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The Prevention of Breast and Endometrial cancer using Total Diet Replacement (PROBE-TDR) Trial; Protocol for a Randomised Controlled Trial.

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The Prevention of Breast and Endometrial cancer using Total Diet Replacement (PROBE-TDR) Trial; Protocol for a Randomised Controlled Trial.

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Keywords: breast, endometrium, cancer, diet therapy, risk reduction

Abstract

Introduction

Worldwide, there is an obesity epidemic. In the United Kingdom 29% of adult women are living with obesity and 31% overweight. Obesity and overweight are strong modifiable risk factors for endometrial and postmenopausal breast cancers. Every 5 unit increase in BMI is associated with a RR of 1.2 (1.15–1.25) for breast cancer and 1.5 (1.42-1.59) for endometrial cancer(1). Bariatric surgery can achieve considerable weight loss and risk reduction of weight-related cancer, but is not a feasible cancer prevention strategy. Total diet replacement can also lead to significant weight reduction. This study aims to examine the cellular and molecular changes in breast and endometrial tissue in high risk women following total diet replacement-induced weight loss.

Methods and analysis

PROBE-TDR: the PRevention Of Breast and Endometrial cancer using Total Diet Replacement is a prospective, non-blinded, randomised controlled trial of 47 women at increased risk of breast and/or endometrial cancer. Randomisation is 2:1 to either an immediate dietary intervention group (n=31) or a delayed dietary intervention control group (n=16). The 12-month weight loss programme incorporates a 3-month period of total diet replacement (TDR) (850 kcal/day) followed by a 9-month food-based (Mediterranean style diet, 1500 kcal/day) continued weight loss or maintenance programme. Menstrual phase matched biopsies of the breast and endometrium will be performed at baseline and after the first 3-months within both groups. This will allow us to assess molecular changes in breast and endometrium with weight loss achieved, compared to the control group following their usual diet.

Ethics and dissemination

HRA and Health and Care Research Wales (HCRW) Approval 20/NW/0095.
Registration details

ISRCTN15358157, assigned 11/05/2020.

Strengths and limitations of this study

- First study to evaluate the impact of diet-induced weight loss using TDR in both breast and endometrial tissues, in high risk women, with a two-stage design assessing tissue changes and longer term adherence.
- Provide proof of principle for weight loss and reduction in breast and endometrial cancer risk through biological measures, adherence and compliance.
- Assessing changes in premenopausal women, further studies are required amongst postmenopausal women.
- Relatively small sample size may preclude adequate assessment of biomarker change if non-adherence to diet allocation or study procedures is greater than expected.
- Women joining this study are likely to be highly motivated and the adherence may not reflect that seen in the wider general population.

Introduction

The 2018 World Cancer Research Fund report concludes there is convincing evidence for positive associations between obesity and twelve cancers (1). Breast cancer (BC) is the commonest cancer of women in the United Kingdom, affecting over 54,000 per year. Endometrial cancer (EC) is the fourth most common cancer in females in the UK, with around 9,500 new cases in 2017 and incidence rates increasing by over 50% since the early 1990s (2).

Maintained weight reductions of ≥5-15% have been shown to reduce the risk of postmenopausal breast (>2-4.5 kg lost: HR = 0.82 [95% CI = 0.70 to 0.96], >4.5-<9 kg lost: HR = 0.75 [95% CI = 0.63 to 0.90], ≥9 kg lost: HR = 0.68 [95% CI = 0.50-0.93]) and endometrial cancers (loss ≥ 5% HR, 0.71 [95% CI, 0.54-0.95(3–5). Furthermore bariatric surgery, which typically achieves an average weight loss between 20 – 30%, has been shown to reduce the risk of breast and endometrial cancer by ~50% (6–8). However bariatric surgery is not thought to be a feasible strategy for cancer prevention due to high patient burden and economic costs.

Low energy formula total diet replacement (TDR) provides around 800 kcal/day and aims to restrict energy intake by around 60% compared to 25% energy restrictions with standard weight loss diets. A number of recent studies have demonstrated the utility of using low energy TDR diets to achieve significant weight loss in the management of obesity (45% participants >10% body weight) and weight loss leading to remission of diabetes (46% achieved remission at 12 months (9,10). INTERCEPT (Impact of Diet-Induced Weight Loss on Biomarkers for Colorectal Cancer) studied an 8-week 800 kcal TDR in participants with a BMI >30kg/m² specifically for the purpose of evaluating risk reduction of colorectal cancer. This study showed that an average 14% weight loss led to improvements in insulin sensitivity, blood lipid profiles as well as significant reductions in colorectal cell proliferation, measured as Ki67 expression, in serial mucosal biopsies (mean change -43.8%; P=0.027) (11).

To date, no clinical trial has evaluated the impact of weight loss on both the breast and endometrium in combination. In a move towards more personalised medicine and a drive to
improve cancer prevention strategies, this study has the potential to act as an alternative or adjunct to cancer prevention modalities already available within clinical practice.

**Ethics and dissemination**

This study was adopted onto the National Institute for Health Research (NIHR) trial portfolio on 22 April 2020 and is sponsored by Manchester University NHS Foundation Trust (MFT). Any planned modifications to the protocol will be approved by the REC before they are adopted into the study. An audit trail of ethical amendments and documentation will be kept to allow monitoring by the research team and external regulatory bodies (table 1). The study was registered with an International Standard Randomised Controlled Trial Number on 11 May 2020.

**Table 1**

| Protocol | Date       | Summary of Changes                                                                 |
|----------|------------|------------------------------------------------------------------------------------|
| V4       | 29.04.2021 | Collection of diet related side effects to include control group.                  |
|          |            | Offer use of Nutritics Ltd app to allow remote function for recording 7-day food diary. |
|          |            | Additional clinicians/research study staff.                                        |
| V3       | 14.10.2020 | Amendment to wording of patient information sheet cover letter to reflect remote review of patients during the covid-19 pandemic. |

Table 1: Summary of ethical amendments

Results will be disseminated through publication in peer-reviewed scientific journals, presentation at conferences and via charity websites.

**Role of the funding source**

This work was jointly funded by NIHR Manchester BRC award [BRC-1215-20007] and Cancer Research UK via funding to the ARCTIC Clinical Academic Training Award [C19941/A28707] and Cancer Research UK Manchester Centre [C147/A25254]. This research is supported by the NIHR Manchester Clinical Research Facility. The funders of the study had no role in the study design or writing of the report. The corresponding author had final responsibility for the decision to submit for publication.

**Methods and Analysis**

Forty seven female participants, at elevated risk of breast and/or endometrial cancer will be recruited primarily from a high risk breast cancer risk prediction and prevention clinic at MFT and also from staff advertisements within MFT and The University of Manchester. This study will use a non-blinded randomised controlled design with participants randomised 2:1 to either an immediate dietary intervention group (n=31) or a delayed dietary intervention (control) group (n=16). The study will recruit between September 2020 and March 2022 and is recruiting at target rate of 1 participant per week.

**Inclusion criteria**
• Women aged 30-50 years.
• BMI ≥30 kg/m² or ≥27.5 kg/m² in Asian women.
• Pre-menopausal, with regular menstrual cycles.
• Ability to use the Oviva UK app OR access to a telephone.
• Willing to follow the TDR programme using Optifast® products.
• Maintain non-hormonal contraception (barrier or abstinence) until all biopsies completed.
• Type 2 Diabetes Mellitus on diet control +/- metformin can be included.
• Participants must be able to read, understand and communicate in English.

Exclusion criteria

• Prior history of breast or endometrial cancer or preinvasive breast disease.
• Hormonal contraceptive use in the preceding 3 months.
• Preventative Tamoxifen or anti-progestin therapy within the last 6 months.
• Carrier of the BRCA 1 or 2 gene.
• Confirmed pregnant at screening, planning pregnancy in the next 12 months, or current breast feeding.
• Taking prohibited medications including: Warfarin or novel anticoagulants (NOAC), low molecular weight heparin (LMWH) or equivalent anti-coagulants, anti-psychotic medication, anti-diabetic medication other than metformin, orlistat or other pharmacological treatments for weight loss and steroids (more than 20mg daily of prednisolone or its equivalent).
• Previous bariatric surgery for weight loss including gastric bypass and sleeve gastrectomy.
• Known hypersensitivity to any of the Optifast® ingredients (e.g. fish, milk, soy) or lactose intolerance.
• Substance abuse or harmful alcohol use as indicated by a score of 16 or above on the Alcohol Use Disorders Identification Test (AUDIT)(12).
• Diagnosis of an eating disorder, or patients with severe binge eating assessed by a score of 27 or more on the Binge Eating Scale (BES)(13).
• Severe depression assessed by a score of 15 or more on the Patient Health Questionnaire-9 (PHQ-9) questionnaire.
• Severe anxiety assessed by a score of 15 or more on the General Anxiety Disorder (GAD-7) questionnaire.
• Participants with psychiatric or physical comorbidity or scheduled for major surgery, which in the opinion of the treating medical physician, or the Chief Investigator (CI), would compromise their safety or adherence to the study.

Patient Involvement

Prior to ethics submission a local patient group advised on suitability, acceptability of the study and readability of the patient information.

Study Objective

To determine the effects of weight loss with 12+/-4 weeks of Total Diet Replacement (TDR) on cancer risk markers in the breast and endometrium of women at increased risk of breast and/or endometrial cancer as compared to a usual diet control group.

Primary outcome:
The change in epithelial cell proliferation (Ki67) of the breast and endometrium from baseline to 12+/- 4 weeks in the TDR versus delayed intervention group.

**Secondary Outcomes:**

To determine changes in the following in the TDR group as compared to the delayed intervention (usual diet) control group over the 12+/- 4 weeks between biopsies

- Weight, body fat and fat free mass (bioelectrical impedance Tanita MC980MA), waist and hip circumference.
- Biomarkers of cancer risk; (serum/plasma) fasting insulin, glucose, lipids, inflammatory markers, leptin, adiponectin, insulin-like growth factor -1 (IGF-1).
- Anxiety (Generalised Anxiety Disorder-7 [GAD-7] scale), depression (Participant Health Questionnaire-9 [PHQ-9]), quality of life (Obesity and Weight Loss Quality of Life [OWL-QOL]) and [EQ-5D-3L]), diet self-efficacy (Weight Efficacy Lifestyle Questionnaire Short Form [WEL-SF]).
- Dietary intake (energy, protein, fat, carbohydrate) [7-day food diary].
- Physical activity (International Physical Activity Questionnaire [IPAQ] short form).

To determine the following during the 12 month weight loss programme in both groups

- Adherence and retention to the programme.
- Anthropometric measures, cancer risk biomarkers and quality of life measures.
- Fidelity of delivery of the 12-month programme by the multidisciplinary team (dietitian, psychologist, clinicians).
- Adverse events.

**Exploratory Outcomes:**

The impact of weight loss with the TDR vs the delayed intervention usual diet control group at 12 weeks on changes in:

- Expression of progenitor markers in breast and endometrial tissues (LGR5, SOX9, KIT)
- Progenitor populations in the breast in response to TDR using FACS analysis and colony formation (mammosphere) assay.
- Immune microenvironment using multiplex immunofluorescence including markers such as CD68, CD56, CD3, CD8, FOXP3 and PD-1).
- Transcriptional changes in both the breast and endometrium using RNA sequencing.
- We will also assess any changes in mammographic density between the baseline mammogram and mammogram at the end of the12 month weight loss programme using quantitative automated techniques (Volpara Health and predicted visual assessment of breast density [VAS]).

**Trial Procedures**

Potential participants will be screened via telephone to confirm they meet the required criteria for a baseline appointment. Consent will be taken by a member of the trial team who is Good Clinical Practice (GCP) trained, experienced and who has been delegated by the Chief Investigator to undertake this activity. Additional verbal/written consent will be obtained prior to any trial-related procedures being undertaken. Screening procedures (see supplementary information) will be
conducted at initial consent appointment and provisionally eligible participants will receive an appointment for a mammogram (if the participant has not had one performed in the preceding 12 months). Once blood and mammogram results are available, eligible participants will be randomised and baseline breast and endometrial pipelle biopsies scheduled [Figure 1]. All screening and biopsies and other assessments are conducted in research, breast and gynacecological outpatient clinics at MFT.

Figure 1: Study schema illustrating participant journey through the screening, total diet replacement and continued weight loss/weight maintenance phases of the study.
Randomisation and blinding

Eligible patients are randomised 2:1 to the immediate or delayed intervention group by a researcher independent of the intervention using a minimisation program stratified on the following criteria:

- Above or below projected median BMI of 35 kg/m²
- Above or below projected median lifetime risk of breast cancer ≥17% remaining lifetime risk (Tyrer-Cuzick), with competing mortality

Due to the nature of the intervention, it was not possible to blind participants and clinicians to the treatment allocation. Clinical assessments were undertaken by an independent research assistant in the clinic. Laboratory results were assessed by independent laboratory staff.

Study follow-up

Trial design overview is shown in figure 1. Participants from both groups will be supported weekly via the virtual platform (Oviva UK app) during the 12-month weight loss programme and will be reviewed every 3 months for assessment of weight, body composition, body measurements, diet and physical activity, quality of life and cancer risk biomarkers.

Participants may withdraw from the study at their own request or at the discretion of the chief investigation. Withdrawal from the study will not affect patient care.

TDR Weight Loss Programme

TDR phase (weeks 0-12)

The TDR provides around 850kcal/day which includes 3 Optifast® products (216 kcal per sachet), 8 x 80g portions of non-starchy vegetables (~150 kcal) and 1 dessertspoon of vegetable oil (60 kcal). Participants are also advised to take a minimum of 2-2.5 litres of energy-free liquids (i.e. water, diet drinks, black tea/coffee).

Diet reintroduction (DR) phase (weeks 12-16)

The DR phase involves the gradual re-introduction of an energy restricted Mediterranean-based diet over 4 weeks. Participants may either follow a total food based approach or they can continue to include 1 or 2 Optifast® products per day. The meal plans will consist of daily energy restricted Mediterranean diet describe below as previously used by Manchester researchers (14,15). Energy intake will be increased in the following manner over the 4 weeks:

a. 1st week 1000kcal
b. 2nd week 1200 kcal
c. 3rd week 1400kcal
d. 4th week 1500kcal

Weight maintenance/continued weight loss phase (weeks 17-52)
Participants who have attained both a weight loss of ≥15% and their target weight (which is likely to be a lower weight than that attained with a 15% weight loss) will be asked to follow their choice of either an isoenergetic intermittent or continuous weight maintenance diet. The intermittent diet includes one day of a food based very-low energy diet (VLED) (~850 kcal), and 6 days of an isoenergetic Mediterranean diet. The energy content of the Mediterranean diet will be determined by the trial dietitian based on the Mifflin equation(16), multiplied by the metabolic equivalents for their self-reported activity levels(17). The Mediterranean diet provides 30% energy from fat (15% monounsaturated fatty acids, 8% polyunsaturated fatty acids, 7% saturated fatty acids), 25% energy from protein and 45% from low glycaemic load wholegrain carbohydrates and includes at least 5 portions of vegetables, 2 portions of fruit/day, low fat dairy products, protein foods including fish, lean meat and pulses. Energy controlled Mediterranean diets are considered optimum for reducing weight, blood pressure and improving lipid profiles and have been linked to lower risk of cancers including breast cancer. The average energy intake over the week is ~ 2000 kcal. Participants will be given the option of including one meal replacement product per day in the first 6 months of the programme.

Participants who have not achieved the trial weight loss goal of ≥15%, or who wish to lose more than 15% will be asked to follow an energy restricted intermittent or continuous energy restricted food-based diet. The intermittent diet will involve two consecutive days of a food-based VLED (~850 kcal) and 5 days of an isoenergetic Mediterranean diet or 7 days of an energy restricted Mediterranean diet. The average energy intake over the week is ~1500 kcal/day.

Relapse management for weight-gain (weeks 12 – 52)

If participants regain ≥2kg (from self-reported weight data) they will be advised they can either resume the initial 850 kcal TDR for 2 weeks followed by 2 weeks of diet reintroduction or replace one meal a day with a meal replacement product. They will receive a booster call from the trial dietitian and additional support from the trial psychologist if required. Meal replacement products and additional support will be offered for the first 2 relapses, and behavioural support only for any subsequent ones.

Physical activity advice and support

Physical activity advice will be delivered by the trial dietitian via phone/video call. The trial dietitian will check the participant’s responses to the Physical Activity Readiness Questionnaire (PAR-Q) (Canadian Society for Exercise Physiology)(18) to assess any contraindications to exercise. GP clearance may be required for certain participants.

Participants who are physically capable will be asked to follow a resistance exercise programme during the TDR phase, comprising two to four sets of 8 to 15 repetitions of arm, leg and trunk exercises three times / week over 12 weeks guided by on line videos (Physiotec UK).

After the TDR phase participants will be encouraged to continue with the resistance exercises and also build up to between 150 – 300 minutes of moderate intensity physical activity/week
e.g. brisk walking to promote health and continued weight loss or weight loss maintenance(19).

Remote behavioural support

Participants will be supported remotely by their allocated dietitian using weekly video calls via the Oviva UK Ltd app. The app also facilitates written messages, self-monitoring of diet, weight, activity levels, blood pressure (where relevant) and an invitation to take part in a peer support group messaging on the app with other participants. Behaviour change techniques include goal setting, self-monitoring, timely personalised feedback on these records, rehearsing successful performance of behaviour, action planning and planning for how to deal with setbacks.

Enhanced psychology support from the trial psychologist is offered to participants with baseline scores indicating moderate binge eating (score 18-26 on BES), moderate depression (score 10-14 on PHQ-9), moderate anxiety (score 10-14 on GAD-7), low self-efficacy (score <45 on WEL-SF) or increasing risk of alcohol dependency (score 8-15 on AUDIT). Psychology support is also available to participants who relapse and those identified by the team during the trial as experiencing difficulties, impairing their ability to adhere to the programme. The psychological intervention will be centred on motivational interviewing, cognitive behavioural therapy, behavioural activation, mindfulness skills, distress tolerance skills and emotional regulation skills.

Protocol delivery fidelity

Dietitian support is conducted by specialist dietitians with experience of conducting dietary intervention studies using TDR and management of cancer risk. Variability in primary outcome assessments (body weight, cancer risk biomarkers) will be minimised by using calibrated equipment and quality-controlled assays.

Measurements

The measurements taken at each stage of the ProBE-TDR study are detailed in the supplementary information.

Physical measurements

Height will be measured to the nearest milimeter, with the Frankfort horizontal plane, using a portable stadiometer (Chasmors Ltd, London). Body weight will be measured to the nearest 0.1 Kg in light clothing without shoes or socks using calibrated bio-impedance scales (Tanita, EU). Waist circumference is measured across the umbilicus and hip circumference will be measured over the participant’s underwear at the widest point over the buttocks to the nearest 0.5cm. Seated blood pressure will be measured in triplicate at rest with legs uncrossed for at least 10 min. Patients will be asked to abstain from alcohol and moderate physical activity for 24 hours prior to their appointment and be asked to attend in the fasted state (10 hour fast).

Blood sampling
This will be performed at baseline to include: fasting insulin, glucose and lipids, leptin, adipokines, inflammatory cytokines including C-reactive protein and HbA1c. These will be repeated after 12+/−4 weeks of intervention/control and at 6, 9 and 12 months for both groups and month 15 for the control group. HbA1c will only be repeated at the end of the TDR phase and 6 months post TDR. Bloods for oestrogen and progesterone will be taken at the time of the breast biopsies only. Urine samples will be obtained at screening (tested for pregnancy and stored for metabolomic analysis) and repeated at each 3 month visit for metabolomic analysis.

Breast and endometrial biopsies

Baseline vacuum assisted breast biopsy (VAB) and blood sampling for oestrogen and progesterone will be undertaken in the luteal phase (week before expected menstruation) of the menstrual cycle, endometrial sampling will be undertaken in the follicular phase (week following menstruation) of the menstrual cycle. The biopsies will be obtained by study clinicians and the bloods by research staff. Breast and endometrial biopsies will be repeated after at least 8 weeks of TDR/usual diet in the same phase of the menstrual cycle as the baseline tests. Participants in the immediate dietary intervention group will remain on the TDR until they have undergone their post TDR breast and endometrial biopsies. Some participants may need to remain on TDR for up to 16 weeks if there are difficulties scheduling the repeat matched biopsies in line with their menstrual cycle.

Laboratory analyses

Change in proliferation from baseline to 3 months will be assessed by percentage epithelial Ki67 expression in the paraffin embedded tissue sections. Breast samples will be digested to single cell suspension and processed for mammosphere assay to provide readout of stem/progenitor activity. FACS analysis of the proportion of luminal progenitors (CD49f+/EPCAM+), differentiated luminal (CD49f-/EPCAM+), myoepithelial (CD49f+/EPCAM-) and adipose mesenchymal stem cells (MSC) (CD49f-/EPCAM-/CD73+/CD90+/CD105+) will determine the change in cell proportions with TDR vs controls. RNA sequencing will be performed on the breast and endometrial tissues to evaluate gene expression changes owing to weight loss.

Sample Size

With 13 controls and 26 subjects in the intervention group, the study will have 80% power at a 5% significance level to detect a difference in Ki67 within the breast and endometrial biopsy samples. Mean luteal breast Ki67 is 4.99 (SD 5.03) within the Breast Cancer Anti-Progestin Prevention Study (NCT02408770). A difference of 50% or more in change in Ki67 between the control and the intervention group has been chosen as this represents a clinically meaningful effect size for a mechanistic study. Incorporating a 20% loss to follow-up rate, these sample sizes increase to 16 controls and 31 in the intervention group (Table 2). The sample size has been chosen to allow the research team to obtain sufficient data on the feasibility and potential efficacy of the entire 12-month managed weight loss programme.
Table 2: Power calculation for ProBE-TDR Study

| Difference | Power | TDR (n) | Control group (n) | TDR (n) | Control group (n) |
|------------|-------|---------|-------------------|---------|-------------------|
| 50%        | 80%   | 26      | 13                | 31      | 16                |

Statistical analysis

The primary data analysis for change in Ki67 will be by intention to treat using an Analysis of Covariance using a 5% level of significance. This will be an unadjusted analysis comparing the outcome measured, controlling for the baseline value. We will also carry out a per protocol analysis as a secondary analysis. Missing data will be imputed via multiple imputation methods. The analysis will be conducted using SPSS and STATA.

The secondary data analysis will be by intention to treat and will not undertake any significance tests to compare the groups. Change scores (95% CI) for the secondary outcomes will be presented from within and between both groups. To determine secondary endpoints of changes in lifestyle/weight loss over time, linear mixed modelling will be used to assess the degree of change over time.

We will assess engagement with the TDR weight loss programme from the receipt of calls and use of Oviva app functions, e.g. self-monitoring.

Data management

The source data which comprises medical notes, electronic data sources (Oviva app, Nutritics app), case report form (CRF) and copies of the participant completed questionnaires are the primary source data. Participant data will be anonymised and will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act (2018) and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations (2006) as defined in the Kings Health Partners Clinical Trials Office Archiving SOP (17 Data Management). Deidentified data will be stored in a study-specific Research Electronic Data Capture (REDCap) database. The sponsor will periodically audit the site study file, a sample of CRFs, consent forms and source data and accuracy of the study database to ensure satisfactory completion.

Patient safety

Some participants may need close monitoring and medication management during the programme due to rapid changes in weight. In keeping with the recommendations for medicines management within the DiRECT study(9) all anti-hypertensive and diuretic drugs will be stopped on the day TDR is commenced [supplementary information]. Blood pressure, for those whose medications have been stopped, will be monitored weekly using home monitoring and the participant can input their result within the Oviva app for the study clinician.
to review. Medication(s) for managing blood pressure will be reintroduced if clinically required and communicated to the GP.

Participants taking metformin will be advised to remain on this medication for the duration of the study.

Adverse events will be monitored and graded monthly during the TDR phase for the immediate and delayed intervention group. Also, during the normal diet phase for the delayed intervention group using the National Cancer Institute CTCAE v5.0(20). This will indicate rates of TDR associated and background rates of adverse events. Serious adverse events will be reported to the Research Ethics Committee (REC) and sponsor and documented within the participants medical record.

Study Management

The study management group comprises the chief investigator, project manager, clinical research fellow, radiology team and psychologist, who will jointly monitor study conduct and progress. All aspects of the study and all study personnel will adhere to the study protocol (version 3.0 or subsequent approved version) and Good Clinical Practice and Data Protection principles. Regular team meetings will ensure quick resolution of recruitment issues, study processes and data collection inconsistencies.

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Authors' contributions

SJH and EJC are the principal investigators. MH, RC, AH, SJH and EJC all contributed to the design of the study. HC is the responsible for the day-to-day running of the study, patient recruitment and consent and collection of gynaecological tissue samples. SJH and EJC
provide study oversight and with BI, provide clinical guidance. MH and CL are responsible for delivery of the dietetic component of the study. AM, YYL, CP and SP are responsible for the collection of breast tissue samples. SK is responsible for study administration and data collection. KS contributed to protocol development and achieving ethical approval. HH is involved in scientific analyses of breast tissue samples.

HC drafted the initial manuscript. All authors critically reviewed and revised the manuscript and have read and approved the final version and contributed to the development and set-up of the study.

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**Conflicts of Interests Statement**

All authors declare no competing interests. MH and BI have received research funding for previous research associated with Nestle Health Care and Oviva, but no financial benefit from this research study.

**Word Count:** 3,995 excluding abstract and references.
### SUPPLEMENTARY INFORMATION 1

#### Screening procedures

Table 1: Screening procedures commenced at baseline appointment

| Study Procedure                                      | Screening |
|------------------------------------------------------|-----------|
| Review & discuss study participation                 | ✓         |
| Review inclusion/exclusion criteria                   | ✓         |
| Obtain informed consent                               | ✓         |
| Height                                                | ✓         |
| Weight                                                | ✓         |
| BMI                                                   | ✓         |
| Body fat & fat free mass (bioelectrical impedance)   | ✓         |
| Waist/hip circumference                               | ✓         |
| Blood pressure                                        | ✓         |
| Blood progesterone/oestrogen                          | ✓         |
| Study bloods                                          | ✓         |
| Urine pregnancy test (if relevant)                    | ✓         |
| Urine sample for metabolomic analysis                 | ✓         |
| Mammogram                                             | ✓         |
| Alcohol use (AUDIT)                                   | ✓         |
| Self-Efficacy (WEL-SF)                                | ✓         |
| Binge Eating (BES)                                    | ✓         |
| Patient Health (PHQ-9)                                | ✓         |
| Anxiety (GAD-7)                                       | ✓         |
| Quality of life score (OWL-QoL)                       | ✓         |
| 7-day paper food diary/Nutritics app                  | ✓         |
| Physical activity score (IPAQ)                        | ✓         |
| Physical activity readiness questionnaire (PAR-Q)     | ✓         |
| Demographic questionnaire                             | ✓         |
| Tyrer-Cuzick Breast Cancer Risk Questionnaire/Score   | ✓         |
### Schedule of Assessments

#### Table 2: Schedule of assessments – Control group

| Appointment Study Procedure                  | Screening | 1 0 Baseline | 2 ~3 months | 3 ~6 months | 4 ~9 months | 5 ~12 months | 6 ~15 months |
|---------------------------------------------|-----------|--------------|-------------|-------------|-------------|--------------|--------------|
| Review & discuss study participation       | ✓         |              |             |             |             |              |              |
| Review inclusion/exclusion criteria         | ✓         | ✓            |             |             |             |              |              |
| Obtain informed consent                     | ✓         |              |             |             |             |              |              |
| Height                                      | ✓         |              |             |             |             |              |              |
| Weight                                      | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| BMI                                         | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Body fat & fat free mass (bioelectrical impedance) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Waist/hip circumference                     | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Blood pressure                              | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Blood progesterone/oestrogen                | ✓         |              |             |             |             | ✓            |              |
| Study bloods                                | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Urine pregnancy test (if relevant)          | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Urine (metabolomics)                        | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Breast VAB                                  | ✓         | ✓            |             |             |             |              |              |
| Endometrial pipelle                         | ✓         |              |             |             |             |              |              |
| Mammmogram                                  | ✓         | ✓            |             |             |             |              |              |
| Alcohol use (AUDIT)                         | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Self-Efficacy (WEL-SF)                      | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Binge Eating (BES)                          | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Patient Health (PHQ-9)                      | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Anxiety (GAD-7)                             | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Quality of life score (OWL-QoL)             | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| 7-day paper food diary/Nutritics app        | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Physical activity score (IPAQ)              | ✓         | ✓            | ✓           | ✓           | ✓           | ✓            | ✓            |
| Physical activity readiness questionnaire (PAR-Q) | ✓ |       |             |             |             |              |              |
| Demographic questionnaire                   | ✓         |              |             |             |             |              |              |
| Tyrer-Cuzick BC risk                        | ✓         |              |             |             |             |              |              |
| Adverse events screening                    | ✓*        | ✓*           | ✓*          |             |             |              |              |

* Screening not mandatory.
| Appointment          | Screening | 1 0 Baseline | 2 3 months | 3 6 months | 4 9 months | 5 12 months |
|---------------------|-----------|--------------|------------|------------|------------|------------|
| Review & discuss study participation | ✔         |              |            |            |            |            |
| Review inclusion/exclusion criteria       | ✔         | ✔            |            |            |            |            |
| Obtain informed consent                        | ✔         |              |            |            |            |            |
| Height                              | ✔         |              |            |            |            |            |
| Weight                              | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| BMI                                 | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Body fat & fat free mass (bioelectrical impedance) | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Waist/hip circumference             | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Blood pressure                      | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Blood progesterone/oestrogen        | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Study bloods                        | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Urine pregnancy test (if relevant)   | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Urine (metabolomics)                | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Breast VAB                          | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Endometrial pipelle                 | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Mammogram                           | ✔         |              |            |            |            |            |
| Alcohol use (AUDIT)                 | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Self-Efficacy (WEL-SF)              | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Binge Eating (BES)                  | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Patient Health (PHQ-9)              | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Anxiety (GAD-7)                     | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Quality of life score (OWL-QoL)      | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| 7-day paper food diary/Nutritics app | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Physical activity score (IPAQ)       | ✔         | ✔            | ✔          | ✔          | ✔          | ✔          |
| Physical activity readiness questionnaire (PAR-Q) | ✔       |              |            |            |            |            |
| Demographic questionnaire           | ✔         |              |            |            |            |            |
| Tyrer-Cuzick BC risk                | ✔         |              |            |            |            |            |
| Adverse events screening           | ✔*        | ✔*           | ✔*         | ✔          | ✔          | ✔          |
## SUPPLEMENTARY INFORMATION 3

### Weight Loss Programme

#### Table 4: Outline of weight-loss programme

| PHASE                      | ADVICE                                                                 | DIET                                                                 | EXERCISE                                           |
|-----------------------------|------------------------------------------------------------------------|----------------------------------------------------------------------|----------------------------------------------------|
| Total Diet Replacement      | Initial advice 60-90 minutes Phone or Face to Face                      | 7 days/week TDR (Optifast® and non-starchy vegetables ~850 kcal/day) | Undertake progressive resistance exercise 3 days/week |
| (TDR) 12+/-4 weeks          | 15-20 minute weekly support via App or phone. Weekly support focussed on adherence, side effects, motivational interviewing and promoting self-monitoring |                                                                      |                                                    |
|                             |                                                                        |                                                                      |                                                    |
| Diet Reintroduction         | Initial advice 40 – 60 minutes Phone or Face to Face 15-20 minute weekly support via App or phone. | Food reintroduction                                                  | Continue with resistance exercise 3 days/week. Build up to moderate intensity physical activity of 150-300 minutes/week |
| Phase (DRP) 4 weeks         |                                                                        | 1st week 1000 kcal                                                  |                                                    |
|                             |                                                                        | 2nd week 1200 kcal                                                  |                                                    |
|                             |                                                                        | 3rd week 1400 kcal                                                  |                                                    |
|                             |                                                                        | 4th week 1500 kcal                                                  |                                                    |
|                             |                                                                        |                                                                      |                                                    |
| Continued Weight Loss       | 10 minutes / week via App or phone Promoting self-monitoring          | Intermittent food based diet: 850 kcal 2 days per week and Mediterranean diet 5 days per week or daily energy restricted Mediterranean diet | Continue with resistance exercise 3 days/week. Moderate intensity physical activity of 150-300 minutes/week |
| Phase (CWLP) 8 months       | motivational interviewing Preparing for future weight loss maintenance |                                                                      |                                                    |
|                             |                                                                        |                                                                      |                                                    |
| or Weight Maintenance       | Up to 2 Relapse advice 40 – 60 minutes Phone or Face to Face Relapse management will include an exploration of the reasons for weight regain, with appropriate signposting for psychological support with the trial psychologist | 2 weeks of TDR followed by 2 weeks of food reintroduction (Optifast® and non-starchy vegetables) ~850 kcal/day |                                                    |
| Phase (WMP) » if attained   |                                                                        |                                                                      | OR Replace one meal a day with Optifast® shake      |
| target weight:              |                                                                        |                                                                      |                                                    |
|                             |                                                                        |                                                                      |                                                    |
| Relapse intervention if     |                                                                        |                                                                      |                                                    |
| weight increases >2kg       |                                                                        |                                                                      |                                                    |
| assessed from self-         |                                                                        |                                                                      |                                                    |
| monitoring during the 8     |                                                                        |                                                                      |                                                    |
| month CWLP/WM               |                                                                        |                                                                      |                                                    |
|                             |                                                                        |                                                                      |                                                    |
| Total weeks of programme    | 52 weeks                                                               |                                                                      |                                                    |
SUPPLEMENTARY INFORMATION 4

Medicines Management

**Background:** Antihypertensive and diuretic drugs will be stopped on the day Total Diet Replacement (TDR) is commenced. This is a safety measure, because blood pressure is likely to fall on the diet. This protocol lays out the standard approach to be followed, as taken from the DiRECT study. Individual clinical decisions may be necessary for a person’s best interest. The level of 140 mmHg is chosen to allow safe decisions during the weight loss period. After the Food Reintroduction period follow usual guidelines for management of hypertension. To simplify decision making, systolic pressure only is used as a guide to therapy even though both systolic and diastolic are relevant to long term benefit.

**Protocol:** When antihypertensive drugs are stopped, re-emphasise the importance of avoiding sodium (salt)

1. In the first 2 weeks after stopping antihypertensives and diuretics: If systolic BP over 165 mmHg on repeated measurement - restart one drug, as below.
2. Thereafter, if systolic BP is >140 mmHg - restart one drug as below.
3. Increase dose weekly to achieve target.
4. If systolic BP remains >140 mmHg on the first drug - add a second drug, as below.
5. Increase dose weekly to achieve target.
6. Repeat as necessary with third, fourth or more drugs (increasing each to maximum dose).

**Order of reintroduction of previously used drugs:**
1. ACE inhibitors (ramipril, lisinopril, perindopril, etc.)
2. Angiotensin receptor blockers (irbesartan, candesartan etc.)
3. Thiazide type (bendroflumethiazide, indapamide etc.)
4. Spironolactone
5. Calcium channel blocker (nifedipine, amlodipine etc.)
6. Beta blocker (atenolol, labetolol etc.)
7. Alpha blocker (doxazosin, prazosin)
8. All others
The ProBE-TDR Study

**Prevention of Breast and Endometrial cancer using Total Diet Replacement**

Participant Information Sheet

We would like you to invite you to take part in our research study that aims to see whether a weight loss programme using low calorie diet replacement drinks can reduce the risk of developing breast and endometrial cancer and help towards achieving long term weight loss.

- If you decide to take part in the study you will be asked to:
  - attend an initial screening and assessment appointment at Wythenshawe Hospital
  - have a biopsy of the breast and a biopsy of the lining of the womb (endometrial biopsy) at the start of the study and 3 months later
  - If you have not had a mammogram (breast x-ray) within the last 12 months you will be asked to attend Wythenshawe hospital for a mammogram. This will be repeated at the end of the study.
  - Follow a dietitian supported 12-month weight loss programme
    - this involves 3 months of low-calorie diet replacement drinks followed by 9 months of a food-based diet and physical activity programme

- 47 women will join the study
  - 31 women will be randomly placed in an immediate diet group who will start the 12-month diet programme at the start of the study.
  - 16 will be in a delayed diet group who will be asked to follow their normal diet for the first three months before starting the 12-month programme.

- You will also be asked to attend Wythenshawe hospital approximately every three months during the course of the study for follow up clinical assessments.

If you have any questions about the study or would like to join please call the research team on 0161 291 4412 or email us at mft.probe-tdr@nhs.net
Before you decide if you would like to take part, it is important for you to understand why the research is being done and what it would involve for you. Please take your time to read the following information carefully. Discuss it with your friends, relatives, or GP if you wish, before you make a decision to be involved. Take time to consider whether or not you wish to take part.

Your participation in the study is **entirely voluntary**, so you do not have to take part if you do not want to and you can opt out of the study at any time without giving us a reason.

Thank you for reading this information; we hope this research will be of interest to you.

**Why are we doing this research?**

Breast cancer is the most common cancer in women and endometrial (womb) cancer is the fourth. Being overweight and not having a healthy lifestyle increases the risk of both breast and endometrial cancer. Compared with a healthy weight woman, those who are 3 stone (20 kg) above a healthy weight have a 20% greater chance of developing breast cancer and three times the risk of developing endometrial cancer. Research has shown that overweight women who manage to lose large amounts of weight with weight loss surgery (typically 4 stone/30 kg), reduce the majority of the weight-associated cancer risk. However, weight loss surgery is not a suitable or appropriate option for most patients.

A maintained weight loss (in the region of 2 stone/15kg) can be achieved with a dietitian-supported weight loss programme. This programme involve 3 months of total diet replacement drinks and vegetables (850 calorie/day), followed by 9 months of a calorie-controlled food-based diet with a physical activity programme to maintain weight loss. A programme like this has been shown to reverse weight-related Type-2 Diabetes. We now wish to see whether this programme could be used to lose weight and reduce cancer risk markers in women and ultimately help prevent breast and endometrial cancer.

**What is the purpose of the study?**

The study has two main aims. Firstly, to measure any changes in cancer risk markers within the tissues taken from biopsies of the breast and endometrium (womb lining), blood and urine at the start of the study. These will be compared to those taken after 3 months of the 850-calorie diet. This will indicate any likely reduction in cancer risk.

Secondly, since maintained weight loss is key for preventing cancer we will also see whether the weight loss achieved at the end of three months of the 850-calorie diet is maintained up to 12 months, with a dietitian supported diet and physical activity programme.

This study will involve 47 women. Thirty-one will be placed in an immediate 850 calorie diet group, and 16 will be placed in a delayed 850 calorie diet group. The delayed diet group will be asked to maintain their normal diet for the first three months until they have had their baseline and 3-month biopsies, before commencing the 12-month weight loss programme. This is to act as a control, to show us if the dietary intervention does indeed have any positive change.

This study will inform the design and possible future large weight loss cancer prevention study.
**Why have I been invited?**
You have been asked to think about participating in this study as you are above a healthy weight and this increases the risk of developing breast and endometrial cancers. We want to study the effect of weight loss on markers of breast and endometrial cancer risk.

**Who can join?**
We are looking for women aged 30-50 years with a body mass index (BMI) of greater than 30 (or greater than 27.5 if Asian or South Asian ethnicity) and having regular periods. Women must be willing to modify their contraception (if applicable) for the first three months of the study, as hormonal contraception will interfere with the phase when the biopsies are taken. We would like participants to have access to a smartphone for using the both the Oviva UK app which allows you to communicate with the dietitians and study team at your convenience and the Nutritics Ltd app, which allows you to input your food diary and offers comprehensive nutritional analysis. If you would prefer to participate by standard telephone calling, or using a paper-based food diary, this is an option.

**Who cannot join?**
Unfortunately, we cannot accept women who have a personal history of previous breast or endometrial cancer, or preinvasive breast disease (such as Ductal Carcinoma in Situ, DCIS), nor women not willing or able to come off hormonal based contraception for 3-6 months.

**Do I have to take part?**
No, you do not have to take part if you do not wish to and your decision will not affect the standard of care you receive. If you wish to take part you will be asked to attend an appointment with a study doctor to read and sign a consent form to show you have agreed to participate. You will keep this information leaflet and one copy of the consent form for yourself. You will be free to withdraw from this study at any time without giving a reason and without it affecting your medical treatment.

**What will happen to me if I take part?**
The study will involve attending around 10 appointments at Wythenshawe Hospital (depending on which group you are in) and 2 appointments at St. Mary’s Hospital. If you have had a mammogram in the previous 10 months, we will ask your permission to review these images. We would also like to review subsequent mammograms you may have in the future to see whether any changes caused by diet replacement reverse as you get older. If you have not had a mammogram in the previous 10 months, an appointment will be made at the Nightingale Centre at Wythenshawe Hospital, for a mammogram before you begin in the study.

If you are interested in taking part you will be asked to attend an **initial assessment appointment to check you can join the study**. Eligible women will be **randomly placed in** one of two groups. You cannot choose which group you are in.

1. **An immediate diet group**
This group begin the 12-month dietitian supported programme immediately after their baseline (first) breast and womb biopsies have been taken. The programme involves 3 months of the total diet replacement followed by 9 months of a dietitian supported programme. This group will be in the study for around 12-13 months.
2. A delayed diet group
This group will be asked to follow their normal diet for the first 3 months until their repeat breast and womb biopsies. They will then begin the 12-month programme (3 months of the total diet replacement followed by 9 months of a dietitian supported programme. This group will be in the study for around 15-16 months.

Both groups will have a number of baseline (at the start) assessments. These include your weight, completion of 6 lifestyle and well-being questionnaires, a mammogram (if you have not had one in the past 12 months), a biopsy of both the womb lining and breast and blood samples. The womb biopsy will be in the first half of your menstrual cycle and the breast biopsy in the second half. The exact timing of the biopsies will be calculated based on your individual menstrual cycle. On the day of the breast biopsy, we will also take a small blood and urine sample to assess markers of cancer risk. The biopsies, blood and urine tests, weight measurements and questionnaires will be repeated around 3 months later. Following the diet replacement phase, you will be reviewed three-monthly by a member of the study team to have repeat blood and urine tests, weight measurements and questionnaires. The ongoing dietitian support will be via the Oviva app/telephone, to minimise appointments for you at the hospital.

The 12-month weight loss programme
This has three diet phases:

Phase 1: 3 months of an 850-calorie total diet replacement using Optifast® liquid meal replacements and 8 portions of vegetables each day. Optifast® ready to drink shakes are nutritionally complete and are delivered free to your home. They are available in a range of flavours, but are not suitable for anyone with allergies to milk, shellfish and soya.

Phase 2: 4 weeks of a food reintroduction with a progressively increased calorie intake food-based diet (1000–1500 calories/day).

Phase 3: The remainder of the 12-month study period involves a food-based calorie controlled healthy Mediterranean diet aimed at either further weight loss or weight maintenance, if you have reached your weight loss goal. A Mediterranean diet includes low fat meats, fish, fruit, vegetables, wholegrain starchy foods (e.g. wholegrain bread and cereals), beans, pulses, low fat dairy products and healthy fats found in foods like nuts, seeds and olive/rapeseed oil. If you gain weight in this time you have the option to return to the 850-calorie total diet replacement for a short time to help you lose the weight you have gained.

Physical activity
You will be encouraged to do some simple specific muscle strengthening exercises 3 times a week during phase 1 of the diet. The remainder of the programme includes the 3 weekly muscle strengthening exercises as well as 150-300 minutes of moderate intensity physical activity a week (i.e. 30-60 minutes 5 times per week).

Advice and support
All participants will receive personalised one to one advice and support from a specialist dietitian throughout the programme. This will be provided by face-to-face and/or remote smartphone/tablet telephone/text reviews using a specialist health care smartphone/tablet app (Oviva app) or via the telephone if you prefer. The app also allows you to record your weight, food intake and any physical activity you have done and send messages to the dietitian. You may also be offered professional support with the emotional
and behavioural aspects of weight loss from a clinical psychologist. The reduction in calories and subsequent weight loss from diet replacement can lower your blood pressure. If you are taking medication to reduce your blood pressure this can lead to you feeling unwell. You may need to have the dose of your blood pressure medication lowered or stopped altogether. If we change or stop a medication, we will advise you as to how frequently you should have your blood pressure monitored and you can submit the readings to the study team via the Oviva app/telephone. Any changes to medicines will be advised by the doctors working on the study and your GP will be kept informed.

**What will happen to me during the biopsies?**

For the biopsy of your breast you will be asked to lie on an examination couch with your breast exposed. An ultrasound machine will be used to highlight the best area of the breast for the biopsy. For the ultrasound some cold jelly will be put on your breast and a small hand-held probe pressed against the skin surface. The probe will be moved over the skin to view the breast from different angles and the pictures displayed on a monitor. The biopsy area will be cleaned with anti-septic and local anaesthetic will be injected. This may sting a little, but it will soon pass and you should not feel anything other than a little pushing for the remainder of the examination. The doctor will then make a small nick in the skin through which the biopsy needle will be inserted. You will hear a whirring noise as the samples are collected and may feel a vibration sensation. The biopsy should only take a few minutes. Once enough samples have been collected the needle will be removed. Pressure will be applied to the breast to stop any bleeding and reduce the chance of bruising, then a dressing will be applied. As soon as the wound is dressed and you feel able, you may return home.

If you experience pain or discomfort after the biopsy you can take simple painkillers such as paracetamol. Please avoid ibuprofen or aspirin which can make the bruising worse. Avoid lifting anything heavy and refrain from sport or housework for 24 hours. This will help the wound to heal. It is advisable to sleep with a non-wired (sports) bra for extra support the night after the biopsy and to keep the wound dry for at least 24 hours.

The endometrial biopsy involves being in a similar position to having a cervical smear test performed. Either lying on an examination couch and bringing your heels to your bottom and letting your legs flop out to the side or resting your legs in stirrups. Firstly, a speculum is inserted into the vagina to allow us to see the cervix (neck of the womb) and then a fine straw-like device is passed through the opening of the cervix into the lining of the womb. Using suction within the straw, a biopsy is then taken. Occasionally, the cervix is positioned at an angle and it may be necessary to apply an instrument to hold the cervix whilst the biopsy is taken. The procedure is generally very safe. The biopsy can cause some mild period type cramping pain, this generally settles once the procedure is finished, but some patients benefit from simple painkillers (paracetamol and ibuprofen) afterwards.

**Other clinic assessments**

Both groups will be asked to attend follow up appointments at Wythenshawe Hospital at months 6, 9 and 12 of the weight loss programme. These will include:

- Assessment of your weight and body fat using a special set of impedance scales
- Waist and hip measurements
- Blood and urine samples to assess cancer risk markers such as C-Reactive Protein and insulin levels
- Your blood pressure
• A seven-day food diary
• Questionnaires on topics such as: your health, physical activity levels and emotional well-being

What are the possible benefits of taking part?
There are no guaranteed benefits to you taking part in the study. You will receive personalised advice and support to undertake a weight loss programme which is not routinely available to women at risk of breast and endometrial cancer. You will be contributing to scientific knowledge about the benefits of weight loss for the prevention of breast and endometrial cancer.

The Oviva UK Ltd app is a novel way to receive frequent support from your healthcare professionals. The support aims to motivate you to get past barriers or difficulties that have previously stood in the way of successful weight loss. With this advice and adherence to the low-calorie diet on the study, you can expect to lose weight which is aimed at reducing your risk of breast and endometrial cancer. Weight loss is also likely to reduce your risk of 10 other weight related cancers and conditions including diabetes, heart disease, stroke and dementia.

The Nutritics Ltd app allows you to submit your food diary to the study team remotely. This can allow the study dietitians to provide quicker and more accurate dietary advice during the study.

The liquid diet replacement products used in this study are provided free of charge to you and delivered directly to your home address by Nestlé Health Science.

What are the possible disadvantages and risks of taking part?
Participation in the study will require around 13-14 hospital appointments over the duration of the study.

Women who have not had a mammogram in the previous 12 months will be asked to undertake one at study entry and all women will have a follow-up mammogram at the end of the 12-month weight loss programme. There is a very small risk of radiation-induced cancer developing later in life due to exposure to x-rays from mammograms. This study has been reviewed by an expert in radiation, who has assessed that it is safe to have two mammograms over a 12-16 month period. This increased frequency may identify cancers earlier but could also lead to additional investigations that turn out not to be cancer.

The breast biopsies may cause:
• Bruising in the breast following the procedure. We try to minimise bruising by placing firm pressure on the breast immediately after the biopsy. About 1 in 4 women develop minor bruising and 1 in 20 will develop moderate to severe bruising.
• Bleeding from the wound site: Rarely the biopsy site can start to bleed after you have left the unit. We will give you instructions on what to do if this happens.
• Infection of the wound site is very rare. The wound will be covered with a sterile dressing after the procedure and we will advise how to look after the area.
• Scarring: There will be a small scar where the needle is inserted but this should fade over time.

The endometrial biopsies can be uncomfortable and patients sometimes find intimate examinations like this embarrassing.
The breast and endometrial biopsies could potentially show that you have very early breast or endometrial cancer that you were not aware of. If this is the case you will be told straight away and you will not be allowed to continue in the study. You will be referred to the appropriate clinical specialist for further testing and treatment as per standard NHS guidance.

The blood samples taken from your arm which could result in slight discomfort or bruising.

There is a chance we could identify Type 2 Diabetes on the baseline blood tests that you were not aware of. A study doctor would inform you of any clinically significant results and ask your GP to manage this. As a result, you will not be able to continue in the study.

You may find the 850-calorie dietary replacement diet difficult to stick to. Our own work and that of others tell us that Optifast® meal replacements are unlikely to make you feel unwell. Some people experience hunger, feeling a little colder, bad breath, headache, light headedness, fatigue, nausea, and constipation. However, if you are well hydrated on these days, these feelings should subside as your body adjusts to the new regime. Other rare side effects reported in studies using Optifast® include diarrhoea (in 3 out of 100 users), dry skin (in 3 out of 100 users), hair loss (6 out of 1000 users) and gallstones (2 out of 1000 users).

Pregnancy
We advise that you should not become pregnant during the study, particularly whilst following the 850-calorie total diet replacement phase. Hormonal contraceptives will cause interference with the biopsy samples taken during the early phase of the study, therefore we will ask you to prevent pregnancy during this phase of the study using either using barrier methods (condoms/diaphragms) or abstinence (not having sexual intercourse). Once you have completed the biopsy phase, you can re-start hormonal-based contraceptives if you wish. If you did become pregnant during the study, you would be asked to stop the weight loss programme, increase your calorie intake and not continue in the study. The pregnancy would be followed up to the point of delivery and outcome of the pregnancy and any birth defects would be recorded and reported to the Sponsor of this study (MFT). The effects of weight loss/calorie restriction on a gestational foetus have not been studied, but severe calorie restriction during pregnancy might be harmful to mother and/or unborn child.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers directly, who will do their best to answer your questions or contact the Chief Investigator, Dr Sacha Howell on 0161 446 8347 or email Sacha.Howell@christie.nhs.uk. If you remain unhappy and wish to formally complain, you can do this by contacting the Manchester University NHS Foundation Trust Patient and Advice Liaison Service (PALS) on 0161 276 8686 who will deal with your complaint or give you further advice. In the event that something does go wrong and you are harmed during the research and this is due to someone’s negligence then you may have grounds for legal action for compensation against Manchester University NHS Foundation Trust, as per the NHS indemnity policy, but you may have to pay your legal costs. The normal NHS complaints mechanism will still be available to you (if appropriate). In the event of harm where there has been no negligence, there are no special arrangements for compensation.

Will my taking part in this study be kept confidential?
The Data Protection Act 2018 (DPA) came into force on 23rd May 2018. The DPA 2018 amends and updates the rights you have in relation to your personal data and what organisations that process your personal data...
are permitted and required to do. This patient information sheet provides you with further information of how Manchester University NHS Foundation Trust, as sponsor and data controller, uses your personal data if we receive any.

What is personal data?
“Personal data” is any information that can directly identify you, like your name or contact information. It can also be information that does not directly identify you, but if put together with other information available, your identity could be guessed. For example, if the information included your postcode, age and gender.

How will we use information about you?
We will need to use information from you (from your medical records and/or your GP) for this research project.

This information will include your:

- initials
- NHS number
- name
- contact details

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure. Once we have finished the study, we will keep some of the data so we can check the results.

What are your choices about how your information is used?

- You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.
- We need to manage your records in specific ways for the research to be reliable. This means that we won’t be able to let you see or change the data we hold about you.
- If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study (all data and samples are stored securely with no identifiable details at Manchester University NHS Foundation Trust and Manchester Cancer Research Centre).

Where can you find out more about how your information is used?
You can find out more about how we use your information

- at [www.hra.nhs.uk/information-about-patients/](http://www.hra.nhs.uk/information-about-patients/)
- our leaflet available from [https://www.hra.nhs.uk/planning-and-improving-research/policies-standards-legislation/data-protection-and-information-governance/gdpr-guidance/templates/template-wording-for-generic-information-document/](https://www.hra.nhs.uk/planning-and-improving-research/policies-standards-legislation/data-protection-and-information-governance/gdpr-guidance/templates/template-wording-for-generic-information-document/)
• by asking one of the research team
• by sending an email to dpo@mft.nhs.uk
• by ringing us on 0161 701 0375

You will have the option of logging into both the Oviva UK Ltd app and the Nutritics Ltd app which use your personal email address and password of your choosing. Your e-mail address will not be used for any other purposes and will not be passed on to any third party. If you agree to use the app you are choosing to share your dieting and health data with us via the app for the purpose of the study. The app is a secure communication channel, so your dieting and health information will only be accessible to the study team and will not be shared with other participants. Oviva UK Ltd and Nutritics Ltd have systems and procedures in place to ensure patient data is safe, and follow all rules of the Data Protection Act 2018. Oviva’s technology is secure with medical-grade encryption and Oviva UK Ltd have NHS Digital Information Governance Toolkit Level 2 Certification, meaning their system can be used by NHS organisations to store patient data. Nutritics Ltd is used already within the NHS and data is protected using 256-bit Secure Socket Layer (SSL) encryption. Other than your name, email address and password no other identifiable data will be stored by Oviva UK Ltd or Nutritics Ltd. Please note, your dieting and health data and messages to the study team are stored within these apps on your mobile device. If your mobile device is lost or stolen, there is a risk your data can be accessed. You will be encouraged to ensure your mobile device is passcode protected.

• Your study records and the content of any communication with your dietitian on the Oviva UK Ltd app will be kept strictly confidential and will not be sold to third parties, nor distributed. Your data on the app will be stored in a secure medical information data centre in Switzerland, which is outside the European Union.
• Data from the Nutritics Ltd app is stored on secure servers based in Ireland and Europe.

The Optifast® liquid meal replacements used in the study are purchased from Nestlé Health Science and will be posted directly to your home address for your convenience by them. We have a strict agreement in place with Nestlé Health Science to ensure that your personal information is used only for the purposes of this research, and that you will not be contacted directly by Nestlé Health Science once the study has finished.

Manchester University NHS Foundation Trust and the University of Manchester will use your name, NHS number and contact details to contact you about the research study and make sure that relevant information about the study is recorded for your care and to oversee the quality of the study. Individuals from Manchester University NHS Foundation Trust and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The only people in Manchester University NHS Foundation Trust and the University of Manchester who will have access to information that identifies you will be people who need to contact you to discuss your participation in the research or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

The University of Manchester and Manchester University NHS Foundation Trust will keep identifiable information about you from this study for 15 years after the study has finished. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study at screening, we will securely destroy any answered questionnaires you have completed. If you withdraw later on in the study, we will keep the
information we have already obtained in a secure database. To safeguard your rights, we will use the minimum personally-identifiable information possible.

The research team may be asked to share your anonymised data with other researchers. This is important to make the best use of your data for advancing knowledge in this area. Data sharing can enable new discoveries, encourage collaborative research and ensure scientific invention informs future healthcare policy. All data sharing uses linked anonymised data.

You can find out more about how the NHS uses your information at https://hra.nhs.uk/information-about-patients/

If you wish to raise a complaint on how the site or the sponsor has handled your personal data, you can contact our Data Protection Officer who will investigate the matter. If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner’s Office (ICO). Our Data Protection Officer can be contacted on: dpo@mft.nhs.uk

**Involvement of the General Practitioner/Family Doctor (GP)**

We will inform your GP of your participation in the study. If the study team need to confirm that it is safe for you to do more physical activity, we will ask you to seek approval from your GP. Results from the study assessments, e.g. blood pressure levels will be fed back to your GP with your consent. If you take medication for blood pressure, the weight loss programme may mean that you can reduce or discontinue these your GP will need to be informed of this. **You can only participate in the study if you agree that your GP can be informed.**

**What will happen to the biopsy and blood samples I give?**

Your breast and endometrial biopsy samples will be looked at under the microscope (histology) and tests will be performed by hospital doctors and scientists who are experts at performing these in routine clinical practice. The only difference is that these scientists and doctors will not know who you are, since your samples will be anonymised (not bear your name, only a number).

We will also ask you for your permission for us to store any tissue samples we take from you (such as blood or biopsy samples) for future research. If you agree they will be stored, without any personal details on, indefinitely at the Manchester Cancer Research Centre and ethical approval will be obtained before the samples are used for another unrelated research project.

**What will happen to the results of the research study?**

The results of this study will inform the basis of a PhD thesis and be published in a scientific journal. You will not be identified in any publication reporting the results of this study. If requested, you will be sent a summary of the results of this study.

**Who is organising and funding the research?**

This study is sponsored by Manchester University NHS Foundation Trust, who have reviewed the study and are happy for it to take place. Funding is from the Manchester Biomedical Research Centre and Cancer Research UK. The doctors, nurses and dietitians involved in this study will not be receiving any payments for including you in this study.
Expenses
We are able to offer reimbursement for reasonable travel expenses (car, bus or tram) linked to visits for this study including standard car park fees. There are no other payments for taking part.

Who has reviewed the study?
All research within the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by North West Preston Research Ethics Committee (Ref 20/NW/0095), IRAS project ID 274621.

Further information and contact details
For further information please contact:
Chief Investigator: Dr Sacha Howell on 0161 446 8347 or email Sacha.Howell@christie.nhs.uk
Clinical Research Fellow: Dr Helen Clarke 0161 291 4412 or email Helen.Clarke@manchester.ac.uk

Thank you for taking the time to read this information and considering whether nor not you would like to take part in this research study.
### BMJ Open

**The Prevention of Breast and Endometrial cancer using Total Diet Replacement (PROBE-TDR) Trial; Protocol for a Randomised Controlled Trial.**

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The Prevention of Breast and Endometrial cancer using Total Diet Replacement (PROBE-TDR) Trial; Protocol for a Randomised Controlled Trial.

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Keywords: breast, endometrium, cancer, diet therapy, risk reduction

Abstract

Introduction

Obesity and overweight are strong potentially modifiable risk factors for postmenopausal breast and endometrial cancer. Bariatric surgery can achieve considerable weight loss and risk reduction of weight-related cancer but is unlikely to be a feasible cancer prevention strategy. Total diet replacement can also lead to significant weight reduction. This study aims to examine the cellular and molecular changes in breast and endometrial tissue in high risk women following total diet replacement-induced weight loss, as well as long-term adherence to a 12-month TDR weight loss intervention.

Methods and analysis

PROBE-TDR; the PRevention Of Breast and Endometrial cancer using Total Diet Replacement is a prospective, non-blinded, randomised controlled trial of 47 women at increased risk of breast and/or endometrial cancer. Randomisation is 2:1 to either an immediate 12-month TDR weight loss programme (n=31) or delayed dietary intervention (control) (n=16). The TDR programme includes an initial 12-week period of total diet replacement (TDR) (850 kcal/day) followed by a 40-week food-based diet based on the nutritional principles of a Mediterranean diet as either continued weight loss (~1500 kcal/day) or weight loss maintenance (~2000kcal/day). Menstrual phase-matched biopsies of the breast and endometrium will be assessed at baseline and at the end of the 12-week TDR in the immediate diet group, compared to women randomised to the control group following their usual diet. The trial will also assess longer term adherence and weight loss success across the 12-month programme in both the immediate and control groups.
Ethics and dissemination

Approval for this study has been obtained from the Health Research Authority and Health and Care Research Wales (HCRW) [Approval 20/NW/0095] and the study has been registered with the International Standard Randomised Controlled Trial Number (ISRCTN15358157, assigned 11/05/2020). Results will be published in peer review journals presented at conferences and shared with trial participants.

Strengths and limitations of this study

- First study to evaluate the impact of diet-induced weight loss using TDR in both breast and endometrial tissues, in high risk women, with a two-stage design assessing tissue changes and longer term adherence.
- Provide proof of principle for weight loss and reduction in breast and endometrial cancer risk through biological measures and long-term adherence.
- Relatively small sample size may preclude adequate assessment of biomarker change if non-adherence to diet allocation or study procedures is greater than expected.
- Women joining this study are likely to be highly motivated and the adherence may not reflect that seen in the wider general population.
- Due to the small, pragmatic nature of the trial, we will not be able to formally address feasibility and cost effectiveness versus surgical management of weight loss.

Introduction

The 2018 World Cancer Research Fund report concludes there is convincing evidence for positive associations between obesity and twelve cancers (1). Breast cancer (BC) is the commonest cancer of women in the United Kingdom, affecting over 54,000 per year. Endometrial cancer (EC) is the fourth most common cancer in females in the UK, with around 9,500 new cases in 2017 and incidence rates increasing by over 50% since the early 1990s (1).

Maintained weight reductions of ~10% have been associated with reduced risk of postmenopausal breast (0.88 [0.79 to 09.98]) and endometrial cancer (0.72 [0.54 to 0.96]) (2). Furthermore, bariatric surgery, which typically achieves an average weight loss between 20 – 30%, has been shown to reduce the risk of breast and endometrial cancer by ~50% (3–5) with associated changes in endometrial cell proliferation (6). However, bariatric surgery is unlikely to be a feasible strategy for cancer prevention due to high patient burden and upfront economic costs.

Low energy formula total diet replacement (TDR) provides around 800 kcal/day and aims to restrict energy intake by around 60% compared to 25% energy restrictions with standard weight loss diets. A number of recent studies have demonstrated the utility of using low energy TDR diets to achieve significant weight loss in the management of general obesity (45% participants >10% weight loss) and weight loss leading to remission of type-2 diabetes (T2DM) (46% achieved remission at 12 months) (7,8). TDR interventions are projected to be cost effective in adults with obesity both with and without T2DM (9), although may be less cost effective than surgery in those with a BMI > 35kg/m² (10).

INTERCEPT (Impact of Diet-Induced Weight Loss on Biomarkers for Colorectal Cancer) studied an 8-week 800 kcal TDR in participants with a BMI >30kg/m² specifically for the purpose of...
evaluating risk reduction of colorectal cancer. At the end of the low energy diet period there was an average 14% weight loss, improvements in insulin sensitivity, blood lipid profiles and significant reductions in colorectal cell proliferation, measured as Ki67 expression, in serial mucosal biopsies (mean change -43.8%; P=0.027) (11).

To date, no clinical trial has evaluated the impact of weight loss on both the breast and endometrium in combination. This pragmatic RCT will assess the proof of principle of a dietitian-supported TDR programme for the prevention of breast and endometrial cancer in women with obesity. Firstly, changes in cell proliferation and other cellular and molecular changes in healthy breast and endometrial tissue at the end of the 12-week low energy diet will indicate any potential cancer risk reduction with energy restriction/weight loss in both organs. Secondly, dietary adherence and weight loss success throughout the 12-month intervention in both groups will inform the potential for longer term risk reduction in this high-risk population.

Role of the funding source

This work was jointly funded by NIHR Manchester BRC award [BRC-1215-20007] and Cancer Research UK via funding to the ARCTIC Clinical Academic Training Award [C19941/A28707] and Cancer Research UK Manchester Centre [C147/A25254]. This research is supported by the NIHR Manchester Clinical Research Facility. The funders of the study had no role in the study design or writing of the report. The corresponding author had final responsibility for the decision to submit for publication.

Methods and Analysis

Forty-seven female participants, at elevated risk of breast and/or endometrial cancer will be recruited primarily from a high-risk breast cancer risk prediction and prevention clinic at MFT and also from staff advertisements within MFT and The University of Manchester. This study will use a non-blinded randomised controlled design with participants randomised 2:1 to either an immediate dietary intervention group (n=31) or a delayed dietary intervention (control) group (n=16). The control (delayed intervention) comparator was included to increase confidence that biomarker changes at 3 months were due to weight loss and not confounders such as variation in the time of the cycle or tissue sampling. A 12-month control group was not justified given the large body of evidence for the efficacy of TDR compared to minimal intervention standard care(12). A 12-month control group was felt to be unethical given the requirement for 4 tissue biopsies, unacceptable to participants and would hinder recruitment. The 2:1 allocation to immediate versus delayed intervention was utilised to boost initial participation in the study.

Biopsies at the end of the 12-week TDR period will show the effects of successful short-term weight loss and the acute effects of energy restriction. Repeat biopsies at the end of weight loss maintenance would inform longer term biomarker effects. However, these measures were not included as they would present a large burden for patients in an already intensive study.

Weight loss across the 12-month intervention will inform long term adherence and success of the programme in our target population of at-risk premenopausal women. A priori criteria for good adherence would be 75% of low energy days undertaken in the initial 12-week TDR, and 75% retention, and 45% losing >10% weight loss at 12 months as reported in previous UK TDR trials(8). This is important as premenopausal women often have poorer weight loss outcomes than postmenopausal women within weight loss programmes due to competing priorities e.g. child care and work.

Clarke H et al (2021)
Inclusion criteria

- Women aged 30-50 years.
- BMI ≥30 kg/m² or ≥27.5 kg/m² in the Asian group.
- Pre-menopausal, with regular menstrual cycles.
- Ability to use the Oviva UK app OR access to a telephone.
- Willing to follow the TDR programme using Optifast® products.
- Maintain non-hormonal contraception (barrier or abstinence) until all biopsies completed.
- T2DM on diet control +/- metformin can be included.
- Participants must be able to read, understand and communicate in English.

Exclusion criteria

- Prior history of breast or endometrial cancer or preinvasive breast disease.
- Hormonal contraceptive use in the preceding 3 months.
- Preventative Tamoxifen or anti-progestin therapy within the last 6 months.
- Known carrier of the BRCA 1 or 2 gene.
- Confirmed pregnant at screening, planning pregnancy in the next 12 months, or current breast feeding.
- Taking prohibited medications including: Warfarin or novel anticoagulants (NOAC), low molecular weight heparin (LMWH) or equivalent anti-coagulants, anti-psychotic medication, anti-diabetic medication other than metformin, orlistat or other pharmacological treatments for weight loss and steroids (more than 20mg daily of prednisolone or its equivalent).
- Previous bariatric surgery for weight loss including gastric bypass and sleeve gastrectomy.
- Known hypersensitivity to any of the Optifast® ingredients (e.g. fish, milk, soy) or lactose intolerance.
- Substance abuse or harmful alcohol use as indicated by a score of 16 or above on the Alcohol Use Disorders Identification Test (AUDIT)(13).
- Diagnosis of an eating disorder, or patients with severe binge eating assessed by a score of 27 or more on the Binge Eating Scale (BES)(14).
- Severe depression assessed by a score of 15 or more on the Patient Health Questionnaire-9 (PHQ-9) questionnaire(15).
- Severe anxiety assessed by a score of 15 or more on the General Anxiety Disorder (GAD-7) questionnaire(16).
- Participants with psychiatric or physical comorbidity or scheduled for major surgery, which in the opinion of the treating medical physician, or the Chief Investigator (CI), would compromise their safety or adherence to the study.

Recruitment opened in September 2020 and closed in October 2021. The planned final participant follow-up visit due March 2023.

Patient Involvement

Prior to ethical submission we conducted a focus group including 4 premenopausal women, not associated with the study or study team, at increased risk of breast cancer to discuss the acceptability of an interventional prevention study including breast and endometrial biopsies.
The women felt that paired biopsies were acceptable but expressed concerns that additional biopsies would probably dissuade them from entering the study. A second group of premenopausal women reviewed the patient information sheet and consent form.

**Study Objective**

To determine the effects of weight loss with 12+/-4 weeks of Total Diet Replacement (TDR) on cell proliferation and other cancer risk markers in the breast and endometrium of women at increased risk of breast and/or endometrial cancer as compared to a usual diet control group.

**Primary outcome:**

The change in epithelial cell proliferation (Ki67) of the breast and endometrium from baseline to 12+/- 4 weeks after 12-weeks of TDR in the immediate diet group versus 12 weeks of normal diet in the control group.

**Secondary Outcomes:**

To determine changes in the following in the TDR group as compared to the (usual diet) control group over the 12+/- 4 weeks between biopsies:

- Weight, body fat and fat free mass (bioelectrical impedance Tanita MC980MA), waist and hip circumference.
- Biomarkers of cancer risk; (serum/plasma) fasting insulin, glucose, lipids, inflammatory markers, leptin, adiponectin, insulin-like growth factor -1 (IGF-1) and DNA methylation.
- Anxiety (Generalised Anxiety Disorder-7 [GAD-7] scale)(15), depression (Participant Health Questionnaire-9 [PHQ-9])(15), quality of life ( Obesity and Weight Loss Quality of Life [OWL-QOL])(17) and [EQ-5D-3L])(18), diet self-efficacy (Weight Efficacy Lifestyle Questionnaire Short Form [WEL-SF])(19).
- Dietary intake (energy, protein, fat, carbohydrate, fibres) [7-day semi-quantified paper food diary] analysed using Nutritics software (Dublin, Ireland).
- Physical activity (International Physical Activity Questionnaire [IPAQ] short form)(20).

To determine the following during the 12-month weight loss programme in both groups:

- Uptake and retention to the programme.
- Dietary adherence as the potential number of low energy days completed within the initial 12-week low energy diet phase.
- Adherence to the Mediterranean diet (energy, protein, fat, carbohydrate, fibre and alcohol) from -day food diaries and physical activity (IPAQ short form)(20) in the continued weight loss/weight maintenance phase.
- Anthropometric measures, cancer risk biomarkers and quality of life measures.
- Fidelity of delivery of the 12-month programme through the number and type of contacts (i.e. video or standard calls, messages) and total contact time per patient for each of the dietitian, psychologist and clinician within the multidisciplinary team.
- Adverse events.

**Exploratory Outcomes:**
The impact of weight loss with the TDR vs the usual diet control group at 12 weeks on changes in:

- Markers of cellular hierarchy in breast and endometrial tissues (LGR5, SOX9, KIT)
- Progenitor populations in the breast in response to TDR using FACS analysis and colony formation (mammosphere) assay.
- Transcriptional changes in both the breast and endometrium using RNA sequencing.
- We will also assess any changes in mammographic density between the baseline mammogram and mammogram at the end of the 12-month weight loss programme using quantitative automated techniques (Breast Imaging-Reporting and Data System [BIRADS], Volpara Health and predicted visual assessment of breast density [VAS]).

**Trial Procedures**

The trial has been developed, conducted and will be reported following the CONSORT guidelines(21). Potential participants were screened via telephone to confirm they met the required criteria for a baseline appointment. Consent is taken by a member of the trial team who is Good Clinical Practice (GCP) trained, experienced and who has been delegated by the Chief Investigator to undertake this activity. Additional verbal/written consent will be obtained prior to any trial-related procedures being undertaken. Screening procedures (see supplementary information) will be conducted at initial consent appointment and provisionally eligible participants will receive an appointment for a mammogram (if the participant has not had one performed in the preceding 12 months). Once blood and mammogram results are available, eligible participants are randomised and baseline breast and endometrial pipelle biopsies scheduled [Figure 1]. All screening, biopsies and other assessments are conducted in research, breast and gynaceological outpatient clinics at MFT.

**Figure 1:** Study schema illustrating participant journey through the screening, total diet replacement and continued weight loss/weight maintenance phases of the study.
Randomisation and blinding
Eligible patients are randomised 2:1 to the immediate diet or control group by a researcher independent of the intervention using a minimisation program (Sealed Envelope, London UK) stratified on the following criteria:

- Above or below projected median BMI of 35 kg/m^2
- Above or below projected median lifetime risk of breast cancer ≥17% remaining lifetime risk, with competing mortality (Tyrer-Cuzick)(22)

Due to the nature of the intervention, it is not possible to blind participants and clinicians to the treatment allocation. The trial endpoints will be assessed by staff who are independent from the research team delivering the intervention and where possible, they will be blinded to group allocation to minimise any potential bias. Laboratory tests will be assessed by staff who are blind to the intervention group and statistical analysis of anonymised data will be performed by staff independent from the research team to minimise any potential bias.

Study follow-up

Trial design overview is shown in figure 1. Participants from both groups will be supported weekly via the virtual platform (Oviva UK app) during the 12-month weight loss programme and will be reviewed every 3 months for assessment of weight, body composition, body measurements, diet and physical activity, quality of life and cancer risk biomarkers.

Participants may withdraw from the study at their own request or at the discretion of the chief investigation. Withdrawal from the study will not affect patient care.

Multi-disciplinary TDR Weight Loss Programme

The programme is described in full within the supplementary information and includes a TDR phase (weeks 0-12) followed by a diet reintroduction (DR) phase (weeks 12-16) and a weight maintenance/continued weight loss phase (weeks 17-52).

Participants who have attained both a weight loss of ≥15% and their target weight (which is likely to be a lower weight than that attained with a 15% weight loss) will be asked to follow their choice of either an isoenergetic intermittent or continuous weight maintenance diet. The intermittent diet includes one day of a food based very-low energy diet (VLED) (~850 kcal), and 6 days of an isoenergetic Mediterranean diet. The energy content of the Mediterranean diet will be determined by the trial dietitian based on the Mifflin equation(23), multiplied by the metabolic equivalents for their self-reported activity levels(24). The Mediterranean diet provides 30% energy from fat (15% monounsaturated fatty acids, 8% polyunsaturated fatty acids, 7% saturated fatty acids), 25% energy from protein and 45% from low glycaemic load wholegrain carbohydrates and includes at least 5 portions of vegetables, 2 portions of fruit/day, low fat dairy products, protein foods including fish, lean meat and pulses as described previously (25,26). Energy controlled Mediterranean diets are considered optimum for reducing weight, blood pressure and improving lipid profiles and have been linked to lower risk of cancers including breast cancer(27,28). The average energy intake over the week is ~2000 kcal. Participants will be given the option of including one meal replacement product per day in the first 6 months of the programme.
Participants who have not achieved the trial weight loss goal of >15%, or who wish to lose more than 15% will be asked to follow an energy restricted intermittent or continuous energy restricted food-based diet. The intermittent diet will involve two consecutive days of a food-based VLED (~850 kcal) and 5 days of an isoenergetic Mediterranean diet or 7 days of an energy restricted Mediterranean diet. The average energy intake over the week is ~1500 kcal/day.

**Relapse management for weight-gain (weeks 12 – 52)**

If participants regain ≥2kg (from self-reported weight data) they will be advised they can either resume the initial 850 kcal TDR for 2 weeks followed by 2 weeks of diet reintroduction or replace one meal a day with a meal replacement product. They will receive a booster call from the trial dietitian and additional support from the trial psychologist if required. Meal replacement products and additional support will be offered for the first 2 relapses, and dietitian/psychologist support only (not meal replacements) for any subsequent relapse to reduce their dependency on the TDR.

**Physical activity advice and support**

Physical activity advice will be delivered by the trial dietitian via phone/video call. The trial dietitian will check the participant’s responses to the Physical Activity Readiness Questionnaire (PAR-Q) (Canadian Society for Exercise Physiology)(29) to assess any contraindications to exercise. GP clearance may be required for certain participants.

Participants who are physically capable will be asked to follow a resistance exercise programme during the TDR phase, comprising two to four sets of 8 to 15 repetitions of arm, leg and trunk exercises three times per week over 12 weeks guided by online videos (Physiotec UK).

After the TDR phase participants will be encouraged to continue with the resistance exercises and also build up to between 150 – 300 minutes of moderate intensity physical activity/week e.g. brisk walking to promote health and continued weight loss or weight loss maintenance(30).

**Psychological Support**

Enhanced psychology support from the trial psychologist is offered to participants described within supplementary information 3. The psychological intervention will be centred on motivational interviewing, cognitive behavioural therapy, behavioural activation, mindfulness skills, distress tolerance skills and emotional regulation skills(31). Participants will be informed that specific issues disclosed to the psychologist will only be shared with the rest of the MDT with their agreement and if clinically relevant.

**Remote behavioural support**

Participants will receive individualised advice and will be supported remotely by their allocated dietitian and psychologist (where relevant) via the Oviva UK Ltd app or standard telephone call. The app facilitates written messages, self-monitoring of diet, weight, activity levels, blood pressure (where relevant) and an invitation to take part in peer support group messaging on
the app with other participants. Behaviour change techniques include goal setting, self-monitoring, timely personalised feedback on these records, rehearsing successful performance of behaviour, action planning and planning for how to deal with setbacks.

**Protocol delivery fidelity**

Dietitian support is conducted by specialist dietitians with experience of conducting dietary intervention studies using TDR and management of cancer risk. Variability in primary outcome assessments (body weight, cancer risk biomarkers) will be minimised by using calibrated equipment and quality-controlled assays.

**Measurements**

The measurements taken at each stage of the ProBE-TDR study are detailed in the supplementary information.

**Physical measurements**

Patients will be asked to abstain from alcohol and moderate physical activity for 24 hours prior to their appointment and be asked to attend in the fasted state (minimum 8 hour fast) but allowed low calorie fluids.

Height will be measured to the nearest millimeter, with the Frankfort horizontal plane, using a portable stadiometer (Chasmors Ltd, London). Body weight will be measured to the nearest 0.1 Kg in light clothing, without shoes or socks, using calibrated bio-impedance scales (Tanita, MC980MA) using a standardised protocol(32). Waist circumference is measured across the umbilicus and hip circumference will be measured over the participant’s underwear at the widest point over the buttocks to the nearest 0.5cm. Seated blood pressure will be measured in triplicate at rest with legs uncrossed for at least 10 minutes.

**Blood and urine sampling**

This will be performed at baseline to include: fasting insulin, glucose and lipids, leptin, adipokines, inflammatory cytokines including C-reactive protein and HbA1c. These will be repeated after 12+/-4 weeks of intervention/control and at 6, 9 and 12 months for both groups and month 15 for the control group. HbA1c will only be repeated at the end of the TDR phase and 6 months post TDR to limit costs. Bloods for oestrogen and progesterone will be taken at the time of the breast biopsies only. Urine samples will be obtained at screening and repeated at each 3 month visit for pregnancy testing and storage for potential future metabolomic analysis.

**Breast and endometrial biopsies**

Baseline vacuum assisted breast biopsy (VAB) and blood sampling for oestrogen and progesterone will be undertaken in the luteal phase (week before expected menstruation) of the menstrual cycle, endometrial sampling will be undertaken in the follicular phase (week following menstruation) of the menstrual cycle. The biopsies will be obtained by study
clinicians and the bloods by research staff. Breast and endometrial biopsies will be repeated after at least 8 weeks of TDR/usual diet in the same phases of the menstrual cycle as the baseline tests. Participants are recruited with a history of regular menstrual cycles. During the first 3-months of the study they self-report their menstrual pattern to researcher over 2-3 cycles to facilitate scheduling of the endometrial biopsies within the follicular phase of their cycle. The phase of cycle is then confirmed through an assessment of endometrial morphology by a Pathologist. Participants in the immediate dietary intervention group will remain on the TDR until they have undergone their post TDR breast and endometrial biopsies. Some participants may need to remain on TDR for up to 16 weeks if there are difficulties scheduling the repeat matched biopsies in line with their menstrual cycles.

Laboratory analyses

Change in proliferation from baseline to 3 months will be assessed by percentage epithelial Ki67 expression in the paraffin embedded tissue sections. Breast samples will be digested to single cell suspension and processed for mammosphere assay to provide readout of stem/progenitor activity. FACS analysis of the proportion of luminal progenitors (CD49f+/EPCAM+), differentiated luminal (CD49f-/EPCAM+), myoepithelial (CD49f+/EPCAM-) and adipose mesenchymal stem cells (MSC) (CD49f-/EPCAM-/CD73+/CD90+/CD105+) will determine the change in cell proportions with TDR vs controls. RNA sequencing will be performed on the breast and endometrial tissues to evaluate gene expression changes owing to weight loss.

Sample Size

Many randomised controlled trials with a continuous outcome adjust for the same variable at baseline using an analysis of covariance (ANCOVA). Thus, using summary data within the Breast Cancer Anti-Progestin Prevention Study (NCT02408770), in which premenopausal women also underwent luteal phase breast biopsies, we can use the mean(SD) luteal breast Ki67 as 4.99 (5.03) together with the sampsi command in STATA with the following code settings: sampsi 2.5 4.99, sd(5.03) pre(1) post(1) r01(.86) ratio(2) p(0.8). This code uses the two means 4.99, 2.5 (reflecting a 50% difference), a common SD of 5.03, a 2:1 allocation, a baseline correlation of 0.86 and power of 80%. With 13 controls and 26 subjects in the intervention group, the study will have 80% power at a 5% significance level to detect an adjusted mean difference in Ki67 at 3 months within the breast and endometrial biopsy samples. A difference of 50% or more in change in Ki67 between the control and intervention group has been chosen as this represents a clinically meaningful effect size for a mechanistic study(33). Incorporating a 20% loss to follow-up rate, the sample size increases to 16 controls and 31 in the intervention group.

Statistical analysis

The primary data analysis for change in Ki67 will be by intention to treat using ANCOVA with a 5% level of significance. This will be an unadjusted analysis comparing the outcome measured, controlling for the baseline value. We will also carry out a per protocol analysis as a secondary analysis. Missing data will be imputed via multiple imputation methods. The analysis will be conducted using SPSS and STATA.
Data analysis of the secondary endpoints will be by intention to treat and will not undertake any significance tests to compare the groups. Change scores (95% CI) for the secondary outcomes will be presented from within and between both groups. To determine secondary endpoints of changes in lifestyle/weight loss over time, linear mixed modelling will be used to assess the degree of change over time.

We will assess engagement with the TDR weight loss programme from the receipt of calls and use of Oviva app functions, e.g. self-monitoring.

**Data management**

The source data which comprises medical notes, electronic data sources (Oviva app, Nutritics [Dublin, Ireland]), case report form (CRF) and copies of the participant completed questionnaires are the primary source data. Participant data will be anonymised and will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act (2018) and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations (2006) as defined in the Kings Health Partners Clinical Trials Office Archiving SOP (17 Data Management). Deidentified data will be stored in a study-specific Research Electronic Data Capture (REDCap) database. The sponsor will periodically audit the site study file, a sample of CRFs, consent forms and source data and accuracy of the study database to ensure satisfactory completion.

**Patient safety**

Antihypertensive and diuretic drugs will be stopped on the day TDR is commenced for those who take these medications due to a pre-existing diagnosis of hypertension. This is a safety measure, because blood pressure is likely to fall on the diet due to rapid changes in weight. Individual clinical decisions may be necessary for a person’s best interest. In keeping with the recommendations for medicines management within the DiRECT study(7) those whose medications have been stopped will be monitored weekly using a home monitor and the participant can input their result within the Oviva app for the study clinician to review. Medication(s) for managing blood pressure will be reintroduced if clinically required [supplementary information 4] and communicated to the GP.

Participants taking metformin will be advised to remain on this medication for the duration of the study.

Adverse events will be monitored and graded monthly during the TDR phase for both groups. Also, during the normal diet phase for the control group using the National Cancer Institute CTCAE v5.0(34). This will indicate rates of potentially TDR associated and background rates of adverse events. Serious adverse events will be reported to the Research Ethics Committee (REC) and sponsor and documented within the participants medical record.

**Study Management**

The study management group comprises the chief investigator, project manager, clinical research fellow, radiology team and psychologist, who will jointly monitor study conduct and progress. All aspects of the study and all study personnel will adhere to the study protocol...
(version 4.0 or subsequent approved version) and Good Clinical Practice and Data Protection principles. Regular team meetings will ensure quick resolution of recruitment issues, study processes and data collection inconsistencies.

**Ethics and dissemination**

This study was adopted onto the National Institute for Health Research (NIHR) trial portfolio on 22 April 2020 and is sponsored by Manchester University NHS Foundation Trust (MFT). Any planned modifications to the protocol will be approved by the REC before they are adopted into the study. An audit trail of ethical amendments and documentation will be kept to allow monitoring by the research team and external regulatory bodies (table 1). The study was registered with an International Standard Randomised Controlled Trial Number on 11 May 2020.

### Table 1

| Protocol | Date     | Summary of Changes                                                                 |
|----------|----------|------------------------------------------------------------------------------------|
| V4       | 29.04.2021 | Collection of diet related side effects to include control group.                 |
|          |          | Offer use of Nutritics Ltd app to allow remote function for recording 7-day food diary. |
|          |          | Additional clinicians/research study staff.                                      |
| V3       | 14.10.2020 | Amendment to wording of patient information sheet cover letter to reflect remote review of patients during the covid-19 pandemic. |

Table 1: Summary of ethical amendments

Results will be disseminated through publication in peer-reviewed scientific journals, presentation at conferences and via charity websites.

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Authors’ contributions

SJH and EJC are the principal investigators. MH, RC, AH, SJH and EJC all contributed to the design of the study. HC is the responsible for the day-to-day running of the study, patient recruitment and consent and collection of gynaecological tissue samples. SJH and EJC provide study oversight and with BI, provide clinical guidance. MH and CL are responsible for delivery of the dietetic component of the study. AM, YYL, CP and SP are responsible for the collection of breast tissue samples. SK is responsible for study administration and data collection. KS contributed to protocol development and achieving ethical approval. HH is involved in scientific analyses of breast tissue samples. JW is responsible for the Psychological intervention and JB developed the statistical basis for the protocol.

HC drafted the initial manuscript. All authors critically reviewed and revised the manuscript and have read and approved the final version and contributed to the development and set-up of the study.

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Conflicts of Interests Statement

All authors declare no competing interests. MH and BI have received research funding for previous research associated with Nestle Health Care and Oviva, but no financial benefit from this research study.

Word Count: 4376 excluding abstract and references.
SUPPLEMENTARY INFORMATION 1

Screening procedures

| Study Procedure | Screening |
|-----------------|-----------|
| Review & discuss study participation | ✔ |
| Review inclusion/exclusion criteria | ✔ |
| Obtain informed consent | ✔ |
| Height | ✔ |
| Weight | ✔ |
| BMI | ✔ |
| Body fat & fat free mass (bioelectrical impedance) | ✔ |
| Waist/hip circumference | ✔ |
| Blood pressure | ✔ |
| Blood progesterone/oestrogen | ✔ |
| Study bloods | ✔ |
| Urine pregnancy test (if relevant) | ✔ |
| Urine sample for metabolomic analysis | ✔ |
| Mammogram | ✔ |
| Alcohol use (AUDIT) | ✔ |
| Self-Efficacy (WEL-SF) | ✔ |
| Binge Eating (BES) | ✔ |
| Patient Health (PHQ-9) | ✔ |
| Anxiety (GAD-7) | ✔ |
| Quality of life score (OWL-QoL) | ✔ |
| 7-day paper food diary/Nutritics app | ✔ |
| Physical activity score (IPAQ) | ✔ |
| Physical activity readiness questionnaire (PAR-Q) | ✔ |
| Demographic questionnaire | ✔ |
| Tyrer-Cuzick Breast Cancer Risk Questionnaire/Score | ✔ |
# Schedule of Assessments

## Table 2: Schedule of assessments – Control group

| Appointment                  | Screening | 0  | 1  | 2  | 3  | 4  | 5  | 6  |
|------------------------------|-----------|----|----|----|----|----|----|----|
| Study Procedure              |           |    |    |    |    |    |    |    |
| Review & discuss study       | ✓         |    |    |    |    |    |    |    |
| participation               |           |    |    |    |    |    |    |    |
| Review inclusion/exclusion   | ✓         | ✓  |    |    |    |    |    |    |
| criteria                     |           |    |    |    |    |    |    |    |
| Obtain informed consent      | ✓         |    |    |    |    |    |    |    |
| Height                       | ✓         |    |    |    |    |    |    |    |
| Weight                       | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  |    |    |
| BMI                          | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Body fat & fat free mass     | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| (bioelectrical impedance)    |           |    |    |    |    |    |    |    |
| Waist/hip circumference      | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Blood pressure               | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Blood                        |           |    |    |    |    |    |    |    |
| progesterone/oestrogen       | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Study bloods                 | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Urine pregnancy test (if    | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| relevant)                    |           |    |    |    |    |    |    |    |
| Urine (metabolomics)         | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Breast VAB                   | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Endometrial pipelle          | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Mammogram                    |           |    |    |    |    |    |    |    |
| Alcohol use (AUDIT)          | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Self-Efficacy (WEI-SF)       | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Binge Eating (BES)           | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Patient Health (PHQ-9)       | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Anxiety (GAD-7)              | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Quality of life score (OWL- | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| QoL)                         |           |    |    |    |    |    |    |    |
| 7-day paper food             | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| diary/Nutritics app          |           |    |    |    |    |    |    |    |
| Physical activity score      | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| (IPAQ)                       |           |    |    |    |    |    |    |    |
| Physical activity readiness  | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| questionnaire (PAR-Q)        |           |    |    |    |    |    |    |    |
| Demographic questionnaire    | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Tyrer-Cuzick BC risk         | ✓         | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| Adverse events screening     | ✓*        | ✓* | ✓* | ✓* | ✓* |    |    |    |
# Table 3: Schedule of assessments – Intervention group

| Appointment          | Screening | 1️⃣ 0️⃣ Baseline | 2️⃣ ~3️⃣ months | 3️⃣ ~6️⃣ months | 4️⃣ ~9️⃣ months | 5️⃣ ~12️⃣ months |
|----------------------|-----------|-------------------|-----------------|-----------------|-----------------|------------------|
| Review & discuss study participation | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Review inclusion/exclusion criteria | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Obtain informed consent | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Height               | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Weight               | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| BMI                  | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Body fat & fat free mass (bioelectrical impedance) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Waist/hip circumference | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Blood pressure       | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Blood progesterone/oestrogen | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Study bloods         | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Urine pregnancy test (if relevant) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Urine (metabolomics) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Breast VAB           | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Endometrial pipelle  | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Mammogram            | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Alcohol use (AUDIT)  | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Self-Efficacy (WEL-SF) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Binge Eating (BES)   | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Patient Health (PHQ-9) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Anxiety (GAD-7)      | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Quality of life score (OWL-QoL) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| 7-day paper food diary/Nutritics app | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Physical activity score (IPAQ) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Physical activity readiness questionnaire (PAR-Q) | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Demographic questionnaire | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Tyrer-Cuzick BC risk | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ | ✔️ |
| Adverse events screening | ✔️* | ✔️* | ✔️* | ✔️* | ✔️* | ✔️* |
### Table 4: Outline of the 52 week weight-loss programme

| PHASE | Dietitian advice/support | Clinical Psychologist support for a subset | Diet | Exercise |
|-------|--------------------------|------------------------------------------|------|----------|
| Total Diet Replacement (TDR) 12+/-4 weeks | Initial advice 60-90 minutes covering TDR, resistance exercises, use of the Oviva app video/standard call or face-to-face. 15-20 minute weekly support via video/standard call. Weekly support focussed on adherence to the TDR & resistance exercises, side effects, motivational interviewing and promoting self-monitoring on the app. | Enhanced psychology support for participants with baseline scores indicating moderate scores for binge eating (score 18-26 on BES), depression (score 10-14 on PHQ-9), anxiety (score 10-14 on GAD-7), low self-efficacy (score <45 on WEL-SF) or increasing risk of alcohol dependency (score 8-15 on AUDIT). | 7 days/week TDR 850 Kcal / day (3xOptifast® and 8 portions of non-starchy vegetables (~150 kcal) and 1 dessertspoon of vegetable oil (60 kcal). A minimum of 2-2.5 additional litres of energy-free liquids (i.e. water, diet drinks, black tea/coffee) Or a nutritionally equivalent food based LED if women are unable to tolerate the 12-week TDR. | Advised to undertake progressive resistance exercise 3 days/week. Week 1-4, 1 set of 10 reps. Week 5-8, 2 sets of 10 reps. Week 9-12, 3 sets of 10 reps. |
| Diet Reintroduction Phase (DRP) 4 weeks | Initial advice 40-60 minutes by video/standard call or face-to-face. 15-20 minute weekly support via App or phone. | Clinical psychologist support where required. | Gradual re-introduction of an energy restricted Mediterranean-based diet over 4 weeks. Participants may either follow a total food based approach or they can continue to include 1 or 2 Optifast® products per day. Energy intake will be increased in the following manner over the 4 weeks: | Advised to continue with resistance exercise 3 days/week. Build up to moderate intensity physical activity of 150-300 minutes/week. |

a. 1st week 1000kcal  
   b. 2nd week 1200 kcal  
   c. 3rd week 1400kcal  
   d. 4th week 1500kcal
| Continued Weight Loss Phase (CWLP) | 36 weeks | or Weight Maintenance Phase (WMP) | » if attained target weight. |
|-----------------------------------|----------|----------------------------------|----------------------------|
|                                   | 10 minutes per week via video/standard call. | Promoting self-monitoring motivational interviewing/managing relapse. | Preparing for future weight loss maintenance. |
| Interim food based diet: 850 kcal 2 days per week and Mediterranean diet 5 days per week or daily energy restricted Mediterranean diet. | Advised to continue with resistance exercise 3 days per week. Moderate intensity physical activity of 150-300 minutes/week. |
| Relapse intervention if weight increases >2kg assessed from self-monitoring during the CWL/WM phase. | Advice 40-60 minutes via video/standard call or face-to-face. Relapse management will include an exploration of the reasons for weight regain, with appropriate signposting for psychological support with the trial psychologist. | Up to 2 relapses; 2 weeks of TDR followed by 2 weeks of food reintroduction (Optifast® and non-starchy vegetables) ~850 kcal/day OR Replace one meal per day with Optifast® shake. | Continue with resistance exercise 3 days per week. Moderate intensity physical activity of 150-300 minutes per week. |
| Total weeks of programme          | 52 weeks |                                  |                            |
SUPPLEMENTARY INFORMATION 4

Medicines Management

**Background:** Antihypertensive and diuretic drugs will be stopped on the day Total Diet Replacement (TDR) is commenced. This is a safety measure, because blood pressure is likely to fall on the diet. This protocol lays out the standard approach to be followed, as taken from the DiRECT study. Individual clinical decisions may be necessary for a person’s best interest. The level of 140 mmHg is chosen to allow safe decisions during the weight loss period. After the Food Reintroduction period follow usual guidelines for management of hypertension. To simplify decision making, systolic pressure only is used as a guide to therapy even though both systolic and diastolic are relevant to long term benefit.

**Protocol:** When antihypertensive drugs are stopped, re-emphasise the importance of avoiding sodium (salt)

1. In the first 2 weeks after stopping antihypertensives and diuretics: If systolic BP over 165 mmHg on repeated measurement - restart one drug, as below.
2. Thereafter, if systolic BP is >140 mmHg - restart one drug as below.
3. Increase dose weekly to achieve target.
4. If systolic BP remains >140 mmHg on the first drug - add a second drug, as below.
5. Increase dose weekly to achieve target.
6. Repeat as necessary with third, fourth or more drugs (increasing each to maximum dose).

**Order of reintroduction of previously used drugs:**
1. ACE inhibitors (ramipril, lisinopril, perindopril, etc.)
2. Angiotensin receptor blockers (irbesartan, candesartan etc.)
3. Thiazide type (bendroflumethazide, indapamide etc.)
4. Spironolactone
5. Calcium channel blocker (nifedipine, amlodipine etc.)
6. Beta blocker (atenolol, labetolol etc.)
7. Alpha blocker (doxazosin, prazosin)
8. All others
**SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents**

| Section/item               | Item No | Description                                                                                                                                                                                                                                                                                                                                 | Addressed on page number |
|----------------------------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| **Administrative information** |         |                                                                                                                                                                                                                                                                                                                                             |                          |
| Title                      | 1       | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym                                                                                                                                                                                                                               | 1                        |
| Trial registration         | 2a      | Trial identifier and registry name. If not yet registered, name of intended registry                                                                                                                                                                                                                                                       | 1                        |
|                            | 2b      | All items from the World Health Organization Trial Registration Data Set                                                                                                                                                                                                                                                                   | NA                       |
| Protocol version           | 3       | Date and version identifier                                                                                                                                                                                                                                                  | 12                       |
| Funding                    | 4       | Sources and types of financial, material, and other support                                                                                                                                                                                                                                                                           | 3                        |
| Roles and responsibilities | 5a      | Names, affiliations, and roles of protocol contributors                                                                                                                                                                                                                                                                                    | 1                        |
|                            | 5b      | Name and contact information for the trial sponsor                                                                                                                                                                                                                                                                                    | 12                       |
|                            | 5c      | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 3                        |
|                            | 5d      | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)                                                                                                                                         | 11                       |
**Introduction**

| 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention |
| 6b | Explanation for choice of comparators |

**Objectives**

| 7 | Specific objectives or hypotheses |

**Trial design**

| 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) |

**Methods: Participants, interventions, and outcomes**

| 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained |
| 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) |
| 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered |
| 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) |
| 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) |
| 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial |
| 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended |
| 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) |
## Sample size

Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations

---

## Recruitment

Strategies for achieving adequate participant enrolment to reach target sample size

---

### Methods: Assignment of interventions (for controlled trials)

#### Allocation:

| Code | Description |
|------|-------------|
| 16a  | Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions |
| 16b  | Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned |
| 16c  | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions |
| 17a  | Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how |
| 17b  | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial |

---

### Methods: Data collection, management, and analysis

| Code | Description |
|------|-------------|
| 18a  | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol |
| 18b  | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols |
| Section                      | Number | Details                                                                                                                                                        |
|------------------------------|--------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data management              | 19     | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol |
| Statistical methods          | 20a    | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol |
|                              | 20b    | Methods for any additional analyses (eg, subgroup and adjusted analyses)                                                                                         |
|                              | 20c    | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) |
| Methods: Monitoring          |        |                                                                                                                                                                |
| Data monitoring              | 21a    | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed |
|                              | 21b    | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial |
| Harms                        | 22     | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct |
| Auditing                     | 23     | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor                           |
| Ethics and dissemination     |        |                                                                                                                                                                |
| Research ethics approval     | 24     | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval                                                                    |
| Protocol amendments         | 25     | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) |
| Item | Description |
|-----|-------------|
| 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) |
| 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable |
| 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial |
| 28 | Financial and other competing interests for principal investigators for the overall trial and each study site |
| 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators |
| 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation |
| 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions |
| 31b | Authorship eligibility guidelines and any intended use of professional writers |
| 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code |
| 32 | Model consent form and other related documentation given to participants and authorised surrogates |
| 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.*
The Prevention of Breast and Endometrial cancer using Total Diet Replacement (PROBE-TDR) Trial; Protocol for a Randomised Controlled Trial.

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| <b>Primary Subject Heading</b>: | Nutrition and metabolism |
| Secondary Subject Heading: | Obstetrics and gynaecology, Oncology |
| Keywords: | NUTRITION & DIETETICS, Gynaecological oncology < ONCOLOGY, Breast tumours < ONCOLOGY |
The Prevention of Breast and Endometrial cancer using Total Diet Replacement (PROBE-TDR) Trial; Protocol for a Randomised Controlled Trial.

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Keywords: breast, endometrium, cancer, diet therapy, risk reduction

Abstract

Introduction

Obesity and overweight are strong potentially modifiable risk factors for postmenopausal breast and endometrial cancer. Bariatric surgery can achieve considerable weight loss and risk reduction of weight-related cancer but is unlikely to be a feasible cancer prevention strategy. Total diet replacement can also lead to significant weight reduction. This study aims to examine the cellular and molecular changes in breast and endometrial tissue in high risk women following total diet replacement-induced weight loss, as well as long-term adherence to a 12-month TDR weight loss intervention.

Methods and analysis

PROBE-TDR; the PRevention Of Breast and Endometrial cancer using Total Diet Replacement is a prospective, non-blinded, randomised controlled trial of 47 women at increased risk of breast and/or endometrial cancer. Randomisation is 2:1 to either an immediate 12-month TDR weight loss programme (n=31) or delayed dietary intervention (control) (n=16). The TDR programme includes an initial 12-week period of total diet replacement (TDR) (850 kcal/day) followed by a 40-week food-based diet based on the nutritional principles of a Mediterranean diet as either continued weight loss (~1500 kcal/day) or weight loss maintenance (~2000kcal/day). Menstrual phase-matched biopsies of the breast and endometrium will be assessed at baseline and at the end of the 12-week TDR in the immediate diet group, compared to women randomised to the control group following their usual diet. The trial will also assess longer term adherence and weight loss success across the 12-month programme in both the immediate and control groups.
Ethics and dissemination

Approval for this study has been obtained from the Health Research Authority and Health and Care Research Wales (HCRW) [Approval 20/NW/0095] and the study has been registered with the International Standard Randomised Controlled Trial Number (ISRCTN15358157, assigned 11/05/2020). Results will be published in peer review journals presented at conferences and shared with trial participants.

Strengths and limitations of this study

- First study to evaluate the impact of diet-induced weight loss using TDR in both breast and endometrial tissues, in high risk women, with a two-stage design assessing tissue changes and longer term adherence.
- Provide proof of principle for weight loss and reduction in breast and endometrial cancer risk through biological measures and long-term adherence.
- Relatively small sample size may preclude adequate assessment of biomarker change if non-adherence to diet allocation or study procedures is greater than expected.
- Women joining this study are likely to be highly motivated and the adherence may not reflect that seen in the wider general population.
- Due to the small, pragmatic nature of the trial, we will not be able to formally address feasibility and cost effectiveness versus surgical management of weight loss.

Introduction

The 2018 World Cancer Research Fund report concludes there is convincing evidence for positive associations between obesity and twelve cancers (1). Breast cancer (BC) is the commonest cancer of women in the United Kingdom, affecting over 54,000 per year. Endometrial cancer (EC) is the fourth most common cancer in females in the UK, with around 9,500 new cases in 2017 and incidence rates increasing by over 50% since the early 1990s (2).

Maintained weight reductions of ~10% have been associated with reduced risk of postmenopausal breast (0.88 [0.79 to 09.98]) and endometrial cancer (0.72 [0.54 to 0.96]) (3). Furthermore bariatric surgery, which typically achieves an average weight loss between 20 – 30%, has been shown to reduce the risk of breast and endometrial cancer by ~50% (4–6) with associated changes in endometrial cell proliferation (7). However bariatric surgery is unlikely to be a feasible strategy for cancer prevention due to high patient burden and upfront economic costs.

Low energy formula total diet replacement (TDR) provides around 800 kcal/day and aims to restrict energy intake by around 60% compared to 25% energy restrictions with standard weight loss diets. A number of recent studies have demonstrated the utility of using low energy TDR diets to achieve significant weight loss in the management of general obesity (45% participants >10% weight loss) and weight loss leading to remission of type-2 diabetes (T2DM) (46% achieved remission at 12 months) (8,9). TDR interventions are projected to be cost effective in adults with obesity both with and without T2DM (10), although may be less cost effective than surgery in those with a BMI > 35kg/m² (11).

INTERCEPT (Impact of Diet-Induced Weight Loss on Biomarkers for Colorectal Cancer) studied an 8-week 800 kcal TDR in participants with a BMI >30kg/m² specifically for the purpose of
evaluating risk reduction of colorectal cancer. At the end of the low energy diet period there was an average 14% weight loss, improvements in insulin sensitivity, blood lipid profiles and significant reductions in colorectal cell proliferation, measured as Ki67 expression, in serial mucosal biopsies (mean change -43.8%; P=0.027) (12).

To date, no clinical trial has evaluated the impact of weight loss on both the breast and endometrium in combination. This pragmatic RCT will assess the proof of principle of a dietitian-supported TDR programme for the prevention of breast and endometrial cancer in women with obesity. Firstly, changes in cell proliferation and other cellular and molecular changes in healthy breast and endometrial tissue at the end of the 12-week low energy diet will indicate any potential cancer risk reduction with energy restriction/weight loss in both organs. Secondly, dietary adherence and weight loss success throughout the 12-month intervention in both groups will inform the potential for longer term risk reduction in this high-risk population.

Role of the funding source

This work was jointly funded by NIHR Manchester BRC award [BRC-1215-20007] and Cancer Research UK via funding to the ARCTIC Clinical Academic Training Award [C19941/A28707] and Cancer Research UK Manchester Centre [C147/A25254]. This research is supported by the NIHR Manchester Clinical Research Facility. The funders of the study had no role in the study design or writing of the report. The corresponding author had final responsibility for the decision to submit for publication.

Methods and Analysis

Forty-seven female participants, at elevated risk of breast and/or endometrial cancer will be recruited primarily from a high-risk breast cancer risk prediction and prevention clinic at MFT and also from staff advertisements within MFT and The University of Manchester. This study will use a non-blinded randomised controlled design with participants randomised 2:1 to either an immediate dietary intervention group (n=31) or a delayed dietary intervention (control) group (n=16). The control (delayed intervention) comparator was included to increase confidence that biomarker changes at 3 months were due to weight loss and not confounders such as variation in the time of the cycle or tissue sampling. A 12-month control group was not justified given the large body of evidence for the efficacy of TDR compared to minimal intervention standard care(13). A 12-month control group was felt to be unethical given the requirement for 4 tissue biopsies, unacceptable to participants and would hinder recruitment. The 2:1 allocation to immediate versus delayed intervention was utilised to boost initial participation in the study.

Biopsies at the end of the 12-week TDR period will show the effects of successful short-term weight loss and the acute effects of energy restriction. Repeat biopsies at the end of weight loss maintenance would inform longer term biomarker effects. However, these measures were not included as they would present a large burden for patients in an already intensive study.

Weight loss across the 12-month intervention will inform long term adherence and success of the programme in our target population of at-risk premenopausal women. A priori criteria for good adherence would be 75% of low energy days undertaken in the initial 12-week TDR, and 75% retention, and 45% losing >10% weight loss at 12 months as reported in previous UK TDR trials(9). This is important as premenopausal women often have poorer weight loss outcomes than postmenopausal women within weight loss programmes due to competing priorities e.g. child care and work.
Inclusion criteria

- Women aged 30-50 years.
- BMI ≥30 kg/m² or ≥27.5 kg/m² in the Asian group.
- Pre-menopausal, with regular menstrual cycles.
- Ability to use the Oviva UK app OR access to a telephone.
- Willing to follow the TDR programme using Optifast® products.
- Maintain non-hormonal contraception (barrier or abstinence) until all biopsies completed.
- T2DM on diet control +/- metformin can be included.
- Participants must be able to read, understand and communicate in English.

Exclusion criteria

- Prior history of breast or endometrial cancer or preinvasive breast disease.
- Hormonal contraceptive use in the preceding 3 months.
- Preventative Tamoxifen or anti-progestin therapy within the last 6 months.
- Known carrier of the BRCA 1 or 2 gene.
- Confirmed pregnant at screening, planning pregnancy in the next 12 months, or current breast feeding.
- Taking prohibited medications including: Warfarin or novel anticoagulants (NOAC), low molecular weight heparin (LMWH) or equivalent anti-coagulants, anti-psychotic medication, anti-diabetic medication other than metformin, orlistat or other pharmacological treatments for weight loss and steroids (more than 20mg daily of prednisolone or its equivalent).
- Previous bariatric surgery for weight loss including gastric bypass and sleeve gastrectomy.
- Known hypersensitivity to any of the Optifast® ingredients (e.g. fish, milk, soy) or lactose intolerance.
- Substance abuse or harmful alcohol use as indicated by a score of 16 or above on the Alcohol Use Disorders Identification Test (AUDIT)(14).
- Diagnosis of an eating disorder, or patients with severe binge eating assessed by a score of 27 or more on the Binge Eating Scale (BES)(15).
- Severe depression assessed by a score of 15 or more on the Patient Health Questionnaire-9 (PHQ-9) questionnaire(16).
- Severe anxiety assessed by a score of 15 or more on the General Anxiety Disorder (GAD-7) questionnaire(17).
- Participants with psychiatric or physical comorbidity or scheduled for major surgery, which in the opinion of the treating medical physician, or the Chief Investigator (CI), would compromise their safety or adherence to the study.

Recruitment opened in September 2020 and closed in October 2021. The planned final participant follow-up visit due March 2023.

Patient Involvement

Previous work from our group indicated that women with BMI >30kg/m² were willing to lose weight (94%), eat healthily (91%) and exercise more (87%) for the purposes of primary endometrial cancer prevention(18). Prior to ethical submission we conducted a focus group including 4
premenopausal women, not associated with the study or study team, at increased risk of breast cancer to discuss the acceptability of an interventional prevention study including breast and endometrial biopsies. The women felt that paired biopsies were acceptable but expressed concerns that additional biopsies would probably dissuade them from entering the study. A second group of premenopausal women reviewed the patient information sheet and consent form.

**Study Objective**

To determine the effects of weight loss with 12+-4 weeks of Total Diet Replacement (TDR) on cell proliferation and other cancer risk markers in the breast and endometrium of women at increased risk of breast and/or endometrial cancer as compared to a usual diet control group.

**Primary outcome:**

The change in epithelial cell proliferation (Ki67) of the breast and endometrium from baseline to 12+-4 weeks after 12-weeks of TDR in the immediate diet group versus 12 weeks of normal diet in the control group.

**Secondary Outcomes:**

To determine changes in the following in the TDR group as compared to the (usual diet) control group over the 12+-4 weeks between biopsies:

- Weight, body fat and fat free mass (bioelectrical impedance Tanita MC980MA), waist and hip circumference.
- Biomarkers of cancer risk; (serum/plasma) fasting insulin, glucose, lipids, inflammatory markers, leptin, adiponectin, insulin-like growth factor -1 (IGF-1) and DNA methylation.
- Anxiety (Generalised Anxiety Disorder-7 [GAD-7] scale)(16), depression (Participant Health Questionnaire-9 [PHQ-9])(16), quality of life (Obesity and Weight Loss Quality of Life [OWL-QOL])(19) and [EQ-5D-3L])(20), diet self-efficacy (Weight Efficacy Lifestyle Questionnaire Short Form [WEL-SF])(21).
- Dietary intake (energy, protein, fat, carbohydrate, fibres) [7-day semi-quantified paper food diary] analysed using Nutritics software (Dublin, Ireland).
- Physical activity (International Physical Activity Questionnaire [IPAQ] short form)(22).

To determine the following during the 12-month weight loss programme in both groups:

- Uptake and retention to the programme.
- Dietary adherence as the potential number of low energy days completed within the initial 12-week low energy diet phase.
- Adherence to the Mediterranean diet (energy, protein, fat, carbohydrate, fibre and alcohol) from -day food diaries and physical activity (IPAQ short form)(22) in the continued weight loss/weight maintenance phase.
- Anthropometric measures, cancer risk biomarkers and quality of life measures.
- Fidelity of delivery of the 12-month programme through the number and type of contacts (i.e. video or standard calls, messages) and total contact time per patient for each of the dietitian, psychologist and clinician within the multidisciplinary team.
- Adverse events.
Exploratory Outcomes:

The impact of weight loss with the TDR vs the usual diet control group at 12 weeks on changes in:

- Markers of cellular hierarchy in breast and endometrial tissues (LGR5, SOX9, KIT)
- Progenitor populations in the breast in response to TDR using FACS analysis and colony formation (mammosphere) assay.
- Transcriptional changes in both the breast and endometrium using RNA sequencing.
- We will also assess any changes in mammographic density between the baseline mammogram and mammogram at the end of the 12-month weight loss programme using quantitative automated techniques (Breast Imaging-Reporting and Data System [BI-RADS], Volpara Health and predicted visual assessment of breast density [VAS]).

Trial Procedures

The trial has been developed, conducted and will be reported following the CONSORT guidelines(23). Potential participants were screened via telephone to confirm they met the required criteria for a baseline appointment. Consent is taken by a member of the trial team who is Good Clinical Practice (GCP) trained, experienced and who has been delegated by the Chief Investigator to undertake this activity. Additional verbal/written consent will be obtained prior to any trial-related procedures being undertaken. Screening procedures [supplementary information 1] will be conducted at initial consent appointment and provisionally eligible participants will receive an appointment for a mammogram (if the participant has not had one performed in the preceding 12 months). Once blood and mammogram results are available, eligible participants are randomised and baseline breast and endometrial pipelle biopsies scheduled [Figure 1]. All screening, biopsies and other assessments are conducted in research, breast and gynacecological outpatient clinics at MFT.

Figure 1: Study schema illustrating participant journey through the screening, total diet replacement and continued weight loss/weight maintenance phases of the study.
Randomisation and blinding

Eligible patients are randomised 2:1 to the immediate diet or control group by a researcher independent of the intervention using a minimisation program (Sealed Envelope, London UK) stratified on the following criteria:

- Above or below projected median BMI of 35 kg/m²
- Above or below projected median lifetime risk of breast cancer ≥17% remaining lifetime risk, with competing mortality (Tyrer-Cuzick)(24)

Due to the nature of the intervention, it is not possible to blind participants and clinicians to the treatment allocation. The trial endpoints will be assessed by staff who are independent from the research team delivering the intervention and where possible, they will be blinded to group allocation to minimise any potential bias. Laboratory tests will be assessed by staff who are blind to the intervention group and statistical analysis of anonymised data will be performed by staff independent from the research team to minimise any potential bias.

Study follow-up

Trial design overview is shown in figure 1. Participants from both groups will be supported weekly via the virtual platform (Oviva UK app) during the 12-month weight loss programme and will be reviewed every 3 months for assessment of weight, body composition, body measurements, diet and physical activity, quality of life and cancer risk biomarkers.

Participants may withdraw from the study at their own request or at the discretion of the chief investigation. Withdrawal from the study will not affect patient care.

Multi-disciplinary TDR Weight Loss Programme

The programme is described in full within supplementary information 2 and includes a TDR phase (weeks 0-12) followed by a diet reintroduction (DR) phase (weeks 12-16) and a weight maintenance/continued weight loss phase (weeks 17-52).

Participants who have attained both a weight loss of ≥15% and their target weight (which is likely to be a lower weight than that attained with a 15% weight loss) will be asked to follow their choice of either an isoenergetic intermittent or continuous weight maintenance diet. The intermittent diet includes one day of a food based very-low energy diet (VLED) (~850 kcal), and 6 days of an isoenergetic Mediterranean diet. The energy content of the Mediterranean diet will be determined by the trial dietitian based on the Mifflin equation(25), multiplied by the metabolic equivalents for their self-reported activity levels(26). The Mediterranean diet provides 30% energy from fat (15% monounsaturated fatty acids, 8% polyunsaturated fatty acids, 7% saturated fatty acids), 25% energy from protein and 45% from low glycaemic load wholegrain carbohydrates and includes at least 5 portions of vegetables, 2 portions of fruit/day, low fat dairy products, protein foods including fish, lean meat and pulses as described previously (27,28). Energy controlled Mediterranean diets are considered optimum for reducing weight, blood pressure and improving lipid profiles and have been linked to lower risk of cancers including breast cancer(29,30). The average energy intake over the week is ~2000 kcal. Participants will be given the option of including one meal replacement product per day in the first 6 months of the programme.
Participants who have not achieved the trial weight loss goal of ≥15%, or who wish to lose more than 15% will be asked to follow an energy restricted intermittent or continuous energy restricted food-based diet. The intermittent diet will involve two consecutive days of a food-based VLED (~850 kcal) and 5 days of an isoenergetic Mediterranean diet or 7 days of an energy restricted Mediterranean diet. The average energy intake over the week is ~1500 kcal/day.

**Relapse management for weight-gain (weeks 12 – 52)**

If participants regain ≥2kg (from self-reported weight data) they will be advised they can either resume the initial 850 kcal TDR for 2 weeks followed by 2 weeks of diet reintroduction or replace one meal a day with a meal replacement product. They will receive a booster call from the trial dietitian and additional support from the trial psychologist if required. Meal replacement products and additional support will be offered for the first 2 relapses, and dietitian/psychologist support only (not meal replacements) for any subsequent relapse to reduce their dependency on the TDR.

**Physical activity advice and support**

Physical activity advice will be delivered by the trial dietitian via phone/video call. The dietitians have previous experience and in-house training in delivering physical activity interventions from in-house physiotherapists and a cancer exercise specialist. The trial dietitian will check the participant’s responses to the Physical Activity Readiness Questionnaire (PAR-Q) (Canadian Society for Exercise Physiology)(31) to assess any contraindications to exercise. GP clearance will be requested where required.

Participants who are physically capable will be asked to follow a progressive resistance exercise programme during the TDR phase, comprising one to three sets of 8 to 15 repetitions of arm, leg and trunk exercises three times per week over 12 weeks. Exercises are described in a written booklet and demonstrated through online videos (Physiotec UK). Progression will be tailored to their previous level of fitness and reviewed during their calls with the dietitian. Adherence to the exercise programme is recorded from self-reporting to the trial dietitian.

After the TDR phase participants will be encouraged to continue with the resistance exercises and also build up to between 150 – 300 minutes of moderate intensity physical activity per week e.g. brisk walking to promote health and continued weight loss or weight loss maintenance(32).

**Psychological Support**

Enhanced psychology support from the trial psychologist is offered to participants described within supplementary information 2. The psychological intervention will be centred on motivational interviewing, cognitive behavioural therapy, behavioural activation, mindfulness skills, distress tolerance skills and emotional regulation skills(33). Participants will be informed that specific issues disclosed to the psychologist will only be shared with the rest of the MDT with their agreement and if clinically relevant.

**Remote behavioural support**
Participants will receive individualised advice and will be supported remotely by their allocated dietitian and psychologist (where relevant) via the Oviva UK Ltd app or standard telephone call. The app facilitates written messages, self-monitoring of diet, weight, activity levels, blood pressure (where relevant) and an invitation to take part in peer support group messaging on the app with other participants. Behaviour change techniques include goal setting, self-monitoring, timely personalised feedback on these records, rehearsing successful performance of behaviour, action planning and planning for how to deal with setbacks.

**Protocol delivery fidelity**

Dietitian support is conducted by specialist dietitians with experience of conducting dietary intervention studies using TDR and management of cancer risk. Variability in primary outcome assessments (body weight, cancer risk biomarkers) will be minimised by using calibrated equipment and quality-controlled assays.

**Measurements**

The measurements taken at each stage of the ProBE-TDR study are detailed in supplementary information 3.

**Physical measurements**

Patients will be asked to abstain from alcohol and moderate physical activity for 24 hours prior to their appointment and be asked to attend in the fasted state (minimum 8 hour fast) but allowed low calorie fluids.

Height will be measured to the nearest milimeter, with the Frankfort horizontal plane, using a portable stadiometer (Chasmors Ltd, London). Body weight will be measured to the nearest 0.1 Kg in light clothing, without shoes or socks, using calibrated bio-impedance scales (Tanita, MC980MA) using a standardised protocol(34). Waist circumference is measured across the umbilicus and hip circumference will be measured over the participant’s underwear at the widest point over the buttocks to the nearest 0.5cm. Seated blood pressure will be measured in triplicate at rest with legs uncrossed for at least 10 minutes.

**Blood and urine sampling**

This will be performed at baseline to include: fasting insulin, glucose and lipids, leptin, adipokines, inflammatory cytokines including C-reactive protein and HbA1c. These will be repeated after 12+/−4 weeks of intervention/control and at 6, 9 and 12 months for both groups and month 15 for the control group. HbA1c will only be repeated at the end of the TDR phase and 6 months post TDR to limit costs. Bloods for oestrogen and progesterone will be taken at the time of the breast biopsies only. Urine samples will be obtained at screening and repeated at each 3 month visit for pregnancy testing and storage for potential future metabolomic analysis.

**Breast and endometrial biopsies**
Baseline vacuum assisted breast biopsy (VAB) and blood sampling for oestrogen and progesterone will be undertaken in the luteal phase (week before expected menstruation) of the menstrual cycle, endometrial sampling will be undertaken in the follicular phase (week following menstruation) of the menstrual cycle. The biopsies will be obtained by study clinicians and the bloods by research staff. Breast and endometrial biopsies will be repeated after at least 8 weeks of TDR/usual diet in the same phases of the menstrual cycle as the baseline tests. Participants are recruited with a history of regular menstrual cycles. During the first 3-months of the study they self-report their menstrual pattern to researcher over 2-3 cycles to facilitate scheduling of the endometrial biopsies within the follicular phase of their cycle. The phase of cycle is then confirmed through an assessment of endometrial morphology by a Pathologist. Participants in the immediate dietary intervention group will remain on the TDR until they have undergone their post TDR breast and endometrial biopsies. Some participants may need to remain on TDR for up to 16 weeks if there are difficulties scheduling the repeat matched biopsies in line with their menstrual cycles.

**Laboratory analyses**

Change in proliferation from baseline to 3 months will be assessed by percentage epithelial Ki67 expression in the paraffin embedded tissue sections. Breast samples will be digested to single cell suspension and processed for mammosphere assay to provide readout of stem/progenitor activity. FACS analysis of the proportion of luminal progenitors (CD49f+/EPCAM+), differentiated luminal (CD49f-/EPCAM+), myoepithelial (CD49f+/EPCAM-) and adipose mesenchymal stem cells (MSC) (CD49f-/EPCAM- /CD73+/CD90+/CD105+) will determine the change in cell proportions with TDR vs controls. RNA sequencing will be performed on the breast and endometrial tissues to evaluate gene expression changes owing to weight loss.

**Sample Size**

Many randomised controlled trials with a continuous outcome adjust for the same variable at baseline using an analysis of covariance (ANCOVA). Thus, using summary data within the Breast Cancer Anti-Progestin Prevention Study (NCT02408770), in which premenopausal women also underwent luteal phase breast biopsies, we can use the mean(SD) luteal breast Ki67 as 4.99 (5.03) together with the sampsi command in STATA with the following code settings: sampsi 2.5 4.99, sd(5.03) pre(1) post(1) r01(.86) ratio(2) p(0.8). This code uses the two means 4.99, 2.5 (reflecting a 50% difference), a common SD of 5.03, a 2:1 allocation, a baseline correlation of 0.86 and power of 80%. With 13 controls and 26 subjects in the intervention group, the study will have 80% power at a 5% significance level to detect an adjusted mean difference in Ki67 at 3 months within the breast and endometrial biopsy samples. A difference of 50% or more in change in Ki67 between the control and intervention group has been chosen as this represents a clinically meaningful effect size for a mechanistic study(35). Incorporating a 20% loss to follow-up rate, the sample size increases to 16 controls and 31 in the intervention group.

**Statistical analysis**

The primary data analysis for change in Ki67 will be by intention to treat using ANCOVA with a 5% level of significance. This will be an unadjusted analysis comparing the outcome measured, controlling for the baseline value. We will also carry out a per protocol analysis as
a secondary analysis. Missing data will be imputed via multiple imputation methods. The analysis will be conducted using SPSS and STATA.

Data analysis of the secondary endpoints will be by intention to treat and will not undertake any significance tests to compare the groups. Change scores (95% CI) for the secondary outcomes will be presented from within and between both groups. To determine secondary endpoints of changes in lifestyle/weight loss over time, linear mixed modelling will be used to assess the degree of change over time.

We will assess engagement with the TDR weight loss programme from the receipt of calls and use of Oviva app functions, e.g. self-monitoring.

Data management

The source data which comprises medical notes, electronic data sources (Oviva app, Nutritics [Dublin, Ireland]), case report form (CRF) and copies of the participant completed questionnaires are the primary source data. Participant data will be anonymised and will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act (2018) and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations (2006) as defined in the Kings Health Partners Clinical Trials Office Archiving SOP (17 Data Management). Deidentified data will be stored in a study-specific Research Electronic Data Capture (REDCap) database. The sponsor will periodically audit the site study file, a sample of CRFs, consent forms and source data and accuracy of the study database to ensure satisfactory completion.

Patient safety

Antihypertensive and diuretic drugs will be stopped on the day TDR is commenced for those who take these medications due to a pre-existing diagnosis of hypertension. This is a safety measure, because blood pressure is likely to fall on the diet due to rapid changes in weight. Individual clinical decisions may be necessary for a person’s best interest. In keeping with the recommendations for medicines management within the DiRECT study(8) those whose medications have been stopped will be monitored weekly using a home monitor and the participant can input their result within the Oviva app for the study clinician to review. Medication(s) for managing blood pressure will be reintroduced if clinically required [supplementary information 4] and communicated to the GP.

Participants taking metformin will be advised to remain on this medication for the duration of the study.

Adverse events will be monitored and graded monthly during the TDR phase for both groups. Also, during the normal diet phase for the control group using the National Cancer Institute CTCAE v5.0(36). This will indicate rates of potentially TDR associated and background rates of adverse events. Serious adverse events will be reported to the Research Ethics Committee (REC) and sponsor and documented within the participants medical record.

Study Management
The study management group comprises the chief investigator, project manager, clinical research fellow, radiology team and psychologist, who will jointly monitor study conduct and progress. All aspects of the study and all study personnel will adhere to the study protocol (version 4.0 or subsequent approved version) and Good Clinical Practice and Data Protection principles. Regular team meetings will ensure quick resolution of recruitment issues, study processes and data collection inconsistencies.

**Ethics and dissemination**

This study was adopted onto the National Institute for Health Research (NIHR) trial portfolio on 22 April 2020 and is sponsored by Manchester University NHS Foundation Trust (MFT). Any planned modifications to the protocol will be approved by the REC before they are adopted into the study. An audit trail of ethical amendments and documentation will be kept to allow monitoring by the research team and external regulatory bodies (table 1). The study was registered with an International Standard Randomised Controlled Trial Number on 11 May 2020.

| Protocol | Date       | Summary of Changes                                                                 |
|----------|------------|------------------------------------------------------------------------------------|
| V4       | 29.04.2021 | Collection of diet related side effects to include control group.                  |
|          |            | Offer use of Nutritics Ltd app to allow remote function for recording 7-day food diary. |
|          |            | Additional clinicians/research study staff.                                         |
| V3       | 14.10.2020 | Amendment to wording of patient information sheet cover letter to reflect remote review of patients during the covid-19 pandemic. |

Table 1: Summary of ethical amendments

Results will be disseminated through publication in peer-reviewed scientific journals, presentation at conferences and via charity websites.

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Authors’ contributions

SJH and EJC are the principal investigators. MH, RC, AH, SJH and EJC all contributed to the design of the study. HC is the responsible for the day-to-day running of the study, patient recruitment and consent and collection of gynaecological tissue samples. SJH and EJC provide study oversight and with BI, provide clinical guidance. MH and CL are responsible for delivery of the dietetic component of the study. AM, YYL, CP and SP are responsible for the collection of breast tissue samples. SK is responsible for study administration and data collection. KS contributed to protocol development and achieving ethical approval. HH is involved in scientific analyses of breast tissue samples. JW is responsible for the Psychological intervention and JB developed the statistical basis for the protocol.
HC drafted the initial manuscript. All authors critically reviewed and revised the manuscript and have read and approved the final version and contributed to the development and set-up of the study.

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**Conflicts of Interests Statement**

All authors declare no competing interests. MH and BI have received research funding for previous research associated with Nestle Health Care and Oviva, but no financial benefit from this research study.

**Word Count**: 4384 excluding abstract and references.
SUPPLEMENTARY INFORMATION 1

Screening procedures

| Study Procedure                                                                 | Screening |
|--------------------------------------------------------------------------------|-----------|
| Review & discuss study participation                                         | ✓         |
| Review inclusion/exclusion criteria                                           | ✓         |
| Obtain informed consent                                                       | ✓         |
| Height                                                                         | ✓         |
| Weight                                                                         | ✓         |
| BMI                                                                             | ✓         |
| Body fat & fat free mass (bioelectrical impedance)                           | ✓         |
| Waist/hip circumference                                                        | ✓         |
| Blood pressure                                                                 | ✓         |
| Blood progesterone/oestrogen                                                   | ✓         |
| Study bloods                                                                    | ✓         |
| Urine pregnancy test (if relevant)                                            | ✓         |
| Urine sample for metabolomic analysis                                         | ✓         |
| Mammogram                                                                      | ✓         |
| Alcohol use (AUDIT)                                                           | ✓         |
| Self-Efficacy (WEL-SF)                                                         | ✓         |
| Binge Eating (BES)                                                            | ✓         |
| Patient Health (PHQ-9)                                                         | ✓         |
| Anxiety (GAD-7)                                                                | ✓         |
| Quality of life score (OWL-QoL)                                                | ✓         |
| 7-day paper food diary/Nutritics app                                          | ✓         |
| Physical activity score (IPAQ)                                                | ✓         |
| Physical activity readiness questionnaire (PAR-Q)                             | ✓         |
| Demographic questionnaire                                                      | ✓         |
| Tyrer-Cuzick Breast Cancer Risk Questionnaire/Score                           | ✓         |
## SUPPLEMENTARY INFORMATION 2

Multi-disciplinary TDR Weight Loss Programme

| PHASE                                  | Dietitian advice/support                                                                 | Clinical Psychologist support for a subset                                                                 | Diet                                            | Exercise                                                                 |
|----------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------------------------------|-------------------------------------------------------------------------|
| **Total Diet Replacement (TDR)**       | Initial advice 60-90 minutes covering TDR, resistance exercises, use of the Oviva app video/standard call or face-to-face. 15-20 minute weekly support via video/standard call. Weekly support focussed on adherence to the TDR & resistance exercises, side effects, motivational interviewing and promoting self-monitoring on the app. | Enhanced psychology support for participants with baseline scores indicating moderate scores for binge eating (score 18-26 on BES), depression (score 10-14 on PHQ-9), anxiety (score 10-14 on GAD-7), low self-efficacy (score <45 on WEL-SF) or increasing risk of alcohol dependency (score 8-15 on AUDIT). | 7 days/week TDR 850 Kcal / day (3xOptifast® and 8 portions of non-starchy vegetables (~150 kcal) and 1 dessertspoon of vegetable oil (60 kcal). A minimum of 2-2.5 additional litres of energy-free liquids (i.e. water, diet drinks, black tea/coffee) Or a nutritionally equivalent food based LED if women are unable to tolerate the 12-week TDR. | Advised to undertake progressive resistance exercise 3 days/week. Week 1-4, 1 set of 10 reps. Week 5-8, 2 sets of 10 reps. Week 9-12, 3 sets of 10 reps. |
| **Diet Reintroduction Phase (DRP)**    | Initial advice 40-60 minutes by video/standard call or face-to-face. 15-20 minute weekly support via App or phone. | Clinical psychologist support where required.                                                                 | Gradual re-introduction of an energy restricted Mediterranean-based diet over 4 weeks. Participants may either follow a total food based approach or they can continue to include 1 or 2 Optifast® products per day. Energy intake will be increased in the following manner over the 4 weeks:  
  a. 1st week 1000kcal  
  b. 2nd week 1200 kcal  
  c. 3rd week 1400kcal  
  d. 4th week 1500kcal | Advised to continue with resistance exercise 3 days/week. Build up to moderate intensity physical activity of 150-300 minutes/week. |
| Continued Weight Loss Phase (CWLP) | 36 weeks or Weight Maintenance Phase (WMP) | 10 minutes per week via video/standard call. Promoting self-monitoring motivational interviewing/managing relapse. Preparing for future weight loss maintenance. | Intermittent food based diet: 850 kcal 2 days per week and Mediterranean diet 5 days per week or daily energy restricted Mediterranean diet. Intermittent food based diet: 850 kcal 1 day per week and Mediterranean diet 6 days per week or isoenergetic Mediterranean diet 7 days per week. Advised to continue with resistance exercise 3 days per week. Moderate intensity physical activity of 150-300 minutes/week. |
|-----------------------------------|------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Relapse intervention if weight increases >2kg assessed from self-monitoring during the CWL/WM phase. | Advice 40-60 minutes via video/standard call or face-to-face. Relapse management will include an exploration of the reasons for weight regain, with appropriate signposting for psychological support with the trial psychologist. | Up to 2 relapses; 2 weeks of TDR followed by 2 weeks of food reintroduction (Optifast® and non-starchy vegetables) ~850 kcal/day OR Replace one meal per day with Optifast® shake. | Continue with resistance exercise 3 days per week. Moderate intensity physical activity of 150-300 minutes per week. |
| Total weeks of programme | **52 weeks** |  |  |

**For peer review only**

ProBE-TDR Study Protocol Paper Supplementary Information

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## Schedule of Assessments

### Table 3: Schedule of assessments – Control group

| Appointment                  | Screening | 0 Baseline | 2–3 months | 3–6 months | 4–9 months | 5–12 months | 6–15 months |
|------------------------------|-----------|------------|------------|------------|------------|-------------|-------------|
| Review & discuss study participation | ✓         |            |            |            |            |             |             |
| Review inclusion/exclusion criteria | ✓         | ✓          |            |            |            |             |             |
| Obtain informed consent      | ✓         |            |            |            |            |             |             |
| Height                       | ✓         |            |            |            |            |             |             |
| Weight                       | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| BMI                          | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Body fat & fat free mass (bioelectrical impedance) | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Waist/hip circumference      | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Blood pressure               | ✓         | ✓          |             |            |            |             |             |
| Blood progesterone/oestrogen | ✓         |             |            |            |            |             |             |
| Study bloods                 | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Urine pregnancy test (if relevant) | ✓         |             |            |            |            |             |             |
| Urine (metabolomics)         | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Breast VAB                   | ✓         | ✓          |             |            |            |             |             |
| Endometrial pipelle          | ✓         | ✓          |             |            |            |             |             |
| Mamammogram                  | ✓         | ✓          |             |            |            |             |             |
| Alcohol use (AUDIT)          | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Self-Efficacy (WEL-SF)       | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Binge Eating (BES)           | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Patient Health (PHQ-9)       | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Anxiety (GAD-7)              | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Quality of life score (OWL-QoL) | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| 7-day paper food diary/Nutritics app | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Physical activity score (IPAQ) | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Physical activity readiness questionnaire (PAR-Q) | ✓         | ✓          | ✓          | ✓          | ✓          |             |             |
| Demographic questionnaire    | ✓         |            |            |            |            |             |             |
| Tyrer-Cuzick BC risk         | ✓         |            |            |            |            |             |             |
| Adverse events screening     | ✓*        | ✓*         | ✓*         |             |            |             |             |
| Study Procedure                                      | Screening | 1 0 Baseline | 2 ~3 months | 3 ~6 months | 4 ~9 months | 5 ~12 months |
|------------------------------------------------------|-----------|--------------|-------------|-------------|-------------|-------------|
| Review & discuss study participation                 | ✓         |              |             |             |             |             |
| Review inclusion/exclusion criteria                   | ✓         |              |             |             |             |             |
| Obtain informed consent                               | ✓         |              |             |             |             |             |
| Height                                                | ✓         |              |             |             |             |             |
| Weight                                                | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| BMI                                                   | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Body fat & fat free mass (bioelectrical impedance)   | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Waist/hip circumference                               | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Blood pressure                                        | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Blood progesterone/oestrogen                          | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Study bloods                                          | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Urine pregnancy test (if relevant)                    | ✓         | ✓            | ✓           |             |             |             |
| Urine (metabolomics)                                  | ✓         | ✓            | ✓           | ✓           |             |             |
| Breast VAB                                            | ✓         | ✓            |             |             |             |             |
| Endometrial pipelle                                   | ✓         | ✓            |             |             |             |             |
| Mammogram                                             | ✓         | ✓            |             |             |             |             |
| Alcohol use (AUDIT)                                   | ✓         | ✓            | ✓           | ✓           | ✓           | ✓           |
| Self-Efficacy (WEL-SF)                                | ✓         | ✓            | ✓           | ✓           |             |             |
| Binge Eating (BES)                                    | ✓         | ✓            | ✓           | ✓           |             |             |
| Patient Health (PHQ-9)                                | ✓         | ✓            | ✓           |             |             |             |
| Anxiety (GAD-7)                                       | ✓         | ✓            | ✓           |             |             |             |
| Quality of life score (OWL-QoL)                       | ✓         | ✓            | ✓           |             |             |             |
| 7-day paper food diary/Nutritics app                  | ✓         | ✓            | ✓           |             |             |             |
| Physical activity score (IPAQ)                        | ✓         | ✓            | ✓           |             |             |             |
| Physical activity readiness questionnaire (PAR-Q)      | ✓         |             |             |             |             |             |
| Demographic questionnaire                             | ✓         | ✓            |             |             |             |             |
| Tyrer-Cuzick BC risk                                   | ✓         | ✓            |             |             |             |             |
| Adverse events screening                              | ✓*        | ✓*           | ✓*          |             |             |             |
SUPPLEMENTARY INFORMATION 4

Medicines Management

**Background:** Antihypertensive and diuretic drugs will be stopped on the day Total Diet Replacement (TDR) is commenced. This is a safety measure, because blood pressure is likely to fall on the diet. This protocol lays out the standard approach to be followed, as taken from the DiRECT study. Individual clinical decisions may be necessary for a person’s best interest. The level of 140 mmHg is chosen to allow safe decisions during the weight loss period. After the Food Reintroduction period follow usual guidelines for management of hypertension. To simplify decision making, systolic pressure only is used as a guide to therapy even though both systolic and diastolic are relevant to long term benefit.

**Protocol:** When antihypertensive drugs are stopped, re-emphasise the importance of avoiding sodium (salt)

1. In the first 2 weeks after stopping antihypertensives and diuretics: If systolic BP over 165 mmHg on repeated measurement - restart one drug, as below.
2. Thereafter, if systolic BP is >140 mmHg - restart one drug as below.
3. Increase dose weekly to achieve target.
4. If systolic BP remains >140 mmHg on the first drug - add a second drug, as below.
5. Increase dose weekly to achieve target.
6. Repeat as necessary with third, fourth or more drugs (increasing each to maximum dose).

**Order of reintroduction of previously used drugs:**

1. ACE inhibitors (ramipril, lisinopril, perindopril, etc.)
2. Angiotensin receptor blockers (irbesartan, candesartan etc.)
3. Thiazide type (bendroflumethazide, indapamide etc.)
4. Spironolactone
5. Calcium channel blocker (nifedipine, amlodipine etc.)
6. Beta blocker (atenolol, labetolol etc.)
7. Alpha blocker (doxazosin, prazosin)
8. All others
## SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item                  | Item No | Description                                                                 | Addressed on page number |
|-------------------------------|---------|-----------------------------------------------------------------------------|--------------------------|
| **Administrative information**|         |                                                                             |                          |
| Title                         | 1       | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1                        |
| Trial registration            | 2a      | Trial identifier and registry name. If not yet registered, name of intended registry | 1                        |
|                               | 2b      | All items from the World Health Organization Trial Registration Data Set      | NA                       |
| Protocol version              | 3       | Date and version identifier                                                  | 12                       |
| Funding                       | 4       | Sources and types of financial, material, and other support                  | 3                        |
| Roles and responsibilities    | 5a      | Names, affiliations, and roles of protocol contributors                      | 1                        |
|                               | 5b      | Name and contact information for the trial sponsor                           | 12                       |
|                               | 5c      | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 3                        |
|                               | 5d      | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 11                       |

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Introduction

Background and rationale 6a Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention 2

6b Explanation for choice of comparators 3

Objectives 7 Specific objectives or hypotheses 5

Trial design 8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) 3, 6

Methods: Participants, interventions, and outcomes

Study setting 9 Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained 6

Eligibility criteria 10 Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) 4

Interventions 11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered 7, supplementary 3

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) 8, supplementary 3

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) 3, 4, 5

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial NA

Outcomes 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended 5, 6

Participant timeline 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) 6
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations |
|-------------|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size |
|             |    | NA |

**Methods: Assignment of interventions (for controlled trials)**

**Allocation:**

| Sequence generation | 16a | Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions |
|---------------------|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned |
| Implementation      | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions |

**Blinding (masking):**

| Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how | 17a | Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how |
| If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial | 17b | NA |

**Methods: Data collection, management, and analysis**

| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol |
|                        | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols |

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Data management

Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol

Statistical methods

Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol

Methods for any additional analyses (eg, subgroup and adjusted analyses)

Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

Methods: Monitoring

Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial

Harms

Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct

Auditing

Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

Plans for seeking research ethics committee/institutional review board (REC/IRB) approval

Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
| Section | Item | Description | Page |
|---------|------|-------------|------|
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 6 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | supplementary file (consent form) |
| Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 11 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 15 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 11 |
| Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | NA |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 12 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | NA |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | NA |

**Appendices**

| Section | Item | Description | Page |
|---------|------|-------------|------|
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Supplementary documents |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | 9,10 |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.*
The TIDieR (Template for Intervention Description and Replication) Checklist:
Information to include when describing an intervention and the location of the information

| Item number | Item | Where located ** |
|-------------|------|------------------|
|             | BRIEF NAME |                 |
| 1.          | Provide the name or a phrase that describes the intervention. | In methods / Multi-disciplinary TDR Weight Loss Programme |
|             | WHY |     |
| 2.          | Describe any rationale, theory, or goal of the elements essential to the intervention. | In methods / supplementary information 3 |
|             | WHAT |     |
| 3.          | Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of intervention providers. Provide information on where the materials can be accessed (e.g. online appendix, URL). | In methods / supplementary information 3 |
| 4.          | Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities. | In methods / supplementary information 3 |
|             | WHO PROVIDED |     |
| 5.          | For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given. | In methods / supplementary information 3 |
|             | HOW |     |
| 6.          | Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group. | In methods / supplementary information 3 |

**Where located**
- **Primary paper**
  - (page or appendix number)
- **Other † (details)**
7. Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features.

WHEN and HOW MUCH

8. Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose.

TAILORING

9. If the intervention was planned to be personalised, titrated or adapted, then describe what, why, when, and how.

MODIFICATIONS

10. If the intervention was modified during the course of the study, describe the changes (what, why, when, and how).

HOW WELL

11. Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.

12. Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.

**Authors** - use N/A if an item is not applicable for the intervention being described. **Reviewers** – use ‘?’ if information about the element is not reported/not sufficiently reported.

† If the information is not provided in the primary paper, give details of where this information is available. This may include locations such as a published protocol or other published papers (provide citation details) or a website (provide the URL).

‡ If completing the TIDieR checklist for a protocol, these items are not relevant to the protocol and cannot be described until the study is complete.

* We strongly recommend using this checklist in conjunction with the TIDieR guide (see BMJ 2014;348:g1687) which contains an explanation and elaboration for each item.

* The focus of TIDieR is on reporting details of the intervention elements (and where relevant, comparison elements) of a study. Other elements and methodological features of studies are covered by other reporting statements and checklists and have not been duplicated as part of the TIDieR checklist. When a randomised trial is being reported, the TIDieR checklist should be used in conjunction with the CONSORT statement (see www.consort-statement.org) as an extension of Item 5 of the CONSORT 2010 Statement. When a clinical trial protocol is being reported, the TIDieR checklist should be used in conjunction with the SPIRIT statement as an extension of Item 11 of the SPIRIT 2013 Statement (see www.spirit-statement.org). For alternate study designs, TIDieR can be used in conjunction with the appropriate checklist for that study design (see www.equator-network.org).