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BAMBINI

Study Protocol
*Ba*riatric surgery vs. *M*edical care for o*be*sity and polycystic ovar*i*an syndrome related i*n*fertil*i*ty:
The BAMBINI randomised-controlled clinical trial

Version 4.0, 5th October 2021

Main Sponsor: Imperial College London
Funder: JP Moulton Charitable Foundation
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Protocol authorised by:

Name & Role	**Date**	**Signature**
Dr Alex Miras Chief Investigator	**5th October 2021**	

Study Management Group

Chief Investigator: Dr Alex Miras

Co-investigators: Prof Harpal S Randeva, Prof Stephen Franks, Prof Carel W le Roux, Mr Sanjay Purkayastha, Mr Vinod Menon, Dr Georgios K. Dimitriadis, Dr Channa Jayasena, Miss Lisa Webber, Dr Tony Goldstone, Mr Ahmed R Ahmed, Mr Krishna Moorthy, Mr Christos Tsironis, Mr Sherif Hakky, Dr David Wingfield and Ms Rachel Morman.

Statistician: not appointed

Study Management: Dr Alex Miras

Clinical Queries

Clinical queries should be directed to Dr Alex Miras 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
Room 215, Level 2, Medical School Building
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Funder

JP Moulton Charitable Foundation

This protocol describes the BAMBINI 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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STUDY SUMMARY

TITLE: Bariatric surgery vs. Medical care for obesity and polycystic ovarian syndrome related infertility: The BAMBINI randomised-controlled clinical trial

DESIGN: Open-label randomised controlled trial

AIMS: To investigate the safety and efficacy of obesity surgery vs. standard medical care for women with PCOS, obesity and oligo or amenorrhea.

OUTCOME MEASURES: Number of ovulatory cycles within the 12 month follow-up period.

POPULATION: Women with polycystic ovarian syndrome and obesity

ELIGIBILITY: Pre-menopausal women ≥ 18 years old, Body mass index (BMI) ≥ 35 kg/m^2 with obesity related complications and a diagnosis of PCOS

DURATION: 3 years

1.1 BACKGROUND

Polycystic ovarian syndrome (PCOS) affects up to 1 in 5 women and is the most common endocrine disorder in women of reproductive potential [1]. There are more than 3 million women suffering from this condition in the UK. PCOS is a heterogeneous disorder characterised by elevated circulating androgen levels, chronic anovulation, and polycystic ovaries. It manifests with hirsutism, acne, oligo or amenorrhea (i.e. few or no menstrual cycles) and subfertility. The difficulty in have children is a major cause of emotional distress. Approximately 50% of women with PCOS are overweight or obese and often have visceral adiposity when compared to age and BMI (body mass index)-matched controls [2]. PCOS complicated by obesity amplifies adverse fertility and metabolic outcomes, doubles the likelihood of developing type 2 diabetes mellitus (T2DM) and is a strong risk factor for endometrial cancer and cardiovascular disease [3]. The annual healthcare burden of PCOS in the UK, based on a conservative estimate, stands at £237 million [4]. If current trends continue without innovative prevention and treatment strategies, the annual cost is predicted to rise to £550 million by 2038.

Weight loss through any means is an effective therapy for improving subfertility in women with PCOS and obesity. Reduction in weight of 5% can help regulate the frequency of menstruation and increase the chances of a pregnancy [5]. Lifestyle modification including healthy eating and increase in physical activity is effective in causing short term weight loss, but unfortunately this is rarely maintained in the long term [6]. Strong physiological factors resist weight loss below a certain weight set point, eventually causing weight regain in more than 80% of patients and loss of any improvement in endocrine function [6]. Pharmacotherapy, including metformin and Orlistat, has a positive, but modest, impact on weight loss, and the metabolic and reproductive manifestations of PCOS [7]. Glucagon-like peptide 1 receptor agonists are more effective but have not been endorsed by NICE due to their high cost [7].

1.2 RATIONALE FOR CURRENT STUDY

In a community-based study, subfertility was reported in 72% of women with PCOS compared with 16% without [8]. Thus, a substantial proportion of women with PCOS and obesity remain sub-fertile and this has a devastating impact on their psychological health [9].

A systematic review and meta-analysis of cohort and case-control studies demonstrated that laparoscopic obesity surgery, like the vertical sleeve gastrectomy (VSG) and Roux-en-Y gastric bypass has a substantial positive impact on all of the manifestations of PCOS [10]. Obesity surgery caused weight loss of 25-30%. The incidence of PCOS preoperatively was 46% and was significantly decreased to 7% at 12-month follow-up. The incidence of pre-operative menstrual irregularity, subfertility and hirsutism were all substantially decreased at 12-month. Obesity surgery has also been shown to reduce overall mortality and the risk of developing endometrial cancer [11], T2DM [12] and cardiovascular disease [13, 14].

Modern laparoscopic obesity surgery is one of the safest operations in the field of surgery and carries similar risks of morbidity and mortality as a laparoscopic cholecystectomy [15]. Young women with PCOS rarely have significant cardiovascular or respiratory disease, making surgery even safer for this cohort. Pregnancies after obesity surgery are associated with reduced risk of gestational diabetes and excessive foetal growth, shorter gestation, but with an increased risk of small-for-gestational-age infants as compared with matched control pregnancies [16].

The knowledge gap is articulated by recent international guidelines for the assessment and management of PCOS which stated that “bariatric surgery should be considered an *experimental* therapy in women with PCOS, for the purpose of having a healthy baby, with risk to benefit ratios currently too uncertain to advocate this as fertility therapy” [17]. National and international guidelines will only change if the safety and efficacy of obesity surgery is demonstrated within randomised controlled trials (RCTs) confirming the encouraging meta-analyses of cohort studies.

This proposal aims to change clinical practice and address the knowledge gap by conducting the first RCT in this field. The ultimate goal is to provide the data for an intervention that would enable women with PCOS have healthy pregnancies and children.

Hypothesis: Obesity surgery will be superior to standard medical care in increasing the number of ovulatory cycles in women with PCOS, obesity and oligo or amenorrhea.

2. STUDY OBJECTIVES

To perform a RCT comparing the safety and efficacy of obesity surgery vs. standard medical care for women with PCOS, obesity and oligo or amenorrhea.

3. STUDY DESIGN

In this prospective open-label RCT we propose to recruit 80 women with PCOS, obesity and oligo or amenorrhea from Imperial College Healthcare NHS Trust, University Hospitals Coventry & Warwickshire NHS Trust, two centres with tertiary obesity and reproductive endocrinology expertise, and the Hammersmith and Fulham GP Partnership.

They will be eligible for obesity surgery based on NICE CG189. Patients will be randomised at a ratio of 1:1, stratified by BMI and trial site to:

- Standard medical care (n=40): combination of an intensive lifestyle intervention ± pharmacotherapy *or*
- Obesity surgery (n=40): standard laparoscopic VSG.

3.1 STUDY OUTCOME MEASURES

Primary outcomes

- Number of ovulatory cycles within the 12 month follow-up period. Ovulation will be defined as a rise in serum progesterone ≥16 nmol/L

Secondary outcomes

Change from baseline to 12 months. For each endpoint, temporal changes, mean levels and peak levels will be analysed as appropriate:

- Number of reported menses
- Body weight
- Waist circumference
- Body composition
- Plasma lipid concentration
- Plasma liver function tests
- Plasma HbA1c
- Arterial blood pressure
- Serum LH
- Serum FSH
- Serum Oestradiol
- Serum SHBG
- Serum Testosterone
- Serum free androgen index
- Serum DHEAS
- Serum Androstenedione
- Serum AMH
- Glucose concentrations at the OGTT
- Hospital Anxiety and Depression Scale score
- Multidimensional Health Profile: Health Functioning questionnaire score
- Social Functioning Questionnaire score

- PCOS Health-Related Quality of Life score
- Modified Ferriman-Galwey hirsutism score
- Ludwig visual score
- Savin Alopecia Scale score
- Cardiff Acne Disability Index
- Number of medications
- Adverse events
- pregnancy rates

Sub-studies
- Energy expenditure
- Sleep-disordered breathing
- Endometrial clonal activity
- Adipose tissue biology
- Brain responses to food

4. PARTICIPANT ENTRY

4.1 PARTICIPANT RECRUITMENT

This will take place from:
- **Outpatient clinics. The direct care team is also the research team. They will identify patients through direct clinical contact but also by looking through the databases and medical records of the obesity and reproductive clinics, and GP practices in the Hammersmith and Fulham GP Partnership.**
- **Online advertising via Clariness (below)**
- **Clariness is an international participant recruitment company for clinical trials. It advertises the trial through an online awareness campaign including search engine marketing, banner advertising on relevant websites and social media. Upon clicking an ad, these candidates will be taken through an online prescreening process. In this prescreening process patients are asked questions about their health based on the trial inclusion and exclusion criteria and those who pass this step will be referred to the investigators.**

  Data that the potential volunteer has provided Clariness via the ClinLife® website with the intention that this information will be forwarded to a participating investigator, are made available to investigators as follows: Data is stationed on a specially-equipped and accordingly-secured server on AWS in Frankfurt, Germany. (Privacy policies and security measures as stipulated by the Data Protection Act can be inserted or displayed where necessary and at any time). Data is not sent, but made available to the investigator in his/her SSL-encrypted and password-protected ClinLife® account. Data will only be available to sites during the enrolment period of the trial. The privacy policies and security measures according to BDSG (Bundesdatenschutzgesetz, based on the General Data Protection Regulation) apply. The appropriate trial site personnel associated with the trial will receive a neutral informative email (no personally identifiable information) requesting him/her to access his/her SSL-encrypted and password-protected ClinLife® account to view the patient's data. All volunteer data provided to the trial site as part of the trial registration process on ClinLife® will be deleted after the statutory retention period, but no later than the end of the study.

4.2 PRE-REGISTRATION EVALUATIONS

Screening will be performed to confirm that patients meet the inclusion criteria for the trial and are safe to undergo treatment for obesity following careful psychological and dietetic assessments. In addition to routine clinical care assessments, physical examination (i.e. modified Ferriman Gallwey score, Ludwig visual score and Savin Alopecia Scale) and investigations, participants will have their reproductive hormone profile checked ± pelvic ultrasonography if necessary. A urine pregnancy test will be performed. The importance of effective non-hormonal contraceptive use for the duration of the trial will be emphasized.

4.3 INCLUSION CRITERIA

- Pre-menopausal women ≥18 years old
- Body mass index (BMI) ≥ 35 kg/m^2 with obesity related complications
- Diagnosis of PCOS based on International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018 that requires two of the following [17]:
  i. for women > 3 years post menarche to peri-menopause: < 21 or > 35 days or < 8 cycles per year
  ii. Hyperandrogenism
    a. clinical hirsutism (Modified Ferriman-Galwey hirsutism score ≥ 4-6) or male pattern alopecia (positive Ludwig visual score)] or
    b. biochemical (raised free androgen index or free testosterone)
  iii. Polycystic ovaries on ultrasound: Using transvaginal ultrasound transducers with a frequency bandwidth that includes 8MHz, the threshold for PCOM should be on either ovary, a follicle number per ovary of > 20 and/or an ovarian volume ≥ 10ml, ensuring no corpora lutea, cysts or dominant follicles are present.

4.4 EXCLUSION CRITERIA

- Type 1 or Type 2 diabetes mellitus
- Specific contraindications to obesity surgery
- Previous obesity surgery
- Inability to maintain adequate contraception
- Medications affecting reproductive function (e.g. oral steroids, hormonal contraceptives) at screening or 3 months previously.
- Other causes of anovulation (e.g. untreated hypothyroidism, adrenal or pituitary disorders)
- Current pregnancy or breastfeeding
- History of any medical, psychological or other condition, or use of any medications, including over-the-counter products, which, in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer.
- Without access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study.

4.5 WITHDRAWAL CRITERIA

All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. Should they wish to withdraw their consent, all of their data to date will be held.

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 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.

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.

5.2.1 Non serious AEs

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

5.2.2 Serious AEs

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

All SAEs should be reported to the London-Dulwich Research Ethics Committee 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.

Pregnancy

We ask participants to use non-hormonal contraception for the duration of the study. This is especially important for those in the surgical arm as pregnancy in the first 12 months following obesity surgery should be avoided due to the risk of nutritional deficiencies and other potential complications.

Contact details for reporting SAEs

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Please send SAE forms to:
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Imperial College London
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Imperial College London at Hammersmith Campus
Du Cane Road, London W12 ONN, UK
Tel: +44 (0)20 8383 3242 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

Baseline and randomisation visit: Patients will be asked to take Medroxyprogesterone to induce a menstrual bleed before the baseline visit. Medroxyprogesterone is used routinely in clinical practice for the same indication. The induction of the bleed will enable all women to be studied at the same phase of their menstrual cycle.

At this visit the reproductive hormone profile will be repeated together with a pregnancy test and Oral Glucose Tolerance Test (OGTT). The participant will be asked to complete the Hospital Anxiety and Depression Scale (HADS), Multidimensional Health Profile: Health Functioning questionnaire (MHP-H), Social Functioning Questionnaire (SFQ), PCOS Health-Related Quality of Life, and Cardiff Acne Disability Index questionnaires. If the participant decides to take part in the mechanistic studies this will also take

place (Appendix 2). Subjects will be randomized via computer software at a 1:1 to either standard medical care or obesity surgery stratified by BMI and trial site.

Interventions
Patients in both groups will be cared for by a multidisciplinary team consisting of endocrinologists, surgeons, dietitians, psychiatrists/psychologists and specialist nurses as per standard NHS practice.

- Standard medical care: patients will undergo lifestyle modification as part of a standard NHS "tier 3" programme [18]. This will be delivered by a dietitian, psychologist and physical activity specialist and will incorporate a personalised approach depending on patient needs and functional impairment. It will include caloric reduction, changes in macronutrient composition, modification of eating behaviour and emotional eating, and increase in physical activity levels. Pharmacotherapy will include the option of metformin and/or Orlistat. Patients in this group will still be eligible for obesity surgery after completing this trial.

- Obesity surgery: patients randomised to this group will undergo standard laparoscopic VSG. Surgery will be performed by experienced consultant obesity surgeons.

With the participants consent, their GP will be informed of any incidental findings.

Clinical trial follow-up
Patients in both groups will be assessed by the research team for 12 months post intervention. In addition to routine clinical parameters the following will be measured/performed:
- On a weekly basis: serum progesterone
- On a monthly basis:
    - Menstrual bleeding using a diary or smartphone application
    - Reproductive profile: serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol, sex hormone binding globulin (SHBG), testosterone, dehydroepiandrosterone sulphate (DHEAS) and androstenedione.
    - Urine pregnancy test
- Every 6 months: OGTT, Anti-mullerian hormone (AMH), modified Ferriman Gallwey score, Ludwig visual score and Savin Alopecia Scale) , Hospital Anxiety and Depression Scale (HADS), Multidimensional Health Profile: Health Functioning questionnaire (MHP-H), Social Functioning Questionnaire (SFQ), PCOS Health-Related Quality of Life, and Cardiff Acne Disability Index questionnaires, blood pressure, plasma lipids, liver function tests and HbA1c.

Sub-studies follow-up
All participants will be given the opportunity to take part in state-of-the-art studies that will investigate the physiological mechanisms underpinning the clinical impact of the two treatments (Appendix 2). These will be performed at the following time points:
- Energy expenditure and sleep-disordered breathing: baseline and 6 months
- Endometrial clonal activity : baseline and 6 months
- Adipose tissue biology: baseline and 6 months
- Functional Magnetic Resonance Imaging (fMRI): baseline and 3 months

Contraception
Participants will be asked to maintain effective contraception for the duration of the study. Effective contraception methods include:

- Barrier methods
- intrauterine device (non-hormone releasing)

- vasectomised partner: this is a highly effective birth control method provided that partner is the sole sexual partner of the trial participant and that the vasectomised partner has received medical assessment of the surgical success.
- sexual abstinence: sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments and when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.

Drop-outs
Subjects will be free to withdraw at any point. In view of the unprecedented impact of the COVID19 pandemic on clinical research, only participants who withdraw because of fears of contracting the virus will be replaced.

Trial Closure
The end of the clinical trial is defined as the last visit of the last patient.

Sample storage
For the clinical trial, blood samples will be sent to NHS labs for processing and handled as per standard NHS care. The samples from the sub-studies will be stored for up to 10 years in freezers located in the Department of Metabolism, Digestion and Reproduction at Imperial College London and the Human Metabolic Research Unit (HMRU) at the University Hospital of Coventry and Warwickshire NHS Trust.

Patient and Public involvement
During the development of this application we organised focus groups and engaged closely with:
- Ms. Rachel Morman, Chair and Trustee of Verity, the largest patient support group for women with PCOS in the UK
- Ms. Georgina Hayman, lead of the British Obesity Surgery Patient Association West London
- Dr Shamil Chandaria, Patron of the National Obesity Forum

Ms. Rachel Morman is a co-applicant and will be invited to be a member of the trial steering committee. Ms. Georgina Hayman will kindly hold support groups for the participants to improve adherence, engagement and retention. This is particularly important for the group that will not have obesity surgery for 12 months. The patient group representatives have agreed to help in the dissemination of the results of this trial to the local and national communities of patient and healthcare professional.

7. STATISTICS AND DATA ANALYSIS

Justification of sample size
Based on the available data on the effect size of lifestyle interventions [5, 19] and obesity surgery [10] on ovulation we estimated that women in the standard medical care group will have a mean of 7 and women in the obesity surgery group a mean of 10 ovulatory cycles in the 12 month follow-up period. With a standard deviation of 3.3 around both means, we will need 33 women in each group to have a 95% power to detect statistically significant differences between the groups at α of 0.05. We will recruit 40 patients in each group to account for a 15-20% drop-out rate based on rates in similar trials we have conducted in this field (LIPOS UTN: U1111-1126-3292 and DOMINO ISRCTN76278694).

Statistical analysis plan
Analysis of the data will only take place after completion of the trial having written a statistical analysis plan and use the intention to treat principle. Data will be summarised using descriptive statistics. Differences between treatment groups will be compared using ANCOVAs and/or chi-square tests adjusting for the randomisation stratification variables and baseline value of the tested parameter.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the London-Dulwich Research Ethics Committee (REC) and Health Regulator 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

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and a minimum of 24hrs 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.

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

The J P Moulton Charitable Foundation is funding the trial. All patients will receive **£400** upon completion of the study as a reimbursement for their time and inconvenience. Patients taking part in the mechanistic studies will receive an additional reimbursement depending on which sub-study/studies they take part in:

- Energy expenditure and sleep studies £100
- Endometrial clonal activity £50
- Adipose tissue biology £50
- Functional MRI scan £200

8.7 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 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 through Dr Alex Miras. A Trial Management Group, Trial Steering Committee (TSC) and a Data Monitoring & Ethics Committee will be established. The trial will be conducted at the NIHR Clinical Research Facility at Imperial according to their SOPs and with oversight of their QA team.

10. PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the TMG and the Data Monitoring Committee will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project. The results are likely to be published within the 12 months following the study in peer-reviewed journals and websites, and presented in medical conferences. Participant confidentiality will be ensured at all times and they will not be identified in any publication as these will be anonymised. A lay summary of the key results from the study will be written and sent and/or presented to them.

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Appendix 1. Summary of investigations and assessments

Assessment													
	Baseline	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	7 mo	8 mo	9 mo	10 months	11 months	12 months
Number of reported menses	X	X	X	X	X	X	X	X	X	X	X	X	X
Body weight waist circumference body composition	X	X	X	X	X	X	X	X	X	X	X	X	X
Plasma lipids HbA1c liver function tests	X						X						X
Blood pressure	X						X						X
Reproductive hormones	X	X	X	X	X	X	X	X	X	X	X	X	X
Anti-mullerian hormone	X						X						X
Urine pregnancy test	X	X	X	X	X	X	X	X	X	X	X	X	X
Progesterone	Weekly throughout trial												
Questionnaires	X						X						X
Hirsutism and alopecia indices	X						X						X
Number of medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Energy expenditure	X						X						
Sleep studies	X						X						
Endometrial biopsy	X						X						
Adipose tissue biopsy	X						X						
Oral glucose tolerance test	X						X						X

Appendix 2

Sub-studies

All participants will be given the opportunity to take part in one or more state-of-the-art studies that will investigate the physiological mechanisms underpinning the clinical impact of the two treatments. These mechanistic studies will be optional and focus on:

- Energy expenditure and sleep-disordered breathing
- Endometrial clonal activity
- Adipose tissue biology
- Functional MRI

Energy expenditure and sleep-disordered breathing

All patients will be offered an optional metabolic study at the Human Metabolic Research Unit (HMRU) for body composition, energy expenditure and sleep study. This metabolic study will take place at baseline and 6 months post-intervention follow-up.

Each HMRU study will commence at 8am with measurement of body composition in the BodPod, which provides an accurate measurement of body composition. Participants will have fasted overnight. Each participant will enter the metabolic chamber at 9:30am for a 24-hour metabolic study to measure energy expenditure profile in real time. Prior to entry into the chamber, each participant will be shown around the chamber by the HMRU nurse, for orientation purposes. Throughout their 24-hour stay within the calorimeters, subjects will be asked to collect their urine in specific containers provided by the research team. These will be collected 8-hourly and used for the assessment of substrate oxidation.

Following chamber entry, the experiment will start at 09:50 am to allow participants to settle in the calorimeter. At 12.00 a standard lunch will be served and subjects will have sequential blood tests every 30 minutes at 12:30, 13:00, 13:30 and 14:00pm to assess glucose metabolism.

Following the post-prandial period from the standard lunch, there will be a standard activity protocol at 15.30 involving stepping for 30 minutes at a rate of 90 beats per minute with one full-step up and down per second. A standard online metronome application will be used and this will enable assessment of standard activity-related energy expenditure. Following this at 18.00, a standard meal will be provided. Subjects following this will be asked to remain seated for 3 hours avoiding any form of activity for the assessment of post-prandial thermogenesis.

There will be a standard snack provided at 21.00, and participants will be requested to sleep from 22.30 with wake-up at 07.00 the next morning. The next morning, subjects will be woken at 07:00 and will be asked to remain on the bed without sleeping or moving during which time, resting metabolic rate is going to be assessed after at least 8 hours fast. At 08:00am a standard 2-hour glucose tolerance test will be performed using 75gr of glucose. Participants will be asked to exit the chamber at 10.00. Assessment of sleep data using complete polysomnography will be carried out by the Lead Respiratory Physician at UHCW NHS Trust (Dr Asad Ali). This will be carried out at baseline and 6 months after randomisation.

Throughout each of the HMRU visits, all subjects will be monitored continuously (including during the whole 24-hours HMRU study). Subjects will be encouraged to report any unusual or unpleasant sensation to the investigator immediately. Any significant adverse effects will lead to discontinuation of the visit after assessment by an investigator. Throughout the study there will be at least one member of the research team available on 24-hour call via a direct line, with a second member on back up, and a secondary direct line to one of the senior investigators. Although we do not anticipate any serious adverse effects based on screening procedures and previous experience with similar studies, participants will be able to contact a member of the research team at any time.

Endometrial clonal activity
Endometrial biopsies will be taken using an endometrial sampler, as used in routine clinical care. Following a transvaginal scan to assess endometrial thickness, the sampler will be introduced into the uterus until resistance from the fundus is felt. The piston will then be withdrawn to generate negative pressure and the device rotated through 360°. Biopsies will be processed as previously described [18]. Biopsies will be performed before and at 6 months after intervention.

Adipose tissue biology
Subcutaneous and omental fat biopsies will be collected from participants at the time of their obesity surgery and under general anesthesia. During operation biopsies will be taken by scissors and/or harmonic scalpel (ultrasound instrument for dissection). Subcutaneous fat tissue can be taken from an incision from a trochar and visceral fat tissue from the major omentum at the greater curvature of the stomach. These procedures should take 15 minutes and will not add any further risk to the operation. Patients in the standard medical care group will only have abdominal subcutaneous fat biopsies performed under a local anaesthetic and by using a trucut biopsy or biopsy punch approach. Follow-up abdominal subcutaneous biopsies will be performed at 6 months in both groups. The use of the local anaesthetic will minimize any potential discomfort. Fat cells will be isolated from the biopsies and plated for culture. The effects of androgen added to the culture will be examined by investigating metabolic pathways including insulin signaling, the action on molecules involved in steroid hormone synthesis and the action and effects on the production of adipokines.

Brain Imaging sub-study (only available at Imperial College London Healthcare NHS Trust)

BACKGROUND
This Brain Imaging sub-study protocol is an optional mechanistic protocol for the BAMBINI clinical trial only for patients recruited into the study at Imperial College Healthcare NHS Trust in London.

The alterations in eating behaviour that occur in obesity and how these are affected by weight loss treatments are poorly understood. It is likely that altered central regulation of food reward plays a key role in weight loss, and that individual psychological and genetic factors modulate these changes.

We hypothesize that there is reduced activation of reward pathways in response to particularly high-energy food stimuli in patients who have had treatment for obesity compared with similarly obese patients to match those of non-obese individuals. Furthermore, we hypothesize that there may be changes in impulsivity and brain responses to non-food reward in obese patients and following treatment for obesity that may contribute to altered eating behaviour. In addition, different surgical, dietary and medical treatments for obesity may have differing effects on food and non-food reward. By visualising activity in brain pathways related to food and non-food reward, we will be able to demonstrate how changes in eating behaviour are altered by weight loss treatments.

This protocol for the non-scan and scan visits is identical to several other studies of appetite after bariatric surgical, medical and endoscopic interventions by the Investigators (10/H0707/60 and 14/LO/0871) (Glaysher MA et al. BMJ Open. 7:e018598, 2017). These have been readily acceptable both in nature and burden to the participants with low drop-out (<10%) for return visits.

1.1.1 OVERALL STUDY DESIGN

In addition to the main study protocol, women with obesity and PCOS will undergo an additional 4 study visits. All visits will involve questionnaires, neuropsychological tests, blood tests and appetite assessments. Two of the visits will include brain scanning - functional magnetic

resonance imaging (fMRI). Two visits (1 non-scan, 1 scan) will be at baseline and another 2 visits (1 non-scan, 1 scan) at around 3 months after standard medical management or laparoscopic vertical sleeve gastrectomy (LVSG). At each time point (baseline, follow-up) the 2 visits will be 3-7 days apart.

Both at baseline and following treatment, eating and psychological profiles will be measured using questionnaires and food diaries, and blood samples taken to measure hormones, adipocytokines and metabolites involved in obesity, its metabolic complications and appetite control (e.g. gut hormones) in addition to routine blood tests (full blood count, urea and electrolytes, liver function tests, plasma glucose, lipids and thyroid function tests). Subjects will also be asked for consent for DNA and RNA samples (e.g. blood). In addition, neuropsychological tests (such as food preference, impulsivity) of appetite, functional and anatomical brain scans will also be performed,

During each fMRI scanning visit, subjects will have changes in brain activity measured whilst completing tasks involving food stimuli (e.g. viewing food pictures) or other cognitive and neuropsychological tests. Some of these tests may also be performed outside the scanner for subjects to practice these on the day of scanning. Blood samples will be taken at separate intervals to measure circulating hormones and metabolites. Appetite is assessed using visual analogue scales and test lunch meals.

1.1.2 PARTICIPANTS

Inclusion criteria

- As per main study

Exclusion criteria

As per main study with additional exclusion criteria for this mechanistic sub-study:

- Vegetarian, vegan, gluten or lactose intolerant (given pictures used in food evaluation fMRI task)
- Current smoker
- History of alcohol dependence, substance dependence (e.g. cannabis, cocaine, opiates), problem gambling
- History of serious mental illness (e.g. bipolar disorder, schizophrenia), current uncontrolled depression
- Significant structural abnormality on magnetic resonance brain scan.
- Claustrophobia.
- Pacemaker, metal implant, clips, implanted device, shrapnel or bullet, metal in eyes that precludes magnetic resonance imaging.

Aim is to recruit at least n=20 participants in each arm of the study.

1.1.3 PROTOCOL

VISIT 2A (baseline), and 4A (post-treatment): NON-SCAN VISITS

(up to 7 hours) at the NIHR/Wellcome Trust Imperial Clinical Research Facility, Hammersmith Hospital

Normal breakfast at home at 7am
Arrive at 09:00
Review study, answer questions, written informed consent
Medical history and observations
Blood sampling (including full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, serial samples over visit for insulin, lipids, glucose, gut hormones and metabolites)

Urine pregnancy test
Measurement of height and weight
Bio-electrical impedance analysis to calculate total fat-free and fat mass
Eating behaviour, food preference, personality and neuropsychological questionnaires and computer tasks
Fixed lunch meal with visual analogue scale ratings of appetite
DNA and RNA sample (blood) - on 1st visit only
Urine and plasma samples for metabonomics

T (Time) = -180 min (09:15): Consent
T = -150 min: Medical history
T = -105 min: Height, weight, pulse, blood pressure, bio-electrical impedance analysis, urine pregnancy test
T = -90 min: Cannula inserted into vein for serial blood sampling
T = -80 min to +120 min: Blood sampling (4 time points)
T = -20 min to +120 min: Visual analogue scales of appetite (6 time points)
T = -120 min (11:30): Delay and probability discounting task for food and money (30 mins)
T = -60 min: Food valuation task (20 mins)
T = -40 min: 4-choice serial reaction time task (25 mins)
T= 0 min (1.30pm): Fixed liquid lunch meal
T = +5 min to + 120 min: Complete questionnaires (60-75 mins)
T = +150 min (16:00) depart

VISIT 2B (baseline), and 4B (post-treatment): SCAN VISITS
(up to 8 hours) at the NIHR/Wellcome Trust Imperial Clinical Research Facility and Clinical Imaging Facility, Hammersmith Hospital

1.1.4

Subjects will be advised to avoid alcohol and strenuous exercise the day before and day of each scan visit, and will be asked to fast from the evening before the visit.

Prior to visit:
3 day food diary (on-line using myfood24)
Collection of stool sample at home

Overnight fast
Arrive at 09:00
Medical history and clinical observations (pulse, blood pressure)
Blood sampling (including sex hormones, and serial samples over visit for glucose, lipids, cortisol, insulin, gut hormones and metabolites)
Urine pregnancy test
Measurement of height and weight
Bio-electrical impedance analysis to calculate total fat−free and fat mass
Eating behaviour, food preference, personality, mood and neuropsychological questionnaires
Neuropsychological computer tasks
Urine and plasma samples for metabonomics
Stool sample for metabonomics and microbiome

T (Time) = -30 min (09:15): Cannula inserted into vein for serial blood sampling
T = 0 min to +340 min: Visual analogue scales of appetite (7 time points)
T = 0 min to +340 min: Blood sampling (5 time points)
T = 0 min (09:45) Kirby delay discounting task (10 mins)
T = +10 min Leeds Food Preference Questionnaire (20 mins)
T = +40 min Practice of fMRI tasks (20 mins)
T = +60 min Start other on-line questionnaires (15 mins)

T = +90 min (11:15: MRI scan lasting up to 90 mins: anatomical and functional MRI scans
T= +220 min (13:25): Ad libitum test lunch meal, including taste ratings
T= +240 min: WTAR intelligence test (baseline only) (10mins)
T = +250 min Mood questionnaire (PANAS)
T = +260 min: Complete any remaining on-line questionnaires
T= +340 min (15:25): Progressive ratio task for sweets (15 mins)
T= +370 min: Complete any remaining on-line questionnaires
T = +405 min (16:30) depart

Questionnaires

At non-scan visits 2A and 4A, subjects will complete validated questionnaires (duration 60-75 mins) to assess eating behaviour and attitudes, and personality measures related to reward sensitivity, addictive behaviours and mood as below. These questionnaires can be completed at home over the internet if they are unable to finish them during the screening visit or finished at the subsequent scanning visit to reduce the burden on individuals at the screening visit.

1. Dutch Eating Behaviour Questionnaire (DEBQ) − to measure restraint, emotional and external influences on eating behaviour (Wardle J Psychosom Res 31:161−9, 1987).
2. Eating Disorder Examination Questionnaire (EDE−Q) – to screen for existing eating disorder (Fairburn CG, Beglin S Int Jour. Eat Dis 16:363−370, 1994).
3. Three Factor Eating Questionnaire (TFEQ) - to measure restraint, disinhibition and hunger (Stunkard & Messick J Psychosom Res 29:71-83, 1985).
4. Binge Eating Scale to assess binge eating severity (Gormally J, et al. Addict Behav. 7:47-55, 1982).
5. Power of Food Scale to assess the psychological influence of the food environment (Lowe MR et al. Appetite 53:114–8, 2009).
6. Yale Food Addiction Scale to measure features of addiction towards particular foods (Gearhardt AN et al. Appetite.52(2):430-436, 2009).
7. Palatable Eating Motives Scale (PEMS) – to measure motivations behind overeating of palatable foods (Burgess EE et al. Appetite. 72:66-72, 2014).
8. Behavioural Inhibition and Activation System (BIS / BAS) scales − to measure punishment and reward sensitivity (Carver & White J Pers Soc Psych 67:319−333, 1994).
9. Eysenck Personality Questionnaire (EPQ−R) − to measure extraversion that correlates with reward drive and sensitivity (Eysenck et al. Person Indiv Diff 6:21−29, 1985; Carver & White J Pers Soc Psych 67:319−333, 1994).
10. Beck Depression Inventory (BDI−II) − to measure levels of depression and anxiety (Beck et al. J Pers Assess 67:588−97, 1996).
11. Hospital Anxiety and Depression Scale (HADS) to assess symptoms of anxiety and depression (Herrmann C. J Psychosom Res.42:17-41, 1997).
12. Barratt Impulsivity Scale − to measure impulsivity (Patton JM et al. J Clin Psych 51:768−74, 1995; Yeomans MR et al. Appetite 50:469−76, 2008).
13. UPPS-P Questionnaire to assess impulsivity, risk taking and sensation seeking seen in addiction (Cyders MA et al. Psychol Assess;19(1):107-18. 2009)
14. Short-Form 36 Health Survey Questionnaire (SF36) to assess quality of life (Stewart AL et al. Med Care. 26:724-35, 1988).
15. Impact of Weight on Quality of Life Questionnaire (IWQoL-Lite) to measure weight-related quality of life in domains of self-esteem, sexual life, public distress, work, physical function, (Kolotkin RL et al. Obes Res 2:49-56, 1995).
16. Pittsburgh Sleep Quality Index (PSQI) to measure quality and duration of sleep (Buysse DJ et al. Psychiatry Res 28:193-213, 1989).
17. DS-R to assess disgust sensitivity, which influences brain responses to unpleasant images (Haidt J et al. Personality Indiv Diff 16:701-13, 1994; Wright P et al. Neuroreport 15:2347-51, 2004; Olatunji BO et al. Psych Assess. 19:281-97, 2007).
18. Handedness inventory - to measure degree of right or left handedness (Oldfield RC

Neuropsychologia 9:97-113, 1971).

19. Arts and Sigstad questionnaires to assess dumping symptoms after eating e.g. nausea, abdominal discomfort (Sigstad H. Acta Med Scand. 188:479-86, 1970; Arts J et al. Clin Gastroenterol Hepatol. 7:432-7, 2009; Scholtz S et al. Gut doi: 10.1136/gutjnl-2013-305008, 2013).
20. International Physical Activity Questionnaire (IPAQ) - to measure physical activity (Craig et al. Med Sci Sports Exerc. 35, 1381−1395, 2003).
21. Magnetic resonance imaging metal check list - to ensure compatibility with MRI scanning (1st visit only)
22. Fagerström Test for Nicotine Dependence (Heatherton TF et al. Br J Addict. 86:1119-27, 1991). to assess past smoking dependence behaviour.
23. Alcohol Use Disorders Identification Test (AUDIT) to determine alcohol use/abuse (Babor TF et al, WHO 2006).
24. Revised Drinking Motives Questionnaire (DMQ-R) to measure motivations behind alcohol consumption (Grant VV et al. Addict Behav. 32:2611-32, 2007).

At scan visits 2B and 4B participants will complete the following questionnaires:

25. Positive and Negative Affect Schedule (PANAS) − to measure tendency to experience positive and negative affect (Watson et al. J Pers Soc Psych 54:1063−70, 1988; Killgore & Yurgelun−Todd Int J Eating Dis 39:357−63, 2006).
26. Mood/Depression Assessment Questionnaire: to assess any changes in mood since the non-scan visit
27. Magnetic resonance imaging metal check list - to ensure compatibility with MRI scanning

Neuropsychological tasks

Several tasks will be performed on each of the *non-scan visits* (Visits 2A and 4A) and *scan visits* (Visits 2B and 4B) outside of the scanner using computers (duration up to 2.5 hours in total per visit):

1. Food valuation task to assess subjective value given to food and non-food items through a Becker-deGroot-Marschak auction in which participants state how much money they would spend on an item (Medic N et al. J Neurosci 34:16856-64, 2014) (*non-scan visit*).
2. 4-choice serial reaction time task to measure waiting impulsivity by detecting premature motor responding (Voon V et al. Biol Psych 75:148-55, 2014 (*non-scan visit*)
3. Leeds food preference questionnaire (LFPQ) Food choice task: to asses liking and wanting for different foods (Finlayson G et al. Appetite. 50(1):120-7, 2008) (*scan visit*).
4. Kirby delay discounting task and adapted delay and probability discounting task for food and money: to assess temporal impulsivity and risk aversion to money and food (Kirby K et al, Psychno Bull Rev 3:100-104, 1996, Kirby KN et al, Addiction. 99(4):461-71, 2004, Perry JL, & Carroll ME. Psychopharmacology 200:1-26, 2008, Weller RE et al. Appetite. 51:563-9, 2008, Davis C et al. Appetite. 54:208-13, 2010; de Ridder D et al. PLoS ONE, 9, e111081, 2014) (*non-scan and scan visit*).
5. Progressive ratio task – to measure food reward in which subjects earn a sweet fatty candy (M&M Crispy; Mars UK Ltd) for their effort in clicking a computer mouse (*scan visit*). On subsequent trials the effort required to earn a candy is increased. The point at which they stop clicking to obtain a reward represents the break point, which is a measure of the reinforcer value (Miras et al. Am J Clin Nutr 96:467-73, 2012). The breakpoint for high-energy foods may be reduced after bariatric surgery.
6. Wechsler Test of Adult Reading (WTAR): to document intellectual and cognitive level (Green RE et al. J Clin Expt Neuropsychology 30:163-172, 2008) (at *1st scan visit* only).

DNA sampling

Blood will be taken at the first scan visit, with specific consent, to extract DNA and RNA to enable examination of genetic variations in candidate genes associated with obesity, insulin resistance,

glucose metabolism, appetite regulation and reward, such as MC4R and FTO, e.g. using DNA sequencing and PCR based SNP analysis (Frayling et al. Science 316:889-94, 1007; Dina et al. Nat Genetics 39: 724-6, 2007; Chambers et al. Nat Genetics 40: 716-8, 2008; Loos et al Nat Genetics 40:768-75, 2008; Xiang et al. Biochemistry 45:7277-88, 2006). Such genetic variations have the potential to alter brain activation to food and non-food reward. DNA and RNA will be analysed through commercial genotyping of single nucleotide polymorphisms using pseudoanonymised samples. This is identical to several other studies of appetite and body composition by the Investigators which already have ethical approval (07/Q0406/19, 08/H0707/163, 08/H0707/139, 07/Q0411/19, 10/H0707/60, 14/LO/0871).

Food diaries
Prior to each scan scan visit participants will be asked to keep a food diary for 3 days using a written booklet or via an on-line record (www.myfood24.org/web/).

Bio-electrical impedance analysis
This painless, safe procedure involves standing upright on a metal platform while holding handles in each hand for up to 1 minute (Tanita).

Urine pregnancy tests
All participants will have a urinary pregnancy test at the 1st non-scan visit and at each scanning visit. If positive they will be excluded from the brain imaging study.

Stool sample
Participants will be supplied with a sealable stool collection container ('Fecotainer' - see supplemental page) at non-scan visits and asked to collect a stool sample either the day prior to or the morning of their scan visit and bring it along. This sample will undergo DNA analysis to identify the species and quantity of gut bacteria present, as well as changes in its chemical composition that may result. Recent studies have found that obesity and bariatric surgery procedures may alter the types and quantity of bacteria that are present in the bowel and the chemicals they produce, and that this itself may contribute to weight loss (Li JV et al. Gut 60:1214-23, 2011). Analysis of samples will be performed in collaboration with Dr Julian Marchesi, Dept. of Surgery and Cancer, and Dr Jia Li, Dept. of Metabolism, Digestion and Reproduction, Imperial College London.

Serial blood sampling
Venous blood samples will be taken at intervals throughout the study mornings. No more than 200 mls will be taken on the non-scan visits and 150 mls on each of the scanning visits, totalling 700 mls over the duration of the study. Plasma levels of glucose and other metabolites, adipocytokines, insulin and other hormones (including ghrelin, GLP-1, PYY) will be measured by immunoassay.

Urine and blood metabonomics
Samples of urine, serum and plasma will be collected for analysis of its chemical composition by mass spectrometry and magnetic resonance spectroscopy (Li JV et al. Gut 60:1214-1223, 2011). This analysis provides information about metabolic changes in obesity and after surgery or dietary changes. This also complements the information obtained from analysis of stool sample bacteria and chemical composition, as different bacteria produce different metabolites that can be measured in urine. Analysis will be performed in collaboration with Dr Jia Li, Dept. of Metabolism, Digestion and Reproduction, Imperial College London.

Visual analogue scales
Visual analogue scales will be used to assess subjective feelings including hunger, nausea, fullness, sleepiness, stress, anxiety, volume of food that can be eaten and food palatability at intervals throughout the study visits (Questionnaire 27).

Magnetic resonance imaging

Participants will have a brain magnetic resonance (MR) scans for up to 90 mins on each scan visit. None of the MR techniques to be used employs ionizing radiation or intravenous contrast agents. Scanning will be performed on the 3.0 Tesla scanner in the Clinical Imaging Centre at the Hammersmith Hospital. Subjects lie supine in the scanner with their head placed in a padded head coil for support. While in the scanner volunteers will have access to a buzzer to sound an alarm, and will be able to hear and respond to instructions from the scanning console. While in the scanner subjects view a mirror reflecting a computer screen mounted above or behind the head coil. Subjects can respond to instructions using a keypad or joystick held in their hand. Subjects can rate their hunger and mood at various times while in the scanner using this device.

It should be noted that these scans cannot be viewed as a comprehensive health screening procedure. However, vary rarely, unexpected information can be detected which may warrant further investigation. In this event, a report will be sent to the volunteer's GP, who will arrange further tests and coordinate further care. Significant structural anatomical abnormalities may also preclude the subject from further participation in the study. The anatomical brain MR scans will be reported by a Consultant Neuroradiologist at Imperial College Healthcare NHS Trust or an equivalently qualified deputy.

The following anatomical brain scans will be collected at one or more of the visits:

(i) anatomical T1 and T2-weighted MR scans to provide structural data on which to overlay the functional data.

(ii) diffusion tensor imaging to examine white matter tracts.

The following resting state functional brain scans will be collected:
(iii) arterial spin labelling to measure resting regional blood flow (Petersen et al. Br J Radiol. 79:688-701, 2006; Paiva et al. NMR Biomed. 20(7):633-42, 2007). A radiofrequency (RF) pulse is applied to the neck so as to 'magnetically-label' blood in vessels that send blood to the brain. Subsequent MR scanning of the brain is therefore able to detect blood flow changes as the labelled blood circulates.

(iv) resting state BOLD functional MRI to measure regional connectivity in activity in different brain regions at rest (Damoiseaux JS et al. Proc Natl Acad Sci 103(37):13848-53, 2006).

The following task-related functional MRI scans will be collected at each visit:
(v) Food pictures: subjects view a variety of different pictures (e.g. food, household objects, animals, blurred pictures as a baseline) and rate how 'appealing' the pictures are using the keypad (Goldstone AP et al, Am J Clin Nutr 99:1319-30, 2014; Scholtz S et al. Gut 63:891-902, 2014; Goldstone AP et al. J Clin Endo Metab 101:599-609, 2016).

(vi) Stress images and emotion recognition tasks: subjects view mildly negatively distressing images and neutral images from the International Affective Picture System (IAPS, NIMH Center for the Study of Emotion and Attention, University of Florida, Gainesville, FL, USA) (e.g. war, accidents) or faces displaying different emotions (e.g. fearful or happy) (Glaysher MA et al. BMJ Open. 7:e018598, 2017; McGonigle J et al. J Psychopharmacol. 2017 Jan;31(1):3-16).

(vi) Stress images and emotion recognition tasks: subjects view mildly negatively distressing images and neutral images from the International Affective Picture System (IAPS, NIMH Center for the Study of Emotion and Attention, University of Florida, Gainesville, FL, USA) (e.g. war, accidents) or faces displaying different emotions (e.g. fearful or happy) (McGonigle J et al. J Psychopharmacol. 2017 Jan;31(1):3-16).

(vii) Monetary incentive delay (MID) task: a game in which subjects need to press a button during a specific time window when given a cue on the computer screen in order to win hypothetical monetary prizes of differing amounts to assess reward responsivity and ventral striatum function (Knutson B et al, J Neurosci 21(16):RC159, 2001, Knutson B et al, Neuroimage. 12(1):20-7, 2000; McGonigle J et al. J Psychopharmacol. 2017 Jan;31(1):3-16).

(viii) Go/NoGo Task: to assess impulsivity via effects on response inhibitory control mediated in the prefrontal cortex-striatum (Horn NR et al, Neuropsychologia. 41(14):1959-66, 2003. Yan P et al, Am J Drug Alcohol Abuse. 35(5):284-9, 2009, Chao HH et al, BMC Neurosci. 14;10:75, 2009, Li CS et al, Neuroscience. 155(4):1142-51, 2008, Perry JL, & Carroll ME. Psychopharmacology 200(1):1-26, 2008 ; McGonigle J et al. J Psychopharmacol. 2017 Jan;31(1):3-16). The task contrasts brain activation during responses to infrequent no go signals (e.g. 'do not press' button when viewing an infrequent red arrow) compared to an implicit go baseline (e.g. 'do press' button when viewing a frequent green arrow).

Some of these tasks will be practiced outside of the MRI scanner on scan visits to habituate the participants to the experimental procedures. Subjects will have continuous heart or breathing rate and pulse oximetry monitoring while in the MR scanner.

Fixed lunch meal

At lunch time at the *non-scan visits* participants will be given a small liquid meal (Fortisip Copmpact Vanilla, Nutricia (250 mL 600 kcal) to assess hormone and glucose changes in the blood after food intake.

Test lunch meal

At lunchtime at the *scan visits*, participants will be presented with a meal consisting of 4 different dishes (2 soups, yoghurt and ice cream), asked to rate their taste using a computer program (www.sipm.co.uk), and then eat until they feel comfortably full.

SAFETY AND PROTECTION OF VOLUNTEERS

During each visit, at least one experienced researcher will monitor the volunteers and subjects will be encouraged to report any unusual or unpleasant sensation to the investigator immediately. Any significant adverse effects will lead to withdrawal of the individual from the study.

Some individuals may find the confined space of the MR scanner intolerable because of claustrophobia. This is therefore an exclusion criterion for recruitment to the study and they will have an opportunity to get used to the procedure during the dummy visit.

If, however, subjects experience discomfort within the scanner despite the measures taken to ensure patient comfort, the patient may request immediate cessation of the procedure with withdrawal from the scanner by ringing the patient alarm bell. The MRI produces a "hammering noise" but subjects wear earplugs and headphones to prevent discomfort or damage to hearing.

INCIDENTAL FINDINGS

It should be noted that the MRI brain scan cannot be viewed as a comprehensive health screening procedure. However, vary rarely, unexpected information can be detected which may warrant further investigation. In this event, the participant will be informed and a report will be sent to their GP, who will arrange further tests and coordinate their further care. In the rare event that we find a significant abnormality on their structural brain scan on the first visit this may exclude them from continuing with the rest of the Brain Imaging sub-study protocol or main study protocol.

OUTCOME MEASURES

Primary outcomes
Change in brain blood-oxygen level dependent (BOLD signal) using functional MRI in response to:
(i) viewing food versus non-food pictures,
(ii) viewing unpleasant vs. control pictures,
(iii anticipation and receipt of non-food monetary reward using functional MRI,
(iv) inhibition of motor actions.

Secondary outcomes
Slope and area under curve to food and money in delay and probability discounting tasks
Breakpoint in Progressive ratio task
Reaction time, choice frequency, and rating of food liking and wanting of high-fat vs low fat and sweet vs. savoury foods in LFPQ task
Food and non-food monetary value in food valuation task
Premature responding rate in 4-choice serial reaction time task

Changes in resting brain activity measured by resting fMRI connectivity and arterial spin labelling

Food intake during test meals
Food intake (total energy and macronutrient composition) recorded at home
Appeal score of food pictures versus non-food pictures
Hunger, appetite, food palatability, fullness scores in visual analogue scales
Lunch taste pleasantness, liking, sweetness, creaminess scores
Gut hormone, leptin, glucose, and insulin levels
Polymorphisms in genes related to obesity, appetite and addiction

Dutch Eating Behaviour Questionnaire (DEBQ) – restraint, emotional and external influences scores
Behavioural Inhibition and Activation System (BIS / BAS) scales – BAS drive, BAS reward responsiveness, BAS fun seeking, BIS scores
Three Factor Eating Questionnaire (TFEQ) restraint, disinhibition and hunger scores
Eysenck Personality Questionnaire (EPQ-R) scores
Positive and Negative Affect Scores from PANAS
Beck Depression Inventory (BDI–II) score
Barratt Impulsivity Scale score
Binge Eating Scale score
DS-R score
Arts and Sigstad questionnaires
Hospital Anxiety and Depression Scale (HADS)
Power of Food Scale
Short-Form 36 Health Survey Questionnaire (SF36)
IWQoL – total energy expenditure
Pittsburgh Sleep Quality Index (PSQI) score
UPPS-P Questionnaire
Alcohol Use Disorders Identification Test (AUDIT)
Yale Food Addiction Scale score and diagnosis
WTAR intellectual level
PEMS Questionnaire score
DMQ-R Questionnaire score

Exploratory outcomes

Changes in gut microbiota
Changes in faecal water, plasma and urinary metabolites from metabonomic studies

DATA HANDLING AND RECORD KEEPING

Personal contact details are required for communication with the subjects participating in the study. This information is held solely for communication between the researchers and participants. Details stored on university computers will be password protected and will only be accessed by the researchers involved in the study.

Information held on NHS computers is solely for the purpose of hospital booking and routine sample collection and analysis (e.g. for medical screening). This information is password protected in a similar manner to that of other hospital patients.

Subjects will be given a personal study code number which will be used throughout the study and in the analysis of data. Coded samples will be treated with confidentiality, similarly to patient samples, when undergoing analysis both in the department and in the hospital laboratory.

Self-reported food consumption data will be recorded using a validated on-line tool at home, called Food24 (www.myfood24.org/web). myfood24 was designed by the University of Leeds and Imperial College London, the software was developed by Rippleffect and is now supported by Calls9 Limited. The hosting is provided by Digital Ocean. Participants using myfood24 can only access their own diary information. They are invited to complete a food diary via an email containing a link that is unique to them. Researchers using myfood24 are only able to access projects created by themselves unless they have been granted access to specific projects by the system administrator. The myfood24 administrator account is strictly controlled and only used for the purpose of system administration and maintenance. Only Calls9 programming staff assigned to the development of myfood24 have access to the application and the hosting servers. Access to myfood24 is only in response to maintenance requirements. Digital Ocean technical support staff do not have access to the backend hypervisors where virtual servers reside nor direct access to the NAS/SAN storage systems where snapshots and backup images reside. Only select engineering teams have direct access to the backend hypervisors based on their role. More information can be found at: https://www.digitalocean.com/legal/data-security/.

Data analysis will take place in the Department of Brain Sciences, and Metabolism, Digestion and Reproduction, Imperial College London and be carried out by the researchers themselves. The data will be kept in a secure environment in these departments, under the authority of Dr. Miras and Dr. Goldstone. Data will be stored for 10 years after completion of the study. Only the researchers will have access.