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Study Protocol
Investigation of the metabolic effects of DuOdenal resurfacing on insulin resistant woMen wIth polycystic ovariaN syndrOme
The DOMINO Trial

Background and rationale

1. Polycystic ovarian syndrome and insulin resistance.

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting women, which causes the dysregulation of the menstrual cycle, excessive actions of the male hormone, testosterone (hyperandrogenism), and polycystic ovaries (1). The prevalence of PCOS is approximately 15%-20% of women (2). Clinical manifestations include reduced or absent menstrual cycles, excess hair growth, and infertility. Moreover, women with PCOS have an increased rate of obesity, with a propensity toward abdominal deposition of body fat (3) and insulin resistance which affects 50%-70% of women with PCOS (4). This insulin resistance occurs mostly in muscle and fat, and results in increased pancreatic insulin secretion to maintain normal glucose levels (4). Hyperandrogenism, obesity and insulin resistance lead to a number of comorbidities including metabolic syndrome, hypertension, dyslipidaemia, glucose intolerance, and type 2 diabetes mellitus (T2DM) (5).

Weight loss is the most effective therapy for restoring fertility in women with PCOS (1). Reduction in weight of as little as 5% can restore regular menses and improve response to ovulation- inducing and fertility medications. However, lifestyle interventions only work in the short term and is very difficult to maintain. Other pharmacological and invasive treatments have high rates of side effects and limited efficacy (1, 6, 7). As a consequence, a large number of these women remain infertile and this has a devastating impact on their psychological health (8-10).

2. Duodenal mucosal resurfacing.

The Fractyl Revita System™ has achieved CE-marking and a number of studies support its safety and effectiveness in T2DM patients (11, 12). So far 102 patients have been treated with this duodenal mucosal resurfacing (DMR) technology around the world with very acceptable rates of complications (11). The procedure is performed endoscopically, without surgery, and under general anaesthesia and in most patients as a day-case. A balloon attached to a catheter is inserted endoscopically and through the use of hot water thermally (by using heat) ablates ~10cm of the duodenal mucosa (11).

The procedure is very effective for the treatment of patients with T2DM with rapid reductions in blood glucose (11). It is thought that it works mainly by increasing insulin sensitivity which the predominant mechanism in both T2DM and PCOS (1).

In this trial we will investigate both whether this non-invasive device indeed increases insulin sensitivity using gold-standard methodologies but also whether it can help women of reproductive age start menstruating. Both the intervention and control group will be given intensive NHS Tier 3 lifestyle advice for weight loss, therefore the control group is expected to benefit from the trial.

If successful, this trial can have a substantial impact on millions of women around the world. The technology could be used as a one-off treatment to enable women with PCOS to get pregnant without exposing them to the side effects of long term medications or the complications of invasive pelvic interventions.

Objectives

Our main objectives are to assess the effect of the Fractyl Revita System™ in women with PCOS in terms of:

1. Insulin sensitivity
2. Ovulation and menstruation

Trial team

The Chief Investigator is Dr Alexander Miras, Senior Clinical Lecturer in Endocrinology at the Department of Investigative Medicine, Imperial College London. Co-investigators and collaborators are Dr Channa Jayasena Dr Dev Bansi, Dr. Belen Perez-Pevida, Miss Vasha Kaur, Professor Harpal S Randeva, Dr Georgios K. Dimitriadis, Dr Barbara Fielding and Dr Bu'Hussain Hayee.

Trial design

This will be a prospective double-blinded randomised controlled clinical trial. The setting will be a multi-entre with tertiary obesity, metabolic medicine and reproductive endocrinology expertise. Thirty female patients will be recruited and randomised to either DMR or the sham procedure. All patients will be registered at Imperial College Healthcare NHS Trust. Patients who have their procedure performed at King's College Hospital NHS Trust will also be registered there.

Both groups will receive standard NHS Tier 3 lifestyle advice and support for the duration of the trial. Lifestyle modification aimed at weight loss will be delivered by a dietician (and psychologist as necessary) in monthly group or individual sessions for a period of 6 months.

All patients will also be followed up for 6 months.

All patients will receive £300 upon completion of the study as a reimbursement for their time and inconvenience. Patients based outside the M25 will also have their travel expenses reimbursed (in addition to the £300).

Inclusion criteria

- Female participants
- Age 18-50
- Body mass index (BMI) ≥ 30 kg/m^2
- Diagnosis of PCOS based on the NIH Criteria. Require ALL of the following:
  a) Menstrual irregularity (anovulation or >35 day cycle)
  b) Clinical or biochemical hyperandrogenism
  c) Exclusion of other causes other aetiologies of menstrual dysfunction (e.g. thyroid dysfunction, hyperprolactinaemia)
- Insulin resistance as defined by a 2-hour oral glucose tolerance test glucose concentration of 7.8 mmol/l and/or HOMA-IR ≥ 3.0.
- Willing to comply with study requirements and able to give informed consent

Exclusion criteria

- Type 1 or Type 2 diabetes mellitus

- 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. These includes:
    - Active H. pylori infection (Participants with active H. pylori may continue with the screening process if they are treated via medication and re-testing verifies the condition has resolved.)
    - Previous gastrointestinal surgery that could affect the ability to treat the duodenum such as subjects who have had a Billroth 2, Roux-en-Y gastric bypass, or other similar procedures or conditions
    - History of chronic or acute pancreatitis
    - Known active hepatitis or active liver disease
    - Symptomatic gallstones or kidney stones, acute cholecystitis or history of duodenal inflammatory diseases including Crohn's Disease and Celiac Disease
    - History of coagulopathy, upper gastro-intestinal bleeding conditions such as ulcers, gastric varices, strictures, congenital or acquired intestinal telangiectasia
    - Use of anticoagulation therapy (such as warfarin) which cannot be discontinued for 7 days before and 14 days after the procedure
    - Use of P2Y12 inhibitors (clopidogrel, pasugrel, ticagrelor) which cannot be discontinued for 14 days before and 14 days after the procedure. Use of aspirin is allowed.
    - Unable to discontinue NSAIDs (non-steroidal anti-inflammatory drugs) during treatment through 4-weeks post procedure phase
    - Taking corticosteroids or drugs known to affect GI motility (e.g. Metoclopramide)
    - Persistent anaemia, defined as haemoglobin<10 g/dl
    - eGFR <30 ml/min/1.73m^2
    - Active systemic infection
    - Active malignancy within the last 5 years
    - Poor candidates for surgery or general anaesthesia
    - Active illicit substance abuse or alcoholism
- Medications affecting insulin sensitivity (oral steroids, metformin, thiazolidinediones, atypical antipsychotics, hormonal contraceptives, weight loss medication) at screening or 2 months previously.
- Other causes of anovulation (e.g. hypothyroidism, adrenal or pituitary disorders)
- More than 6 menstrual bleeds within the previous 12 months
- Current pregnancy or breastfeeding at screening or 6 months previously
- Smoking at screening or 6 months previously
- Without access at home to a telephone or other factor likely to interfere with ability to participate reliably in the study.
- Donated blood during the preceding 3 months or intention to do so before the end of the study
- Any other mental or physical condition which, in the opinion of the Investigator, makes the subject a poor candidate for clinical trial participation

Patient identification

The routes of identifying potential participants will be:

- Contacting the patient after review of their medical history by the direct clinical care team. This will take place at Imperial College Healthcare NHS Trust by the direct clinical care team members that are also research team members and at other research and PIC sites (Chelsea and Westminster Hospital NHS Foundation Trust, West Middlesex University Hospital and University Hospitals Coventry and Warwickshire NHS Trust). Patients who are willing to take part will be asked for their permission for their clinical information to be passed on to the Imperial research team.

- Clariness which is an international patient recruitment provider for clinical trials. Clariness will run a campaign to advertise this clinical trial using online outreach strategies, such as search engine marketing, banner advertising on relevant websites, and social media advertising. Data is stationed following the privacy policies and security measures as stipulated by the (EU) General Data Protection Regulation (GDPR) and the German Data Protection Act (BDSG).
- Posters: These will be placed in areas where potential participants are routinely cared for and they will contain the clinical research team contact details

Screening visit
This will be performed after the research team have made first contact with the participant. All participants will be screened to assess whether they meet inclusion criteria and this process will comprise a medical history, routine physical examination, and the following investigations:

- Full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, lipid profile, vitamins, minerals and metabolites, electrocardiogram and a pregnancy test. To confirm anovulation, a serum reproductive profile will be performed: serum progesterone, LH, FSH and oestradiol (E2).
- Assessment of insulin secretion – oral glucose tolerance test: An oral glucose load of 75 grams of glucose will be consumed by the patients. This will be followed by measurements of glucose, insulin, c-peptide and metabolites for 3 hours, where time zero is the time of administration of the glucose.
- Urine pregnancy test

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.

Baseline visit
This visit will take place approximately 4 weeks before participants undergo the intervention.

Assessment of insulin sensitivity - euglycaemic hyperinsulinaemic clamp
Patients will be asked to refrain from alcohol and strenuous physical activity for 48 hours before the study. They will also be asked to consume a standardised meal the evening before the study and only consume fluids from 10pm onwards. The visit will be performed in early follicular phase in any patients who have resumed menses. Patients will attend the Imperial NIHR clinical research facility on the day of the clamp procedure. Two venous catheters will be inserted. The first cannula will be used for infusions and the other for blood sampling. An insulin infusion may be commenced to keep their blood glucose stable between 4.0-6.0 mmol/l. A primed continuous infusion of [6, 6-$^{2}H_{2}$] glucose, a stable isotope tracer, will be started and maintained for ~7 hours. Two hours later a two-stage hyperinsulinaemic-euglycaemic clamp procedure will be started and continued for ~5 hours. During stage 1 of the clamp procedure, in which hepatic insulin resistance is assessed, insulin will be infused at a low dose (depending on patient's weight/body surface area) for 2 hours. During stage 2 of the clamp procedure, in which peripheral insulin resistance is assessed, insulin will be increased to a higher dose (depending on patient's weight/body surface area) for 2 hours. Euglycaemia will be maintained by infusing 20% dextrose at a variable rate. Blood samples will be taken every 5-10 minutes to measure blood glucose concentration and the dextrose infusion will be adjusted accordingly. The exogenous glucose infusion will be enriched with 6, 6 $^{2}H_{2}$ glucose to prevent a fall in plasma tracer enrichment and underestimation of endogenous glucose production rate. Regular glucose monitoring is necessary to ensure safety and avoid the small risk of hypoglycaemia.

Blood samples will be obtained before the start of the tracer infusions, every 10 min during the final 30 min of the basal period and stages 1 and 2 of the clamp procedure and every 30 minutes between these

periods to determine glucose enrichment and concentration and insulin. The same time points participants will be asked to complete appetite visual analogue scales.

At the end of the study, participants will undergo an oral glucose tolerance test as described above. The maximum amount of venesected blood will be 180 mls. Blood samples will be centrifuged and the separated plasma kept in a -20°C or -80°C freezer. The isotopic enrichment of plasma glucose will be determined by gas chromatography mass spectrometry (GCMS) at the Wolfson Centre for Translational Research, Postgraduate Medical School, University of Surrey.

The stable labelled isotope tracer [6, 6 $^{2}H_{2}$] glucose is not a drug, but a naturally occurring metabolite which has been labelled with a stable and non-radioactive label. Stable isotope tracers are widely and safely used in metabolic research by groups throughout the UK and worldwide. All labelled isotope tracers are ordered from Cambridge Isotopes Ltd through their UK suppliers CK Gases Ltd. They are prepared as sterile solutions suitable for intravenous use by the Pharmacy Production Unit at Guys & St. Thomas' NHS Trust to ensure they are safe for the participants. The products are supplied with the appropriate certificate of analysis and MSDS. We have used the same manufacturer to ensure the quality of the products and the supporting documentation. They will be stored at the Imperial NIHR clinical research facility.

Additional assessments

The following assessments will also take place on or around the baseline visit:

- Body weight and body composition using bioelectrical impedance
- Full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, lipid profile, iron indices, vitamins, minerals and metabolites.
- Reproductive profile: serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, E2, sex hormone binding globulin (SHBG), testosterone, dehydroepiandrosterone sulfate (DHEAS), androstenedione.
- Urine pregnancy test
- Blood pressure and pulse
- Total caloric intake and macronutrient composition will be assessed through the use of food diaries. These will be given to the participants at the visit prior to this one and returned to the investigators on the day of the visit.
- Number of medications
- Energy expenditure: All patients, including those who crossover at the end of the trial, 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 fat and lean mass through air displacement (on principle of Boyles Law). Participants will have attended fasting since 22:00 pm previous night and a fasting blood sample will then be taken and spun for serum and plasma, to be stored at -20°C and then transferred to -80^{o}C freezers. Each participant will then 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 and will be trained on the use of a portable sleep machine. Participants will be also provided with theatre scrubs to ensure clothing standardisation whilst in the calorimeters, and will have two urine pregnancy tests. 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. This period allows equilibration of and participant familiarity with the chamber environment. At 12.00 hrs 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. Serum samples will be analysed for insulin, adiponectin and other adipokines, glucose and lipid profiles. These samples taken during HMRU will be anonymised. They will be stored for 10 years and analysed by the clinical research team.

Following the post-prandial period from the standard lunch, there will be a standard activity protocol at 15.30 hrs 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 hrs, a standard evening meal will be provided. Subjects following this will be asked to remain sited 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 hrs, and participants will be requested to sleep from 22.30 hrs with wake-up at 07.00 hrs the next morning. Prior to going to sleep, participants will be requested to fix their portable sleep machine in place (as explained to them by the HMRU nurse prior to entry into the chamber). The next morning, subjects will be woken at 07:00am 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 blood test will be carried out; sleep machines will be removed and standard breakfast will be provided at 08.30 hrs. Participants will be asked to exit the chamber at 10.00 hrs. Assessment of sleep data using complete polysomnography will be carried out by our Lead Respiratory Physician at UHCW NHS Trust (Dr Asad Ali).

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. Subjects will be supervised throughout their HMRU visits by a member of the research team. 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 via phone after clear instructions that, if they feel unwell, they should call us.

Randomization

Subjects who meet all criteria after screening will be randomized via computer software 1:1 (DMR to sham). This will take place at the baseline visit. The patient, the research and clinical team, except for the endoscopist, will be blinded to the type of intervention that has been performed, unless clinical need and procedure dictates the un-blinding of the clinical team (e.g. development of a complication). Unblinding will take place at the end of the trial.

Intervention

Duodenal mucosal resurfacing: The Fractyl Revita System consists of two main components: the Revita Catheter and a console.

Revita Catheter: The Revita Catheter is a sterile, single use device that performs two functions: 1) it injects saline into the submucosa of the duodenum to create a thermal barrier while also lifting the mucosa with saline to create a more uniform surface for ablation; and 2) ablates the mucosal surface using heated water recirculating inside a balloon. To achieve its function, the Revita Catheter is

constructed of a multi-lumen shaft with a balloon affixed to its distal end. Affixed to the outside of the balloon are three narrow shafts with a port that are used to draw a vacuum when placing the saline during the mucosal lifting portion of the procedure. Within each shaft is a fluid lumen with a miniaturized needle affixed to the distal end. Each needle is wholly constrained within the port ensuring its safe use. During the mucosal lift, the tissue is drawn into the needle port, and saline is injected into the submucosal space through the needles. The proximal end of the shaft is fitted with a handle and saline and vacuum lines that are affixed to a console unit to control its function. The catheter will be available with a 24 mm outer diameter balloon.

Console: The console is a reusable electro-mechanical piece of equipment and provides functionality to the submucosal lift and hot fluid ablation steps of the procedure. It is controlled through the use of a software user interface monitor. Prior to use, it is fitted with a sterile single use line set that serves as the pathway for the saline to be placed into the duodenal submucosa during the procedure.

The DMR procedure using the Revita System is completed in the endoscopy suite using general anaesthesia. The patient is positioned in the left lateral decubitus position used for endoscopic procedures or preferred position as dictated by the site's requirements for endoscopic procedures. A standard endoscope is used to complete an initial endoscopic evaluation and a guidewire is delivered past the ligament of Treitz to assist in delivering the catheter. Anti-peristaltic agents may be used during the procedure. Catheter delivery and device location for treatment is verified using fluoroscopic guidance. The use of fluoroscopy is limited to use during catheter placement and verification of location during treatment. Based on data collected during earlier clinical investigations, the duration of radiation exposure is approximately equivalent to that delivered during an endoscopic retrograde cholangiopancreatography procedure, which is a common endoscopic procedure with an acceptable safety profile. A lead apron drape will be placed on the abdomen and pelvis during the procedure to protect the reproductive organs. The total procedure time is less than 70 minutes.

The Revita catheter is placed in the proximal duodenum distal to the papilla. Using the console interface, the balloon is inflated and vacuum delivered to draw the intestinal mucosal tissue onto the ports located on the balloon. The actuator on the handle is moved to advance the needle into the submucosal space within each of the ports. The console delivers saline into the submucosa through the needles within the lumens of the catheter resulting in complete circumferential lift of the mucosa. Once complete, the ablation cycle is started and hot water is circulated into the balloon to complete an ablation of the previously expanded tissue. The balloon is deflated and the catheter repositioned distally to the next segment to be treated. The Revita catheter and endoscope are then removed. The number of ablations completed in the duodenum is determined by the distance between the papilla and the Ligament of Trietz and may be variable based on the individual anatomy.

Sham

The sham procedure will consist of placing the DMR Catheter into the duodenum under general anaesthesia and leaving it in place for a minimum of 30-45 minutes and then removing it from the patient.

Unforeseen events (findings or procedures) may occur during either the DMR or sham procedure. These unforeseen events are those that are not planned as part of this procedure (e.g. a drop in oxygen saturation or evidence of intestinal bleeding, etc.). Unforeseen events that are emergent in nature should be recorded as adverse events and the investigator should reassess the subject's suitability for continued participation in this study.

Post-Procedure Care & Discharge

Immediately following the procedure, the subject is transported to the recovery area and monitored according to the hospital/physician protocol for endoscopic procedures. The subject may be released from the recovery room to the nursing unit when they have met the hospital's criteria for discharge from the recovery area. Immediate postoperative care is dictated by the hospital or physician's standard care protocol regarding post-anaesthesia recovery.

Prior to discharge, all subjects are examined and evaluated for the presence of any adverse events that may have occurred between the procedure and discharge. A subject's hospital stay can be extended based on need as determined by the Investigator. Subjects are eligible to be discharged when they meet the criteria following the local sedation protocol and discharge requirements.

Following intervention patients in both groups will be asked to consume the same low-calorie diet for approximately 14 days. Participants will be informed as to how this liquid diet will be consumed by the research team.

Early post-procedure mechanistic visit
This will take place within 14 days after the intervention. The following assessments and procedures will be performed:

- Clinical assessment
- Full blood count, urea and electrolytes, liver function tests, HbA1c, lipid profile
- serum LH, FSH, progesterone, E2, SHBG, testosterone, DHEAS, androstenedione
- Urine pregnancy test
- Oral glucose tolerance test
- Body weight and body composition using bioelectrical impedance
- Blood pressure and pulse
- Total caloric intake and macronutrient composition will be assessed through the use of food diaries. These will be given to the participants at the visit prior to this one and returned to the investigators on the day of the visit
- Adverse events

12-week mechanistic visit
The following assessments and procedures will be performed:

- Clinical assessment
- Full blood count, urea and electrolytes, liver function tests, thyroid function tests, HbA1c, lipid profile, vitamins, minerals and metabolites.
- Reproductive profile: serum LH, FSH, progesterone, E2, SHBG, testosterone, DHEAS, androstenedione
- Urine pregnancy test
- Euglycaemic hyperinsulinaemic clamp as described above
- Oral glucose tolerance test as described above
- Body weight and body composition using bioelectrical impedance
- Blood pressure and pulse
- Total caloric intake and macronutrient composition will be assessed through the use of food diaries. These will be given to the participants at the visit prior to this one and returned to the investigators on the day of the visit.
- Number of medications
- Adverse events

Reproductive assessments weeks 12-24
During the duration of the study, information about self-reported menstrual bleeding will be collected.

The reproductive assessments that will be performed from weeks 12 to 24 will include:

- Weekly pelvic ultrasound scans: The ultrasonographer will be blinded to treatment for all subjects. During each scan, the following parameters will be measured: endometrial thickness (in millimetres), mean ovarian volume (in cubic centimetres), mean follicle number, and maximum diameter of largest follicle in each ovary (in millimetres). Ovulation will be defined as a rise in serum progesterone >10 nmol/L together with suggestive radiological features (visualization of a dominant follicle with subsequent appearance of a preovulatory follicle and/or corpus luteum). The ultrasounds will be either transabdominal or transvaginal depending on views obtained and patient preference.
- Measure serum progesterone 7-10 days later (i.e. mid-luteal phase).
- Once-weekly progesterone/E2 ratio, LH, FSH, E2 measurement.

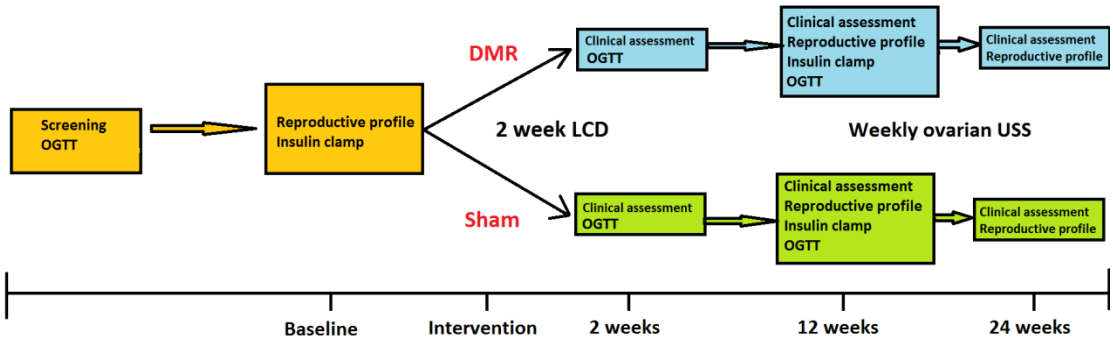
Patients recruited at University Hospitals Coventry and Warwickshire NHS Trust will be offered to have this follow-up done locally.

6-month clinical visit

The clinical assessments will include:

- Body weight and body composition using bioelectrical impedance
- Blood pressure and pulse
- Blood tests: full blood count, urea and electrolytes, liver function tests, thyroid function tests, glucose, insulin, c-peptide, HbA1c, lipid profile, vitamins, minerals and metabolites
- Reproductive profile: serum LH, FSH, progesterone, E2, SHBG, testosterone, DHEAS, androstenedione
- Urine pregnancy test
- Number of medications
- Adverse events
- The energy expenditure study follow-up will be conducted for those patients who have opted to have it done.

Trial flow diagram



Primary outcomes:

- Metabolic outcome: The change from baseline in total insulin sensitivity at 12 weeks. Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity
  Reproductive outcome: The number of menses during 24 weeks.

The trial will be viewed as positive if statistical significance is obtained for either of the two primary endpoints.

Secondary outcomes

- Change in hepatic insulin sensitivity from baseline at 12 weeks
- Change in peripheral insulin sensitivity from baseline at 12 weeks
- Number of ovulatory cycles defined by an increase in serum progesterone and / or ultrasound evidence of ovulation followed by menstrual bleeding between weeks 12-24
- Change in fasting and post-prandial concentrations of glucose, insulin and c-peptide from baseline at 2 weeks
- Change in fasting and post-prandial concentrations of glucose, insulin and c-peptide from baseline at 12 weeks
- % body weight loss from baseline at 24 weeks

Exploratory Endpoints

There will be no formal treatment comparisons on the following exploratory endpoints.

- Change from baseline to week 24/ For each endpoint, temporal changes, mean levels and peak levels will be analysed:
    - Plasma lipid concentration
    - Plasma liver function tests
    - Arterial blood pressure
    - Serum LH
    - Serum FSH
    - Serum Oestradiol
    - Serum SHBG
    - Serum Testosterone
    - Serum free androgen index
    - Serum DHEAS
    - Serum Androstenedione
    - Energy expenditure
    - Apnoea hypopnoea index
    - Body composition

- Change from week 12 to week 24/ For each endpoint, temporal changes, mean levels and peak levels will be analysed:
    - Endometrial thickness
    - Ovarian volume
    - Follicle number
    - Diameter of largest follicle in each ovary

Risk Analysis

There are certain residual risks associated with the use of the Fractyl Revita System™ and the DMR procedure. As with any endoscopic procedure, there are risks that are associated with interventional procedures in the duodenum. Below is a listing of these risks and the means by which they may be minimized.

Procedure Risks

There are risks related to the endoscopic procedure in general, as well as, risks specific to the Fractyl Revita System™ procedural treatment (in alphabetical order):

- abdominal tightness, cramping, pain
- diarrhoea

- difficulty swallowing
- infection
- mucosal injury to GI tract
- pancreatitis
- perforation
- sore throat
- stricture
- transient bleeding
- worsening diabetic symptoms including hypoglycaemia

Many of these risks and complications associated with the procedure would be similar to those associated with other commonly performed endoscopic procedures such as duodenal biopsies and endoscopic mucosal resection.

Device Risks
In addition to the risks listed above, the Fractyl Revita System may have unique risks associated with its catheter and console used to complete the procedure. This includes risks associated with the materials selected, its design and construction. These risks include:

- Allergic reaction to the device materials or endoscopic labelling dye or injectate
- Component degradation
- Control module delivers incorrect ablation time and temperature profile
- Device breakage
- Disarticulation of components from the device
- Device/Component lost in GI tract or wall
- Hole in hot fluid catheter balloon resulting in leakage of hot fluid
- Lost catheter component in the GI tract or wall
- Thermal damage to the duodenum wall or surrounding structures
- Unforeseen adverse events

General anaesthesia risks
These are rare, occurring in less than 1 in every 10,000 cases. They include:

- a serious allergic reaction to the anaesthetic (anaphylaxis)
- an inherited reaction to the anaesthetic that causes breathing difficulties
- waking up during the intervention – this is rare, and the amount of anaesthetic given will be continuously monitored to help ensure this does not happen
- death – this is very rare, occurring in 1 in every 100,000 to 1 in every 200,000 cases

Minimizing Study Risks
The following steps have been taken to minimize risks associated with the procedure and the use of the Fractyl Revita System™:

- The tissue or fluid contacting materials used in the construction of the Revita Catheter are known medical grade materials that are well characterized and have a long history of use. In addition, biocompatibility testing has proven that the materials are safe.
- The device design uses known technologies including sub-mucosal injection and hot fluid balloon to complete the procedure. Similar technologies are currently in use for such accepted procedures as endoscopic mucosal resection and treatment of menorrhagia.
- The device design has been rigorously tested in the laboratory, animal models and clinical trials to characterize its performance and confirm the safety and performance of the procedure.
- All investigators receive detailed training in the use of the Fractyl Revita System and the DMR procedure. The training includes hands on use of the system in a lab setting.

- The anaesthetist will review the patient's medical history and adjust the anesthesia so that its risks are minimised

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. Drop-outs taking place up to and including the intervention will be replaced. Drop-outs following the intervention will not be replaced.

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

Sample size calculations
Assumptions of effect size for the primary efficacy endpoints in the treatment arm was derived from previous publications in which insulin sensitising medications were administered in similar groups of women [1, 2]. The assumption made was that the insulin sensitising effect of DMR would be similar to the effect observed with those medications.

It is assumed that:
(a) a difference in mean change in total insulin sensitivity between treatment and control of 5.6 µmol/kg.min at 12 weeks with equal variance in both groups (standard deviation of 4.5). Total insulin sensitivity is the sum of hepatic and peripheral insulin sensitivity.

(b) a difference in the number of menses between treatment and control of 1.0 over 24 weeks with equal variance in both groups (standard deviation of 1.0)

The weighted Hochberg procedure to adjust for multiple endpoints is described in Section 6.5. Under this procedure 24 randomised subjects (12 per group) provides at least 94% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.050 significance level, and provides at least 88% power that the benefit of DMR treatment over sham will be found for at least one primary endpoint when testing using an overall one sided 0.025 significance level.

Thirty patients will be randomised to account for potential patients lost to follow up prior to the primary endpoint assessment. Patients who for technical reasons cannot have the DMR will be replaced.

Statistical analysis plan
This is detailed in the Statistical Analysis Plan document 8th April 2019 Version 1.0).

Procedure for emergency un-blinding

The randomisation lists will be created and held by Professor Tricia Tan, Professor in Endocrinology, Imperial College London, in a secure area within the centre (this copy to be held as code-break envelopes).

In the case of a medical emergency or in the event of a serious medical condition, when knowledge of treatment allocation is essential for the clinical management or welfare of the subject, an investigator or other physician managing the subject may decide to un-blind that subject's treatment code. They should therefore request and obtain the relevant code-break envelope.

The investigator must sign and date the open un-blinding envelope, as soon as is reasonably possible, and at the very least within 24 hours of the code break. The reason for the code break must be documented on the envelope. The Investigator will also record the date and reason for revealing the blinded treatment assignment for that subject in the clinical research facility and in the subject's medical notes.

Definitions of Adverse Events and Reactions

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 AEs

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

Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to non-obesity or diabetes related causes, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs. All SAEs should be reported to the REC where in the opinion of the Chief Investigator, the event was:

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

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

SAEs must be reported to the Chief investigator and the Sponsor within 24hrs of becoming aware of the event:
CI details: Fax: 0208 383 8320, attention of: Dr Alexander Miras
Sponsor details: Tel: 0207 594 9459 or jrco@imperial.ac.uk
Please send SAE forms to: Section of Investigative Medicine, Division of Diabetes, Endocrinology & Metabolism, Imperial College London
Tel: 0208 383 3242 (Mon to Fri 09.00 – 17.00) or 07551266480 (24 hours, 7 days a week).

Follow-up of AEs and SAEs

After the initial AE report, the Chief Investigator or appropriately qualified designee will proactively follow the subject at subsequent visits and contacts. Follow up information about a previously reported SAE must be reported to the Trial Management Group and Sponsor within 24 hours of receiving it. AEs and SAEs will be followed until they resolve, stabilise to a level acceptable to the Investigator or delegates even after the reporting period or the subject is lost to follow-up. Additional measures may be carried out by the Investigator to elucidate as fully as possible the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations or consultation with other health care professionals. In the event that a subject becomes pregnant, the follow-up period will be deemed to have ended when the health status of the child has been determined on its birth.

Monitoring

The principal investigator is responsible for monitoring arrangements. 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 for Health and Social Care Research.

Regulatory issues

Ethics and regulatory approvals: The Chief Investigator has obtained approval from the London-Dulwich Research Ethics Committee and the HRA. 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.

Consent: The study will be conducted in accordance with applicable regulatory requirements, with International Conference on Harmonization "Good Clinical Practice" (GCP), with all applicable subject privacy requirements, and with the guiding principles of the Declaration of Helsinki. Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. If a participant, who has given informed consent, loses capacity to consent during the study they would be withdrawn. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

Confidentiality: The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act. All patients will be registered with Imperial College NHS Trust or King's College Hospital NHS Trust and their personal data will be kept on password protected NHS computers. Personal addresses, postcodes, faxes, emails or telephone numbers will be used to enable the research team to contact participants during the trial. A paper copy of these data will be placed in the individual patient folders and the study Master File. These folders will be kept in locked storage at the Imperial NIHR clinical research facility or Dr Bu'Hussain Hayee's NHS office at King's College Hospital NHS Trust for the duration of the trial.

All other study documentation and data stored on other non-NHS computers will use only the study code, without any personal data, and hence will be anonymised. Subjects will be given a personal study code number which will be used throughout the study and in the analysis of data. Anonymised samples will be stored for 10 years at the Department of Investigative medicine laboratories and analysed by the clinical research team. Anonymised samples of isotopic enrichment quantification will be transferred to the University of Surrey for analysis. Anonymised samples may be sent for analysis outside Imperial College London, the United Kingdom, to the European Union, USA or commercial companies.

Transfer of electronic personal or clinical non-anonymised data between the research sites will only take place via the secure nhs.net email system.

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

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

Funding: Fractyl® is funding this study through its investigator-initiated study programme.

Audits: The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, and other also by other regulatory bodies to ensure adherence to GCP and the UK Policy for Health and Social Care Research

Study management, data monitoring and ethics
A Trial Steering Committee (TSC) and a Data Monitoring & Ethics Committee will be established.

Quality Control and Quality Assurance
The trial will be adopted by the NIHR Clinical Research Facility at Imperial and will fall under their QC/QA regime.

References

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