PROTOCOL

The effects of types of fruits and vegetables on vascular function in prehypertensive participants (CIRCUS): a pilot study

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Short title: Effects of types of fruits and vegetables on vascular function (CIRCUS)

Long title: The effects of types of fruits and vegetables on vascular function in prehypertensive participants: a pilot study

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Sponsor

The University of Cambridge is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager at:

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1. STUDY SUMMARY

AIM To evaluate the effects of increased intakes of citrus fruits and cruciferous vegetables on vascular function in untreated, prehypertensive participants

DESIGN Randomised controlled, cross-over trial with three 14-day dietary intervention periods, preceded by one week run-in period and separated by one week wash-out periods, 9 weeks in total. The diet will be fully controlled and provided by the researchers. Participants will receive in random order three interventions:

1: 1 portion of fruits plus 1 portion of vegetables, of commonly consumed types per day. Intakes are at 25th percentile of UK consumption and will exclude citrus fruits, cruciferous and allium vegetables.

2: 4 portions of fruits plus 4 portions of vegetables, of commonly consumed types per day excluding citrus fruits, cruciferous and allium vegetables.

3: 4 portions of citrus fruits plus 4 portions of cruciferous vegetables per day.

Numbers of fruit and vegetable portions for the treatment groups are according to recommendations of the Dietary Approach to Stop Hypertension (DASH) diet.[1] Diet and macronutrient balance will be based on UK average habitual diet (NDNS). Body weight will be maintained by tailoring the diet to individual energy requirements.

POPULATION 36 apparently healthy men and women, aged 40 to 65 years, with upper levels of prehypertension (systolic BP 125-160 mmHg), not using antihypertensive, other cardiovascular, or diabetic medication, with a body mass index between 20 and 35 kg/m$_2$, with a habitual dietary pattern low in plant foods (≤4.2 portions per day) and with sufficient available food storage at home will be recruited.

Exclusion criteria include smoking, pre-existing morbidity including diabetes mellitus, hypertension, cardiovascular or other metabolic diseases, ≥10 hours per week of moderate to vigorous physical activity, excessive habitual alcohol intake of >21 units per week (females) or >28 units per week, adherence to a prescribed diet, pregnancy or lactation, use of dietary supplements, food sensitivities, or vegetarian/vegan diet by choice.

TREATMENT **7-day run-in/washout periods:** Participants will be asked to consume the run-in or washout (control) diet for 7 days. A run-in period is to minimize the effect of habitual diet intake before start of intervention, a washout period is to ensure that potential cardiometabolic effect of the previous intervention diet is eliminated.

14-day dietary intervention periods: Participants will consume the control or intervention diet, weekly delivered at their home. Participants will be asked to visit the Cambridge Epidemiology & Clinical Trials Unit testing sites 7 times in total (every 1st and 15th day of each intervention sequence) in fasted condition.

Day 1 and day 15 of 14-day dietary intervention periods: Participants will be asked to arrive in fasted condition and stay at the testing site until the last measurement has been completed. Participants will bring 24-hr urine and stool collections. The following measurements will be done: anthropometrics, bioelectrical impedance, blood pressure,

pulse wave velocity, and cognitive function. Samples of fasting blood will be taken. Participants will complete questionnaires to assess maintenance of lifestyle factors e.g. physical activity and appetite profiles throughout the study. Breakfast will be provided.

DURATION Participants will be involved for 9 weeks in total:

Week 1: Run-in period
Week 2-3: 14-day dietary intervention
Week 4: Wash-out period
Week 5-6: 14-day dietary intervention
Week 7: Wash-out period
Week 8-9: 14-day dietary intervention

OUTCOME MEASURES

Primary outcome: Changes in systolic and diastolic blood pressure (mmHg)

Secondary outcomes: Changes in:

- Arterial stiffness assessed by pulse wave velocity and pulse wave analysis
- Cardiovascular risk factors measured in fasted blood samples
- Markers of endothelial function measured in fasted blood samples
- Markers of low-grade inflammation measured in fasted blood samples
- Urinary and circulatory metabolite profiles and individual metabolite levels
- Established objective urinary and circulatory markers of intakes of fruits, vegetables, and other foods
- Composition of gut microbiota
- Cognitive function
- Average total, daytime, night time 24-hr ambulatory blood pressure (in subsample)
- Average objective physical activity intensity and sleep time (in subsample)

2. INTRODUCTION

2.1 Background

Elevated blood pressure (BP) is a leading population-wide modifiable risk factor of cardiovascular diseases (CVD), which contributes yearly to ~31% of all deaths globally.[2] Hypertension (≥140/90 mmHg[3,4]) is prevalent in 40% of adults, while a billion of individuals have prehypertension (120-139/80-89 mmHg[3]) and are at higher risk to develop hypertension and CVD. The strong, well-established evidence on the adverse relationship between higher BP and risk of CVD events emphasizes the need for targeted, practical lifestyle modifications to maintain healthy BP levels to prevent future CVD events.

2.2 Rationale of current study

Low fruit and vegetable consumption have been identified as major risk factors contributing to hypertension and CVD. Meta-analyses of prospective cohort studies showed strong evidence that eating higher amounts of fruit and vegetables lowers the risk of CVD by ~20% in a dose-response manner.[5-8] Two landmark large intervention studies demonstrated that untreated mildly hypertensive participants who increased their daily fruit and vegetable intakes significantly reduced their average systolic BP by 2.8 mmHg systolic and diastolic by 1.1 mmHg compared to the control group with average US diet, with stronger BP-lowering effects in hypertensive participants. [1,9] These findings underlie the evidence of the current dietary recommendations to eat at least 5 (80 gram) portions of a variety of fruits and vegetables per day with a limit of 150 ml 100% pure, unsweetened juice counting as one portion.

Fruits and vegetables are however biologically and chemically complex and vary largely in amounts and types of nutrients and bioactive compounds. Prospective cohort studies have only since 2010 started to investigate the influence of eating higher amount of specific types of fruits and vegetables on the risk of CVD. Meta-analyses of prospective cohort studies indicate that higher intakes of apples and pears, citrus fruits, carrots, cruciferous vegetables (e.g. broccoli and cabbage), grapes, green leafy vegetables, raw vegetables, and tomatoes have been suggested to lower the risk of CVD by 14-22%.[8] Few cross-sectional[10] and prospective cohort studies[11,12] have been published on associations of types of fruits and vegetables with BP or risk of hypertension, with inconsistent findings.

Subsequent dietary intervention studies attempted to demonstrate BP-lowering effects of specific types of fruit and vegetables, but with inconsistent findings. So far, interventions have been mainly focused on fruits and vegetables abundant in a single nutrient e.g., flavonoids[13], lycopene[14], potassium[15], or nitrate[16]. Acknowledging the complexity of foods, we have only recently understood that we need to look at whole foods as the constituents act in combination and in synergy on health.[17] Only by investigating the whole food will improve our understanding of potential synergistic and underlying mechanisms of BP-lowering effects of specific types of fruits and vegetables, which can be used to develop understandable, targeted dietary strategies to lower BP at a population-wide level.

The main limitation of nutritional research is the use of traditional, self-reported dietary assessment methods with high prevalence of misreport leading to over- or underestimation of dietary intakes and associations and thus misinterpretation of findings. Established biomarkers to assess fruit and vegetable intakes include urinary potassium and plasma carotenoids, flavonoids, and vitamin C. These biomarkers measure intakes of nutrients from various dietary sources and are nowadays often supplemented to foods, which may overestimate calculation of fruit and vegetable consumption. A new approach is metabolomic profiling of biofluids using Nuclear Magnetic Resonance (NMR) spectroscopy and/or mass spectrometry (MS) to measure many small compounds in biological fluids (urine and blood) simultaneously. Recent advances demonstrated that metabolomic strategies can be used to develop objective biomarkers of dietary intake[18] and at once comprehensively characterises diet-related metabolic responses in human biofluids.[19] Urinary biomarkers reflect the end product of metabolism. Urinary biomarkers combined with

assessment of compounds in food, blood (controlled to maintain homeostasis), and changes in gut microbial composition will provide a systematic approach to aid identification of underlying mechanisms of fruit and vegetable-induced BP effects.

Using metabolomic strategies, small, short-term intervention studies have identified potential biomarkers for citrus fruits (proline betaine) and broccoli (S-methyl-L-cysteine sulfoxide (SMSCO)) in healthy participants.[20,21] In a large-scale epidemiologic cohort study, the PI investigated their validity by combined analyses from high quality dietary data (24-hour dietary recalls), measured BP data and 24-hr urine collections measured by NMR spectroscopy from 2,032 US and 449 UK participants. In both populations and across clinic visits, consistent and strong correlations were found for intakes of citrus fruit with proline betaine (0.72), 4-hydroxyproline betaine (0.63) and several co-metabolites (0.22-0.49) related to the same metabolic pathway and for cruciferous vegetables with S-methyl-L-cysteine sulfoxide (SMSCO, 0.39). These biomarkers will be highly valuable as objective assessment of citrus fruits and cruciferous vegetable intake, as unbiased estimates of associations with disease outcomes in large-scale epidemiological studies, and as objective measures of compliance in intervention studies.

Although meta-analyses of prospective cohort studies indicate that higher intakes of citrus fruits and cruciferous vegetables lower the risk of CVD[8], limited evidence exists from intervention studies. Few small, acute interventions showed inconsistent findings on BP and endothelial function after supplementation of flavanones and/or orange juice[25-27], while little evidence exist on cardioprotective effects of cruciferous vegetables. [28-30] Based on the evidence from prospective cohort studies and recent identification of strong, specific, and validated urinary markers for these metabolites, we will test increased intakes of citrus fruits and cruciferous vegetables on vascular function in this intervention study as proof of concept to develop future, larger intervention studies. We will not only test increased intakes of citrus fruit and cruciferous vegetables against low fruit and vegetable consumption, but also against increased intakes of other fruits and vegetables to rule out the effect of the higher amount consumed.

In this pilot study, we will take into account the complexity of the whole food and will test the effects of higher intakes of citrus fruits and cruciferous vegetables on vascular function and cardiometabolic risk factors using predefined novel biomarkers to measure compliance. The simultaneous approach of measuring metabolites in various biofluids enables us to further validate these biomarkers and to assess changes with other urinary and circulatory metabolites to improve our understanding of potential underlying mechanisms and pathways. Additionally, we will also test cognitive function reported to relate to improved blood flow and neural activity following higher fruits and vegetable intakes.

3. STUDY DESIGN

This is a fully controlled randomised cross-over trial with three 14-day dietary intervention periods, preceded by one week run-in period and separated by one week wash-out periods, 9 weeks in total, in 36 apparently healthy, untreated, prehypertensive participants aged between 40 and 65 years.

3.1 Study objectives and outcome measures

To assess the effect of types of plant foods on vascular function in untreated prehypertensive participants.

Primary outcome

Changes in systolic and diastolic blood pressure (mmHg) measured by automated oscillometric device (average of 3 measurements).

Secondary outcomes

Changes (end minus baseline values) in:

1. Arterial stiffness assessed by pulse wave velocity and pulse wave analysis
2. Cardiovascular risk factors measured in fasted plasma samples:

   a. Lipid profiles: total cholesterol, high-density lipoprotein cholesterol, triacylglycerides (low-density lipoprotein cholesterol will be calculated using the Friedewald formula)
   b. Insulin resistance: glucose, insulin, HbA_{1c} (HOMA-IR will be calculated using fasting glucose and insulin values)
3. Markers of endothelial function measured in fasted plasma samples (e.g. C-reactive protein)
4. Markers of low-grade inflammation measured in fasted plasma samples (e.g. Interleukin-6)
5. Urinary and circulatory metabolite profiles and individual metabolite levels
6. Established objective urinary and circulatory markers of food intake:
   a. Fruits and vegetables: vitamin C, carotenoids, potassium, nitrate
   b. Salt: sodium
   c. Protein and muscle mass turnover: urea nitrogen and creatinine
7. Composition of gut microbiota to be measured from stool samples
8. Cognitive function measured by six computerised cognitive tests to assess four main cognitive domains: memory, processing speed, attention, and executive function
9. Self-rated general health and mental well-being
10. Average total, daytime, night time 24-hr ambulatory blood pressure (in subsample)
11. Average objective physical activity intensity and sleep time (in subsample)

3.2. Study methodology

The study involves two screening moments (one screening questionnaire/interview to complete at home and one screening visit) followed by enrolment onto a 9-week randomized, placebo-controlled, cross-over intervention study. Each intervention period will be preceded by one week run-in period and separated by one week wash-out period in which participants will consume an average UK diet, low in plant foods. During the three 14-day intervention periods, participants will consume an average UK diet with increased amounts (8 servings per day) of specific types of fruits and vegetables (**Table 1**) or low in fruits and vegetables (control group, ≤2 servings per day). The diets will be fully controlled, provided by the researchers and delivered at the participants' home once a week. Participants will be involved for 9 weeks in total and will be invited to attend the Cambridge Epidemiology & Clinical Trials Unit testing sites 7 times in total.

Screening questionnaire/interview (15 minutes)
Consent/Screening visit (clinic visit, 2 hours)
Week 1: Run-in period
Week 2-3: 14-day dietary intervention
Week 4: Wash-out period
Week 5-6: 14-day dietary intervention
Week 7: Wash-out period
Week 8-9: 14-day dietary intervention

Screening questionnaire/interview (15 min)

Respondents will be send by mail a screening questionnaire. Participants will be invited to complete and to return the screening questionnaire in the provided envelope to the researchers. If preferred, respondents can be contacted by telephone to assess suitability. The researchers will assess eligibility using the returned questionnaires and will contact all respondents by telephone to inform them if eligible. Eligible respondents will be sent the patient information sheet. Having read the information sheet, those who wish to continue the enrolment process will be invited to attend the Cambridge Epidemiology & Clinical Trials Unit testing sites for a consent visit and to continue screening.

Consent/screening visit (clinic, 2 hours)

Potential participants will be asked to visit the Cambridge Epidemiology & Clinical Trials Unit testing sites. The respondent will receive detailed information of the study protocol and their eligibility before obtaining informed consent. Questions and concerns will be discussed with the researchers before giving formal consent. Potential participants will then be eligible to continue the screening process.

Potential participants will be asked to complete a general health and lifestyle questionnaire. Automated office BP (twice with >30 min apart), anthropometrics and body composition will be measured. If eligibility criteria are met, the participant will be enrolled and randomly allocated to one of the intervention sequences. The participant will be asked to complete food diaries for 3 days (2 week and 1 weekend days) and a salt intake questionnaire to assess habitual dietary patterns and to return to the researcher by post for analyses. In the event of any abnormalities of test results, the participant will be informed and will be advised to discuss with their GP.

3.3. Dietary intervention

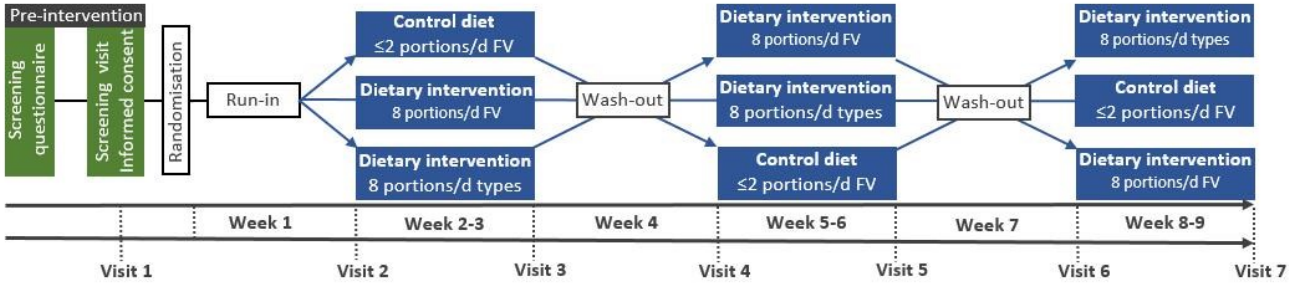

Figure 1: Schematic representation of the study design and clinic visits

The dietary interventions will be based on an average UK diet with similar macronutrient content:

Control group: 1 portion of fruits plus 1 portion of vegetables, of commonly consumed types per day. Intakes are at 25th percentile of UK consumption (NDNS) and will exclude citrus fruits, cruciferous and allium vegetables.

Treatment 1: 4 portions of fruits plus 4 portions of vegetables, of commonly consumed types per day excluding citrus fruits, cruciferous and allium vegetables.

Treatment 2: 4 portions of citrus fruits plus 4 portions of cruciferous vegetables per day.

The control group and treatment 1 will be provided with similar types of fruits and vegetables, but in different amounts, to investigate the importance of amount consumed (**Table 1**). Treatment 3 contains citrus fruits and cruciferous vegetables to test the importance of targeted types of fruits and vegetables consumed. Numbers of fruit and vegetable portions for the treatment groups are according to recommendations of the Dietary Approach to Stop Hypertension (DASH) diet.[1] Fruits and vegetables will be provided as snack, salad, cooked in mixed dishes, as 100% juice, or as soup. Allium vegetables (onion, garlic, leek, etc) will be restricted throughout the study as these vegetables also contain SMSCO. Also any other fruit or vegetable containing food will be restricted e.g., jam and fruit cakes. The diets will be matched for servings of other food groups and macronutrient balance according to average UK habitual diet. Duplicates of daily provided diets will be collected to analyse average nutritional compositions of the diet.

Meals and snacks covering 90% of daily energy needs will be delivered once a week at the participants' home according to a weekly menu cycle for each intervention sequence and accompanied by daily menus. Each day, 10% of the calorie intake is free for the participant to choose from a list of food or beverage options (to be provided by participant). This may include a maximum of one daily unit of alcohol, equalling 10 to 15 gram of ethanol. A run-in period (control diet) is to minimize the effect of habitual diet intake

before start of intervention, washout period (control diet) precedes each intervention sequence to ensure that potential cardiometabolic effects of previous diet or treatment are eliminated.

Table 1. Types of fruits and vegetables provided in each intervention sequence

Control & Treatment 1	Treatment 2
Common fruits	**Citrus fruits (Rutaceae)**
Apple	Clementine
Banana	Grapefruit
Grape	Lemon
Pear	Lime
	Mandarin
	Orange
Common vegetables	Tangerine
Carrot	**Cruciferous (Brassicaceae Oleracea) vegetables**
Cucumber	Broccoli
Sweet pepper	Brussels sprouts
Tomato	Cabbages, including savoy, white and red
	Cauliflower
	Collard greens
	Kale
	Kohlrabi

Diet control for weight maintenance is a crucial part throughout this study. Diets will be tailored to individuals' energy requirements measured by bioelectrical impedance analysis and the validated Mifflin-St Jeor equation that estimates basal metabolic rate.[31] Body weight and appetite profiles will be measured at start and end of each intervention sequence to check weight maintenance and feelings of hunger and satiation. Menu cycles at various caloric levels will be developed, and if needed, to match energy expenditure to maintain body weight. Participants will be asked to maintain usual lifestyle habits including physical activity levels throughout the study. Participants will be asked to complete a validated questionnaire at each visit to measure any changes in physical activity. The researchers will have weekly telephone contact with participants to discuss issues relative to the provided diet or if any lifestyle changes.

Compliance to dietary intervention

To aid compliance, all meals and snacks will be delivered on a weekly basis to participant's home accompanied by instructions, daily menus and food checklists. The participant will be asked to complete the food checklists daily to report compliance to the intervention diet, the food choice for the remaining 10% kcal, and any additional foods consumed not provided by the study. Participants will be asked to return the completed checklist and uneaten foods at the next clinic visit. Compliance will also be checked with objective established and novel dietary biomarkers.

3.3. Study measurements

Participants will be invited to attend the Cambridge Epidemiology & Clinical Trials Unit testing sites for clinical measurements at days 1 and 15 of each intervention sequence having fasted from 10 pm the previous day. Participants will be provided with containers and instructions for urine and stool sample collection. At each clinic visit, participant will bring 24 hour urine and a stool samples that were collected on the previous day, as well as completed food checklists and a questionnaire reporting any medications or dietary supplements used during the specimen collection. The participants will be reminded by text message to complete these collections and questionnaires.

At arrival, a fasting venous blood sample will be taken with a volume that will not exceed 26 ml (156 ml for the whole study: see overview of fractions of blood samples in Table 3). Participants will be asked to void a spot urine for dipstick analyses to check pathological changes in the urine. Automated office BP, pulse wave

velocity, anthropometrics and body composition will be measured. Breakfast will be provided after completion of the measurements. Cognitive test will be completed at the laptop after breakfast which requires 12 to 15 minutes. We will use the same cognitive tests as currently used for the Airwave Health Monitoring study (MREC/13/NW/0588). 24-hr ambulatory blood pressure devices and AX3 acccelerometers became available at a later stage of the study. Therefore, these measurements will be conducted in a subsample of participants with main aim to validate the office blood pressure measurements and subjective physical activity questionnaire data. A subsample of the CIRCUS study participants will be invited to wear a 24-hr ambulatory blood pressure monitoring device at each visit and an accelerometer throughout the study period while performing their routine work and daily activities. The 24-hr ambulatory blood pressure monitoring device is worn around the waist which is connected with a wire to the cuff along the upper arm. The accelerometer is worn around the wrist as a watch. For the 24-hr ambulatory blood pressure measurement, participants will be trained to install the device themselves or will briefly attend the clinic the day before the actual clinic visit to have the device fitted by a trained researcher. After being notified by the device with a minimum inflation of the cuff, the device will automatically start measurement of blood pressure every 30/60 minutes for 24 hours. Participants who are willing to wear the accelerometer will be fitted or replaced the wrist band at each visit by a trained researcher.

Table 2. Summary of assessments

Assessment	Screening 1 Visit 1	Intervention period 1 Visit 2	Intervention period 1 Visit 3	Intervention period 2 Visit 4	Intervention period 2 Visit 5	Intervention period 3 Visit 6	Intervention period 3 Visit 7	Total
Informed consent	X							1x
Screening questionnaire	X							1x
Health & lifestyle questionnaire	X							1x
Salt questionnaire	X						X	2x
Food diaries	X							3x
Anthropometrics	X	X	X	X	X	X	X	7x
Bioelectrical impedance	X	X	X	X	X	X	X	7x
Blood pressure	2x	X	X	X	X	X	X	8x
Pulse Wave Velocity		X	X	X	X	X	X	6x
Fasting blood sample		X	X	X	X	X	X	6x
24-hr urine		X	X	X	X	X	X	6x
Spot urine & dipstick test		X	X	X	X	X	X	6x
Faeces sample		X	X	X	X	X	X	6x
Cognitive tests		X	X	X	X	X	X	6x
Questionnaire including physical activity, dietary behaviour and appetite		X	X	X	X	X	X	6x

Table 3. Summary of blood samples

Blood sample	Quantity	Vacutainer	Purpose
Serum Separator Tube (SST)	6 mL	Red	Serum for biochemistry, metabolomics
Serum Separator Tube (SST)	5 mL	Gold	Serum for lipid profiles, hs-CRP, insulin
Lithium Heparine	6 mL	Green	Plasma for nutritional biochemistry (Vit C)
Fluoride Oxalate	2 mL	Grey	For glucose
EDTA	6 mL	Purple	Plasma and buffy coat for haematology and HbA1C
Total blood sample	**25 mL**		

At the end of the study, collected samples will be kept in locked freezer storage managed by the MRC Epidemiology Unit which hosts the Cambridge Epidemiology & Clinical Trials Unit.

4. PARTICIPANT ENTRY

4.1. Recruitment of participants

We envisage that we will need to screen many people to find 36 eligible prehypertensive participants willing to participate in this study. We will therefore use various ways to recruit respondents:

- by contacting the general pool of volunteers registered at the NIHR/Wellcome Clinical Research Facility and the Imperial College London, Division of Nutrition, and those registered at the Cambridge Epidemiology & Clinical Trials Unit of the MRC Epidemiology Unit, who participated in previous studies and who gave consent to re-contact
- by advertisement, e.g. posters and online media, in staff rooms of wards, common areas, and waiting rooms of all the trust hospitals (Charing Cross, Hammersmith, and St Mary's hospitals), and Addenbrookes Hospital in Cambridge, Prince of Wales Hospital in Ely, North Cambridgeshire Hospital in Wisbech and Imperial College London/University of Cambridge campuses – and via staff newsletters.
- by contacting participants of the Airwave Health Monitoring Study who consented to be approached for recruitment of subsequent studies. The Airwave Study is an epidemiological occupational cohort study of the police forces (officers and staff) in mainland Great Britain to investigate long-term health effects associated with use of the TETRA radio system.[32] The Airwave Study obtained ethical approval from the National Health Service Multi-site Research Ethics Committee (MREC/13/NW/0588). Airwave participants who consented to be approached for recruitment to subsequent studies, who reside in London and Cambridgeshire, and who are identified eligible based on cardiometabolic risk factors measured at baseline will be approached.
- If necessary, we will extend our recruitment strategy to advertise at waiting rooms of local GP practises and/or direct recruitment via GPs.
- When the study runs at the proposed new location, the Cambridge Epidemiology & Clinical Trials Unit, we aim to use the local Eastern CRN to recruit volunteers via searches of GP patient databases in Cambridge. Posters and adverts will be used if the GP surgeries does not create enough interest.

4.2 Pre-randomisation evaluations

The screening phase to assess suitability for the study consists of a short screening questionnaire telephone interview and a consent/screening visit. Details of procedures are described in the Study methodology section and briefly presented here:

1. **Screening questionnaire/interview:** Respondents will be invited to complete and return by mail the participant information sheet and a screening questionnaire to assess suitability. If preferred, the screening questionnaire can be done by telephone interview. Eligible participants who wish to continue the enrolment process will be invited to attend the Cambridge Epidemiology & Clinical Trials Unit testing sites for a consent/screening visit.
2. **Consent/screening visit:** After receiving consent information and signing the informed consent the following assessments will be done to assess eligibility: general health and lifestyle questionnaire, automated office BP, and anthropometrics and body composition. The researchers will assess the results of both screening tests and will decide if the potential participant can enter the study. The participant will be asked to complete food diaries for 3 days (3 week and 1 weekend days) and a salt intake questionnaire in between visits to assess habitual dietary patterns. Eligible participants will be enrolled and randomly allocated to one of the intervention sequences. In the event of any abnormalities of test results, the participant will be informed and advised to discuss their results with their GP.

Inclusion and exclusion criteria

To limit potential confounding or misleading results, volunteers will be recruited according to inclusion and exclusion criteria as outlined in Table 4.

4.2. Randomisation

If eligible, the participant will be enrolled and randomly allocated to one of the intervention sequences. Randomisation will be carried out by an independent statistician.

4.3. Withdrawal criteria

The safety of the study participants takes priority. Any significant adverse event (as assessed by the researchers) will halt the study and the ethics committee and sponsor will be informed as per standard protocol. All adverse events will be recorded and investigators will review each adverse event as it arises. In addition, participants will be free to withdraw at any time and are not required to give a reason.

Table 4. Inclusion and exclusion criteria

Inclusion criteria:	
Age	40 to 65 years
Automated office systolic BP	Average systolic BP of 125-160 mmHg at 2 measurements seperated by >30 min
Antihypertensive medication use	No use less than 3 weeks before screening
Apparently healthy condition	No reported current or previous diabetes mellitus, (secondary) hypertension, or metabolic, cardiovascular, renal, liver, thyroid, gastrointestinal diseases
Body Mass Index	20 to 35 kg/m^2
Smoking status	Non-smoker (not smoked within last 6 months)
Low habitual fruits and vegetable intake	Average fruits and vegetables intake of <4 portions per day (UK average, 40-65 yr) or willing to reduce habitual daily fruit and vegetable intake ≤2 weeks before enrollment
Exclusion criteria:	
Use of other medications	Any medications likely to interfere with energy metabolism, appetite regulation and hormonal balance, including inflammatory drugs or steroids, antibiotics, androgens, phenytoin, erythromycin or thyroid hormones and use of antibiotics.
Alcohol intake	> 21 units per week (females) or >28 units per week (male) and not willing to limit alcohol intake to maximum 1 unit/day ≤2 weeks before and during enrollment
Physical activity	≥10 h/week of moderate to vigorous physical activity
Recent weight loss or gain	≥3 kg in the preceding 3 months
Use of dietary supplements	Unwillingness to stop supplement use ≥2 weeks before enrollment and during intervention
Other medical factors	Pregnancy or lactation
Intervention specific factors	Unable or unwilling to consume provided diets during the intervention Unsufficient storage space for provided diets Food sensitivities or vegetarian/vegan diet by choice Participation in another intervention study at the same time Living >15 miles from the Cambridge Epidemiology & Clinical Trials Unit testing sites, or not willing to travel more then 15 miles No signed informed consent

5. ADVERSE EVENTS

5.1. Definitions

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical study subject.
Serious Adverse Event (SAE): Any untoward and unexpected medical occurrence that:

- results in death

- is life- threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- requires hospitalisation, or prolongation of existing inpatients' hospitalisation.
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise

the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. Reporting procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non-serious AEs

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

Serious AEs (SEAs)

An SAE form should be completed and faxed to the Chief Investigator within 24 h. However, relapse, death and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

5.3. Referral procedures

All SAEs should be reported to the Research Ethics Committee where in the opinion of the Chief Investigator the event was:

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

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

Local investigators should report any SAEs to the sponsor and their Local Research Ethics Committee and/ or Research and Development Office.

Contact details for reporting SAEs, for attention of Dr Linda Oude Griep –
FAX: 01223 769133 Tel: 01223 769220
Email: Linda.OudeGriep@mrc-epid.cam.ac.uk
(Mon to Fri 09.00- 17.00)

6. STATISTICS AND DATA ANALYSIS

The statistical power calculation is based on BP-lowering effects (-7.6±1.4/-5.3±1.4 mmHg) of a DASH fruit and vegetable diet (+4.5 servings/day) vs average US diet in 15 obese hypertensive participants with an average BP of 136/90 mmHg in a 3-wk cross-over study with nutritional advice (no controlled diet).[33] Assuming a conservative detectable effect size (-1 mmHg SBP) considering differences in design and type of participants, 18 participants would be sufficient to detect a difference of 1 mmHg in SBP between groups (two-sided test at 5%, SD SBP change of 1.4 mmHg, 80% power). As we use block randomisation with 6 intervention sequences, internal independent statisticians have advised to randomise a substantial number of participants per sequences. To further strengthen the study design, we will recruit and randomise (stratified by gender) 36 participants in total – with 6 participants in each group.

Treatment effects will be calculated as the changes between values at start and end of each intervention sequence. Treatment effects will be tested by comparing changes of each of the increased fruit and vegetable interventions with changes during low fruit and vegetable period (control group), and of the citrus fruit and cruciferous vegetables period with increased commonly consumed fruit and vegetable intervention. Linear mixed models will be used for repeated measures to compare changes. Treatment and period will be set as fixed effects and subject as random effect. To test for carryover effect, the previous treatment will be included in the model. Statistical significance will be set at 2-sided P-value of 0.05. The researcher performing the statistical analyses will be blinded to the treatment groups.

7. REGULATORY ISSUES

ETHICS APPROVAL

This study has been ethically approved by the London Brent Research Ethics Committee (17/LO/0862). The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. Amendments to the protocol should be approved by the sponsor before being sent to ethics. After ethical approval, all amendments must have Trust R&D approval before they can be implemented.

CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In such cases, the participants remain within the study for the purposes of follow-up and data analyses. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants in the study and is registered under the Data Protection Act.

INDEMNITY

The University of Cambridge holds adequate provision for insurance or indemnity to cover liabilities which may arise in relation to the design, management and conduct of the research project.

SPONSOR

The University of Cambridge will act as the main sponsor for this study.

FUNDING

This research project is funded by the Academy of Medical Sciences. Travel expenses on public transport to and from the Cambridge Epidemiology & Clinical Trials Unit testing sites will be reimbursed up to a maximum of £20 per day for all visits (including screening visit) for both eligible and non-eligible volunteers. There will be no additional reimbursement during the intervention, however all meals and snacks during the 9 week intervention will be provided by the research team and delivered at participant's home.

AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by The University of Cambridge under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

8. STUDY MANAGEMENT

The day to day management of the study will be co-ordinated by Dr Linda Oude Griep.

9. PUBLICATION POLICY

The findings of the research will be published in peer-reviewed journals and will be presented at targeted academic meetings to ensure wide reach of the results. In addition, we will be collaborating with patient groups and professional groups to disseminate the findings via multiple media channels such as patient association publications, print and broadcast media.

10. REFERENCES

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