University of East Anglia

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The impact of Cranberries On the Microbiome and Brain in healthy Ageing sTudy (COMBAT)

A FEASIBILITY INTERVENTION

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Summary

Protocol Title	The impact of Cranberries On the Microbiome and Brain in healthy Ageing sTudy (COMBAT): a feasibility intervention
Protocol Number	Version 4.0, 7th May 2019
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Objectives	i) Investigate the impact of cranberry intervention on gut microbiome composition and metabolism after a 12-week intervention. ii) Investigate the role of microbiome modulation and metabolism in mediating the effect of the cranberry intervention on cognition and brain physiology (MRI)
Outcomes	1. Gastrointestinal microbiota analysis 2. Cognitive function 3. Neuroimaging: MRI 4. Biochemical assessment: to include flavonoid metabolites
Design	Single-centre randomised, double-blind, placebo-controlled parallel intervention trial.
Participants	60 healthy adults (i.e. n=30 in treatment and control groups) aged 50 to 80 years.
Product to be Tested	Cranberry Institute freeze-dried cranberry powder estimated to contain 500mg plant-derived polyphenols per day.
Treatment Details	Twice-daily oral consumption of freeze-dried cranberry powder or matched placebo powder for 12 weeks.

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2. Abbreviations

ACE-III …… Addenbrooke's Cognitive Examination III

BDNF …… Brain-Derived Neurotrophic Factor

BOLD …….. Blood-Oxygen-Level Dependent imaging

BPQ …….. Body Perception Questionnaire

CBI …….. Cambridge Behavioural Inventory

CCI …….. Cognitive Change Index

CRF …….. Clinical Research Facility

ELISA …… Enzyme-Linked Immunosorbent Assay

FDR …… False Discovery Rate

GAD7 …… Generalised Anxiety Disorder questionnaire 7

GCP …….. Good Clinical Practice

IPAQ …….. International Physical Activity Questionnaire

KSS …….. Karolinska Sleepiness Scale

MRI …… Magnetic Resonance Imaging

MS …….. Mass Spectrometry

NNUH …….. Norfolk and Norwich University Hospitals

PHQ-9 …….. Patient Health Questionnaire 9

PI …….. Principal Investigator

PIS …….. Participant Information Sheet

PSQI …….. Pittsburgh Sleep Quality Index

RCFT …… Rey Complex Figure Test

SCFA …….. Short Chain Fatty Acid

TMT …….. Trail Making Test

UEA …… University of East Anglia

3. Background

The human microbiome has recently emerged as a significant contributor to nutrition, health and incipient disease development. This microbial impact is, however, not limited to the gut, instead the human microbiome interconnects the gastrointestinal tract with the central nervous system via a complex series of highly interactive and symbiotic host-microbiome signalling systems (Bhattacharjee & Lukiw, 2013). In particular, gut bacteria may contribute to brain function through the cumulative effects of microbial metabolites (Cryan & Dinan, 2012), such as bile acids, choline and short-chain fatty acids (SCFAs) that are essential for health (Nicholson et al., 2012). Microbiome composition is relatively stable throughout adulthood, though changes in dietary composition and diversity are considered the main drivers of the shifts in microbiome structure and activity (Claesson et al., 2012). Such findings argue in favour of an approach of modulating the microbiome and indirectly brain functions with dietary interventions containing defined nutrients and food bioactives designed to promote healthier ageing. Amongst those nutrients, polyphenols have been consistently reported to play a protective role against cognitive decline (Vauzour, 2014) and have the ability to modify the microbiome composition and metabolism (Tuohy, Conterno, Gasperotti, & Viola, 2012). However, although it has become increasingly clear that the metabolic activities of the gut microbiome provide a mechanistic connection between environmental factors and brain functions (Hsiao et al., 2013), there have been no previous studies examining gut bacterial composition and metabolism in older adults following Cranberry intake and their impact on cognitive functions. Such a Cranberry-mediated cognitive resilience would be highly relevant for the increasing ageing population, for whom signs of cognitive decline are often the harbinger of an incipient dementia. More specifically, ageing related cognitive deficits are usually starting after the age of 65 when the incidence of dementia rises as well. In particular, medial temporal lobe brain regions are highly sensitive to ageing effects, resulting in subtle memory and navigation problems, which are mediated via these brain regions. The proposed project tries to rectify the shortcoming in our knowledge of how Cranberries impact on gut-brain axis and therefore cognition. Success in this project would have important implications for an ageing population where an increase in healthy ageing is greatly desired. As such, it is predicted to considerably advance the scientific knowledge based in the area and to establish whether the microbiome is an important therapeutic target when designing intervention and novel foods that aim to improve cognition. The potential benefits in terms of quality of life are relevant to the population as a whole, as are the potential savings in health care costs. It is estimated that the total annual cost of dementia will escalate to 1.7 trillion euros by 2030. In addition to the general consumer and in particular the elderly, the data resulting from this proposal will also inform future pharmacological and non-pharmacological interventions in the prevention of dementia with the gut-brain axis as its main target.

4. Trial Objectives

We propose for the project to:

i) Investigate the impact of a cranberry intervention on gut microbiome composition and metabolism after a 12-week intervention.

ii) Investigate the role of microbiome modulation and metabolism in mediating the effect of a cranberry intervention on cognition and brain physiology.

5. Trial Design

The study will be a single-centre, 12-week, double-blind, placebo-controlled parallel intervention to investigate the impact of cranberry on the gut microbiome, cognition, and brain physiology. All participants will attend three study visits: one screening visit, one baseline visit at the beginning of the 12-week intervention, and one post-intervention follow-up visit.

6. Participants

This study will include 60 (i.e. n=30 control and treatment groups) healthy older adults. Volunteers aged 50-80 years old with no memory complaints will be recruited. Married couples who live together will be particularly targeted to reduce the variability in background diet patterns.

6.1 Recruitment

Participants will be identified through existing studies being conducted at UEA where participants have agreed to be contacted about relevant studies, community- based advertising (e.g. recruitment posters, leaflets, talks) and local participant databases. We will also be using 'Join dementia research' (JDR) as a recruitment tool. This is an on-line self-registration service that enables volunteers with memory problems or dementia, carers of those with memory problems or dementia, and healthy volunteers to register their interest in taking part in research. The purpose of JDR is to allow such volunteers to be identified by researchers as potentially eligible for their studies. Researchers can then contact volunteers, in line with the volunteers preferred method of contact, to further discuss potential inclusion. JDR is funded by Department of Health working in partnership with the charities Alzheimer's Society and Alzheimer's Research UK and is Health Research Authority (HRA) endorsed. The on-line service and all associated documentation, methods of contacting volunteers and handling of data, were reviewed by a specially convened HRA committee which included experts in research ethics, data protection and information governance. Formal endorsement was issued by the HRA in a letter dated 20 May 2014. The study has also been adopted by the NIHR Clinical Research Network Portfolio database, which can also assist with recruitment of research participants as it allows access to wider recruitment databases.

6.2 Inclusion Criteria

Participants are included in the study if they have all of the following:

- Aged between 50 and 80 years old.

- Male and female.
- Are generally fit and healthy.
- Willing and able to provide written informed consent.
- Fluent in written and spoken English.
- Normal or corrected to normal vision and hearing.
- Understands and is willing and able to comply with all study procedures.

The age range of 50 to 80 year is critical as cognitive deficits can commence in people in their 50s when people do not show yet significant structural brain changes, which typically occur with more advanced age (>80 years). Hence any dietary intervention needs to target people at early yet increased risk (>50) but with brain correlates still intact (<80) as to improve brain and cognitive functioning.

6.3 Exclusion Criteria

Participants will be excluded from the study if they have any of the following:

- Diagnosis of any form of dementia or significant neurological condition.
- Significant memory complaints.
- History or MRI evidence of brain damage, including significant trauma, stroke, learning difficulties or serious neurological disorder, including a loss of consciousness for more than 24 hours.
- Currently smoking or ceased smoking less than 6 months ago.
- Chronic fatigue syndrome, liver disease, diabetes mellitus, or gall bladder abnormalities.
- History of alcohol or drug dependency.
- Clinically diagnosed psychiatric disorder.
- Existing diagnosed gastrointestinal disorder.
- Known allergy to the intervention supplement.
- Any significant medical condition likely to affect participation.
- Currently a participant or have been a participant in any other study involving an investigational product within the last 4 weeks.
-
- Major cardiovascular event, such as myocardial infarction, within the last 12 months.
- A current diagnosis of cancer, or treatment for cancer that has concluded less than 12 months prior.

Participants will be eligible for the study if they are on blood pressure lowering medication only if the dose has been stable for 2 months.

Participants will not be eligible for the study if they are currently prescribed anticoagulant medicine such as warfarin.

Regarding supplements and diet, participants will not be eligible if they take and/or unwilling to stop taking the following:

- Flavonoid containing supplements (and unwilling to cease intake during, and 1 month preceding the trial) or unwilling to maintain existing intake of other supplements.
- High flavonoid intake defined as > 15 portions of flavonoid rich foods per day.
- Any other supplements that could have a significant impact on the outcome measures.

Participants will not be eligible to undergo the neuroimaging component of the study if they have any of the following:

- Cardiac pacemaker
- Claustrophobia

To ensure safety within the MRI scanner, access to medical records or further inquiry will be required if participants indicate that they may have had any of the following:

- Heart surgery
- Brain, head, spine or eye surgery
- Aneurysm clips
- Hydrocephalus shunt
- Metal dust or fragments in the eye
- Metal injuries (eg. shrapnel, bullets, pellets)
- Electronic, mechanical, or magnetic implants
- Operations involving metal implants, plates, clips, stents, bands or expanders
- Operations within the previous 8 weeks
- Kidney problems
- Have had liver transplant or waiting for a liver transport
- Fits, blackouts, or epilepsy
- Piercings, hearing aids, dentures (to check if they are removable)
- Medication patches, tattoos, permanent eyeliner or makeup

For female participants:

- Chance of pregnancy
- Breast-feeding
- Intrauterine contraceptive device (IUD)

For cases of operations, implants, or devices a surgeon's report will be requested from the participant's medical records, which will then be reviewed by the radiologist at NNUH before proceeding with the MRI scan.

If participants are unable or unwilling to undergo an MRI scan, they are still able to participate in other components of the study.

6.4 Contact with Potential Participants

The initial approach to potential participants who have expressed interest in research participation provides the opportunity for information to be provided and to ask questions about the study before informed consent is obtained. Where participants have provided consent to be contacted regarding this study, written information (i.e. PIS and an invitation letter) will be provided to the participant. Potential participants who are identified from the pre-existing databases will first be phoned to be

screened for study eligibility and verbally provided with information about the study procedures. Potential participants will then be mailed the written information if they have not already received this. After sending the PIS and invitation letter, potential participants will be again contacted at least one week later, giving them sufficient time to read the information, consider their participation, and consult with others. Following verbal consent to participate, when they come to their first trial visit at a mutually agreed date and time, the researcher will go through the study verbally and with written information and give further opportunity for any questions to be answered. Participants are then asked to sign a consent form of which the contents are outlined under the section 12.3 Consent.

6.5 Withdrawal of Participants

Participants may be withdrawn from the study for the following reasons:

- At their own request
- If continuation of the study would be detrimental to their wellbeing
- Adverse Event (AE) or Serious Adverse Event (SAE)
- Non-compliance
- Loss of mental capacity of consent

As soon as the research team became aware that a participant had lost the capacity to consent or has met one of the exclusion criteria, then that participant would be fully withdrawn from the study such that no further samples nor cognitive assessment data would be collected. As we are targeting healthy volunteers with no diagnosis of dementia or neurological condition, the loss of capacity during the study is extremely unlikely.

If a participant is prematurely withdrawn from the study for any reason after product administration, the investigator must make every effort to perform these Follow-Up Visit assessments: Review of concomitant medications, and review adverse events. This information should be recorded in the case report form. In all cases, the reason for withdrawal must be recorded in the case report form.

6.6 Reimbursement

Participants will be financially reimbursed £25 for participation in this study. Attendance at all research visits will be required in order to receive the full amount, and this is agreed to on the participant consent forms.

Additionally, should any forms or questionnaires need to be posted to the research team, the participants will be supplied with reply paid envelopes which will cover the cost of postage.

7. Intervention

All participants will attend for three clinical visits: one screening visit, one baseline visit and one follow-up visit after the 12-week intervention.

7.1 Pre-Treatment Screening

At the first contact with potential participants, individuals will be screened over the telephone according to the inclusion and exclusion criteria listed above. Participants will also provide verbal consent to continue with the study over the phone before written consent is obtained at the first screening visit.

Participants will be invited for a screening visit at the Clinical Research Facility prior to the treatment phase of the study. During this first screening visit participants will provide informed consent to the study and will undergo screening measures for cognition and indications of health, including blood pressure.

All participants will be screened via cognitive screening tests (ACE-III score > 88/100), as well as general physical health (normal blood pressure & haemoglobin levels, no psychiatric or alcohol history).

Screening of plasma biochemistry

A fasted blood sample will be taken at the screening assessment visit, using a standard gauge needle and vacutainer system; 22mL (10mL in SST tube, 8mL in CPT tube and 4ml in EDTA tube) and sent to the accredited pathology laboratories for determination of markers of general health.

The following will be measured by the accredited pathology laboratories, following standardised procedures:

- Full blood count analysis (white cell count (lymphocytes, neutrophils, monocytes, eosinophils, basophils), red cell count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelet count).
- Urea and electrolytes (sodium, potassium, bicarbonate, urea and creatinine).
- Liver function (total bilirubin, total protein, albumin, globulin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT)).

Urinalysis

The midstream spot urine sample collected at the clinical screen will be tested to assess pH, the presence of protein, glucose, ketones and blood, as indicators of general health (using a Combur 9 Test®, Roche Diagnostics Ltd., or similar dipstick method).

7.2 Treatment Period

Treatment to be Administered

After screening and enrolment, each dyad will be assigned to the intervention (CI freeze-dried whole cranberry powder) or the control group (CI Placebo powder, matched for appearance and taste to the cranberry powder) using a randomisation algorithm. It is proposed that the active group will be supplemented with 500mg total polyphenols per day, consisting of 280 mg/d proanthocyanidins, 81 mg/d flavonols and 54 mg/d anthocyanins. This dose has been selected to be physiologically relevant and shown previously to independently impact on systemic vascular function and cognitive performance (Neshatdoust et al., 2016; Rodriguez-Mateos et al., 2016). The control intervention will be matched for energy and macronutrients content.

This intervention will be provided in the form of a freeze-dried powder which can be incorporated into beverages and food with the daily dose spread over two servings in order to maximize the physiological impact (based on our current understanding of bioavailability). Participants will be advised not to alter their usual calorie intake, asked to document any unusual symptoms or side effects and to keep a diary of illness and medication. Habitual dietary intake will be assessed using a validated, semi-quantitative, food frequency questionnaire (FFQ) based upon the SLAN study (Harrington et al., 2011).

Assigning Subjects to Treatment Groups

To minimise bias this study will employ both randomisation and blinding. Eligible, recruited participants will be assigned a participant number. The participant will receive the treatment that has been randomly allocated to that participant number.

Randomization codes will be kept in a sealed envelope in a secure filing cabinet. While the study is a double blind trial, study investigators will know the location of, and have access to, the sealed randomisation code envelope. This is in the event of an emergency where the content of the treatment is needed to be known. In the event that the code break envelope is opened, the ethics committee will be informed. Each participant's un-blinding would be considered on a case-by-case basis following authorisation by the Safety Committee which includes the study research nurses, the chief investigator (Dr Vauzour) and advice from study members responsibly for medical oversight.

Prior and Concomitant Therapy

Participants will not be allowed to enter the trial if they are regularly taking the medications listed in 6.3 Exclusion Criteria. All participants will be informed at the beginning of the trial to maintain their normal medication regimen, if possibly (unless medically necessary), for the duration of the trial.

Participants who are irregular users of vitamin or mineral supplements will be asked to maintain their regular habits during the trial. Those taking flavonoids containing supplements (such as berry extracts or herbal supplements) will be asked to cease taking them 1 month prior to beginning the study and for the duration of the 12-week intervention. Participants will also be encouraged not to commence taking new over-the-counter supplements while partaking in the trial, unless instructed

to by their GP, and to inform the study team at any time if they change their medication for any reason.

Any changes to medication, including over-the-counter medications, will be documented in the participant's case report form.

Table 1: Overview of research activities at each time point.

Research Activity	Site	First Contact	Screening Visit	Baseline	During Intervention	After Intervention
Telephone Screening	Phone	X				
Participant Information Sheet	Post	X				
Informed Consent	CRF		X			
Background Questionnaire	Post		X			
QRisk3	Post		X			X
CBI	Post		X			X
CCI	Post		X			X
GAD-7	Post		X			X
PHQ-9	Post		X			X
IPAQ	Post			X		X
PSQI	Post			X		X
Sunlight Exposure	Post			X		X
KSS	CRF			X		X
FFQ	Post			X		
BPQ	Post			X		X
ACE-III	CRF		X			X
Digit Span	CRF			X		X
Interoception	CRF			X		X
RCFT	CRF			X		X
Sea Hero Quest	CRF			X		X
Supermarket Test	CRF			X		X
TMT	CRF			X		X
Actigraphy	Home			X		X
Adverse Events Reporting	CRF, NNUH		X	X	X	X
Blood Pressure	CRF		X	X		X
Physical Measurements	CRF		X	X		X
Blood Sample	CRF		X	X		X
Urine Sample	Home		X	X		X
Stool Sample	Home			X		X
Neuroimaging	NNUH			X		X

Treatment Compliance

Participant treatment compliance and any medical events or general comments about their experience taking the treatment will be monitored through regular phone contact with the investigators and recorded appropriately in the case report form.

Participants will be asked to return all remaining cranberry sachets at the end of the 12-week treatment period. The remaining sachets will also be an indicator of compliance.

Flavonoid metabolite concentrations will be quantified at baseline and following the 12-week intervention, and will be used to determine observance to the treatment.

Lifestyle Restrictions

In addition to the lifestyle exclusion criteria, for the duration of the 12m intervention and one month prior to baseline the participant will be asked to restrict the intake of particular foods as per Table 2 below.

Table 2: Food intake guidance

The following is a list of foods and beverages, which should be consumed less often during the study. Please try and check whether the foods to avoid are part of ready-made and packet meals. Other foods can be consumed without limitations.

Food	Guidance for Intake	Alternative Foods
Berries, red/black/purple grapes or foods containing them	Please try to avoid during the study	banana, orange, mango, melon, , peach, pear, nectarine
Dark chocolate (e.g. >70% cocoa solids)	Please try to avoid during the study	milk chocolate, white chocolate
Cocoa/dark chocolate drink	Please try to avoid during the study	malted drink such as Horlicks
Tea (including herbal), *medium mug*	Try to limit to 4 or fewer mugs per day	malted drink such as Horlicks, coffee, sugar-free soft drinks, water

On the day before the clinical visits at for screening, baseline and follow-up visits participants will be required to fast from 10pm the day before. For the 24 hours prior to clinical assessment participants are requested to refrain from exercising and alcohol and to consume their intervention food with breakfast on the day before the clinical visit.

7.3 Adverse Event Monitoring and Medication Changes

Procedures for recording and reporting adverse events are in place, with an adverse event (AE) being classified as any compromise of participant safety, and a serious adverse event (SAE) being defined as a compromise of participant safety that results in death; is life-threatening; requires hospitalisation or prolongation of existing hospitalisation; or results in persistent/significant disability/ incapacity. All AE's will be recorded and handled in accordance with Good Clinical Practice (GCP) guidelines. All AE's occurring after the subject has signed the informed consent must be fully recorded in the subject's case report form. Documentation must be supported by an entry in the subject's file. A laboratory test abnormality considered clinically relevant, e.g. causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product.

At each assessment visit and phone contact, participants will be asked if they have experienced any AE's or (SAE's) since consenting to participate in the trial. Any adverse or serious adverse event data will be collected on the appropriate documentation. Trends in AE's and SAE's reporting will be monitored across all volunteers. Additionally, participants will be asked to report if they have started (or amended) any medications during the trial which will be noted on the case report forms.

If participants feel in anyway adversely affected by any treatment (including placebo), or the principal investigator feels an AE necessitates cessation, the participant would be advised not to continue and the appropriate measures will be taken (i.e. case report forms, contact research nurse and principal investigator if deemed necessary).

The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study subject presenting for medical care, or upon review of subject data by a study monitor. Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it will be recorded as an AE. All AEs must be graded for severity and relationship to study product.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity:

- **Mild**: events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate**: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe**: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Product: The clinician's assessment of an AE's relationship to test article is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to study product assessed by the PI using the following guidelines:

- **None**: The AE is definitely not associated with the study product administered.
- **Remote**: The study product is not likely to have had an association with the observed AE.
- **Possible**: The AE follows a reasonable temporal sequence from the study product, but could have been produced by the study subject's clinical state or other modes of therapy administered to the study subject.
- **Probable**: The AE follows a reasonable temporal sequence from study product administration, abates upon discontinuation of treatment, and cannot be reasonably explained by known characteristics of the study subject's clinical state.
- **Highly Probable**: The AE follows a reasonable temporal sequence from study product administration, abates upon discontinuation of treatment, and is confirmed by reappearance of the AE on repeat exposure (rechallenge).

Once a participant has reported experiencing an AE, participants will be followed up through regular telephone calls. Participants will be asked about their symptoms, and if medical advice has been sought.

All AEs including local and systemic reactions not meeting the criteria for "SAEs" will be captured on the appropriate case report form. Information to be collected includes event description, date and time of onset, clinician's assessment of severity, relationship to study product, and time of resolution/stabilization of the event. Additional information to be collected, if applicable, includes the treatment administered. All AEs occurring during the study must be documented appropriately regardless of relationship. All AEs will be followed until adequate resolution.

All adverse events occurring during the study will be reported and recorded whether or not they are considered to be non-serious, serious and/or related to the treatment. The following information will be required in each case:

- Subject and date
- Description of event
- Duration
- Frequency
- Intensity
- Seriousness
- Action taken
- Outcome and sequelae
- Relationship to test product

Documentation of all adverse events includes completion of the appropriate section of the case report form (AE).

Documentation of a serious adverse event requires that a separate form (SAE) be completed by the investigators in each case.

In addition, the ethics committee will be notified by the investigator of serious adverse events. Such events should be reported immediately by phone and followed by a faxed SAE form within 48 hours.

The risk of an AE or SAE arising from any of the research activities involved in the study is extremely low. Decisions regarding the causality of such events will be made at the relevant site by a medically qualified person associated with the study and will also involve the PI. In the case that an SAE is decided to be related to the study protocol, it will be recorded and reported using the non-CTIMP SAE reporting template provided by the HRA, and the final report will be completed and signed by the CI and sent to the relevant research ethics committee that issued the favourable opinion for the study.

8. Data Collection Methods

8.1 Assessment Questionnaires

Telephone Screening

The initial screening of potential participants will be done during the first contact, and will typically be conducted over the phone. The screening will be done using the telephone screening questionnaire produced for this study. This telephone screen will take between ten and fifteen minutes to complete.

Background Questionnaire

A background questionnaire produced for this study will be provided to participants to collect demographics and general health information about the participant. The background questionnaire will take approximately fifteen minutes to complete.

Cognitive Change Index (CCI)

The CCI (Rattanabannakit et al., 2016) is a brief self-completed measure of the participant's perceived cognitive status and decline over the past five years. The CCI takes approximately five minutes to complete.

Cambridge Behavioural Index (CBI)

The CBI (Wedderburn et al., 2008) is a brief self-completed measure of changes in the participant's behaviour. The behavioural domains measured include memory and orientation, everyday skills, self-care, abnormal behaviour, mood, beliefs, eating habits, sleep, stereotypic and motor behaviours, and motivation. The CBI measures the frequency of these behaviours over the past month on a five-point scale, from 'never' to 'constantly'. The CBI takes approximately five minutes to complete.

Patient Health Questionnaire 9 (PHQ-9)

The PHQ-9 (Löwe, Unützer, Callahan, Perkins, & Kroenke, 2004) is a 9-item self-report measure of the severity of depressive symptoms in the participant, and takes less than five minutes to complete

Generalized Anxiety Disorder Questionnaire 7 (GAD-7)

The GAD-7 (Spitzer, Kroenke, Williams, & Löwe, 2006) is a 7-item self–complete questionnaire measuring for screening symptoms of generalized anxiety disorder in the participant. The GAD-7 takes less than five minutes to complete.

Body Perception Questionnaire (BPQ)

The short BPQ (Porges, 1993) is a 46-item self-completed questionnaire measuring the participant's body awareness. The short BPQ takes approximately five minutes to complete.

Food Frequency Questionnaire (FFQ)

The FFQ used in this study (Harrington et al., 2011) will provide a detailed self-reported measure of the participant's dietary intake over a given time period which allows for a thorough breakdown of micronutrient and macronutrient intakes. This questionnaire takes approximately fifteen minutes to complete.

International Physical Activity Questionnaire (IPAQ)

The IPAQ is a reliable and well validated measure (Craig et al., 2003) which assesses physical activity across 5 domains including; leisure time physical activities, domestic and gardening activities, work-related physical activity, transport-related physical activity.

The QRisk3 Cardiovascular Screen (QRisk3)

The QRisk3 is a well-established screening tool (Hippisley-Cox, Coupland, & Brindle, 2017) for identifying individuals at high risk of developing cardiovascular disease.

Sunlight Exposure Questionnaire

Participants will be asked to complete a brief questionnaire (Macdonald et al., 2011) regarding their degree of sunlight exposure over the last three months, at baseline and at follow-up.

Karolinska Sleepiness Scale (KSS)

The KSS (Åkerstedt & Gillberg, 1990) is a single 9-point question asking participants to rate their current level of alertness. This question will be given at the beginning and end of cognitive testing sessions.

Pittsburgh Sleep Quality Index (PSQI)

The PSQI (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) is a self-report questionnaire asking participants about their sleep habits and quality over the past month.

8.2 Cognitive Assessment

Participants will undergo at screening, baseline and follow-up a cognitive testing battery to examine general cognitive profiles, including language, memory, executive and visuospatial function. Cognitive tests are well-established validated tests used in the dementia research clinic of Prof Hornberger. Participants will further undergo cognitive testing at baseline and at the end of intervention for medial temporal lobe specific tests, tapping into verbal and nonverbal memory tests as well as allo- and ego-centric navigation performance. In particular, navigation has been shown to be highly sensitive to detect incipient cognitive changes in ageing and dementia. Therefore, participants will be tested on novel virtual reality navigation tests, including Sea Hero Quest (http://www.seaheroquest.com/en/) which has population level navigation data for over 2.7 million people in 193 countries and ages 19-95 years and was co-developed by Prof Hornberger. Critically, navigation tests show little practice effects and are therefore ideal to measure cognitive improvements after the 12-week intervention period.

Addenbrooke's Cognitive Examination III (ACE-III)

The ACE-III (Hsieh, Schubert, Hoon, Mioshi, & Hodges, 2013) is a measure of global cognitive functioning which covers domains including attention and orientation, memory, fluency, language, and visuospatial function. This test takes approximately fifteen minutes to complete.

The Trail Making Test (TMT)

The TMT (Reitan, 1992) is a short test of processing speed, attention, and set-shifting. This test takes less than five minutes to complete.

Digit Span

Digit span is a subtest from the Weschler Adult Intelligence Scale – third edition (WAIS III) (Scale & Edition, 1997) that assesses attention and short term memory. Digit span is composed of two tasks administered independently of each other: digits forwards and digits backwards. For each digits forwards item, participants are presented with a series of digits in increasing length, and must immediately repeat them to the examiner in the same order as presented. For digits backwards, the participant is required to repeat the number sequence in the reverse order. This test takes approximately five minutes to administer.

Rey Complex Figure Test (RCFT)

The RCFT (Meyers & Meyers, 1995) is a short measure of visual memory and visuospatial constructional ability. This study will include the copy and three-minute recall trials of the test, and takes approximately ten minutes in total to complete.

Sea Hero Quest

Sea Hero Quest is a novel computer, tablet, or virtual reality measure of spatial navigation. The configuration of levels chosen for this study will take approximately fifteen minutes to complete.

The Supermarket Test

The Supermarket Test (Tu et al., 2015) is a computer- and tablet-based assessment of spatial orientation which uses an ecological shopping environment. This test takes approximately fifteen minutes to complete.

Interoception Task

The Interoception Task is a brief pilot task that assesses participant's ability to estimate the number of their own heartbeats within several time intervals of varying lengths. This test takes approximately ten minutes to complete.

8.3 Blood Pressure and Heart Rate

Brachial blood pressure will be calculated with the participant seated and following a five-minute rest period. Measurements will be calculated using a sphygmomanometer and an appropriately sized cuff. To ensure the accuracy of the assessment, blood pressure will be taken three times and averaged.

A 24-hour ambulatory blood pressure will also be assessed. Six blood pressure measurements will be made each hour, and as many observations as practicable will be made. A record will be kept by the participant of their sleeping pattern and any notable events likely to affect blood pressure during the 24 hours. Advice will be provided regarding the maintenance and safe application (i.e. switching off whilst driving) of the monitor. The monitor will be worn on the same arm before each assessment visit.

8.4 Actigraphy

Participants will be provided with an ActiHeart unit (https://www.camntech.com/actiheart-combined-actigraph-and-heart-rate-monitor) to wear continuously for one week between the screening and baseline visit and one week during the end of the 12-week main study. These devices collect measures of heart rate and energy expenditure. The devices will be provided in the first instance at the screening visit and collected a week later at the baseline visit, and then posted for the second measurement at the end of the 11th week of the study and collected at the follow-up visit.

8.5 Physical Measurements

Height and weight will be taken at each study visit. Participants will be fully clothed for these measurements, however may be asked to remove shoes to improve accuracy. Body mass index will also be calculated from the measurements taken.

8.5 Biological Samples

The collection of blood, stool, and urine samples is central to the goals of COMBAT. Biomarkers will be especially valuable, together with neuroimaging and cognitive tests, in the evaluation of potential health-promoting and disease modifying nutritional strategies. Such markers will include indications of neuronal activity (eg. neurotrophins), inflammation (eg. cytokines, eicosanoids), cognitive performance (BDNF), gut homeostasis (i.e. LPS, LBP, IgL), and gene variants associated with neurodegenerative conditions and cognitive decline (eg. APOE, BDNF).

A total of 86ml of blood will be drawn during the entire study (22ml at screening, 32ml at baseline and follow-up visits).

Test Type	Screening	Baseline (0 weeks)	Follow-Up (12 weeks)
Clinical Labs	1x10ml SST 1x4ml EDTA	1x5ml SST 1x6ml EDTA	1x5ml SST 1x6ml EDTA
Gene Variants	1x8ml CPT		
Biomarkers	1 urine	1 urine 2x5ml SST 1x5ml Heparin 1x6ml EDTA	1x urine 2x5ml SST 1x5ml Heparin 1x6ml EDTA
Total blood collected:	22ml	32ml	32ml
Total tubes:	4	7	7

Genetic Variants

A 8mL fasted blood sample will be taken into an Cell Preparation Tube (CPT) to provide a buffy layer (containing most of the white blood cells and platelets after blood sample centrifugation). The buffy layer will be collected, genomic DNA will be extracted using Qiagen's DNA extraction kit, following the manufacturer's instructions and genetic variants associated with increased risk of cognitive decline will be assessed.

There is potential for DNA sequencing to reveal incidental information that has potential health importance, but is not related to the primary research question. It would be inappropriate to give genotyping results to the study participants as this may result in unnecessary anxiety. Thus, individual DNA results will not be shared with the participants by the study team, but rather provided to their GP to communicate to them if the participant has consented to this.

Blood Biomarkers

32 mL of fasting blood will be drawn for analytical purposes at baseline and follow-up visits using a standard butterfly system into vacutainer tubes. Blood will be drawn using approved venepuncture techniques by suitably qualified staff (e.g. research nurses, phlebotomists) during participants' study visits at the Clinical Research Facility.

At each blood draw, a total blood of 32 mL will be collected, as follows (in this order):

I. 2 x 6mL EDTA tubes
II. 1 x 5mL heparin tube
III. 3 x 5mL SST tubes

Blood samples will be analysed to determine the chronic effects (pre- versus post-intervention) of the intervention on a range of health biomarkers and flavonoid metabolism to include a number of, but not limited to, the biomarkers listed below.

Specifically, the fasted samples will be utilised to assess biological parameters associated with biomarkers of vascular and microvascular function, inflammatory status, gene expression profiling, and for assessment of circulatory concentrations of flavonoids (and metabolites) and other nutrients (eg. vitamin D).

Samples from the EDTA and heparin tubes will be separated by centrifugation (at a speed / force, and temperature, consistent the future assessment of each analyte) and then aliquoted into sub-fractions into polypropylene tubes (to provide sufficient material for duplicate analysis). Similarly for serum separation, blood will be left to coagulate (in clot activating gel tubes) following manufacturers guidelines (i.e. ≥ 30mins) to be followed by centrifugation and removal of the resultant serum into sub-fractions. The erythrocyte fraction will also be collected for fatty acid analysis. Aliquoted plasma, serum and erythrocytes fractions will be stored at −80°C for later analysis (as described in subsequent sections). Peripheral blood mononuclear cells (PBMCs) will also be isolated for purposes of gene expression profiling for endpoints associated with inflammation, vascular and cognitive function. Samples collected will be labelled with volunteer identification code, age, gender, visit number and time and date of collection.

Spare plasma and serum will be collected as a backup resource and will be used to repeat data analysis and stored for future assessment / studies of emerging biomarkers of vascular function and cognition (as they become available).

Biomarkers of (micro) vascular and cognitive function

Commercially available enzyme linked immunosorbent assay (ELISA) kits, or similar validated analytical kits, will be used. ELISA kits will be stored in accordance with manufacturer recommendations and purchased in bulk so that the lot numbers are the same throughout the study.

Markers of Neuronal Activity and Cognitive Decline (BDNF, GDNF)

Whilst some biomarkers directly reflect the pathology of neurodegeneration by providing evidence of the presence of key proteins, other biomarkers provide less direct or non-specific evidence of neurodegeneration by tracking a variety of indices of neuronal injury.

Markers of inflammation (IL-6, IL-6RC, TNF-a, IL-1b, Nitric oxide, α-ACT, hs-CRP, H2S)

Degenerating tissue and the deposition of highly insoluble abnormal materials are classical stimulants of inflammation. Likewise, in the ageing brain damaged neurons and neurites provide obvious stimuli for inflammation.

Markers of microvascular changes (MR-proANP, MR-proADM, CT-proET-1)

Since vascular changes are hypothesized to precede the onset of age-related neurodegeneration, the measurement of microvascular cerebral damage could provide a sensitive instrument for the

early detection of these changes. A consistent pattern of elevated vasodilators (MR-proANP and MR-proADM) and a decreased vasoconstrictor (CT-proET-1) has been found to be altered in patients with dementia when compared to healthy controls. These results indicate an altered expression of microcirculation parameters and support the hypothesis of a disturbed microvascular homeostasis.

Markers related to lipid metabolism (total-, LDL-, HDL-Cholesterol, triglyceride, Apo E, Apo E genotype, Clusterin (Apo J))

Changes in the cholesterol equilibrium across the whole body may, to some extent, cause alterations in sterol recycling within the central nervous system (CNS), which, in turn, may affect neuronal and myelin integrity.

Assessment of flavonoid parent compounds and metabolites

Flavonoid parent compounds and their metabolites will be assessed using our in-house validated methods (Czank et al., 2013). Briefly, serum will be attained from whole blood samples (after clotting, followed by centrifugation), with the resultant serum acidified to pH 2.4 with formic acid to prevent degradation of certain class of flavonoids prior to storage at -80. HPLC-MS/MS analysis techniques will be then be employed.

Urinary flavonoid analysis

For all participants, a minimum of two urine samples will be collected (baseline and follow-up) for purposes of assessing treatment adherence. All participants will be asked to provide the first morning void and to collect at least 100 mL of urine in a provided 200 mL container. Urine samples will be aliquoted in the laboratories and acid stabilisers added (i.e. Hydrochloric acid, ascorbic acid). Compliance will be monitored by quantifying 5 (total) key phenolic metabolites of flavanols and anthocyanins for comparison to levels in pooled baseline samples. Samples will be analysed by HPLC-MS using published methods (Czank et al., 2013).

Faecal biomarkers of gut microflora

Faecal samples will be collected during the study to determine whether the speciation and metabolism of the gut microbiota is associated with cognition at baseline and differential responses to the intervention. In total, two faecal samples will be collected (within 48 hours prior to baseline and follow-up visits), using the collection vessels provided by the research team (NHS approved Easy sampler collection kit supplied by Cover them Limited). The storage container will then be placed in a cool dry location prior to returning to the research facility at the earliest opportunity (i.e. at the study visit). The remaining sample will be disposed of via normal sanitation route, as the collection material is water soluble.

To account for potential changes to the gut microflora during the study, which are unrelated to the chronic intervention, a participant's use of medications known to affect the microbiota (i.e. antibiotics) and experience of stomach complaints (i.e. diarrhoea) will be routinely collected through adverse event documentation.

Microbiome Community Analysis

To examine species composition we will perform community analysis via 16S rRNA sequencing. DNA will be extracted from faecal samples and a 250bp region from the V4 hyper-variable region amplified and sequenced on the Illumina MiSeq platform. Principle co-ordinate analysis of UniFrac distances and ANOSIM/PERMANOVA tests will be used to identify differences in microbial community structure between control and treatment groups and across time points (0 and 12 weeks). Phylogenetic features differing between classes will be identified by LEfSe (linear discriminant analysis effect size). In addition, Random Forests will provide a complementary approach to identify highly predictive OTU that discriminate between class labels (e.g. control and treated).

Metabolomic Assessment and Biomarkers of Gut Permeability

Untargeted high resolution proton Nuclear Magnetic Resonance spectroscopy (1H NMR) will identify and quantify the metabolites present in serum/plasma and faecal samples. The metabolome of the participants will be monitored at baseline and after completion of the Cranberry intake or placebo (12 weeks). Metabolites covered (~50 for each type of sample) include amino acids, carbohydrates, amines, nucleotides, organic acids, short-chain fatty acids and phenolic compounds. Additionally, we plan on measuring by Mass Spectrometry (MS) targeted compounds relevant to gut microbiome metabolism (e.g. TMAO, hippurine etc.). Dimensionality reduction techniques (e.g. PCA, PLS-DA, tSNE) and clustering will be performed to reveal phenotype stratification of the study groups.

8.6 Neuroimaging

MRI scans will be conducted in all participants at baseline and end of intervention on the in-house 3T research MRI (GE healthcare). Neuroimaging sequences and analyses pipelines are established by Prof. Hornberger and are commonly used in his elderly patient and control cohorts. Each MRI session will take 30 minutes and include sequence to measure structural (gray matter – MRPAGE; white matter – DTI, FLAIR) as well functional (BOLD) brain integrity measures. The structural brain measures will be used to establish brain integrity in participants, whereas the functional brain measures (BOLD) will allow determining the Cranberry intervention effect. More specifically, baseline vs. end of treatment BOLD changes will allow estimating how brain connectivity has changed due to Cranberry intake, while controlling for structural brain integrity across participants. Finally, cognitive changes will be cross-correlated against metabolomic and neuroimaging measures. Principal component/correspondence analysis and Procrustes superimposition will enable an assessment of concordance of microbiome, neuroimaging and cognitive measures.

Structural MRI

In order to monitor structural brain information across the study a T1-weighted 3D gradient-echo MR sequence will be conducted at each testing visit A T2-weighted scan will be also conducted during the study visits.

Arterial Spin Labelling (ASL)

Arterial Spin Labelling (ASL) has previously been used to monitor changes in cerebral blood flow in Alzheimer's disease and MCI patients. At both baseline and post-treatment, ASL will be used to obtain cerebral blood flow information.

Functional Imaging

It is also proposed that a functional imaging run be included, in order to monitor treatment-related changes to functional brain activity.

9. Locations

Screening, baseline, and follow-up visits will be conducted at the Norwich Clinical Research Facility (CRF), Quadram Institute.

Participant data will be stored within the Bob Champion Research Enterprise, UEA.

Neuroimaging will be conducted using the imaging facilities at the Norfolk and Norwich University Hospital (NNUH).

Biological samples including blood, urine and stool will be stored long-term at the Norwich Biorepository. Analyses of these samples will be conducted in laboratories based in the Bob Champion Research and Education Building (BCRE), UEA Biomedical Research Building (BMRC), the Quadram Institute based in the Norwich Research Park, Imperial College London, and the University of Parma.

10. Data Analysis

Statistical Analyses

For each outcome measure, data will be manually screened and (only) data deemed 'invalid' will be omitted from analyses. Data will be deemed invalid if a biological rational sustains this assumption, e.g. non physiological values. These invalid data and the reason for exclusion will be reported in the final report.

Parametric statistical analyses will be performed, except where normality cannot be achieved, in which case equivalent non-parametric analyses will be performed.

Demographic, behavioural and biological (neuro-imaging, clinical and biochemical) data will be analysed using the Statistical Package for the Social Sciences (SPSS; v16.0), applying standard statistical thresholds ($p<0.05$), corrected for multiple comparisons where appropriate.

The impact of treatment on the outcomes of interest will be established using repeated measures ANOVA with time and treatment as independent variables. Individual means will be obtained for each outcome measure and group means, collapsed across the treatment arms, will be calculated to

determine treatment effects. Relationships between lifestyle, cognitive, biochemical and cardiovascular variables, and establishment of the main determinants of the response to intervention will be performed using correlation and regression analysis

Analysis Populations

All participants selected for analyses must have completed the whole study. Any participants with data that inexplicably varies widely from the rest of their treatment group will be excluded from analyses.

In addition, any of the following will be considered a major protocol violation:

- Violation of inclusion or exclusion criteria that are deemed to affect efficacy.
- Non-compliance with assigned treatment regimen.
- Use of prohibited treatment or medication before or during the study, which is felt to affect the assessment of efficacy.
- Not receiving randomised treatment.

Missing and Spurious Data

Where appropriate, missing values will be marked and explained in individual data tables. When one or more outliers are defined, scientific evidence or explanations will be provided to justify the exclusion of the subject's data from the statistical analysis. In case of outliers, the corresponding statistical analysis will be provided with and without these values.

Power Calculation

For the analysis of the microbiome, we estimated the sample size based on the false discovery rate (FDR) control (Pawitan, Michiels, Koscielny, Gusnanto, & Ploner, 2005). We used the following assumptions: 50% of microbiome profiles are not associated with the case-control status and at least 80% power. Our calculation indicates that with a sample size of 30 in each experimental group, we expect to control FDR between 0.5% and 4%. FDR of 4% is considered low and hence we propose that a sample size of 30 in each group is adequate to control FDR at reasonable levels. This number is consistent with other studies investigating changes in the gut microbiome associated with diabetes (a risk factor for cognitive decline) (He, Shan, & Song, 2015) and with the only study evaluating the impact of cranberry on cognitive functions (Crews et al., 2005).

11. Handling of Tissue

A suitably qualified member of the research team will obtain a 22ml venous blood sample at the initial screening visit and 32ml again at each of the baseline and follow-up visits. Someone competent in taking blood samples will undertake the collection of blood. Blood will be collected

and temporarily stored at CRF during clinic visits, then transported and stored long-term at the Norwich Research Park Biorepository.

If participants consent to having stool samples collected, these samples will be collected as close to the study as possible, within 48 hours. Participants will be provided with the necessary equipment including the sample jar, collection plate device, freezerbag, and gloves. Participants will be provided with an information form with clear instructions outlining how to collect and store the sample. Collection of these samples will be conducted by a member of the research team upon arrival at each study day. These samples will then be stored in short-term storage freezers at the CRF before transported and stored at the Norwich Biorepository.

If participants consent to having urine samples collected, they will be provided with the necessary equipment for collection including the sample jar and bag. These samples will be usually collected during the screening and follow-up research clinic visits. These samples will then be transported and stored long-term at the Norwich Biorepository.

The biological samples (including the DNA) collected during the intervention will be kept at the Norwich Biorepository for up to 10 years as the samples could be invaluable for future research in this area. Any subsequent analyses would be conducted in accordance and agreement with local ethical procedures. Volunteers will be asked if they agree to their anonymous samples being stored for future analysis as part of the consenting process.

12. Ethical Considerations

12.1 Ethical Conduct of the Study

The procedures outlined in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by GCP Guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an inspection by the sponsor representatives and/or Regulatory Authority representatives at any time. The investigator must agree to the inspection of study-related records by the Regulatory Authority/sponsor representatives, and must allow direct access to source documents to the Regulatory Authority/sponsor representatives.

The investigator may implement a deviation form, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior EC/IRB/Sponsor approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the EC/IRB. Any deviations from the protocol must be fully explained and documented by the investigator.

12.2 Confidentiality and Data Storage

We follow standard good research practices within our departments, to ensure confidentiality of electronic and hard copy data, in keeping with the European Union General Data Protection

Regulation (2018). In accordance with good practices locally, we will hold non-identifiable data for a minimum of 10 years within the Norwich Medical School, UEA. Individual data will be anonymised and indexed by an alphanumeric reference code, which is kept separately from other data, with the link to identifiable information retained only by the study team. It will not be possible to identify participants' individual data without this code. All non-identifiable data will be kept within a locked building with security access doors. Anonymised electronic data will be stored and managed using databases encrypted with industry standard cryptographic methods and protected by passwords. Hard copy data will be stored in a locked cabinet in a locked room to which only the study team have access.

MRI data held by the NNUH MRI facility will remain identifiable and stored indefinitely on a secure picture archiving and communication system (PACS). Identifiable participant information will also be sent to NNUH Radiology staff prior to scanning including name, date of birth, and GP details.

Blood, urine, and stool samples will be held in long-term storage at the Norwich Research Park Biorepository at -80 C in secure freezers. These samples will only be used for purposes to which the participant has consented that they may be used for. In the case of sample disposal, samples will be destroyed according to the standard operating procedures of the biorepository.

Data entered into the case report form will either be verifiable against source documents, or directly entered into the case report form, in which case the entry will be considered as the source data. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. As per GCP guidelines, upon request of the monitor, auditor, IRB/IEC, or regulatory authority, the investigator should make available for direct access all requested trial-related records, including source documents. The study file and all source data will be retained for a period of 15 years.

12.3 Quality Control and Quality Assurance

In order to comply with GCP guidelines, monitoring and auditing procedures will be followed. The study will be monitored at regular intervals to ensure compliance with the study protocol, GCP and legal aspects. This may include on-site checking of the case report form for completeness and clarity, cross-checking with source documents, and clarification of administrative matters. Standard Operating Procedures (SOP's) will be in place to ensure the trial is conducted in accordance with GCP. Additionally, there will be an SOP for administering and scoring each task to ensure production of quality data. The clinical trials co-ordinator will routinely monitor the trial and ensure the trial is being conducted in accordance with the protocol and GCP requirements. Additionally, the principal investigator, clinical trials co-ordinator and research assistants/PhD students involved in this study will have weekly meetings to discuss the progress and any issues that arise during the course of the study.

12.4 Use of Data and Publication

This study will be published in international peer reviewed journals and presented at scientific conferences and congresses.

12.5 Consent

Written consent for participation in the research study will be obtained at the beginning of the first screening visit before any further research activities take place. Should the dyad be eligible for and express interest in joining any of the other studies being conducted by the research group or collaborators, consent will be sought separately for these on a project-by-project basis. It will be made clear to participants that participation in the study is entirely voluntary and anonymous, and that they can withdraw from the study or change their decision at any time without needing to provide a reason and without it affecting their usual healthcare or other studies they may be enrolled in.

Participants will be asked to sign a consent form, which includes:

- Confirmation that they have, or had read to them, understood and accept what the study involves as written in the PIS for the study, and have had the opportunity to ask questions and that any questions have been answered to their satisfaction.
- Confirmation that the procedures required for the study and the time involved have been explained to them, including possible risks, side effects, and benefits.
- Indication that they agree to take part in the cognitive and scientific testing conducted by the research team, as described in the PIS.
- Indication that they agree to undergo magnetic resonance imaging (MRI) scanning to take images of their brain.
- Indication that they are aware that their GP will be informed of their participation in the study and that they may be contacted regarding their clinical result as well as any information that raises concerns about their wellbeing or that of those close to them.
- Confirmation that they understand that involvement is voluntary and will be kept strictly confidential, and that personal data will be accessed by study and clinical staff only for purposes relating to the conduct of the study and understand that any research data gathered from the results of the study may be published, however this will not include any information that can identify them.
- Confirmation that they understand that their data and samples may be accessed by other studies being conducted in collaboration with this study within the University of East Anglia and affiliated NHS trusts, and which they have also consented to participate in to access and share my individual results across studies, and that these results will not be shared until their consent for this has been obtained for all studies involved.
- Indication that they agree to the research team accessing my medical records for purposes relating solely to the conduct of the study.
- Indication that they agree to be contacted by the study team about research opportunities which might arise in the future.

In addition, participants will be consented using the Norwich Biorespository's consent forms regarding the collection, storage, and analysis of biological samples. These consent forms have separate REC approval to this study (IRAS no. 130478; REC reference: 08/h0304/85+5), and include a section where all samples being collected for research are indicated, including genetic (DNA and/or RNA) studies and for the possible development of commercial products for the improvement of

patient care, from which the participant would receive no financial benefit. On these forms, participants consent to the following:

- That the samples become the property of the Norfolk & Norwich University Hospitals NHS Foundation Trust ("the Trust")
- The Trust may store these samples in a tissue bank / biorepository
- The Trust may use these samples at its discretion in properly approved research programmes
- The Trust may pass on these samples to other approved tissue banks and/or companies, which may be in this country or abroad, in properly approved research programmes (participants can indicate 'Yes' or 'No').
- Genetic material and donated sample(s) may be stored for an indefinite amount of time for future research projects, which may include whole genome sequencing.
- Information about the participant's case may be kept on the Norwich Biorepository database.
- Anonymous data derived from the sample(s) may be placed in an international database for future research.
- Such information may be passed in an anonymous form to persons outside the Trust In connection with research and may be published with any research findings.
- That appropriately qualified staff employed by the Trust may review my hospital Medical records, including case notes, as appropriate, for the purposes of research using the donated samples.
- These samples may be used in ethically approved animal research.
- These samples may be used in ethically approved cloning research
- That they have read and understood the relevant version of the Norwich Biorepository's Information Sheet for Patients.
- The issues have been explained to them, and that they have had the opportunity to ask questions.
- In addition to the removal of tissue, blood or other fluid samples as a necessary part of my procedure, I also consent to the removal of additional tissue, blood or other fluid samples from the operation site during my procedure PROVIDED THAT SUCH REMOVAL CAUSES ME NO HARM now or in the future, is limited to what I and the doctor treating me (or a research nurse or nurse practitioner delegated by him/her) have discussed and agreed, and which is specified below. (A list of additional samples for research would be provided here).

Participants will be made aware that they do not have to consent to all areas of the study, and provision is made for this on the consent form. The consent form is signed and dated by the participant, and then by a member of the research team who has been trained and approved to obtain informed consent. The participant will be provided with a copy of their signed consent form for their records, and a copy of the signed consent form will also be sent to the participant's GP with the notification of participation letter. Consent will be considered an ongoing process, and willingness to continue with the study will be confirmed at each point of contact.

Careful checks will be made to ensure that the individual has capacity to consent to participate in the study by a team member trained in Valid Informed Consent and the Mental Capacity Act (2005). As the study is focusing on healthy individuals with no history of neurodegenerative condition, it is not expected that it is likely that a potential participant will lack capacity to consent. Ability to provide informed consent will be assumed until proven otherwise. Individuals who are found not to

have capacity to consent for themselves will not be eligible for the study. Capacity to consent will be treated as ongoing process throughout the duration of the participant's involvement in the study and will be reviewed at each point of contact. If a participant is found to have lost capacity to consent during the course of the study, they will be immediately withdrawn.

12.6 Safety and Comfort

Fatigue during cognitive testing will be closely monitored in participants. The maximum length of any cognitive assessment is 2 hours, including breaks. Participants are informed before testing begins to notify the examiner if at any point the participant feels tired or distressed, and hence would like to take a break or discontinue. Participants are then asked throughout the assessment following each test whether they are happy to continue, or whether they would like to take a break - after which the participants are asked whether they would like to continue testing. The participant in response to frequent monitoring by the assessor therefore determines the frequency and length of breaks.

Researchers will use risk assessment and management skills as appropriate to maintain participant comfort and safety during all sessions. Participants will be given information to signpost them to sources of support should difficulties arise between research visits. In the eventuality that additional risks are noted which fall beyond the scope of the study intervention (e.g. safeguarding or environmental issues), the researcher will exercise a professional duty of care and will make onward referrals to appropriate services for further input. The participant's GP will be kept informed of their involvement in the study, and of any concerns that arise during the study regarding the wellbeing of participants. The researcher will also monitor the participant's mood and levels of fatigue, and will terminate the session if the participant appears distressed, in pain, or excessively fatigued.

Information regarding the safety and comfort relating to MRI scanning will be provided in the PIS, and participants will have the option to consent to this component of the study in the consent forms. If participant select to undergo the MRI scan, they will be screened prior to scanning for any metal in the body or other contraindications to scanning, including claustrophobia. The scanning procedure will involve participants having their upper bodies scanned within the MRI machine. They will be provided with earplugs and additional ear protection from the noise of the scanner, as well as pillows, foam padding, and blankets to ensure comfort during the scan. They will be provided with a button to alert MRI staff of any complications or discomfort during the scan, and the scan will be aborted immediately at any time if the participant wishes. There are no known adverse side effects with having a single or multiple MRI scans of any length, or even repeated scans after short intervals, provided that the participant does not have any metal within or on the body. The MRI scans do not involve injection of radioactive compounds or exposure to potentially harmful radiation.

The collection of blood samples will only be undertaken by someone trained and competent in collecting blood samples. These samples will be collected away from public areas. The collection of these samples will be aborted if the participant begins to feel unwell or distressed.

Participants will also be provided with an information sheet and collection equipment for collecting the stool and urine samples to minimise contamination, and clear instruction on how to safely store these prior to collection by the research team before transferred to long-term storage at the Norwich Research Park Biorepository.

12.7 Complaints

A system is in place that enables complaints to be directed either the Chief Investigator or NNUH's independent patient liaison service (PALS). Contact details for all of these sources are provided on the PIS.

13. Indemnity

UEA has appropriate insurance policies in place to provide professional indemnity and public liability cover to UEA staff. Medical personnel on the study are also advised to have additional personal medical indemnity.

14. Sponsorship

This study will be sponsored by UEA, Norwich, United Kingdom.

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