

Formulation and validation of Reducose as a functional food ingredient to improve metabolic health

Protocol for twelve-week study on glucose, insulin, haemoglobin A1c, satiety, body composition, lipid profile, inflammatory markers and changes in gut microbiome in overweight individuals

Oxford Brookes Centre for Nutrition and Health (OxBCNH)
Faculty of Health and Life Sciences
Oxford Brookes University
Gipsy Lane Campus
Headington
Oxford OX3 0BP

Tel: 01865 483297

Email: OxBCNH@brookes.ac.uk

<http://www.brookes.ac.uk/oxbcnh>

Aim

The aim of this placebo-controlled study is to examine the effect of mulberry leaf-derived food ingredient (Reducose®) on glucose levels, insulin, Haemoglobin A1c (HbA1c), satiety, lipid profile, body composition, blood pressure, inflammatory markers and changes in gut microbiome in overweight individuals.

Study design

A randomised, placebo-controlled parallel design trial will be used to study the effect of twelve-week consumption of mulberry leaf-derived food ingredient (Reducose®) on glucose levels, insulin, HbA1c, satiety, lipid profile, body composition, inflammatory markers and changes in gut microbiome.

The trial will be conducted by the Oxford Brookes Centre for Nutrition and Health (OxBCNH). Ethical approval for the trial will be obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University. Participants will be given full details of the study protocol and the opportunity to ask questions. All participants will give written informed consent prior to participation.

Participants

A sample size of 50 overweight participants aged 18-60 years will be recruited in each group aiming to have a complete data set for 40 participants. Participants will be excluded from the study if they meet any of the following criteria:

- Aged < 18 or > 60 years
- Body mass index (BMI) <25 kg/m² or >29.9 kg/m²
- Body fat percentages < 20%¹ for males, <30% for females
- HbA_{1c} >6.5%
- Any known food allergy or intolerance to the placebo or test product
- Medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- Known history of diabetes mellitus or the use of antihyperglycaemic drugs or insulin to treat diabetes and related conditions

¹ Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR & Sakamoto Y. Healthy percentage body fat ranges: An approach

for developing guidelines based on body mass index. *American Journal of Clinical Nutrition* 2000; 72(3):694–701.

- Major medical or surgical event requiring hospitalization within the preceding 3 months
- Use of steroids, protease inhibitors or antipsychotics (all of which have major effects on glucose metabolism and body fat distribution)
- Volunteers self-reporting as currently dieting (including ketogenic) or having lost >5% body weight in the previous year
- Volunteers who have significantly changed their physical activity in the past 2-4 weeks or who intend to change during the study
- Participation in another experimental study or receipt of an investigational drug/product within 30 days of the screening visit
- Participants with restricted or abnormal eating behaviour
- Regular intake of a food supplement with effects on glucose metabolism and satiety (e.g. prebiotics, proteins, chromium, cinnamon, berberine, biotin)
- Smoker in the last 3 months
- Heavy alcohol consumers, more than 14 units per week (14 units is equivalent to 6 pints of average-strength beer or 10 small glasses of low-strength wine).
- Have access to an iPhone 7 or above, or an android 5.0 with NFC (Near-Field Communication – able to make contactless payments).

In addition, participants will be excluded if they are unable to comply with experimental procedures or do not follow testing guidelines.

Products

The placebo (cellulose) and test product (mulberry leaf-derived food ingredient (Reducose®) will be administered to the participants twice a day (250 mg each) before the biggest meal of the day and before dinner.

Protocol

The study will be conducted by Good Clinical Practice (GCP) certified researchers at OxBCNH and will comprise a twelve-week intervention period. Prior to the start of the intervention, a screening visit will be arranged for participants to ask any questions and to complete the consent form. During this session, after giving consent, participants will be asked to complete a health questionnaire as part of the screening. Their height, weight, BMI and body composition (fat and muscle percentage) will be measured to determine their eligibility to take part. A 3 ml venous blood sample will be drawn to check for HbA1c. The screening visit will last for approximately 30 minutes.

If eligible to take part in the study, the participants will be randomly assigned to one of the two parallel groups according to the randomization order provided by Phynova Ltd. Participants will be provided with a 12-week supply of the placebo/test product. They will be instructed to take two daily doses of the test product/placebo every day, once at dinner and once at the next largest meal of the day, for 12-weeks. Participants will also be instructed to keep the study product packaging and bring them to their study visits in order to assess compliance. Additionally, the researchers will make telephone calls or send text message reminders to the participants 3-4 times in a week to confirm compliance. The participants are not required to change their usual diets, however they will be asked to keep a food diary using Nutritics Libro App and limit their total calories to maximum of 2000kcal/day for women and 2500kcal per day for men.

There will be five study visits in total (Week 1, Week 2, Week 6, Week 10 & Week 12) at the laboratory

at OxBCNH. In Week 1, Week 6 and Week 12, a blood sample will be taken to test glucose, insulin, HbA1c, lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), and inflammatory markers. Blood pressure, body weight, waist and hip circumference and body fat measurements will also be taken during the three study visits in Week 1, Week 6 & Week 12. The participants will be fitted with a continuous glucose monitoring system [CGMS) during their first visit to monitor their glucose levels during the first two weeks of the study period. They will be asked to manually scan the sensor daily at least once every 8 hours to capture the 24-hour available glucose data. There will be visits to OxBCNH, at the end of Week 2 to remove the CGMS from the first visit and in Week 10 to fit the CGMS for the last two weeks. Participants will also receive standard nutrition counseling on their first visit. They will consume a standardised breakfast, before 9am, followed by a standardized lunch at noon, with controlled levels of carbohydrate, fat and protein content, on day 2 and the designated satiety test days in Week 1, Week 6 and Week 12. During the Week 1, Week 6 and Week 12 satiety test days, the participants will take Reducose® before breakfast and dinner. They will complete questionnaires to measure satiety during those days. Glucose data will also be collected for the first and last two weeks of the study period to compare any differences between the placebo and test product groups. The participants will also record daily food diaries during this period when glucose data is collected. To determine any changes in gut microbiome, stool samples will be collected by participants and sent off for analysis in Week 1 and Week 12.

Study visit 1 in Week 1

Participants will be instructed to observe a 10-12 hour overnight fast prior to attending study visit 1, although water will be allowed in moderation, they will be advised not to consume more than one litre. They will be asked to restrict their intake of alcohol and caffeine-containing drinks and to restrict their participation in intense physical activity (for example, long periods at the gym, excessive swimming, running and aerobics) for 24 hours prior to this visit. Additionally, they will be asked to keep their mode of transport to OxBCNH the same on the visit days. On arrival (day 1), a 15 ml venous blood sample will be drawn to measure HbA1c, blood lipids, glucose, insulin and inflammatory markers. Blood pressure readings, waist and hip circumference will also be taken and participants' body fat and body weight measures will be taken using bioelectrical impedance analysis (BIA). The participants will then be fitted with a continuous glucose monitoring system (CGMS), which will be activated before sending them home with a standardised breakfast and lunch for the next two days. They will be given a supply of either placebo or test product for the first 6 weeks of the study. On day 2, the participants will consume the standardised breakfast and lunch. On Day 3, the participants will start consuming the placebo/test product daily for 12 weeks. The participants will take a stool sample at home to be sent for microbiome analysis between day 1 and day 3. On day 3, the participants will consume the standardised breakfast and lunch and complete the satiety questionnaire during the day. The participants will continue to wear the CGMS and record daily food diaries for the first two weeks to collect glucose data. Participants will receive nutritional counseling and be instructed to limit their daily calorie intake to a maximum of 2000 kcal for women and 2500 kcal for men. They will track their intake using the Nutritics Libro app.

Visit 2 in Week 2

The participants will return to OxBCNH to have their CGMS removed after the first two weeks and return the empty product packaging to assure compliance.

Visit 3 in Week 6

The participants will return to OxBCNH and have their body weight, blood pressure, waist and hip circumference and body fat measurements taken following a 10-12 hour fast. A venous blood sample will be taken to measure HbA1c, blood lipids, glucose, insulin and inflammatory markers and they will receive a supply of either placebo or test product for the final 6 weeks of the study as well as a standard breakfast and lunch. They will repeat the satiety questionnaire following a standardised breakfast and lunch on the day following the study visit.

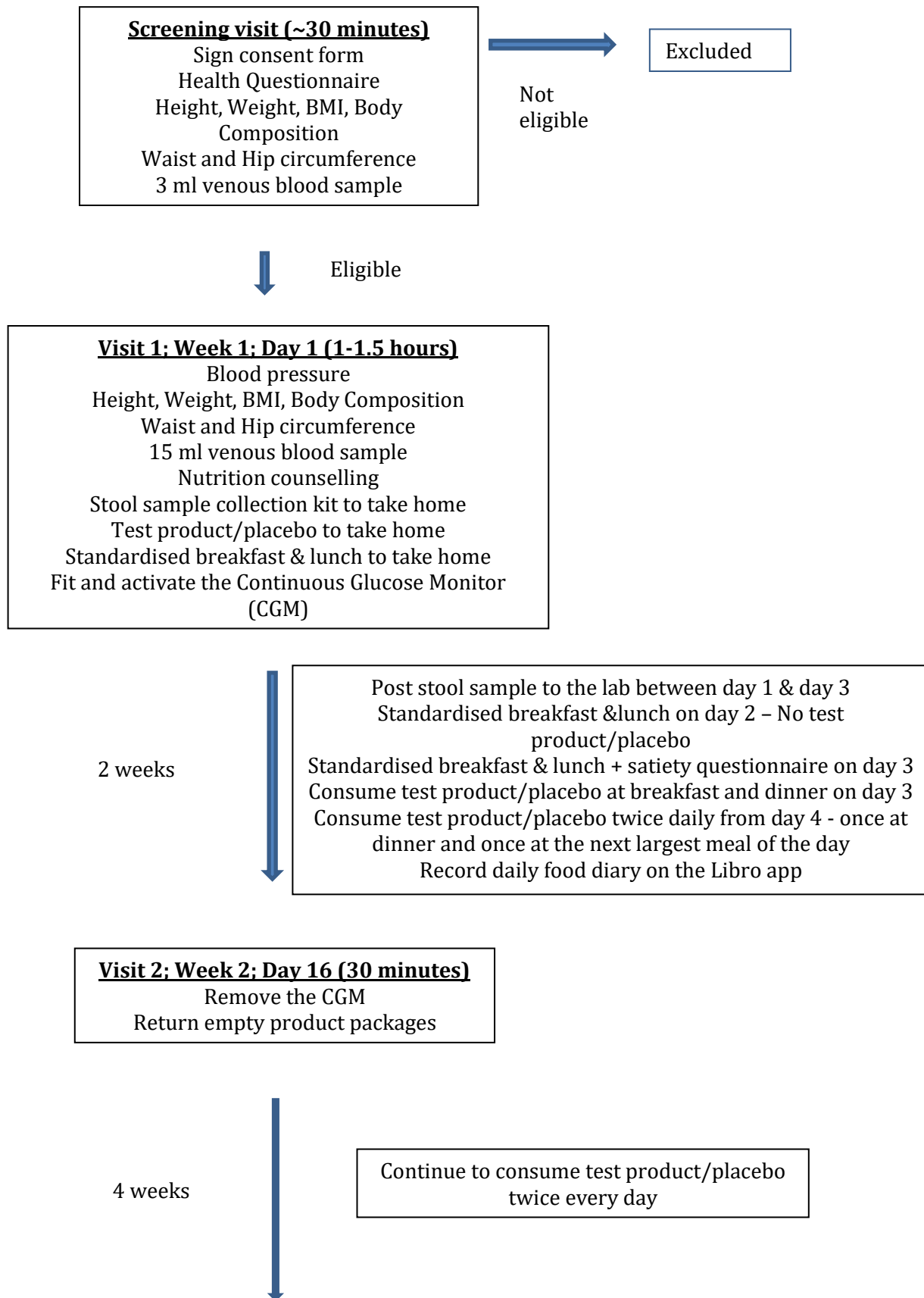
Visit 4 in Week 10

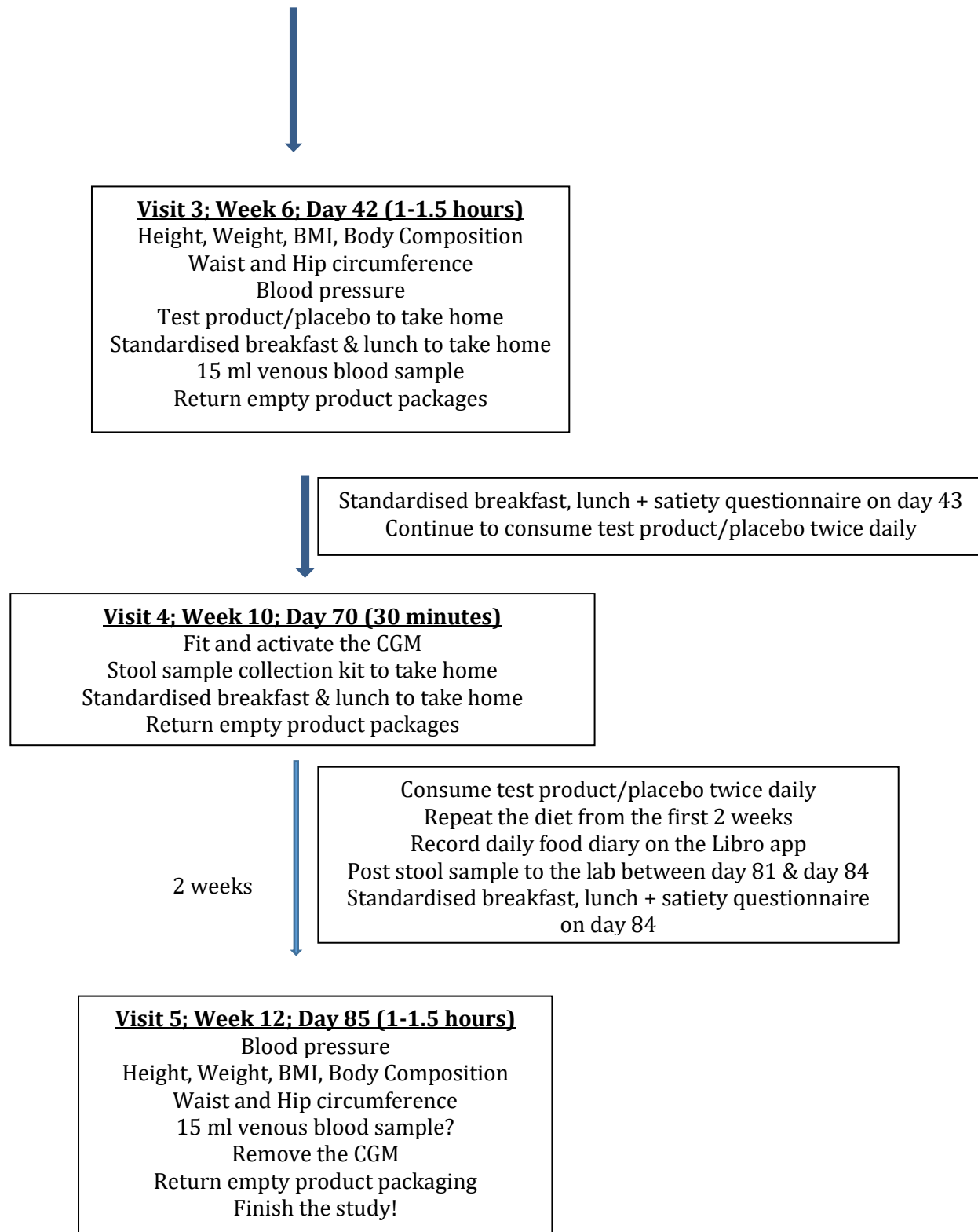
The participants will return to OxBCNH to have their CGMS fitted to collect glucose data for the last two weeks of the trial and return the empty product packaging from weeks 6-10. In these weeks participants will be instructed to match their diets from the first two weeks as recorded in their food diaries. They will be sent home with the standardised breakfast and lunch for the last satiety test as well as the stool sample collection kit for week 12.

Study visit 5 in Week 12

The participants will arrive after a 10-12 hour fast and provide a venous blood sample to measure HbA1c, glucose, insulin, blood lipids and inflammatory markers. Blood pressure readings, waist and hip circumference, body weight and body fat measures will also be taken and the CGMS will be removed. The participants will take a stool sample at home to be sent for microbiome analysis. They will also repeat the measurement of satiety using questionnaires following a standardised breakfast and lunch, on the day before visit 5. They will also need to return their remaining empty product packaging from weeks 10-12,

A flow diagram of the study protocol is given below





Statistical analysis

Data will be analysed using the IBM Statistical Package for the Social Sciences (SPSS, version 25 or above). Data will be presented as mean, standard deviation (SD) and standard error of the mean (SEM) values. Prior to statistical analysis, the normality of the data will be tested using the Shapiro-Wilks statistic. Parametric or non-parametric tests will be used as appropriate to compare the following results between placebo and test product groups:

- Body weight, body fat, waist and hip circumference
- Glucose concentrations, iAUC
- Blood pressure
- Satiety questionnaire
- HbA1c, lipid profile, insulin, glucose, leptin and adiponectin profile and inflammatory markers (CRP, TNF- α , IL-6)
- Microbiome profile
- Microbiome metabolites

Comparisons for the above results will be made:

- i) Between Week 1, Week 6 and Week 12 within each parallel group
- ii) Between the changes from Week 1 to Week 6 and Week 1 to Week 12 in the two parallel groups. Statistical significance will be set at $P < 0.05$.
- iii) Between each group and their respective baseline

Outcomes

- The primary outcome will be the changes from Week 1 to Week 12 in glucose parameters, between the two groups after consumption of placebo or test product for twelve weeks.
- The secondary outcomes will be changes from Week 1 to Week 12 in , satiety, HbA1c, lipid profile, insulin, blood pressure, glucose time in range, inflammatory markers, waist and hip circumference, body fat, microbiome profile and microbiome metabolites after consumption of placebo or test product for twelve weeks.