You are invited to take part in a research study. Before you decide whether to volunteer, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Please do not hesitate to contact us (see below) if there is anything that is not clear or if you would like more information.

**What is the purpose of this study?**
High blood sugar levels (hyperglycaemia) increases the risk of developing diabetes and cardiovascular disease (CVD), and a healthy diet is essential to keep blood sugar levels normal. Eating vegetables have been suggested to reduce the risk of getting diabetes and CVD, however few studies have looked at this in detail, and none have focused on women. This study aims to see if eating vegetables can help reduce the risk of diabetes and CVD in women.

**Who can participate?**
Healthy women between the ages of 18-35 years and 40-75 years with a BMI between 25-30 kg/m² may be eligible to participate. Anyone who fulfils all the criteria listed below are qualified to be included in the study. We will confirm eligibility at the study screening session.

- No known illnesses
- If you are 18-35 years old, have regular menstrual cycles for at least 3 months prior to the beginning of the study.
- You do not have polycystic ovary syndrome (PCOS).
- You are not on prescription medications such as hormonal contraceptives, thyroid medications or hormonal replacement therapy.
- You are not allergic/intolerant to any of the foods in the study
- You do not have any eating disorders such as anorexia, bulimia, binge eating or night eating syndrome.
- You are not breastfeeding or pregnant

**Do I have to take part?**
No, it is up to you to decide whether or not to take part. If you do decide to take part, you will be asked to sign a consent form. If you initially think you would like to participate but change your mind at any time, tell us and we will withdraw you from the study immediately. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

**What will happen to me if I take part?**
All study activities will take place at the Human Nutrition Unit (HNU) at the Rowett Institute (RI). A diagram of the study protocol can be found below:

Screening Visit (approximately 1 h)

During the screening session we will provide a detailed explanation of the study and give you the opportunity to ask any questions you may have. If you are happy to continue, we will then ask you to complete a consent form. The following will be carried out to assess your suitability for the study:

- Complete a health questionnaire and sign a consent form
- Measure height, weight and calculate BMI
- Measure blood pressure and glycosylated haemoglobin (as a marker of diabetes) and total cholesterol levels analysis (as a marker of cardiovascular health)

If you fulfil all inclusion and exclusion criteria you will be invited to participate in the study. This will involve you coming for four test sessions; the 1st session will be an Oral Glucose Tolerance Test (OGTT) and the remaining three will be intervention sessions (please see below for details). You would be free to select the days you would like to attend the sessions, however if you are pre-menopausal, all sessions will have to be at the same phase of your menstrual cycle and therefore separated by around 4 weeks. If you are post-menopausal, all sessions would have to be separated by a minimum of 1 week.

Study visit 1 - OGTT (approximately 2.5 hours)

The evening before this session, we will provide you with dinner. On the day of the visit, you would have to arrive in the morning in a fasted state (i.e. not having taken any food and drinks except water after 10 pm the previous evening). We will ask you to complete two questionnaires; on stress and on physical activity and we will measure your weight, waist and hip circumference and body fat composition. We will then insert a cannula in one of your arms. After obtaining a blood sample, we will give you a glucose drink to consume. Following this, we will collect blood samples at 15, 30, 45, 60, 90 and 120 min after drinking the glucose drink (Figure...
1. The total amount of blood sampled during OGTT session will be approximately 2 tablespoons. At the end of this, you would be free to leave HNU.

**Figure 1. Schematic of the OGTT study session**

![Schematic of the OGTT study session]

**Study Visits 2,3,4 - Intervention sessions (approximately 7½ hours)**

Two days prior to each intervention session we will ask you to follow a diet low in green leafy vegetables. We will provide you with a food diary, scales and with a list of foods that you can eat, and those that you should avoid. Depending on your habitual intake, this diet might have an effect on your bowel habits although we don’t foresee this due to its short duration.

**Day before the intervention session (approximately 90 minutes)**

The afternoon before each intervention session, you would have to come to the HNU between 15:00 and 17:00 for the insertion of a continuous blood glucose monitoring (CGM) sensor. We will insert a sensor (which is like a small piece of string) just under your skin in the abdomen area using a special insertor. The insertion is rapid and causes minimal discomfort and will be carried out by a trained researcher/nurse. We will ask you to wear the CGM device for approximately 24 hours; i.e. until the end of each test session. Wearing the sensor, which is about the size of a £2 coin, causes minimum interference with your daily activities (including sleeping and having baths). The CGM is commonly used by diabetics to track their blood glucose levels daily. Therefore it is designed for daily use and for minimal discomfort. If the sensor causes you any pain, tenderness or undue discomfort then it will be removed. After insertion you will have to remain in the HNU for one hour in order to take a finger prick blood sample to calibrate the sensor. We will provide you with instructions and give you a standard blood glucose meter to take home to carry out further calibrations. Before leaving, we will provide you with dinner to take home and consume.

**On the intervention day:**

On the day of the session, you would have to arrive in the morning in a fasted state (i.e. not having taken any food and drinks except water after 10pm the previous evening). You will complete three questionnaires; on stress, on physical activity and one on satiety levels. We will measure your weight and repeat the body fat composition measurement only if you weight has increased by a minimum of 3 kg.

Following these measurements, we will insert a cannula in one of your arms and take a blood sample. We will also ask you to provide a breath sample, which will involve blowing into a tube using a straw. We will be taking this measurement to see how long it takes the food to empty from your stomach. Following these samples, we will give you one of the 3 test breakfast meals to consume within 10 minutes. This will be either
rice, rice with spinach, or rice with bak choi. Blood samples will be taken at 15, 30, 45, 60, 90, 120, 180 and 240 min after the breakfast consumption as the figure illustrates below. In total, 9 blood samples will be taken during these intervention visits; the total amount of blood sampled will be approximately 4 tablespoons. In addition to the blood samples, we are going to ask you to provide breath samples every 15 min providing in total 16 breath samples (Figure 2).

Four hours following breakfast, we will remove the cannula. We will provide you with a lunch and a questionnaire to complete. We will ask you to stay in the RI for 3 hours following lunch until we remove the CGM. After CGM removal, you will be free to leave the HNU.

**Figure 2. Schematic of the intervention study sessions**

What will happen to the samples I give?
The samples will be used to measure levels of metabolites related to diabetes and CVD. Each participant will be assigned a unique anonymising code that will be used to label all samples and no names will be used. Only the research team will have access to your samples. All data will be treated confidentially. All data and study information will be stored in password protected University computers in a locked office. Hard copies will be stored in locked drawers with access only to research staff.

Expenses and payments
We will reimburse a maximum of £20 for any travel costs to and from your home/ place of work to the Rowett. As a way to thank you for your time and participation you will be given £30 on your last study visit.

What are the possible benefits of taking part in the study?
At the end of the study, we will give you a profile of your health status based on the measurements we obtained at your screening visit.

This study may not help you personally but the information you provide will help us understand the health effects of eating vegetables and how it may contribute to reducing disease risk. The information will be used to develop public health strategies for improving the health status of the Scottish and wider UK population. In addition the data once published will be found useful also outwith the UK.

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 (contact details below) who will do their best to answer your questions. Alternatively, you could contact the Chair of the Human Studies Management Committee, Prof. Baukje de Roos, University of Aberdeen, Rowett Institute, Foresterhill, Aberdeen AB25 2ZD (01224 438636, b.deroos@abdn.ac.uk) or the Director of the Rowett Institute, Professor Peter Morgan (p.morgan@abdn.ac.uk, 01224 438642).

**Who has reviewed this study?**

This study has been reviewed and approved by the Human Studies Management Committee of the Rowett Institute, and received ethical approval from the Rowett Ethics Committee.

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

The study is being organised by the Rowett Institute, University of Aberdeen. It is funded by the Scottish government (RESAS) and the University of Aberdeen.

**Will my taking part be kept confidential?**

Yes, your participation in the study will be kept confidential.

**CONTACTS FOR STUDY**

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