The BETA Study

Broccoli Effect on Glycated Haemoglobin (HbA1c)

A randomised double-blind crossover study

QIB Chief Investigator

Dr. Paul Kroon

QIB Principal Investigator

Dr. Jennifer Ahn-Jarvis

Registered Nutritionist

Prof. Ailsa Welch

Study Sponsor:
Quadram Institute Bioscience, Norwich Research Park, Norwich, Norfolk, NR4 7UQ

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This protocol has been approved by The Quadram Institute Biosciences Human Research Governance Committee (HRGC)

Signature:

Name: Dr Antonietta Hayhoe

Role: QIB Human Study Lead

Date: 21 January 2022

I have fully discussed the objectives of this trial and the contents of this protocol with the Sponsor's representative. I understand that the information in this protocol is confidential and should not be disclosed other than to those directly involved in the execution or ethical review of the trial.

I agree to conduct this trial according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practices (GCP) and with the applicable regulatory requirements.

Chief Investigator name and address: Dr Paul Kroon
Quadram Institute Bioscience
Norwich Research Park

Signature:

Date: 11th January 2022

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Executive Summary

Abstract

Diets rich in cruciferous vegetables such as those including broccoli, have been linked with a reduction in risk and incidence of cancer. These effects have been largely linked to the presence of the breakdown products of the sulfur metabolite glucosinolates. However, the role of the sulfur metabolite S-methyl cysteine sulfoxide (SMCSO) is relatively unexplored in human health and disease, even though it is found in a much higher concentration in broccoli compared to glucosinolate content.

There have been a small number of human intervention trials that have examined the effects of broccoli consumption on blood glucose and cancer. A previous intervention conducted at the Quadram Institute Bioscience, Effect of Sulforaphane on prostate CAncer PrEvention (ESCAPE trial, IRAS project ID: 124967, NHS REC reference number:13/EE/0110, ClinicalTrials.gov Identifier: NCT01950143), utilised a 3-arm parallel study design that investigated the effects of the consumption of low, intermediate, and high glucoraphanin broccoli with similar SMCSO content on health parameters in a prostate cancer cohort on active surveillance. Alongside blood, urine, and prostate biopsy tissue, fasting plasma glucose was measured. The study observed that all 3 variants of broccoli reduced the patients fasting plasma glucose over 24 months, but the reason behind this reduction is inconclusive.

As glucose drives cancer progression, we propose to undertake a randomised, double-blind, placebo-controlled, two-arm crossover intervention to investigate the effects of broccoli on glucose metabolism further. The study aims to test the hypothesis that SMCSO-rich broccoli soup will result in a reduction of glycated haemoglobin (HbA1c) levels, compared with a similar soup containing no broccoli. We will recruit 35 men and women aged 18 and over who have pre-diabetes (HbA1c between 42 – 47 mmol/mol) with the aim that 30 will complete. The study will involve blood collections at the Quadram Institute Clinical Research Facility (QI CRF), urine collections and questionnaires that will assess energy and SMCSO metabolism alongside transcriptomic analysis of blood.

The study will consist of an eligibility screening visit followed by two 12-week intervention phases separated by a 12-week washout phase. To confirm pre-diabetes, HbA1c screening will be conducted at QI CRF. Eligible participants will be randomly allocated to one of two arms either ‘SMCSO-rich broccoli soup’ or ‘Placebo (courgette) soup’ for intervention phase 1. After the washout phase, they will crossover onto the other arm in the second intervention period. The participants will eat 3 soups-a-week per 12-week intervention period and are designed to be consumed as part of the participant’s usual diet. The soups will be double-blinded, assigned a 3 digit alpha or numeric code, and similar in colour, texture, and taste to ensure blinding. The participants must be willing to restrict consumption of cruciferous and alliaceous foods such as broccoli and cauliflower to 3 portions per week during the soup intervention periods, as this will influence metabolic analysis. The outline of the study can be seen in Figure 1.

This will be the first study that will explore the long-term intermittent effect of the consumption of an SMCSO-rich broccoli soup on energy metabolism in individuals with pre-diabetes.

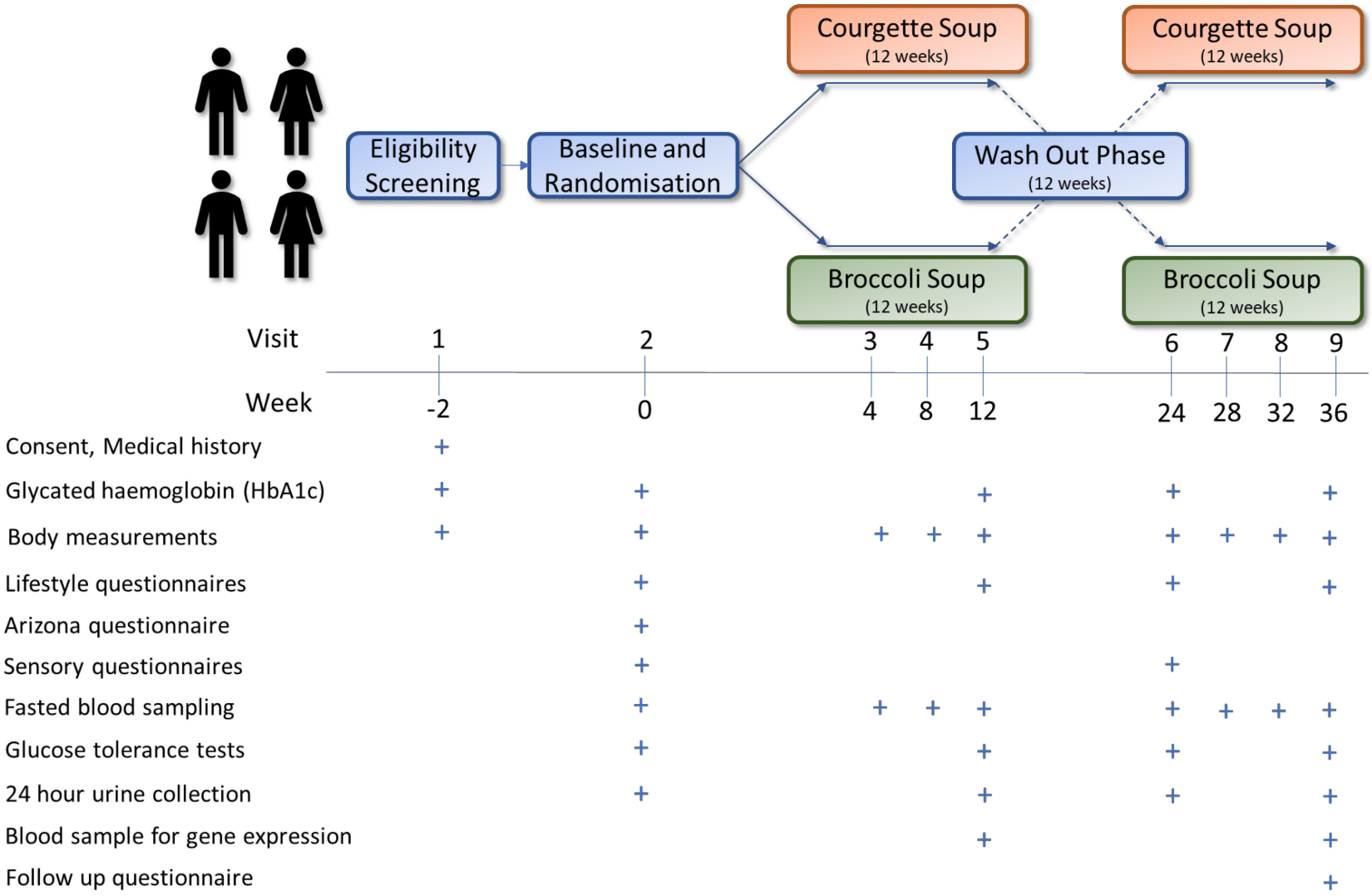

Figure 1. Summary of the study involvement for each participant. Body measurements include height, weight, BMI, waist and hip circumference, blood pressure and pulse.

1. Scientific Background

1.1 Prediabetes and its Impact on Human Health and Disease

The incidence and prevalence of metabolic syndromes, such as pre-diabetes, is rapidly increasing as a result of poor diet and sedentary lifestyles in Western populations (1). Pre-diabetes can be described as having higher than normal blood glucose level but not high enough to be classed as type 2 diabetes (2). HbA1c is typically measured over a period of 8-12 weeks and is diagnosed through measuring fasting plasma glucose and/or glycated haemoglobin (HbA1c) through a fasting blood test and/or oral glucose tolerance test (3). A HbA1c level between 42 and 47 mmol/mol or fasting plasma glucose (FPG) between 5.5 – 6.9 mmol/L is typically classified as pre-diabetes. A HbA1c below 42 mmol/mol or FPG below 5.5 mmol/L is considered as healthy, while and a HbA1c of 48 mmol/mol or over or FPG of 7.0 mmol/L or higher is considered as type 2 diabetes (2,4).

It is estimated that in the UK alone, around 7 million people have prediabetes (2). The cause of prediabetes is largely unknown, though genetic and environmental factors such as being inactive and overweight, have been linked with the development (5). However, it is possible to reverse prediabetes with a change in diet and increase in exercise. Without these lifestyle changes, it is very common for an individual with prediabetes to progress to type 2 diabetes (6). High blood glucose has been shown to have more direct complications including cardiovascular disease and neuropathy (7) but also increased risk, prevalence and growth of cancers including pancreatic, bladder and breast (8). Even though men with diabetes have a lower risk of prostate cancer compared to individuals without diabetes (9), the role of high blood glucose remains uncertain in prostate cancer. Finding a way through dietary intervention to reduce high blood glucose levels and prevent the progression of prediabetes to type 2 diabetes, is a vital area of metabolic syndrome and cancer research.

1.2 Sulfur-metabolites present in cruciferous vegetables

Diets high in cruciferous vegetables are beneficial to health with anti-inflammatory, anti-cancer, and anti-diabetic effects (10). Cruciferous vegetables such as broccoli, cauliflower and cabbage accumulate secondary sulfur metabolites which have been associated with these health benefits, in particular the breakdown products of glucosinolates. However, the role of another metabolite that also accumulates in cruciferous vegetables, S-methyl cysteine sulfoxide (SMCSO), is relatively unexplored compared to the glucosinolates.

In the plants, the secondary sulfur metabolites present are produced as a defence mechanism against insects and pathogens (11). These sulfur containing compounds give cruciferous vegetables the pungent smell and taste which can be released during food preparation. When eaten, glucosinolates are broken down into their active constituents by the plant myrosinase enzyme, or microbial thioglucosidase present in the human gut microbiota (12). The most abundant glucosinolate in broccoli is glucoraphanin, which is metabolised to the isothiocyanate, sulforaphane. This microbial breakdown varies between individuals but on average is ~10-30% conversion rate of glucoraphanin to sulforaphane (11-13). Sulforaphane has been heavily researched in clinical studies which has highlighted the potential in preservation of human health and mitigation of diseases such as cancer (13, 15-17) and diabetes (14, 18-20).

Whilst much of the current evidence regarding the benefits for cruciferous vegetable consumption relates to the breakdown products of glucoraphanin, there is limited evidence regarding the secondary metabolite S-methyl cysteine sulfoxide (SMCSO). SMCSO is metabolised by β-conjugate cysteine lyases, either plant or microbial based (21), which leads to the formation of several bioactive products S-methyl methanethiosulphinate (MMTSI) and S-methyl methanethiolsulphonate (MMTSO) (22,23).

Evidence from animal studies have indicated that SMCSO consumption by diabetic rats led to blood glucose and lipid controlling effects (24-26), potentially due to the presence of thio-sulphinate and thiol functional groups present in MMTSI and MMTSO respectively (23). An interventional study at QIB, Sulphate Accumulation in Prostate (SAP) Study (IRAS project ID: 197753, NHS REC reference number:16/EE/0054, Clinical Trials Identifier: NCT02821728) investigated the influence of a four-week parallel single blinded high-dose broccoli soup in men scheduled for transperineal prostate biopsy. The study found that SMCSO accumulated in the prostate tissue as well as sulphate and ADP; further cell-based assays demonstrated SMCSO reduced ATP present in cells (27). Although SMCSO role in metabolic syndrome prevention remains relatively unknown, this study suggests a potential mechanism for SMCSO in cancer cell metabolism. Considering the abundance and distribution of SMCSO in a wide range of vegetables (22,23), the role of this sulfur metabolite in glucose metabolism and cancer still remains unknown, thus further studies are warranted.

1.3 Glucose metabolism, cancer risk and sulfur-metabolites

Epidemiological and clinical studies have indicated a negative association between consumption of broccoli and cancer risk, incidence, and progression (13). Within the tumour environment, there is an altered glucose metabolism requiring an increased glucose uptake that favours aerobic glycolysis to provide additional energy to promote cancer cell proliferation and support the tumour growth, known as the Warburg effect (28). Therefore, as glucose drives cancer progression, human studies exploring the link between the effect of broccoli consumption on glucose metabolism are needed.

A small number of human intervention trials have examined the effects of broccoli consumption on blood glucose. One three-arm parallel randomised control study (Clinical Trails Identifier: IRCT138901181640N2, registered in the Iranian Registry of Clinical Trials), showed that 10 g/day broccoli sprout extract (BSE) for four-weeks led to a reduction in fasting glucose (1.9 mmol/l) and a significant reduction in serum insulin concentration (0.85 mU/l) in a type 2 diabetic cohort (29). One other randomised double-blinded placebo-controlled study (ClinicalTrials.gov Identifier: NCT02801448), demonstrated that 5 g/day broccoli sprout extract containing 5410 ppm of sulforaphane, reduced fasting blood glucose by 0.7 mM and reduced glycated haemoglobin (HbA1c) by 4 mmol/mol in an obese type 2 diabetic cohort (14).

More recently, the Effect of Sulforaphane on prostate CAncer PrEvention (ESCAPE trial, IRAS project ID: 124967, NHS REC reference number:13/EE/0110, ClinicalTrials.gov Identifier: NCT01950143), a previous intervention study conducted at QIB, investigated the influence of once-a-week consumption of broccoli soup on health parameters in a prostate cancer cohort on active surveillance. The study investigated the effect of broccoli variants with different glucoraphanin but similar SMCSO content (21) and utilised a three-arm parallel design. The glucoraphanin content of each soup was as follows: Soup X; 72 micromoles/300 g soup; Soup

Y; 214 micromoles/300 g soup; Soup Z; 492 micromoles/300 g soup (30). The study observed that all 3 variants of broccoli overall reduced the patients fasting plasma glucose over 24 months compared with baseline: Soup X; 0.51 mmol/l, Soup Y; 0.25 mmol/l and Soup Z; 0.67 mmol/l (Figure 2, 31), and attenuated changes in gene expression and pathways associated with the development and progression of prostate cancer. As glucose levels fell across all arms, other phytochemicals present in the broccoli such as SMCSO could be responsible for this decrease in fasting plasma glucose. However, since there was no comparison group not supplemented with SMCSO, further human studies on SMCSO metabolism and its influence on blood glucose are needed to estimate any causal effect between SMCSO-rich broccoli and glycaemia.

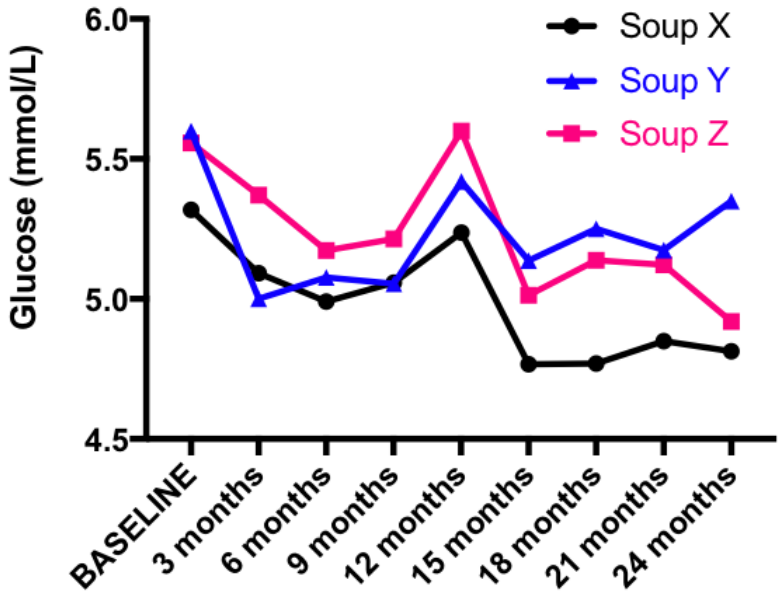

Figure 2. Fasting blood glucose levels from the Effect of Sulforaphane on prostate CAncer PrEvention (ESCAPE trial) where men on active surveillance participated in a 12-month dietary intervention with 3 variants of broccoli soups. All 3 variants of broccoli (Soup X; standard, Soup Y; Beneforte®, Soup Z; 1086) reduced fasting blood glucose over 24 months (31).

Despite the current evidence discussed, there is only one other human intervention study currently underway exploring the effect of broccoli sprout extract on blood glucose in individuals with pre-diabetes (ClincalTrials.gov Identifier: NCT03763240).

Therefore, we plan to undertake a randomised, double-blind, placebo-controlled, two-arm crossover study to explore further whether a long-term intermittent intervention with SMCSO-rich broccoli soup, can reduce glycated haemoglobin (HbA1c) levels in individuals with pre-diabetes. Included in the study is a comparison soup not supplemented with SMCSO (courgette soup) which we will be able to attribute any difference seen to SMCSO. This will provide further information on how dietary sulfur metabolites influence blood glucose, highlighting SMCSO (Figure 1). The study could contribute towards current dietary recommendations for individuals with pre-diabetes.

2. Hypothesis, Aims and Objectives

2.1 Hypothesis

In men and women with pre-diabetes, consumption of SMCSO-rich broccoli soup for 12 weeks, may lower glycated haemoglobin (HbA1c), compared with a similar soup not containing broccoli, and affect gene expression in cancer-related pathways.

2.2 Primary Aim

- To estimate the effect of 12 weeks of incorporating SMCSO-rich broccoli soup, three times per week, on glycated haemoglobin (HbA1c), compared to 12 weeks of incorporating a similar soup but without broccoli.

2.3 Secondary Aims

- To estimate the effect of SMCSO-rich broccoli soups on:
    - ❖ glucose tolerance measured by finger prick capillary glucose tests.
    - ❖ differences in energy metabolism markers obtained from fasted venous blood sampling (venepuncture).
    - ❖ sulfur metabolite differences in urine, between control and active treatment periods, using targeted metabolite analysis.
    - ❖ changes in blood gene expression using next generation RNA sequencing.

2.4 Objectives

We will undertake a randomised, double-blind, placebo-controlled, two-arm crossover intervention study. The primary endpoint of the study will be to evaluate the effect of the incorporation of SMCSO-rich broccoli soups on glycated haemoglobin (HbA1c) measured using venous blood samples. Secondary endpoints of the study will be to evaluate glucose tolerance response from capillary blood measurements. Conventionally, metabolic markers are measured using venous blood by venepuncture or cannulation. However, capillary blood sampling obtained using a finger-prick is considered a valid means to measure acute postprandial glucose response and shows similar variance to venous blood measurements (32). Therefore, more invasive measurement methods are not required to answer the secondary objective in this study. The glucose tolerance will assess time specific glycaemia rather than average glycaemia over the previous period. We will assess fasting blood parameters: fasting plasma glucose, fasting insulin, fructosamine and total/HDL/LDL cholesterol and triglycerides in venous blood samples. We will also quantify the bioactives from the soup consumption in urine through targeted metabolite analysis of glucoraphanin and its metabolites and SMCSO and its metabolites, to detect more systemic changes and effects of the soup consumption. Participants will be asked to complete a soup record sheet to measure adherence to the study. We will also estimate how gene expression is altered by the soups through blood RNA sequencing analysis.

3. Study Design

3.1 Study Team

The study will be led by Dr Paul Kroon (QIB Food, Innovation and Health Group Leader). All aspects of the study will be managed by Dr Jennifer Ahn-Jarvis (QIB research scientist). A delegation log will be used for recording the roles and responsibilities of the local research team and the authorisation of the Chief Investigator (CI). All analysis of data will be performed by Dr Jennifer Ahn-Jarvis; metabolite detection will be undertaken by Dr Shikha Saha (QIB analytical chemist), and RNA sequencing data analysis will be undertaken with the assistance of Dr Perla Troncoso-Rey (QIB bioinformatician) and Dr Maria Traka (QIB scientist). Statistical analysis will be undertaken with the assistance of Dr George Savva (QIB statistician).

The study will be carried out in collaboration with the Clinical Research Facility (CRF) based at the Quadram Institute (QI). The QI CRF is an NHS-governed facility managed. All clinical visits and clinical procedures for this study will be carried out by the QI CRF team following Norwich and Norfolk University Hospital (NNUH) standard operating procedures. Clinical assessment and procedures will be performed by two members of the QI CRF team when research participants are attending the QI CRF. This will include a Healthcare professional trained in NNUH emergency procedures such as a registered nurse, and a second designated member of staff to provide support.

3.2 Study Summary

The study team will aim to recruit 35 participants with the expectation ≥30 participants will complete the study (anticipated dropout ~10-15%). The study will consist of eligibility screening followed by two 12-week intervention phases separated by a 12-week washout phase. Eligible participants will conduct a study talk over the phone followed by a screening visit and 8 further intervention visits at the QI CRF over the course of 36-week intervention. Participants recruited into the study will be randomly allocated to into one of two arms (SMCSO-rich broccoli soup or placebo soup) for intervention period 1. After the washout phase, participants will move onto the other arm for intervention period 2. ***NB:*** previous broccoli crossover studies have used much shorter washout phases although not in individuals with pre-diabetes (33,34); however, 12 weeks will minimise the presence of a carry-over effect. During each intervention period participants will consume 3 soups-a-week per 12-week period, as part of their usual diet.

Participants will be asked to give a total of 9 blood samples: 5 will be analysed for HbA1c including screening, 1 will be analysed for full blood count (FBC) at screening, 8 will be analysed for energy metabolism markers and 2 will be analysed for blood gene expression. Four glucose tolerance tests will be conducted. ***NB:*** we will be taking pre-treatment as well as post-treatment measurements for the energy metabolism markers and the glucose tolerance tests. This is due to using a complex food for the control and treatment and, if no difference is observed post-treatment we can compare to baseline values. Participants will be asked to complete lifestyle questionnaires and one cruciferous vegetable food frequency questionnaire (FFQ) whilst at the study visits 2, 5, 6 and 9. Participants will be asked to provide four 24-hour urine collections and asked to complete a soup record sheet for adherence throughout the study. At the end of the study, participants will be asked to complete a follow up questionnaire to assess soup taste. Summarised in Figure 1 and 3.

Study Talk over the phone

- A member of the study team will discuss the PIS and answer any questions.

Visit 1 (Screening Visit)

- A member of the study team will explain the study details and answer any further questions.
- The participant will sign consent for study participation.
- A medical questionnaire will be completed by QI CRF research nurse and participant.
- BMI, waist and hip circumference, blood pressure and pulse will be measured.
- Venous blood sample will be collected for glycated haemoglobin (HbA1c) and full blood count (FBC) analysis, followed by a complimentary breakfast/snack.
- If deemed eligible, 24-hour urine collection pot given to participant and asked to collect all urine that passes 24-hours prior to next visit.

Visit 2 (Week 0)

- Participants will be asked to arrive at the QI CRF between 7.30 - 8.30am fasted for at least 10 hours.
- A study team member will complete the assessment day checklist and go through details of the day.
- 24-hour urine collection pot given to member of the study team, aliquoted and stored appropriately.
- BMI, waist and hip circumference, blood pressure and pulse will be measured.
- Fasted venous blood samples will be collected for analysis.
- Participants will conduct glucose tolerance test: baseline finger prick measurements will be taken at -15, -10, -5 min.
- At 0 minutes, the participant will consume the 250 mL sugary drink in 15 minutes.
- Participants will do finger prick measurements at 15, 30, 45, 60, 90, 120, 150, 180, 210 min.
- Whilst at this study visit, lifestyle questionnaires to assess normal diet and exercise, and at Visit 2 only, a food frequency questionnaire to assess cruciferous vegetable intake will be completed.
- Participants will be randomised to one of two soup arms for Intervention Period 1.
- Participants will be given a complimentary lunch.

Visit 3 (midpoint of period 1)

- Participants will be asked to arrive at the QI CRF between 7.30 - 8.30am fasted for at least 10 hours.
- A study team member will complete the assessment day checklist and go through details of the day.
- BMI, waist and hip circumference, blood pressure and pulse will be measured.
- Fasted venous blood samples will be collected for analysis.
- Participant will be given a complimentary breakfast/snack.

Visit 4

- As described in Visit 3.
- 24-hour urine collection pot given to participant.

Visit 5

- As described in Visit 2.
- 24-hour urine collection pot given to participant.

Washout Phase

- Participants will eat their normal diet during the 12-week washout phase.

Visit 6 (Start of Intervention period 2)

- A member of the study team will welcome the participant back and answer any further questions.
- As described in Visit 2.
- Participants will be given the alternative soup to that in Intervention Period 1.

Visit 7

- As described in Visit 3.

Visit 8 (midpoint of period 2)

- As described in Visit 4.

Visit 9

- As described in Visit 2, excluding randomisation of soups.
- Participants will be asked to return soup packets.
- Participants will complete a follow-up questionnaire.
- End of Study.

Figure 3. Study diagram

4. Participant Eligibility Criteria

4.1 Inclusion Criteria

- Individuals with prediabetes (confirmed through screening)
- Aged 18 and older.
- Body Mass Index (BMI) between 18.5 – 35 kg/m^2.
- Living within 40 miles from the Norwich Research Park.

4.2 Exclusion Criteria

The participant will not be able to take part if they('re):

- Screening test results indicate they are not suitable to take part in this study such as HbA1c level at screening visit of < 42 mmol/mol (healthy) and > 47 mmol/mol (diabetes) and BMI at screening visit < 18.5 kg/m^2 (underweight) and > 35 kg/m^2 (extremely obese).
- Have a known allergy to any of the components (broccoli, courgette, milk, lactose, or gluten) of the test soups.
- Have been diagnosed or have a history of blood or clotting disorders such as anaemia or thrombosis.
- Have been treated for heart disease, cancer, or diabetes.
- Are immunocompromised due to medications or viral infection such human immunodeficiency virus (HIV).
- Have low or high blood pressure with hypertension medication (≤90/60, ≥160/100 respectively would be classed as abnormal).
- Have any acute or chronic illnesses that affects the outcome of the study such as a gastrointestinal disorder. This will be assessed on a case by case basis by QI medical advisor.
- Plan to become pregnant during the study duration, pregnant or breastfeeding.
- Frequently take medications that may interfere with sugar metabolism or absorption such as laxatives, steroids, dietary supplements, or anti-inflammatory medications. This will be assessed on a case by case basis by QI medical advisor.
- Drink more than 14 alcohol units/week.
- Smoke socially or on occasion more than 12.5 grams or 20 cigarettes/week
- Vegan or any dietary restrictions that prevent the consumption of study soups or follow a diet programme which requires fasting for multiple days.
- Are a registered blood donor and have donated a large quantity of blood within the last 16 weeks. Registered blood donors should abstain from blood donations for the duration of the study.
- Are unable to give written or verbal informed consent
- Unable to provide your GP contact details.
- Are participating in another dietary intervention study nor given blood in another dietary study in the last 3 months.
- Are related to or living with any member of the study team or part of the management/supervisory structure of the Chief Investigator.
- Have symptoms of COVID-19, been asked to self-isolate, or have been diagnosed with COVID-19 in the last 14 days.

5. Study Procedure

5.1 Recruitment Strategy

We aim to recruit 35 participants with the expectation ≥30 participants will complete the study (anticipated dropout ~10-15%).

The primary route for recruitment will be through GP surgeries whereby participants will be diagnosed with pre-diabetes (HbA1c level between 42 – 47 mmol/mol). However, the participants will be screened via a HbA1c blood test at QI CRF, prior to participating in the study. The study team will send study packs to the GP surgeries which will include an invitation letter (Annex 1) and Participant Information Sheet (PIS, Annex 3A) to discuss with and give to potential participants. The participants can then contact the principal investigator or member of the study team for more information about the study and arrange a study talk over the phone, return the completed response slip to the study manager or complete the expression of interest form on the QI study webpage, if interested in taking part in the study. Informed consent for the study will be taken on Visit 1 (Annex 4). The participant is free to ask any questions throughout the study.

While the primary route of recruitment will be through GP surgeries, we will also use the QIB database. We will send an invitation letter (Annex 2) and PIS (Annex 3A) to potential eligible participants on the database. Although HbA1c is not recorded on the database, we can assess the individuals BMI (as being overweight/obese is linked with elevated blood glucose levels), invite these individuals for a study talk over the phone and subsequent screening onto the study.

We may also use ethically approved posters and flyers (Annex 5) in this study as well as social media platforms such as Facebook and Twitter. The posters advertising this study will have tear off contact slips attached to the poster to facilitate participant recruitment. A member of the study team will send interested responders a letter of invitation (Annex 6) and a copy of the PIS (Annex 3A) with accompanying response slip and pre-paid envelope in which to return the completed response slip to the study manager if interested in taking part in the study. They can also complete the expression of interest form on the QI study webpage.

Any screening results which fall outside of the study criteria will be assessed by the QI medical advisor. The GPs of those successfully recruited onto the study will be informed of their patient's participation in the study by letter (Annex 7), along with the PIS (if required, Annex 3A). We are unable to discuss test results with the participant; however, they will be advised to speak to their GP about the results if deemed necessary.

5.2 Eligibility Assessment

Participants who have responded positively to the study talk over the phone, will be invited for Visit 1 where eligibility will be assessed. A blood sample will be taken for screening purposes to give the glycated haemoglobin (HbA1c) value of the participants. This will be taken by QI CRF research nurse and sent for analysis prior to participating in the study. The QI CRF research nurse will then measure height and weight, and BMI will be calculated, waist and hip circumference, blood pressure and pulse. ***NB:*** 3 measurements of blood pressure will be taken on each arm, so an average can be taken. The QI medical advisor will

be responsible for confirming eligibility. If the participants are deemed eligible from the screening blood sample at QI CRF, they will be invited for Visit 2.

In an event of an abnormal finding at the eligibility assessment, the participants GP will be informed of the reason why the participant did not qualify for the study (Annex 13). A copy of all the eligibility screening results (HbA1c results, full blood count (FBC), blood pressure, and BMI) will be made available to the GP via the hospital electronic system and/or by letter (Annex 14).

5.3 Randomisation Process

Block randomisation will be employed to ensure that groups are balanced in number and are balanced over the study period. Participants will be randomised consecutively in blocks of two. Blocks allocation sequence will be generated by the R package 'randomizeR' by an independent QIB researcher.

5.4 Blinding Process

This study will be double blinded. This guarantees the study remains blinded to the study team as well as the study participants. Chief Investigator, Principial Investigator and Study Scientists will remain blind during the allocation throughout the study, as well as during the analyses of data from participants so an unbiased approach is adopted to the evaluation of results. However, the code may be broken in the event of a medical emergency as seen as necessary and appropriate by the QI CRF Research Nurse. All personal information will be kept confidential and known only to the CI, members of the study team, QI CRF Research Nurse and participants' GP.

The SMCSO-rich broccoli soups and placebo soups will be prepared, packaged and given a 3 digit alpha or numeric code at the QI CRF kitchen in a hygienic and food-safe manner, and stored in a food grade freezer. Both soups will look identical in colouring and texture and will be packaged in matching packaging. Depending on the randomisation, Dr Jennifer Ahn-Jarvis will then allocate participants to either study arm.

Participants will be blinded to their glucose readings to prevent modification of lifestyle and food habits during the study and washout phase, excluding the glucose tolerance tests where the participants will fill in their own values into the glucose record booklet (Annex 15). Participants will be able to contact their GP surgeries for the blood test results after the study has completed. If there is an abnormal measurement, the participant will be contacted by the GP and potentially asked to withdraw from the study, however this will be assessed on an individual basis.

5.5 Allocation concealment

Allocation concealment will be achieved by the initial randomisation of 'SMCSO-rich broccoli soup' and 'Placebo soup' to either 'Soup 1' or 'Soup 2' conducted in house QI scientists handled hygienically and safely. This will be followed by a QI scientist, who is not part of the study team, who will re-label the labelling of soups with another labelling system such as 3 digit alpha or numeric code, this way the QI CRF research nurse randomising the participant does not know what the next treatment allocation will be, this is important for preventing bias when giving the participants the soups.

5.6 Study visits

The study will consist of a study talk over the phone followed by a screening visit and 8 further assessment visits to the QI CRF. Participants will be asked to consume no more than 3 portions of cruciferous and alliaceous vegetables whilst part of the two 12-week intervention periods (24-week total dietary restriction). Such foods include broccoli, cauliflower, Brussel sprouts, onion and garlic. Participants will receive a list of the vegetables they will be required to restrict in Annex 18. If participants don't cook for themselves or go out for a meal, it will be more difficult to track what vegetables they have eaten, however we can suggest to participants to read the ingredient list or ask the restaurant what dishes contain cruciferous and alliaceous vegetables.

Participants will not restrict their diet during the 12-week washout phase, and they will consume their normal diet with as many cruciferous vegetables as they wish. The dietary restriction for the two 12-week periods is to standardise the number of cruciferous vegetables eaten whilst part of the intervention periods. During the broccoli soup 12-week intervention, participants could be consuming as much as 6 portions of cruciferous vegetables per week (3 portions within the soup and 3 portions within their diet). During the two 12-week intervention periods, they can achieve the government recommended guidelines of 5-a-day with the following examples of fruits and vegetables to achieve a healthy, balanced diet: oranges, tangerines, apples, bananas, mango, passionfruit, apricots, avocado, blackberries, strawberries, raspberries, blackcurrants, blueberries, melon, cherries, figs, grapefruit, grapes, kiwi, olives, peaches, pear, pineapple, plums, pomegranate, prunes, rhubarb, courgettes, lettuce, cucumber, peppers, tomatoes, eggplant, carrots, peas, artichokes, beetroot, mushrooms, okra, potatoes, sweet potatoes, pumpkin, butternut squash, sweetcorn. Some examples have been included in the participant information sheets.

5.6.1 Study Talk over the Phone

After receiving documents and information about the study, and the participant feels they meet the criteria for the study, participants will have returned the response form included in the PIS (Annex 3A) to the QIB using the freepost envelope provided, completed the expression of interest form on the QI study webpage or contacted a member of the study team. They will be invited to a study talk over the phone. With the study talk, the principal investigator or member of the study team will talk through the study and answer any questions they may have so that they are fully clear with what the study entails. They will be made aware that withdrawal from the study can occur at any time.

Participants will be given a 'consideration period' of 24-hours whereby they will not be contacted. If they decide to participant in the study, we will then arrange to meet them at the QI CRF for Visit 1, where screening will take place.

5.6.2 Procedure of Visit 1

This visit will last approximately 30-60 minutes. ***NB:*** As HbA1c is typically measured over a period of 8-12 weeks (2-3 months), if the participants fail the first HbA1c screening, the earliest the participants will be invited back for re-screening will be 8 weeks from the date of their failed HbA1c measurement. Similarly, those participants who have not started the study by 8 weeks, will be re-screened for HbA1c as well. ***NB:*** Participants do not have to fast for the screening visit.

Consent
Participants who have verbally agreed to proceed after the consideration period will be invited for Visit 1. At this visit, the participant will be invited to the QI Clinical Research Facility (CRF) where they will be welcomed and will be walked through the study and answer any further questions. To participate in the study, informed consent will be taken which must be obtained before undergoing any procedures (Annex 4).

Medical questionnaire and body measurements
A QI CRF research nurse will complete a medical questionnaire which will include discussing any medications they are taking and inform us about their general health and past and current medical history (Annex 8). They will be advised on the study talk to please bring details of medications with them on this visit. At this visit, body measurements will be taken by QI CRF research nurse as described in section 5.2: eligibility assessment.

Blood sampling
A trained QI CRF research nurse will proceed to insert a needle into a vein in their forearm. A maximum of 20 mL of blood will be taken for screening blood measurements. We will send copies of the screening results to the participants' GP (Annex 13 and 14).

Urine collection pot
If the participants are deemed eligible from the screening blood sample, participants will be given a specially designed 24-hour urine collection pot. They will be asked to collect all the urine that passes 24-hours prior to Visit 2. This information is included on an instruction sheet given to the participants to prepare for their next study visit (Annex 18).

Breakfast/snack on study day
At the end of these visits, we will give the participant a complimentary breakfast/snack. The research participants will be offered to consume the snack in the QI CRF lounge area.

5.6.3 Procedure of Visits 2, 5, 6 and 9

These visits will last approximately 4-5 hours.

Arrival
Participants will be asked to arrive at the QI CRF between 7.30 and 8.30am fasted for at least 10 hours. If they have not fasted, then we will have to reschedule the visit to another day. They will be welcomed again and will be walked through to a clinical room.

Urine sample collection
On arrival at the QI CRF for Visit 2, 5, 6 and 9, the 24-hour urine collection pot will be handed to the study scientist for appropriate preparation and storage for analysis.

At Visit 5, participants will be given another 24-hour urine collection pot and asked to collect all the urine that passes 24-hours prior to Visit 6. This information is included on an instruction sheet given to the participants to prepare for their next study visit (Annex 18). These samples will subsequently be used for metabolic analysis.

Completion of assessment day checklist and body measurements
A QI CRF research nurse will ask the participant if there have been any changes in their health and will discuss any changes to medications they are taking and update the record on

the assessment day checklist (Annex 19). At these visits, body measurements will be taken by QI CRF research nurse as described in section 5.2: eligibility assessment.

Blood sampling
A trained QI CRF research nurse will proceed to insert a needle into a vein in their forearm for blood measurements and sent for analysis. No more than 35 mL of venous blood will be taken: no more than 20 mL for energy metabolism markers: HbA1c, fasting plasma glucose, fasting insulin, and cholesterol/triglycerides analysis, no more than 10 mL for blood RNA sequencing analysis and no more than a 5 mL aliquot for fructosamine analysis.

Glucose tolerance tests
The participants supported by a member of the study team will conduct glucose tolerance tests. Initially, three fasting capillary blood baseline measurements, using a finger prick device, will be taken at times: -15, -10 and -5 minutes. At 0 minutes, the participant will then be asked to consume the 250 mL sugary drink in 15 minutes (See section 7.6: Glucose drink used in glucose tolerance testing for information on the sugary drink used). Capillary blood glucose concentration will be measured 9 times from baseline up to 3.5 hours, giving a total of 12 measurements. Measurements, using finger prick device, will be taken over 15-minute intervals for the first hour and over 30-minute intervals after: 15, 30, 45, 60, 90, 120, 150, 180, 210 minutes, to ensure their blood glucose returns to their normal level. Participants can conduct the finger pricking themselves or can be assisted by the study scientist.
Participants will fill in their own capillary blood measurements into the glucose record booklet provided (Annex 15).

Completion of lifestyle questionnaires
There will be idle times during the glucose tolerance testing that we will ask the participant to complete three questionnaires regarding their physical activity and dietary pattern. The following is a summary of the questionnaires used for the study:

- Arizona cruciferous vegetable food frequency questionnaire (Annex 9) collects specific information regarding their habitual consumption of cruciferous vegetables for 90 days before the enrolment in the study (20 to 25-minute duration), completed at visit 2 only.
- Vioscreen food frequency questionnaire (Annex 10) collects information about foods that make up their habitual diet over 90-day intervals for this study (20 to 30-minute duration).
- International physical activity questionnaire (IPAQ, Annex 11) collects information of physical activity from lifestyle behaviours and from exercise (20 to 25-minute duration).

The international physical activity questionnaire (IPAQ) and Vioscreen food frequency questionnaire will be completed using a tablet whereas the Arizona cruciferous vegetable food frequency questionnaire will be completed using a paper survey. They will be provided with a clean, sanitised tablet and pencil to complete the questionnaires during the visits. All three questionnaire have been used in previous research and studies, although not validated in the UK. Note: the questionnaires are not optional, and the participant will complete most of these questionnaires whilst at the study visits 2, 5, 6 and 9 to reduce burden, and may reduce some of the boredom they may have from the visits.

We will also ask the participants to complete sensory evaluation questionnaires at home during the first week of eating the soups. The sensory evaluation questionnaire (Annex 12) collects information on their impressions of the study soups in how they taste. This will be completed at home (5 to 10-minute duration).

Randomisation
At Visit 2, the participants will be randomised to one of two soup arms for intervention period 1. At Visit 6, participants will be given the alternative soup to that in intervention period 1 to consume for intervention period 2. The participant or the study scientists will not be aware of which are they are in.

Collection of soups
Participants will receive soups in batches of 14 at the relevant timings throughout the study (2 extra soups will be included in the batches to allow for any unforeseeable issues). They will receive a total of 3 batches for each intervention period. Participants will also receive a soup information sheet with information about the nutrition, ingredients, allergens, storage and cooking instructions of both soups (Annex 16), and soup record sheet to record consumption of soups and vegetables (Annex 17). At the end of each intervention, they will be asked to give the completed soup record sheet and return the soup containers.

Completion of follow-up questionnaire
Participants will be asked to complete a brief follow-up questionnaire (Annex 20) collect anonymous comments on the soups and the study during the final study visit (visit 9, 10 to 15-minute duration).

Lunch on Study Day
At the end of each visit, we will give the participant a complimentary lunch. The research participants will be offered to consume the lunch in the QI CRF lounge area.

5.6.4 Procedure of Visits 3, 4, 7 and 8
These visits will last between 30-60 minutes.

Arrival
On the scheduled days for Visit 3, 4, 7 and 8, the participant will be welcomed again and will be walked through to a clinical room in the QI CRF. For these visits, they will be asked to be fasted for at least 10 hours. If they have not fasted, then we will have to reschedule another day.

Completion of assessment day checklist and body measurements
They will be asked if they are happy to continue participating in the study and the QI CRF research nurse will ask the participant a series of questions about their health and discuss any changes to medications they are taking and record these on the assessment day checklist (Annex 19). At these visits, body measurements will be taken by QI CRF research nurse as described in section 5.2: eligibility assessment.

Blood sampling
Once completed the assessment day checklist, a trained QI CRF research nurse will proceed to insert a needle into a vein in their forearm for energy metabolism blood measurements and sent for analysis. No more than 25 mL of venous blood will be taken: no more than of 20 mL for energy metabolism markers: fasting plasma glucose, fasting insulin, and cholesterol/triglycerides analysis, and no more than a 5 mL aliquot for fructosamine analysis.

Urine collection pot
At Visit 4 and 8, participants will be given another 24-hour urine collection pot and asked to collect all the urine that passes 24-hours prior to Visit 5 and 9 respectively. This information is included on an instruction sheet given to the participants to prepare for their next study visit (Annex 18). These samples will subsequently be used for metabolic analysis.

Breakfast/snack on study day
At the end of these visits, we will give the participant a complimentary breakfast/snack. The research participants will be offered to consume the snack in the QI CRF lounge area.

5.6.5 Washout Phase
After completion of intervention period 1, the participants will conduct a washout phase for 12 weeks prior to intervention period 2. During this washout phase, participants will be asked to continue their normal diet and to not consume any of the soup products during this time. During this period, they can contact a member of the study team and ask any questions they may have about intervention period 2. Further study appointments can be scheduled in the washout phase if needed.
After the washout phase, if participants HbA1c reduces to a normal level, they will continue on the study unblinded. However, if the participants HbA1c increases to a diabetes level, they will be asked to withdraw from the study and their GP will be contacted.

6. Post-Study Follow Up
The participants can contact their GP surgeries for further follow-up information and advice, however there is no routine follow up appointment as part of this study.

7. Study Food, Handling and Distribution
7.1 Broccoli Powder
Broccoli florets contain a range of sulfur compounds such as glucoraphanin and SMCSO, we aimed to find a commercially available broccoli powder that delivered high SMCSO and low glucoraphanin/sulforaphane. Within this broccoli powder there are consistent levels of glucoraphanin and SMCSO. Hydrolysis of glucoraphanin to sulforaphane was conducted at room temperature and at 100˚C; both yielded very low levels of sulforaphane. The product does not preserve the full extent of the enzyme myrosinase so a very small fraction of the glucoraphanin in the SMCSO-rich broccoli is metabolised to sulforaphane, therefore is relying on the human gut microbial breakdown, which is variable but on average is low (~10- 30% conversion rate), indicating high delivery of SMCSO and low delivery of sulforaphane to participants.

The broccoli powder will be purchased from Sussex Wholefoods. The dried broccoli powder origin is *Brassica oleracea* and is manufactured using a freeze-dried process that extends the shelf life of the product. For the SMCSO-rich broccoli soup, a base vegetable soup will be supplemented with the freeze dried broccoli powder (see section 7.4).

7.2 Courgette Powder

Courgette powder will be substituted instead of broccoli powder in the placebo soup. Courgette has a similar but not the same carbohydrate, protein and fibre content to the broccoli powder and is absent from all sulfur compounds including glucosinolates and SMCSO. The powders differ in the fat and salt content: broccoli powder has 0.7g salt / 100g and 3.5g fats / 100g whereas the courgette powder has 0.05g salt / 100g and 6.2g fats / 100g. However, as the carbohydrate, protein and fibre content are similar, if we observe a difference between the results, they can be eliminated from the factors which could affect blood glucose. Pea powder, cauliflower and steamed broccoli powder were also considered for the placebo soup alternative; however, the carbohydrate, protein and fibre content were very different, and some considerations still contained sulfur compounds even in low amounts, that could affect the study data.

The courgette powder will be purchased from Sussex Wholefoods. It is manufactured using a freeze-dried process that extends the shelf life of the product. For the placebo soup, base vegetable soup will be supplemented with the freeze dried courgette powder (see section 7.4).

7.3 Base Vegetable Soup

The base vegetable soup used in the study will be Batchelors golden vegetable soup, which is low in calories, saturated fat, and sugar content, relatively low in salt and does not contain broccoli. The soup is suitable for vegetarians but not for vegans as it contains milk. If the participant is a vegan or lactose intolerant, they will be asked in the medical questionnaire (Annex 8) and will be told that this dietary study is not suitable for them. This cup of soup also contains wheat, barley, and celery so those with an allergy to these would be advised not to participate in the study. Even though the base vegetable soup contains swede, leek and onion, from analysis the base vegetable soup contains 0.00 ± 0.00 micromoles of glucoraphanin and 16.2 ± 1.11 micromoles of SMCSO which is minimal. The glucosinolate and SMCSO levels of the base vegetable soup and for the combined dried soup will be determined for each batch and taken into account during analysis.

7.4 Dried Soup composition

The base vegetable soup will be supplemented with either sixteen grams of Sussex Wholefoods freeze dried broccoli powder for the SMCSO-rich broccoli soup or Sussex Wholefoods freeze dried courgette powder for the placebo soup. The soups will be similar in colour and consistency to ensure blinding and taste similar to a standard vegetable cup of soup. The preparation of the soups will be handled hygienically and safely.

One broccoli soup will contain 146 ± 1.85 micromoles of glucoraphanin and 1816 ± 80.8 micromoles of SMCSO. Through glucosinolate hydrolysis, the glucoraphanin is converted to 0.16 ± 0.01 micromoles of sulforaphane. Each broccoli soup will contain a similar amount to what would be consumed in two standard broccoli portions. When compared to the Sulphate Accumulation in Prostate (SAP) study, one portion of broccoli soup contained 280 ± 8.8

micromoles of glucoraphanin and 1513 ± 36.8 micromoles of SMCSO (26). Therefore, the broccoli soups in this study have safe levels of sulfur metabolites for human consumption.

The soups are designed to be consumed as part of the participant's usual diet. Participants will be asked to consume 3 soups-a-week per 12-week intervention and will consume both the broccoli and courgette soups throughout the study. As the study aims to bring about sustained metabolic changes from the sulfur metabolites, when and how the soup should be consumed (with a meal and/or standalone) is not necessary. The putative mechanism of action is a sustained change in glucose metabolism. In fact, metabolic effects following a broccoli intervention were reported in Armah et al., 2015 (35) without 'prescribing' a specific time. The kinetics of sulforaphane production is delayed (plasma peak at ~6-8 hours characteristic of microbial absorption) and SMCSO peaks at around 1.5-2 hours.

Participants will be given a soup information sheet with information about the nutrition, ingredients, allergens, storage, and cooking instructions of the soups (Annex 16) and a soup record sheet to record when the soup and vegetables are consumed (Annex 17).

7.5 Soup safety, microbiological testing, and distribution

The intake of cruciferous vegetables such as broccoli is considered safe, and consumption has not been associated with any serious adverse effects (29,36). The base vegetable soup, courgette powder and broccoli powder will be commercially available and deemed safe for human consumption. The safety information sheets for the ingredients of the soups (broccoli powder, courgette powder and base vegetable soup) that includes microbiological characteristics and allergen information is included as Annex 23. The current specification are examples provided by the companies, but new specification sheets will be requested when the new batch of vegetable powders are received for soup preparation.

The soups will have documentation evidencing: microbiological safety and nutritional composition and will be prepared in the QI CRF by a member of the research team with formal Level 2 training in food safety and the QIB in-house food hygiene training module included in the QIB Human Studies Training Programme, in compliance with Environmental Health Guidelines to ensure food grade standards. Standard operating procedures (SOPs) for the preparation, storage and delivery of the soups to the study participants will be employed to minimise the risk of COVID19 infection for both staff and research participants. A HACCP plan for low risk food will be created for the study product; HACCP plans are implemented as part of GMP for food manufacturing and catering.

Even though all test ingredients are commercially available and safe for human consumption, as they will be combined at the QI CRF, a subset of soups will then be sent to ALS Laboratories for microbiological and chemical testing to ensure the soups are free from contaminants, and therefore safe for human consumption. The soups will be made closer to the intervention start date then the subset of soups will be sent for microbiological and chemical testing. A list of the microbiological analysis and the nutritional values of the soups that will be assessed can be found in Figure 4A and B respectively.

(A)

QUOTATION OF ANALYSIS **28 June 2019**

Sample Matrix: Food

Microbiological Analysis			
Analysis	Method Reference	Cost Per Sample (ex VAT)	UKAS Accredited
TVC (30°C)	ESGMM300	£3.50	Yes
E.coli (presumptive)	ESGMM304	£3.50	Yes
Enterobacteriaceae (presumptive)	ESGMM303	£3.50	Yes
Bacillus cereus (presumptive)	ESGMM319	£3.50	Yes
Yeast & Mould (DRBCA)	ESGMM308	£3.50	Yes
Clostridium perfringens (presumptive)	ESGMM310	£3.50	Yes
Salmonella (per 25g)**	ESGMM515	£10.00	Yes
Coagulase Positive Staphylococci	ESGMM307	£3.50	Yes

**Confirmation of presumptives are charged additionally per colony isolate £15.00 (ex VAT)

(B)

Chemistry Analysis	
Analysis	Cost Per Sample (ex VAT)
Group Two Nutritional:	
Moisture	
Fat	
- of which saturated	
- of which monounsaturated	
- of which polyunsaturated	
Total carbohydrate	£105.00
Available carbohydrate	
Total sugars	
Protein	
Dietary fibre	
Ash	
Sodium	
Energy – Kjoule / 100g	
Energy – Kcal / 100g	

Figure 4: Analysis of the test soup product to ensure food safe and GMP standards (A) List of the microbiological analysis of the soups (B) List of nutritional analysis of the soups.

Prior to distribution, each soup will be assigned a unique code to effectively blind the participants (see section 5.4: Blinding procedure). Specification sheet of test food including packaging, labelling requirements, storage condition, stability and shelf-life testing will be requested when the batch of soups are prepared ready for distribution to the participants. The soups will then be packaged in food grade recyclable plastic tamperproof containers with a lid and the labelling found in Figure 5. All soups will be stored in batches of 14 ready for distribution. The soups will be stored at QI CRF at -20°C in a food grade freezer until distribution to the participants. SOPs and kitchen use plan will be used according to the QI CRF code of practice. An inventory log and administration record will be kept by the study team to keep track of the number of soups distributed and to which participant. When the soups are received by the participants, the soups can be stored at room temperature in a cool, dry place.

Figure 5: Labelling for soup containers which will be used to link the participant code and soup ID.

7.6 Glucose drink used in glucose tolerance testing

The sugary beverage (glucose drink) is purchased from a medical supplier since the amount of glucose needs to be prepared under strict pharmaceutical standards. GlucosePro, is a pleasant commercially available raspberry-flavoured beverage. The specification sheet of ingredients and nutritional information can be found in Annex 23. The level of sweetness is similar to that of a fizzy drink or hot chocolate. The participant will have the choice to consume the beverage chilled (recommended) or at room temperature. The key to a successful OGTT is for the sugar drink to be consumed quickly as possible so a straw will be provided. They will need to consume the entire 250 mL (8 cups) within 15 minutes.

8. Assessment of Adherence

Participants will not know whether they are consuming the broccoli or placebo soup but, will receive a soup information sheet to take with them with information about the nutrition, ingredients, allergens, storage, and cooking instructions of the soups (Annex 16). Participants will be asked to record consumption of the soups and vegetables on the soup record sheet provided (Annex 17) and return any unused soup packets when they attend their final visit at the QI CRF. This is intended to aid adherence with the soups.

Participants will give 24-hour urine samples for assessment of adherence and detectable levels of sulfur metabolites. The measurement in the urine will assess whether sulfur compounds specifically glucosinolates, sulforaphane and SMCSO are present within the urine sample given. This will be analysed using analytical methods such as liquid chromatography- tandem mass spectrometry (LC-MS/MS) to measure the metabolites present.

Each participant will be asked to complete online and paper questionnaires (Annex 9, 10 and 11) whilst at the study visits 2, 5, 6 and 9. This will assess the participant's habitual diet and exercise over the intervention period and whether their dietary and lifestyle habits correlate with, or are reflected in, the outcome. It will also give an indication of any participants that consume a diet naturally high in sulfur compounds, though the participants must be willing to restrict consumption of cruciferous and alliaceous foods to 3 portions during the soup intervention periods, as this will influence metabolic analysis.

If the participants go on holiday during the intervention period, this will be allowed if they will still be able to consume the soups into their daily diet and complete the soup record. The soups do not require any specialist storage such as freezing or refrigeration, and therefore should not influence the adherence or outcomes in such cases. However, if the participants are looking to travel for longer than one month, they will not be able to participate in the study due to the scheduled visit timings.

9. Sample Processing and Experimental Methods

The samples will be collected at QI CRF and, either analysed by study scientists at QIB or at the Norfolk and Norwich University Hospital (NNUH) Pathology Laboratories. The blood tests results will be logged on the hospital electronic system where the participant's GP can access them if needed. The remaining blood samples will be stored appropriately until analysis. The stored samples will not carry any personal information, but instead will be labelled with the participant's unique code. After analysis, the remaining samples will be destroyed.

9.1 Soup Analysis

The amount of glucoraphanin, sulforaphane and SMCSO within the soups are analysed. To measure glucoraphanin, a model 1100 High Performance Liquid Chromatography (HPLC) system will be used. To measure sulforaphane and SMCSO, a model Agilent 6490 Triple Quadrupole LC/MS system will be used. Both machines will be equipped with a binary pump, cooled autosampler, degasser, column oven and diode array detector. The column temperature and autosampler temperature will be maintained at 20 °C and 4 °C respectively. The injection volume will be 2 µl per sample.
Glucoraphanin sample separation will be carried out with MilliQ water (mobile phase A) and LC-MS grade acetonitrile (mobile phase B). For analysis, the flow rate will be set at 1 ml/min with a maximum pressure 300 bar and a gradient of increasing mobile phase B from 5% to 90% over 32 minutes. This was reduced to 5% for 14 minutes preceding re-equilibration. Samples will be separated using Waters Spherisorb ODS2 (4.6 x 250 mm id, 5 µm particle size) column equipped with a Waters Spherisorb guard column (Waters), by positive ion atmospheric pressure chemical ionisation (APCI+) LC-MS. Quantification will be conducted from the chromatogram data measured at the absorbance 229 nm, and the ratio comparison of the peak of interest and the internal standard (16 mM sinigrin).

Sulforaphane sample separation will be carried out with 0.1 % ammonium acetate adjusted to a of pH 4 with 0.1 % acetic acid (mobile phase A) and 0.1 % acetic acid in acetonitrile (mobile phase B). For analysis, the flow rate will be set at 0.25 mL/min. Samples will be separated on Phenomenex Luna 3u C18(2) 100A (100 x 2.1 mm) column, using a programmed gradient mobile phase, by multiple-reaction monitoring (MRM) mode.

SMCSO sample separation will be carried out with 10 mM ammonium acetate and 0.05% hetaflurobutyric acid in water (mobile phase A), and 10 mM ammonium acetate and 0.05% hetaflurobutyric acid diluted in 10 % water with the remaining volume made up with 100 % methanol (mobile phase B). For analysis, the flow rate will be set at 0.3 mL/min. Samples will be separated using Agilent SB-AQ 1.8 µM (100 x 2.1 mm) C18 column with an Agilent Zorbax guard column, using a programmed gradient mobile phase and ESI positive MRM.

For sulforaphane and SMCSO, identification will be achieved based on retention time and product ions. Quantification will be performed by the use of calibration standards and internal standards.

9.2 Blood sampling and processing

During the study, venous blood sampling will be taken on the screening visit (no more than 20 mL) and at 8 study visits (no more than 35 mL). The blood sample will be taken from the arm by a trained QI CRF research nurse. No more than 25 mL of blood will be sent and tested at the Norfolk and Norwich University Hospital (NNUH) Pathology Laboratories for assessment of HbA1c, fasting plasma glucose, fasting insulin, fructosamine, total/HDL/LDL cholesterol and triglycerides. The screening blood sample will also assess full blood count (FBC). The remaining blood sample (10 mL) will be stored for blood gene expression analysis (see section 9.4) and we may also conduct future experiments that may investigate levels of inflammation from plasma samples using enzyme-linked immunosorbent assay (ELISA) assay kits.

Anonymous data via participants' codes will be made available for the study team for analysis. Eligibility screening results will be made available to the GP via the hospital electronic system and/or by letter (Annex 14).

The capillary blood finger samples will be taken on four visits during the study and will assess the capillary glucose levels. The study participants will be shown how to take the samples, but a study scientist will be present to also take the sample if needed. Participants will be given instructions within the glucose record booklet (Annex 15) if they wish to take the sample themselves. For each sample, a needle will prick the end of a finger. A drop of blood from the prick will be put onto a strip of paper and placed into a machine that will give a fasting blood glucose reading within a few minutes. The lancet and strip will be disposed of in the correct clinical waste bin. The glucose reading will be recorded on the glucose record booklet (Annex 15) by the participant. From this data, we will test whether the consumption of broccoli soup, has built up resilience in the body and shows a better glucose tolerance response after repeated consumption.

9.3 Urine processing and analysis

Urine samples will be collected by the participant on four occasions over the study prior to study day (24-hour collection), into the 24-hour urine collection pots with added preservative ascorbic acid. Following receipt of the samples, these will be divided into aliquots and placed on dry ice for transportation from QI CRF to QIB where it will then be stored at -80°C until analysis. Analysis of the urine samples will be conducted following QIB standard operating procedures and will use analytical methods to measure the metabolites in the urine.

SMCSO and its metabolites (MMTSO and MMTSI), and glucoraphanin/sulforaphane and its metabolites (SF-NAC, SF-Cys, SF-Cys-Gly and SF-glutathione) will be separated, detected, and analysed in the urine using the same method as described in section 9.1. The reduced analogues of SMCSO (S-methyl cysteine) and glucoraphanin (erucin) which are often produced via bacterial-mediated reduction reactions (21), will also be measured.

9.4 Gene expression analysis

As described in section 9.2, two 10 mL blood samples will be used to undertake gene expression analysis (global and targeted will be employed, if appropriate).

The blood sample will be stored at -80°C until RNA extraction. According to the manufacturer's instructions, a total RNA extraction will be performed on the samples using Qiagen RNeasy kits. RNA of sufficient quality will be sequenced by Illumina sequencing. Target analysis will be used as well if selected genes are identified through the global approach. Bioinformatic pipelines available in our laboratory group will be used in this analysis and will explore areas such as cancer gene expression pathways and pro-/anti-inflammatory markers.

9.5 Questionnaire data

NB: All questionnaires completed will also be used as an assessment of adherence. The questionnaires are not optional, and the participant will complete most these questionnaires whilst at the study visits 2, 5, 6 and 9 to reduce burden, and may reduce some of the boredom they may have from the visits.

Viocare® is the company that will provide Vioscreen site for the lifestyle questionnaires: the food frequency questionnaire (FFQ, Annex 10) and international physical activity questionnaire (IPAQ, Annex 11). Dietary intake will be analysed using the nutrition analysis software based on USA food composition tables. Although validated in the US, the Viocare FFQ has not been validated in the UK. Whilst other FFQ have been considered, this was found to be the most appropriate FFQ for the study as the diet in the US is very similar to the diet in the UK (Western diet), been used in hundreds of human studies with numerous cohorts including those with pre-diabetes and it has standardised software for analysis, reducing data-interpretation bias.

The FFQ provides information of the participant's habitual diet through online questions to assess food group intake. For the Viocare FFQ, the time interval for assessment can be customised. For this study, we plan to use the FFQ over a 90-day interval. Each completed FFQ generates four clinical reports. The first report is a detailed personal health and nutrition summary report detailing body measures, nutrition and food consumed. The second report is the healthy eating index (HEI) feedback report detailing current intake and recommended intake of food group areas. The third report is a top foods report detailing macronutrients and micronutrients consumed. The final report is a dietary inflammation index (DII®) report detailing areas of the diet that have inflammatory potential. The DII® may be linked with the gene expression analysis to see if the score estimates are associated with pro- and/or anti-inflammatory gene regulation in blood.

The IPAQ provides information of the participant's habitual exercise through online questions of exercise type and quantity. Each completed IPAQ generates one clinical report. This report details such as average daily metabolic equivalents (METS) in exercise of the participants.

The Arizona Cruciferous Vegetable Food Frequency Questionnaire is a 6-page questionnaire that assesses cruciferous vegetable intake and assesses other foodstuffs which are known to be sources of sulfur metabolites (Annex 9). The Arizona questionnaire has been specifically developed by the University of Arizona to assess cruciferous vegetable intake over the time period the participants have been asked to review. In this case, this will

be 90 days before the enrolment in the study. The questionnaire has previously been shown to provide a reproducible, valid estimate of cruciferous vegetable exposure in studies at QIB. The data obtained in the questionnaire will be analysed by a research group at the University of Cancer Centre (US). The Arizona FFQ is much more comprehensive, so will be complementary to the Vioscreen FFQ.

Variability in dietary adherence in chronic feeding studies is largely influenced by personal preferences towards the palatability of the study foods used. Therefore, we will measure if differences in the two soups are noticeable and if this affected their palatability (acceptance). A sensory evaluation questionnaire with a nine-point hedonic scale will be used to determine any differences between the two soups in overall acceptability based on specific attributes detailed in Annex 12. A Just-About-Right (JAR) scale with five anchor points will be used to measure if there are significant deficiencies in soup palatability. Participants will be asked to give their opinion on the intensity of specified product attributes in relation to their JAR level. Penalty analysis determines how the overall acceptability (palatability) was affected by a given attribute where it was scored not to be optimal or JAR, and so identifies those attributes that are most penalising to the product performance. To carry out Penalty Analysis, we will combine overall acceptability (from the 9-point hedonic scale) and JAR scale responses. JAR and overall acceptability data must be collected from the same respondents. The attribute lexicons were derived from previously published papers pertaining to cruciferous vegetables and soups made with broccoli (36,37).

The follow-up questionnaire will give a qualitative analysis on the soups and study and will be assessed on the comments given rather than quantification.

10. Statistical Analysis

The data analyst will be blind to the conditions of the study until all analysis if fully completed.

10.1 Sample size

There is no prior data on the effect of broccoli consumption on blood glucose in individuals with pre-diabetes, and little prior data on its within-patient variability. To calculate a sample size for the current study we used data obtained from an ongoing study of HbA1c using the Clinical Practice Research Datalink, in which a population representative sample of 75,000 people with non-diabetic hyperglycaemia (NDH) (Savva, personal communication) and from previously published data (30).

We will aim to recruit 35 participants with the expectation ≥30 participants will complete the study. This will give us a 90% power to detect a difference in 0.22% in HbA1c. Within person standard deviation estimated using a mixed model was 0.24%, therefore the difference between two HbA1c measurements in the same individual is likely to have standard deviation of $\sqrt{(2)}$*0.24% = 0.34%, and so the estimate of treatment effect will have an expected standard error of 0.058%, and the 95% confidence interval for treatment effect will have an expected total width of 0.232%.

10.2 Data analysis

Statistical analysis will focus on providing estimation of effects with confidence intervals rather than hypothesis testing, but p-values corresponding to hypothesis tests will also be provided.

10.2.1 Primary Outcome

To determine if the dietary intervention affects the primary outcome measure HbA1c, an analysis of covariance (ANCOVA) will be applied, estimating the within-person differences at the end of each treatment period, with the baseline values (measured at the start of each period), the period effect and calendar data included as covariates.

10.2.2 Secondary Outcomes

We will estimate how the effect of the broccoli soup on energy metabolism markers (fasting plasma glucose, fasting insulin, cholesterol and triglycerides), changes with time since the start of the dietary intervention, using a linear mixed regression model to estimate the time by treatment interaction effect.

The effects of soup consumption on levels of bioactives in the urine will be estimated using regression analysis as for prim.

For glucose tolerance testing, the incremental area under the blood glucose response curve (iAUC) for data collected from capillary blood during the 3.5-hour period will be calculated to measure differences in the glucose response over time to test if soup consumption has an influence on glucose response will be compared using a paired t-test. Non-parametric Mann-Whitney U test may be used to identify differences between men and women.

The effect of soup consumption will be tested using developed informatics tools such as *edgeR* and *Limma* to fit linear models to gene expression data. False discovery rate will be corrected using the Benjamin Hochberg procedure.

Dietary data from the questionnaires may be used as covariates to characterise the blood sampling data and distinguish between changes in glucose levels that occur naturally and those that are diet induced.

The difference between clinical parameters such as BMI and waist and hip circumference between the two groups will be assessed by using ANOVA corrected for multiple testing by Tukey's multiple correction test.

11. Completion of the Study

Upon completion of the study, the general findings will be reported back to the participants as a basic summary of the intervention. This will be anonymised, and no individual data or results will be presented to the participants.

12. Withdrawal from the Study

Participants will be withdrawn from the study if the participant, for any reason, loses the capacity to consent during the study and their medical situation changes significantly. If the participants are unable to collect blood samples and capillary blood, they may be asked to withdraw from the study or to re-schedule their study visit, this will be assessed on an

individual basis. If at any point during the study the participant wishes to withdraw, they may do so without giving a reason and their clinical care and participation in future studies at QIB will not be affected. The participant will be sent a withdrawal letter (Annex 21) explaining this and thanking them for their participation so far. Again, any sample or data collected up to the point of withdrawal will be kept and used in the study if possible. The patients' GP will also be informed that they are no longer taking part in the study (Annex 22). The participant will be replaced to maintain the balance of the block system.

13. Participant Payment and Travel Expenses

The participant will receive £210.00 as an inconvenience payment if the study is completed; if the participant withdraws or is excluded from the study, payment will be *pro-rota*, as follows £10 for screening visit (visit 1), £40 for study visits 2, 5, 6 and 9, and £10 for study visits 3, 4, 7 and 8.

Participants will be reimbursed travel expenses to and from the QI and car parking/public transport costs, if required. This will be reimbursed at the QIB's current mileage rate or by reimbursing public transport costs on production of a ticket or receipt. If participants require transport by taxi, this will be arranged to be pre-paid.

14. Safety Measures in response to COVID-19

QIB as research Sponsor has been working with the Norfolk and Norwich University Hospital to put in place appropriate measures to ensure the safety of research participants, NNUH and QIB staff in response to the COVID-19 outbreak. QIB researchers are asked to follow specific standard operating procedures (SOPs) for delivering face-to-face study appointments at the QI CRF. The study team will implement the following safety measures:

<u>Before the appointment:</u>

✓ All participants will be given the option to decline to attend their appointments if they are worried about associated increased risk of COVID-19 infection.

✓ Participants will be contacted within 7 days of any scheduled appointments at QI CRF and asked not to attend their appointment if they can respond "yes" to any of the following questions:

a) Have you been advised that you are extremely vulnerable from COVID-19 and are therefore shielding?

b) Have you or has anyone in your household received a positive test for COVID-19, or awaiting results of a test?

c) Have you or has anyone in your household had any of the symptoms of coronavirus in the last 14 days? (a high temperature, a new continuous cough, a loss or change to your sense of smell or taste).

d) Are you or is anyone in your household self-isolating as a result of the NHS test and trace programme?

✓ Responses to the questions above will be recorded in the study log with the initials of the research team member who contacted the participant and the date the participant was contacted.

✓ Participants will also be sent these questions in a reminder message or by email the day before their appointment and asked to review their responses before attending their appointment.

✓ Appointments will be scheduled to reduce contact with other participants and members of staff.

At the appointment:

✓ Before a scheduled appointment, the research team will disinfect the common surfaces of the appointment room that the participant may come into contact with during their visit (chair, desk, door handles). Disinfectants will be provided by the CRF team in line with NNUH Infection control guideline.

✓ Participants will not be allowed to wait at the QI CRF reception for more than 10 minutes before their scheduled appointments.

✓ Participants will be given the choice to wear personal protective equipment (PPE) for their appointments and they will be offered these if they have not brought personal items. Research staff will wear PPE in accordance with NNUH guidelines.

✓ Research staff will adhere to current government social distancing guidelines wherever possible. However, it is acknowledged that during physical measurements and while participants having their bloods taken, this may not be possible. Staff conducting this aspect of the appointment will be NNUH staff and will be adhering to the latest NHS guidelines on how to conduct this aspect of the study as safely as possible.

15. Risk and Mitigation for a full UK lockdown

We will commence the study as described above with socially distanced measures employed, however in the case of a full UK lockdown due to COVID-19; we will move to a remote study protocol to ensure comparable data will be collected. Note: if full UK lockdown is announced and the participant is 10-12 weeks through a face-to-face intervention period, we may ask them to reschedule their appointment and come in earlier to complete the end-point data. If the participant has just started a face-to-face intervention period, we may ask the participant to restart the intervention period once lockdown is lifted.

Recruitment of individuals with pre-diabetes will follow the same procedure as the face-to-face protocol through GP surgeries, QIB participant database and advertisement (if required). Participants will receive a modified version of the participant information sheet for the remote study (Annex 3B).

Remote screening including informed consent and the medical questionnaires will be conducted via a well-established online platform such as Zoom or Microsoft teams with a QIB study team. The participant will be given different options to conduct the study talks including videocall, conference call without video and telephone calls and their preference will be recorded in the consent form. Participants will be given the option to complete a paper or electronic version of the consent form with a member of the research team over

their preferred contact method. Part of the medical questionnaires and assessment day checklists require body measurements including height, weight, waist and hip circumference, blood pressure and pulse, for these measurements the participant will receive a tape measure, weighing scales and a blood pressure monitor. We will ask them via to conduct the measurements via their preferred contact method. Participants will receive a screening home testing kit provided by Medichecks® to confirm pre-diabetes through measurement of HbA1c. The QIB medical advisor will assess and confirm eligibility. Any screening results which fall outside of the study criteria will be assessed by the QI medical advisor.

Medichecks® will provide home testing kits and be putting together a bespoke array of markers for the study to remotely measure HbA1c, fasting plasma glucose, total/HDL/LDL cholesterol and triglycerides. We may not be able to measure all the biomarkers with Medichecks®, however we will prioritise accordingly to the study aims. Participants will still be required to fast for at least 10 hours before taking the blood sample at home. Participants will receive a detailed set of instructions (Annex 24) of how to take their blood and what to do with the sample when completed. The QI CRF medical advisor and QI research nurse have approved the use of Medichecks® for carrying out remote activities.

Soups and urine collection pots will be delivered to the participant's home. To ensure safety, we will leave packages at the doorstep where appropriate and move back before the participants collect their soups and urine pots. Through the secure study website, participants will be able to view soup information and record when they have consumed the soups. We will make arrangements for a study scientist to collect the 24-hour urine collection pot.

The lifestyle and cruciferous vegetable questionnaires will be uploaded to a study website with secure links to the online forms for completion. The follow up questionnaire will be available on the secure study website for completion remotely.

We will not be able to conduct glucose tolerance tests and insulin assessment nor take blood samples for blood gene expression analysis remotely.

The participant will receive the same inconvenience payment as for the face-to-face protocol, see section 13: participant payment and travel expenses.

16. Data Protection and Participant Confidentiality

QIB is the Sponsor for this study based in the United Kingdom. For any (personal) information collected from participants and/or their medical records to undertake this study, QIB will act as the data controller. This means that we are responsible for looking after their information and using it properly. QIB will keep identifiable information about the participants for 15 years after the end of the study in a secure archive at the QIB or designated secure off-site location. The participants rights to access, change or move their information are not affected, as we need to manage their information in specific ways for the research to be reliable and accurate. If the participant withdraws from the study, all personal and identifiable data will be removed, but fully anonymised study data and samples already collected with consent will be retained and used in the study. No further data or samples will be collected, or any other research procedures carried out in relation to the participant.

All information collected about the participant during this study will be kept strictly confidential. We follow Ethics and Research Governance and Good Clinical Practice (GCP)

requirements. The collection, storage, processing, and disclosure of the study data will be managed by the study team in adherence with EU General Data Protection Regulation (GPDR) and UK Data Protection Act 2018, with regards to the collection, storage, processing and disclosure of personal information and will adhere to the GDPR and DPA core principles to maintain confidentiality.

The legal bases used under the regulation that we employ to process the participant's personal information is for tasks carried out in the public interest, which this study and associated research is. Their personal information will be stored in lockable filing cabinets at the QIB. Suitable security measures and precautions are also taken for any confidential or personal data process or stored electronically. Their data will be pseudo-anonymised with a unique, study-specific code which cannot be linked to the participant and is stored on a password-protected data file. All biological samples collected will be known by the assigned code.

The trial has a study specific email address (BETA@quadram.ac.uk) and specific contact number (07733 699117), that the research team will use for recruitment purposes and for study-related correspondence with the research participants. The study email address will be a shared QIB account with restricted access to the research team only. We will include an automatic management (timely deletion) of emails and phone logs to ensure participant data protection.

During the study, the participant will be asked to use external services to record study information. The accounts will be created for the participant (as per the process described above). The data collected from the blood samples will be identifiable by the NNUH team at the QI CRF and will be transferred from NNUH Pathology department to QIB for analysis. The data collected from the questionnaires, with no personal or identifiable information, will be transferred to a third party (outside of the EEA) to be analysed by the University of Arizona or the company Viocare®. All the data collected from the questionnaires by will be kept anonymous (no personal identifying information), so the data will not need to be stored on an EU server and no data management agreements are required.

If the participant was to enrol onto the remote study protocol, the additional external service provided by Medichecks® will be used. The kits provided will be sent to QIB's address from Medichecks®, and the study team will process and send the kits to the participant's address with their unique study code. Due to the nature of the service, the participant's age and gender will be shared with Medichecks®. All data are pseudo-anonymised to the study team and anonymised to Medichecks®. A data management agreement in place from Medichecks® can be found at https://medichecks.com/pages/practitioner-terms-and-conditions. Further information on how Medichecks® handle/process their data can be found via their privacy policy at https://medichecks.com/pages/privacy-policy.

The results of the study including analysed are expected to be published, fully anonymised, for use by the wider scientific community.

To safeguard the participants rights, we will use minimum personally identifiable information possible. A NBI Data Protection Adviser (dpa@nbi.ac.uk) has reviewed the proposed system in terms of adherence with EU GDPR/UK DPA 2018.

17. Data Management Plan

17.1 Description of the data

Our data will include health questionnaires, food frequency questionnaires, exercise questionnaires, results from the analysis of biological specimens (urine and blood), which will be transcribed and maintained in spreadsheet form, and stored as described above.

17.2 Specific management of personal data

Participants who are successfully recruited onto the study will be assigned a unique code number which will be kept in a secure file and will not be linked to their name outside of a file kept locked away. A lockable filing cabinet or cupboard will be used to keep paper documents that include the file linking the participant code and personal information. Manual files/folders will consist of separate named and numbered files for each participant. No data with the participants' name will be filed in the numbered file and vice versa. Only the study scientists will have access to the file linking personal data to the participants' unique code. Confidential data will be accessed only by the study team at the investigator site. All electronic data will be stored on an encrypted password protected shared data file. Only the study team will have access to these data. Data will be stored for 15 years after completion or discontinuation of the study in a confidential archive. At 15 years, all information about the participant will be destroyed. Archived data will not be used for contacting participants after the end of the study. Access to archived data will be limited to the study scientists and chief investigator (CI) of the study or the CI's successor. The quality assurance auditors may also be allowed access with the permission, and in the presence of, the CI. Any information collected prior to consent (such as patient identification from out-patient clinics, or responses to the PIS) will be used solely for the purpose of the study and will be handled in adherence with the QIB Data Protection Policy in order to protect and respect participants' privacy. This process has been reviewed and authorised by a NBI Data Protection Adviser (dpa@nbi.ac.uk). Data will be managed by the study team in compliance with EU General Data Protection Regulation (GDPR) and UK Data Protection Act 2018.

17.3 Specific management of samples

All biological specimens collected as part of the study will be known only by their code number. All data collected will also be identified by code only. Laboratory results will be maintained in a spreadsheet form and will be in file formats that can be shared. Only anonymised individual-level data will be shared within study team members.

17.4 Data collection / generation

Data will be collected by trained researchers onto study-specific forms and then uploaded into electronic data sets. Methods used to generate data from this study will be fully described in standard operating procedures (SOPs). The study record will include a detailed description of data collection and coding system.

All raw data will be collected on a continuous basis and supplemented with relevant additional information, such as identity of researcher collecting and entering data, date of collection). Most of the data will be in digital form; however, some data will originally be recorded in hardcopy-form and later transcribed to digital copies. Working copies of all

datasets will be kept in an encrypted format on the institutional network (QIB) which has shared access with appropriately authorised research staff working on the project.

We will ensure that clear audit trails link secondary processed information to primary data and will be audited in compliance with International Good Clinical Practice (GCP) standards.

17.5 Data sharing and access

The research protocols will be registered in a publicly accessible database after gaining favourable ethical opinion. Registration to ClinicalTrials.gov Protocol Registration and Results System (PRS) using QIB account will allow us to be transparent in our work. The study team will ensure fully adherent with the standards required for deposition of information in any relevant public databases. Anonymised individual-level datasets will be shared outside the team only after obtaining explicit consent of research participants. Consent forms clearly state the data sharing procedures for data generated from this study.

17.6 Relevant institutional polices on data sharing and data security

All data will be managed, protected and shared in accordance with the requirements of the QIB Quality Code of Practice, the QIB Policy on Safeguarding Good Scientific Practice and the BBSRC Data Sharing Policy. All study collaborators will adhere to the same rigorous standards for data management.

18. Definition of End of Study

The end of the study will be the date when all samples have been collected from all participants, and questionnaires from all participants have been completed. The participant will receive a summary of the results at the end of the study, and their personal data (contact details) will be used for this purpose.

19. Ethical and Regulatory Considerations

19.1 Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki. The proposed research will be conducted in accordance with the conditions and principles of the International Conference on Harmonisation Good Clinical Practice (ICH GCP), and in adherence with national law. The research will meet the requirements of the new EU General Data Protection Regulation (GDPR), UK Data Protection Act 2018 and relevant Sponsor's policies.

19.2 Approvals

The protocol, informed consent form, participant information sheet and any other documents use for this study will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written approval. The study protocol and associated documents will be reviewed by the Human Research Governance Committee (HRGC) at QIB and approved by the QIB statistician prior to submission to the REC. QIB Human Research Governance Committee adheres to the UK Policy Framework for Health and Social Care Research. Copy of the HRGC approval, and any correspondence with the committee will be available to the REC and HRA, if requested.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

19.3 Informed Consent

To ensure participants can make an informed decision as to whether they wish to take part on the study they will be provided with a PIS (Annex 3A or 3B) and invited to an informal study talk by phone. Written consent will be obtained by a member of staff who is GCP trained and experienced in conducting human intervention trials. The participant must have the ability to make and communicate their decision, can give informed consent from the information given about the study, from the participants ability to understand the information and exercising the right to choose to participate. All participants are free to withdraw at any time from the study without giving reasons.

19.4 Adverse events and serious adverse events

This study will comply with the NNUH Trust system for reporting adverse events (AEs) and will adhere to NNUH SOP 206 Adverse Events: Identifying, Recording and Reporting adverse events for Non-CTIMP Non-Device Healthcare Research Studies, version 2, dated 18/06/2020. In brief, AEs will be evaluated for seriousness, causality and expectedness by the QI CRF research nurse and QI medical advisor. All AEs that are not considered serious will be documented on the relevant case report form (CRF) and filed accordingly (Annex 25). The completed form will be filed along with the other CRFs for the study and a copy provided to the Sponsor. Serious adverse events (SAEs) will be reported on the NNUH SAE form (Annex 26). SAEs will be notified by the Chief Investigator (CI) to the Sponsor within 24 hours of the CI becoming aware of the event. This will be followed up within 48 hours of becoming aware of the event by a detailed, written report by the QI medical advisor. SAEs will be notified by the CI to the Research Ethics Committee (REC) using the HRA report of SAE form found on HRA website: https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/safety-reporting where in the opinion of the CI it was possibly, probably or definitely related, within 15 days of the CI becoming aware of it. The CI will report all logged events to the REC annually as a Safety Report; a copy of this report will be provided to QIB HRGC. The CI will report all logged events to the NNUH R&D Department. All SAEs will be followed up by the QI medical advisor until satisfactory resolution, and this would be recorded as a follow up report on the SAE report form, and on the SAE log. At each stage of follow up the QI medical advisor will sign and date the form.

An adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical study subject.

A Serious Adverse Event (SAE) is defined according to ICH GCP as an untoward occurrence that:

- Results in death
- Is life threatening*
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapability
- Is a congenital anomaly or birth defect
- Is otherwise considered medically significant by the investigator

* Life-threatening, in the definition of an SAE, refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might

have caused death if it were more severe. Medical judgement should be exercised in deciding whether an adverse event is serious in other situations. Important adverse events that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

A planned hospitalisation for a pre-existing condition, or a procedure required by the trial protocol, without a serious deterioration in health, is not considered to be a serious adverse event unless specified in the clinical trial protocol. The participant will only be deemed to have suffered an adverse event or serious adverse event if the participant has taken part in any stage of the intervention.

19.5 Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

19.6 Procedures for any harm experienced by the participants

If, throughout the period of this human intervention trials, any participant is harmed by taking part, there are no exclusive compensation privileges. If, due to negligence, harm is caused to the participant and there are grounds for legal action, the participant will likely have to pay for these legal costs. We appreciate that under specific circumstance, participants may still wish to file a complaint, and in this case, a confidential service that is designed to support patients, relatives and carers will be available to them. This service is the Patient Advice and Liaison Service (PALS), and the website can be found here: http://www.nnuh.nhs.uk/patients-visitors/help-support/pals/. QIB has liability insurance (Annex 27) with regards to research involving human participants. Please note that the Institute will not fund any legal costs arising from any action unless awarded by a court.

Furthermore, as this study involves the QI CRF, which is an NHS facility, indemnity is provided through NHS schemes.

19.7 Participant wellbeing throughout the study

Throughout the study, the participant will be asked monthly by one of the study scientists, how the intervention is going. This will usually be when they come to QI CRF as part of the study visits. If any participant becomes unwell at any stage of the study, the first action will be for them to see their GP or A&E. GPs will be informed about the participants' involvement in this study by letter and will receive copies of the PIS (Annex 3). The PIS will advise participants to contact the emergency service (via 999) in case of a medical emergency and ensure that the study team is contacted as soon as practically possible. The decision to exclude the participant from the study will be taken by the QI medical advisor.

19.8 Soup safety

QIB and soup provider follows standard operating procedures for the preparation, delivery, and storage of food for research participants. In agreement to Environmental Health Guidelines, these standard operating procedures will be adhered to when processing and handling our food items. The base vegetable soup complies to food standard guidelines. All staff handling, preparing, or delivering the soups for participants will have completed Level 2 Food Safety and Hygiene Course for Catering.

19.9 Toxicity

Consumption of broccoli is safe and there is no evidence from human and animal studies that it is harmful to health (17,38), unless a participant has an allergy to broccoli. Each soup will contain a similar amount of broccoli to what would be consumed in two standard broccoli portions. The base vegetable soup, broccoli powder and courgette powder are available to purchase and safe for human consumption. If a participant has an allergy to any of the ingredients, they must not consume the soups.

19.10 Allergies

If it is known the participant has an allergy to broccoli or any of the ingredients used in the intervention soups, they will be unable to participate in the study. At each visit to QIB, the participants will be asked about any known allergies that they have may or have development over the duration of the study. However, if the participant develops an allergy to broccoli or any of the ingredients in the intervention, they will be withdrawn from the study. Information on allergies will be documented within the medical screening questionnaire.

19.11 Risks with blood sampling

The participants may endure a little pain or discomfort from the blood and finger prick sampling, but once this is completed there should be minimal or no pain. Where the needle is inserted, there may be a small bruise develop but most bruises fade within a 3-4 days. The blood sampling will be conducted by QI CRF research nurses who are fully trained and experienced in taking blood, minimising the risks associated with the procedure. If there are any issues regarding blood sampling on the day, the QI medical advisor can be contacted and can provide medical support, as the wellbeing of the participant is vital.

19.12 Audits and Inspections

The study may be subject to inspection and audit by QIB under their remit as Sponsor, and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

19.13 Publication Policy

The research protocol will be registered to ClinicalTrials.gov Protocol Registration and Results System (PRS) using QIB account, which is a publicly accessible database after gaining favourable ethical opinion.
The findings of the research will be published in an open-access, peer-reviewed journal. Participants' research data collected during the study may be used to support other research in the future and may be shared anonymously with other research groups. In addition, we may collaborate with patient groups and professional groups to share the findings through multiple media channels including public engagement events and social media.

19.14 Sponsor and Funding

QIB will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study. The study will be funded by Biotechnology and Biological Sciences Research Council (BBSRC) Institute Strategic Programme, Food Innovation and Health, reference BB/R012512/1. Participants will

be paid for their time; see section 13: participant payment and travel expenses for further details.

20. Indemnity

QIB has £10 million commercial insurance for human trial studies/clinical trials, including no fault liability insurance. This includes the remote study activities proposed. The study will be covered by the current QIB Insurance as documented in Annex 27 which gives further details of QIB liability insurance.

NHS bodies are legally liable for the negligent acts and omissions of their employees. If they are harmed whilst taking part in a clinical research study as a result of negligence on the part of a member of the study team this liability cover would apply. Non-negligent harm is not covered by the NHS indemnity scheme.

21. Dissemination Policy

This study will be registered on a publicly accessible database after obtaining ethical approval. Registration on the ClinicalTrials.gov website using the QIB account will enable us to be transparent in our work and to meet legal requirements. Data arising from the study will be owned by QIB. Data will be disseminated in the form of scientific presentations/abstracts and publication in a scientific journal. Authors will acknowledge that the study was funded by the BBSRC in all publications. Participants will also be informed of the outcome of the study.

22. References

1. Wu, Y, Ding, Y, Tanaka, Y, & Zhang, W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. International journal of medical sciences. 2014;11(11):1185-1200.
2. Diabetes UK. 2019; Available from: https://www.diabetes.co.uk/pre-diabetes.html
3. Khan RMM, Chua ZJY, Tan JC, Yang Y, Liao Z & Zhao Y. From Pre-Diabetes to Diabetes: Diagnosis, Treatments and Translational Research. Medicina. 2019;55(9):pii: E546.
4. National Institute for Health and Care Excellence (NICE) guidelines. Type 2 diabetes: prevention in people at high risk. Published 2012, Updated 2017. ISBN: 978-1-4731-2636-7. Available from: https://www.nice.org.uk/guidance/ph38
5. Kolb H & Martin S. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Med. 2017;15(1):131.
6. Bansal. Prediabetes diagnosis and treatment: A review. World Journal of Diabetes. 2015;6(2):296-303.
7. Leon BM & Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World Journal of Diabetes. 2015;6(13):1246-1258.
8. Huang Y, Cai X, Qiu M, Chen P, Tang H, Hu Y et al. Prediabetes and the risk of cancer: a meta-analysis. Diabetologia. 2014;57(11):2261-9.
9. Dankner R, Boffetta P, Keinan-Boker L, Balicer RD, Berlin A, Olmer L et al. Diabetes, prostate cancer screening and risk of low and high-grade prostate cancer: an 11 year historical population follow-up study of more than 1 million men. Diabetologia. 2016;59:1683-91.
10. Traka MH, Saha S, Huseby S, Kopriva S, Walley PG, Barker GC, et al. Genetic regulation of glucoraphanin accumulation in Beneforté® broccoli, New Phytologist, 2013;198(4):1085-95.

11. Traka, M and Mithen, R. Glucosinolates, isothiocyanates and human health. Phytochemistry Rev, 2009;8(1):269-282.
12. Barba, FJ, Nikmaram, N, Roohinejad, S, Khelfa, A, Zhu, Z, & Koubaa, M. Bioavailability of Glucosinolates and Their Breakdown Products: Impact of Processing. Frontiers in Nutrition. 2016;3,24.
13. Livingstone TL, Beasy G†, Mills RD, Plumb J, Needs PW, Mithen R et al. Plant Bioactives and the Prevention of Prostate Cancer: Evidence from Human Studies. Nutrients. 2019;11(9):2245, † - Joint first author.
14. Axelsson AS, Tubbs E, Mecham B, Chacko S, Nenonen HA, Tang Y et al. Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. Sci Transl Med. 2017;9(394). pii: eaah4477.
15. Bayat Mokhtari, R, Baluch, N, Homayouni, TS, Morgatskaya, E, Kumar, S, Kazemi, P et al. The role of Sulforaphane in cancer chemoprevention and health benefits: a mini-review. Journal of cell communication and signaling. 2018;12(1):91-101.
16. Jiang, X., Liu, Y., Ma, L., Ji, R., Qu, Y., Xin, Y., et al. Chemopreventive activity of sulforaphane. Drug design, development and therapy, 2018;12:2905-2913.
17. Yagishita, Y, Fahey, JW, Dinkova-Kostova, AT, & Kensler, TW. Broccoli or Sulforaphane: Is It the Source or Dose That Matters?. Molecules. 2019; 24(19):3593.
18. Kim JK, Park SU. Current potential health benefits of sulforaphane. EXCLI J. 2016;15:571–577.
19. Nagata N, Xu L, Kohno S, Ushida Y, Aoki Y, Umeda R et al. Glucoraphanin Ameliorates Obesity and Insulin Resistance Through Adipose Tissue Browning and Reduction of Metabolic Endotoxemia in Mice. Diabetes. 2017;66(5):1222-1236.
20. Teng W, Li Y, Du M, Lei X, Xie S, Ren F. Sulforaphane Prevents Hepatic Insulin Resistance by Blocking Serine Palmitoyltransferase 3-Mediated Ceramide Biosynthesis. Nutrients. 2019;11(5):1185.
21. Kellingray L, Le Gall G, Doleman JF, Narbad A, Mithen RF. Effects of in vitro metabolism of a broccoli leachate, glucosinolates and S-methylcysteine sulphoxide on the human faecal microbiome. European Journal of Nutrition. 2020.
22. Neequaye, M. Thesis: A Study of Genetic Regulation of Sulfur Metabolism in Brassica oleracea. 2020.
23. Edmands WMB, Gooderham NJ, Holmes E & Mitchell SC. S-Methyl-L-cysteine sulphoxide: the Cinderella phytochemical? Toxicology Research. 2013;2:11-22.
24. Kumari K, Mathew BC, Augusti KT. Antidiabetic and hypolipidemic effects of S-methyl cysteine sulfoxide isolated from Allium cepa Linn. Indian J Biochem Biophys. 1995;32(1):49-54.
25. Sheela CG, Kumud K, Augusti KT. Anti-diabetic effects of onion and garlic sulfoxide amino acids in rats. Planta Medica. 1995;61(4):356-7.
26. Kumari K & Augusti KT. Anti-diabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (Allium cepa Linn.) as compared to standard drugs in alloxan diabetic rats. Indian J Exp. Biol. 2002;40:1005-1009.
27. Coode-Bate J, Sivapalan T, Melchini A, Saha S, Needs PW, Dainty JR, et al. Accumulation of Dietary S-Methyl Cysteine Sulfoxide in Human Prostate Tissue. Mol Nutr Food Res. 2019;63(20):e1900461.
28. Liberti MV & Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? Trends Biochem Sci. 2016;41(3):287.
29. Bahadoran Z, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. Int J Food Sci Nutr. 2012 Nov;63(7):767-71.
30. Sivapalan T, Melchini A, Saha S, Needs PW, Traka MH, Tapp H et al. Bioavailability of Glucoraphanin and Sulforaphane from High-Glucoraphanin Broccoli. Mol Nutr Food Res. 2018;62(18):e1700911.
31. Traka MH, Melchini A, Coode-Bate J, Al Kadhi O, Saha S, Defernez M, et al. Transcriptional changes in prostate of men on active surveillance after a 12-mo glucoraphanin-rich broccoli intervention—results from the Effect of Sulforaphane on

prostate CAncer PrEvention (ESCAPE) randomized controlled trial. Am J Clin Nutr. 2019;109(4):1133-1144.
32. Kuwa K, Nakayama T, Hoshino T & Tominaga M. Relationships of glucose concentrations in capillary whole blood, venous whole blood and venous plasma. Clin Chim Acta. 2001;307(1-2):187-92.
33. Riso P, Vendrame S, Del Bo' C, Martini D, Martinetti A, Seregni E, et al. Effect of 10-day broccoli consumption on inflammatory status of young healthy smokers. Int J Food Sci Nutr. 2014;65(1):106-11.
34. Abukhabta S, Ghawi SK, Karatzas KA, Charalampopoulos D, McDougall G, Allwood JW et al., Sulforaphane-enriched extracts from glucoraphanin-rich broccoli exert antimicrobial activity against gut pathogens in vitro and innovative cooking methods increase in vivo intestinal delivery of sulforaphane. European Journal of Nutrition. 2020; https://doi.org/10.1007/s00394-020-02322-0.
35. Armah CN, Derdemezis C, Traka MH, Dainty JR, Doleman JF, Saha S et al., Diet rich in high glucoraphanin broccoli reduces plasma LDL cholesterol: Evidence from randomised controlled trials. Mol Nutr Food Res. 2015;59(5):918-926.
36. Swegarden H, Stelick A, Dando R & Griffiths PD. Bridging sensory evaluation and consumer research for strategic leafy Brassica (Brassica oleracea) improvement. Journ Food Sci. 2019;84(12):3746-62.
37. Lafarga, T, Acién-Fernández, FG, Castellari, M, Villaró, S, Bobo, G & Aguiló-Aguayo, I. Effect of microalgae incorporation on the physicochemical, nutritional, and sensorial properties of an innovative broccoli soup. LWT. 2019;111:167-174.
38. Shapiro TA, Fahey JW, Dinkova-Kostova AT, Holtzclaw WD, Stephenson KK, Wade KL, et al. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. Nutr Cancer. 2006;55(1):5.