric emptying – this will be carried out by adding 1500mg of paracetamol to the test meal. Levels will be measured at all time-points.
- ✓ Assessment of gut hormones – we will measure ghrelin, CCK, PPY. GLP-1.
- ✓ Assessment of appetite – this will be completed using visual analogue scale (VAS) followed by a meal given in excess at 180 minutes.

## 9. APPENDIX 1: Blood Sampling Schedule

## 10. STATISTICS AND DATA ANALYSIS

## 11. REGULATORY ISSUES

## 12. REFERENCES

## 1. SUMMARY OF STUDY

**TITLE:** The Acute Effects of Food Structure on Appetite Regulation.

**AIMS:** To investigate the effects of different food structures on appetite signals from the upper gastrointestinal tract.

**DESIGN:** A randomised, controlled, clinical trial.

**POPULATION:** Healthy adults (no diagnosed diseases) aged between 18 and 65 years.

**INTERVENTION:** Solid meal group.

Milled (intact-cell) meal group.

Milled (destroyed-cell) meal group.

All of which contain the same foods (chickpeas, carrots, sesame oil, and apple)

**DURATION:** One day on four occasions.

**OUTCOME MEASURES:**

- Difference in gastric emptying rate
- Difference in appetite regulatory hormones (CCK, PYY, GLP-1 and ghrelin) levels and release time
- Difference in energy intake at a given ad-libitum meal
- Difference in appetite regulation as measured by VAS

## 2. INTRODUCTION

The prevalence of overweight and obesity in adults in the European Union Countries was estimated to be 30-70% and 10-30%, respectively. This epidemic is rising in infants and children as well; over 60% of children are overweight (The Obesity Epidemic, CDC, 2011; WHO Europe, 2007). There is a substantial evidence that obesity signifies a burden on healthcare systems in developed and most developing countries, a dramatic increase in the incidence of coronary heart diseases, hypertension, cancer, and diabetes has been linked to obesity, in fact, obesity has become a main risk factor of many non-communicable diseases (WHO headquarters, 2010; Reilly, 2003; Mokdad, 2003; Bray, 2004).

The challenge with modern dietary regimens is that they largely comprise meals that provide a poor sense of satiety. Appetite plays a crucial role in regulating energy balance and glucose haemostasis. Hunger sensation and appetite are mainly regulated by a number of gut hormones that function in either stimulating appetite or suppressing it. Traditionally, macronutrient content has been responsible of the release of these appetite-regulatory hormones. however here is little work on food structure. Structure of food is customarily referred to its appearance and texture of food (Lundin et al, 2008).

Humans have developed very efficient methods to ensure maximal nutritional intake, leading to health problems in today's society where energy and nutrient dense food is readily available. Food consumed by our ancestors (hunter gatherers), were rich in unprocessed fish, meat, vegetables, fruit, fungi and nuts. These foods therefore had many intact structural elements which control the release of nutrients (Jew et al, 2009; Frassetto et al, 2009). Recent work has shown that almonds are resistant to digestion and only release about 60% of their calorific content, due to the resistance to digestion of plant cell walls (Mori et al, 2011). It is likely that these nutrients are released slowly as the structures are gradually digested. This slow release of nutrients helps maintain satiety due to nutrient status and prolonged release of gut hormones (Jonsson et al 2010). Therefore, to maintain a healthy energy and glucose homeostasis, we need to consume foods capable of regulating gut hormone release. This project aims to explore the impact of food structure on appetite regulation.

### Rationale

There is very little evidence at the present time in the role of food structure in energy homeostasis. There is circumstantial evidence that foods consumed intact have great effects on suppressing appetite.

### Hypothesis

Cell wall structure will influence appetite and subsequent energy intake independent of nutritional content.

### Aim

To test the effect of three structurally-different meals with the same nutritional content and volume on appetite regulation. Each participant will receive the following meals:

- Solid meal group.
- Milled (intact-cell) meal group.
- Milled (destroyed-cell) meal group.

- All of which will contain the same macro and micro nutrients from the same food sources.

## 3. OBJECTIVES AND OUTCOME MEASURES

### Objectives

1- To measure gastric emptying rate after each meal
2- To measure and compare hunger, satiety and palatability, individually
3- To investigate energy intake at a meal given in excess
4- To investigate differences in gut hormones levels and release time – ghrelin, CCK, PPY and GLP-1 with each test-meal

### Primary outcome measure

A decrease in energy intake at a meal given in excess between the solid and puree meal.

### Secondary outcome measures

- Variance in gastric emptying rate as measured by Paracetamol plasma levels.
- Difference in gut hormones levels.
- Change in hunger and palatability as measured by VAS.

## 4. STUDY DESIGN

This will be a randomised, controlled clinical trial of a dietary intervention comparing three structurally different meals investigated separately over one day. The study will be carried out on four separate occasions one week apart. Participants will be recruited from a variety of settings including hospitals, universities. The inclusion and exclusion criteria are outlined in details in table 2 below. The aim is to recruit 15 participants for this study that meet the inclusion recruitments and deemed fit for the study. All participants will be offered £25 for each completed study visit, plus transportation to and from the research unit.

This randomised, controlled clinical trial of a dietary intervention will performed at the hospital's research unit under the supervision of the researcher. No doubt that a hospital-based investigation on food intake has its respective limitation; however, factors impacting on food intake are taken under account and minimised. The impact of a laboratory/hospital-based investigation came to light as several researches have explored the impact of surroundings on an individual's food intake. In addition to the impact of social surroundings and home environment, sensory factors such as visual, olfactory, auditory, and textual also influence individual's food intake (Dupertuis et al 2003; Stroebele et al 2004). These factors are shown to have an impinging potential to alter motivation, mood, and behaviour of an individual and hence impact on food intake (Stroebele et al 2004). Therefore to minimise any disturbance to the individual's normal food intake, an initial "dummy" visit to allow the subject to become familiar with the surrounding and despite given a restricted and controlled dietary intervention no records of food intake will be taken. Additionally, the environment of the research unit is also adjusted to mimic a home environment as much as possible. It is designed as a home-style living room that comprises of small dinning tables, chairs, TV, and iPads. The research unit will not only host our participants but also other participants from different studies within the research department. This is a positive addition to our study as it will enhance the social interaction and minimise the hospital-environment impact on normal food intake.

The four phases that our participants undergo will include *dummy-meal phase*, *solid-meal phase*, *Milled (intact-cell) meal* and *Milled (destroyed-cell) meal phase*. Each phase is to be investigated separately one week apart from each other. In order to maintain a controlled variable in this study, the same controlled dietary intervention will be introduced during each phase however in different structural format. My proposed plan is to start with the trial-meal phase, then participants will go through simple randomisation to start with the solid-meal phase, then the puree meal phase, or vice versa.

Table (1) outlines the dietary content that will be introduced to the participants at every phase. The portion size, weight, and calorific value are included within the table and this will remain constant throughout the different phases and throughout the study. This is to ensure that a constant variable is controlled and data retrieved from the study are comparable and unbiased.

Each meal form will consist of standard breakfast (table 1) then a lunch given in excess.

| Portion | Weight (gram) | Calories |
|---|---|---|
| Chickpeas (boiled) | 200 | 325 |
| Sesame oil | 13.6 | 120 |
| Apple | 180 | 90 |
| Total | 493.6 | 570 |

***Table1*: Test-meal content**

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## 5. PARTICIPANT ENTRY

### Recruitment

Study advert posters will be placed in hospitals and universities with contact details.

### Inclusion and Exclusion Criteria

Table (2) explains the inclusion and exclusion criteria that were considered in participants' recruitment.

| Table 2. INCLUSION AND EXCLUSION CRITERIA | |
|---|---|
| **Inclusion Criteria** | **Exclusion Criteria** |
| Gender: male and female<br><br>Age ≥ 18 years ≤ 65 years<br><br>Normal weight as classified by BMI 20-<br><br>29.9 kg/$m^2$<br><br>Healthy, not diagnosed with any chronic diseases.<br><br>Assessed as appropriate for inclusion, based on the pre-study screening.<br><br>Willingness and ability to understand, participate and to comply with the study requirements<br><br>Willingness and ability to give written informed consent | Any one who:<br><br>Has thyroid defects<br><br>Under hormone or steroids therapy<br><br>Is pregnant or lactating (female)<br><br>Had given birth within the past year (female)<br><br>Is taking drugs that could affect appetite or plasma glucose levels.<br><br>Is taking natural remedies that modulate appetite or plasma glucose levels.<br><br>Has excessive alcohol intake<br><br>Had blood donation within 12wks prior to start date<br><br>Psychiatric illness<br><br>Smokers<br><br>History of any disease with unknown outcome<br><br>Has diabetes<br><br>Has nut allergy |

**Screening**

Participants' inclusion will be based on two screening phases, the first phase is telephone screening, where we take initial information to identify participants who fit our criteria, if so the participant will be asked to visit the facility for a screening visit that will last approximately one hour for each participant. See Box 2 for information that will be gathered in the screening visit.

Once medical history, biochemistry, and anthropometric measurements have confirmed participant eligibility, a 7 days food-diary will be given to the participant to be completed the week prior to attending the first study visit (dummy visit) and prior to each subsequent visits.

This will allow us to form a more in depth assessment of the participant nutritional intake and dietary preferences.

| **BOX. 2 SCREENING INTERVIEW DATA COLLECTION** |
| --- |
| • **Informed consent**: requested and given<br>• **Personal details**: name, address, telephone number, gender, date of birth, demographical data<br>• **Anthropometry**: weight, height, calculation of BMI<br>• **Medical history**: current medications, allergies/drug reactions, past medical history [no prior CVD event, IBD], operations/surgeries, use of natural remedies and/or supplements, blood donation<br>• **Blood pressure**<br>• **ECG:** to record electrical activity of the heart<br>• **Blood tests**:<br>• **Glycaemic control**: HbA1c<br>• **CVD risk**: blood tests: lipid profile [total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides]<br>• **Renal function**: urea and electrolytes (sodium, potassium, chloride and bicarbonate), creatinine, estimated glomerular filtration rate<br>• **Liver function**: alanine transaminase, aspartate aminotransferase, alkaline phosphatase, albumin, total protein, bilirubin.<br>• **Female participants**: questioned on pregnancy in past year, current pregnancy status lactation. |

**Withdrawal Criteria**

- Significant deviation from the approved protocol
- The participant no longer fits the inclusion criteria
- Pregnancy (female)

- The participant withdraws consent

### Participant Reimbursement

All participants will be offered £25 for each study visit plus transportation to and from the research unit. This does not include screening visits.
If a participant withdraws from the study at any point, they will be reimbursed for the part they completed.

### Power Calculation

We aim to detect a variance of 200kcal in energy intake with a SD of 220kcal, based on a previous trial by Batterham (2003). Assuming a power of 90% and alpha of 0.05 we will require a sample of 15 subjects.

## 6. DIETARY INTERVENTION

All subjects will receive four different meals that contain the same components served in different forms (Solid, Milled (intact-cell), Milled (destroyed-cell)). These will be commercially available food items. As for the dummy visit, the participants will be given a standardized solid meal with the same components as the other test meals.

## 7. ADVERSE EVENTS

### Definitions

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

**Serious Adverse Event (SAE):** any untoward and unexpected 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.

**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 Adverse Event**

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

**Serious Adverse Event**

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

All SAEs should be reported to the xxx 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 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**

**Fax:** 020 8383 8320 **attention** Gary Frost

**Please send SAE forms to:** G.Frost@imperial.ac.uk

**Tel:** 07872850308 (Mon to Fri 09.00 – 17.00)

## 8. STUDY STRUCTURE

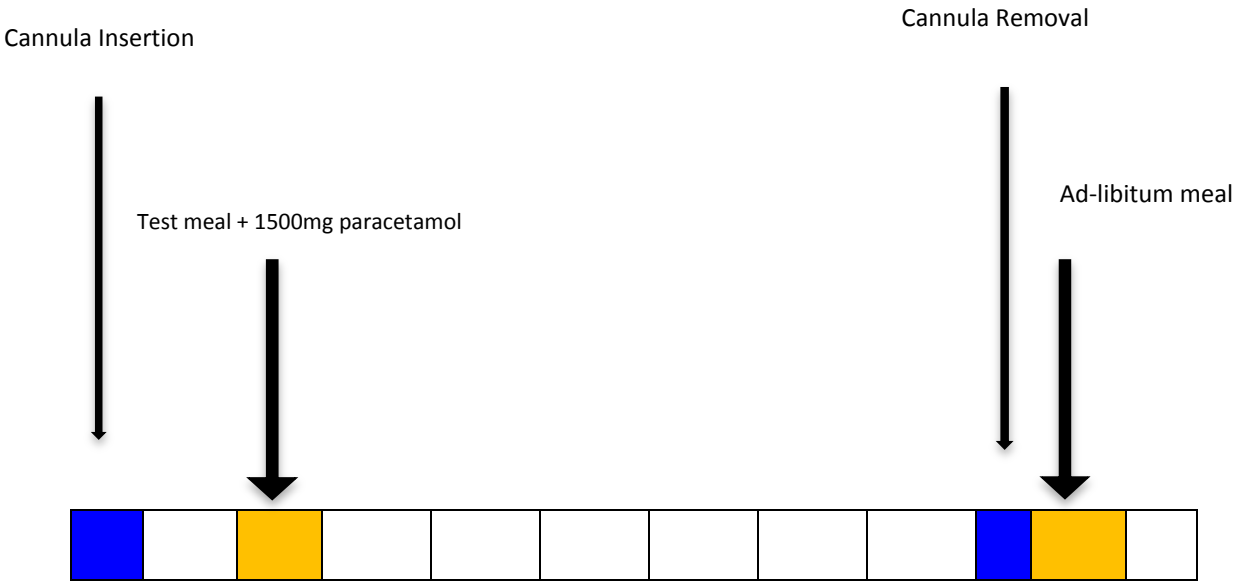
Following successful completion of screening phase, participants will go through four phases. The same course of action will take place in all four phases of the study; once the participant arrive to the CRF unit, the participant will be asked to step into the bioelectrical impedance machine to measure their weight, height, and body fat percentage. After that a cannula will be placed into the participant's arm to take blood samples starting from 10 minutes prior to the test meal. The participant will also be asked to do a visual analogue scale to measure satiety and hunger. Next blood sample will take place 10 minutes later along with introducing the test meal. The participant will be asked to consume the test meal within 10 minutes. Blood samples will take place subsequently at 15, 30, 60, 90, 120, and 180. This will make up a total of 110mls blood samples drawn. The cannula will be removed at this point, then an open buffet homogeneous pasta meal will be introduced to the participant to eat comfortably.

The first visit will be a dummy-visit, for the purpose of familiarizing the participant with the new ambience. After the dummy visit, participants will be randomly allocated (using a computer randomization program) to one of the three test meals on each visit; ‘solid’, ‘milled-destroyed cell’ or ‘milled-intact cell’ meal. Hence each participant will be through all 4 phases by the end of the fourth study visit. Participants will be instructed to avoid strenuous exercise, caffeine, and alcohol on the day prior to each study day. See Figure 1 for a detailed overview of the whole study design.

Figure 1: Schematic Representation of Study period


- **Biochemistry:** All visits will take place in the NIHR/Welcome Trust Imperial CRF. Fasting blood samples (110 ml) will be taken during the screening visits. During the study visits, a total of 110ml blood samples will also be taken to measure the following parameters: insulin, glucose, PYY, CCK, ghrelin, GLP-1 and paracetamol levels. Assays for routine bloods will be performed by the Department of Chemical Pathology at Imperial College Healthcare NHS Trust. Assays for gut hormones will be performed at Imperial College London, Hammersmith Site.
- **Gastric emptying rate:** to measure gastric emptying rate the participant will be given 1500mg of Paracetamol with the test meal, then plasma levels will be measured at each time point, see figure 2. Paracetamol administration will be carried out by a qualified researcher from the research unit.

**Fig 2. Schematic representation of appetite study activities**




| Time (min) | -10 | 0 | 15 | 30 | 60 | 90 | 120 | 180 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Blood | x | x | x | x | x | x | x | x | | |
| VAS | x | | x | x | x | x | x | x | | x |

- **Food Diaries Record and Analysis:** Participants will be required to keep a food diary throughout the study period, although they will be required to record their dietary intake for 7 days before each study visit only. This will allow for a comparison of energy and macronutrient intake. The diaries will be analysed using dietary analysis software.

**Appendix 1: Blood Sampling Schedule for the investigation**

| | Time | PYY | GLP-1 | CCK | Ghrelin | Glucose | Insulin | Paracetamol | VAS |
|---|---|---|---|---|---|---|---|---|---|
| | -10 (1) | 600 µl | 400 µl | 1000 µl | 450 µl | 500 µl | 500 µl | 500 µl | + |
| Breakfast | 0 (2) | 600 µl | 400 µl | 1000 µl | 450 µl | 500 µl | 500 µl | 500 µl | |
| | 15 (3) | 600 µl | 400 µl | 1000 µl | 450 µl | 500 µl | 500 µl | 500 µl | + |
| | 30 (4) | 600 µl | 400 µl | | | | | | + |
| | 60 (5) | 600 µl | 400 µl | 1000 µl | 450 µl | 500 µl | 500 µ | 500 µ | + |
| | 90 (6) | 600 µl | 400 µl | | | | | | + |
| | 120 (7) | 600 µl | 400 µl | 1000 µl | 450 µl | 500 µl | 500 µl | 500 µl | + |
| | 180 (8) | 600 µl | 400 µl | 1000 µl | 450 µl | 500 µl | 500 µl | 500 µl | + |
| **Cannula removed** | | | | | | | | | |
| Ad Libitum Meal | | | | | | | | | |
| | | | | | | | | | + |

## 9. STATISTICS AND DATA ANALYSIS

Once all the required data is collected, statistical analysis will be completed by Microsoft Excels software, using ANOVA test.

## 10. REGULATORY ISSUES

### Safety and Protection of Participants

National Institute for Health Research/Wellcome Trust Imperial CRF provides a safe environment for all studies that take place in the unit. The safety of all participating subjects is ensured by a well-trained staff and qualified researchers. This study will only include healthy volunteers, so the risk is limited to bruising from cannula insertion, and the possibility of blood loss, which will be taken into consideration in the screening phase. Participants who gave blood during the three months prior to the start date will be excluded.

### Ethics Approval

(The Chief Investigator has obtained approval from the XXXX Research Ethics Committee. The study will be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. 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. Any amendments to the protocol will be approved by the sponsor, prior to being submitted to ethics. On receiving ethical approval, all amendments will need further Trust R&D approval, before they can be implemented at any Trust site.)

### Consent

A consent form must be sought to each participant who enters the study, after providing them with a detailed explanation of the study plus an information leaflet including the purpose and the entire stages of the study. Signed consent should be obtained. All subjects have the right to refuse to take place in the study without giving any reasons. 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. In case a participant wants to withdraw at any point they will have the right to do that without giving reasons.

## Confidentiality

The chief investigator of the study will preserve confidentiality of all subjects participating in the study and is registered under the Data Protection Act. Personal information and contact details of subjects will be exposed only to the researchers. All the data will be recorded anonymously in the university computers without any personal data. Subjects' personal data will only be kept on NHS computers, which are password required. Each participant will be assigned to an individual code will be used to identify the participant during the entire duration of the study, including lab blood analyses handling, and the data analysis afterwards, same confidentiality guidelines will apply to these individual codes. Data analysis will take place in the MRC Clinical Science Centre and investigative Medicine. The department provides a secure setting for keeping confidential information and data under the authority of Professor Gary Frost.

## Indemnity

(Imperial College London holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this study. Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study. In addition, volunteers are registered as patients so that they have a right to all the associated health benefits this confers. Therefore, in addition to the Imperial College "no fault" indemnity scheme, normal NHS indemnity rules will apply)

## Sponsorship

Imperial College London will be the main sponsor of this study.

## Funding

Royal Embassy of Saudi Arabia-Cultural Bureau in London (King Saud bin Abdul-Aziz University for Health Sciences) will be funding this study.

## Audits

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).

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