University of East Anglia

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

Participant Information Sheet

You are invited to take part in a research study being conducted at the University of East Anglia. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

This information sheet tells you about the research project. It explains the tests and treatments involved. Knowing what is involved will help you decide whether you want to take part in the research. Please read this information carefully, and ask questions about anything that you do not understand or want to know more about. Before deciding whether you would like to take part, you may wish to talk about it with a relative, friend or your local doctor.

Participation in this research is entirely voluntary. If you do not wish to take part, there is no obligation. If you would prefer not to participate, it would not affect any medical care or other studies in which you are currently participating.

If you decide you want to take part in the research project, you will be asked to sign a consent form. By signing the consent form you are telling us that you:

- understand what you have read;
- consent to take part in the research project;
- consent to have the tests and treatments that are described;
- consent to the use of your personal and health information as described.

Who is organising this study?

This study is being conducted by Dr David Vauzour, Prof Michael Hornberger, and Emma Flanagan in the Faculty of Medicine and Health Sciences, University of East Anglia (UEA). Neuroimaging will take place at the Norfolk and Norwich University Hospital (NNUH).

What is this study about?

This study is a 12-week randomised placebo-controlled trial which will test whether cranberry improves brain function and reduce disease-causing mechanisms in married pairs of healthy older individuals.

Why is this study important?

In an ageing population the incidence of dementia is rapidly increasing, and poses a significant financial, societal, and above all personal burden. Although some drugs exist for

these conditions, they treat the symptoms rather than reverse or slow the progression of the underlying disease. Research studies are now focusing on alternative strategies to prevent cognitive decline. Nutrition is considered important for brain function throughout life, and findings from recent laboratory and human observation studies have suggested that flavonoids can improve brain function. Flavonoids are a group of nutrients found in all fruit and vegetables, and a significant concentration of these nutrients are found in cranberry. Furthermore, gut bacteria in the human microbiome has recently emerged as significant contributor to nutrition and health, and been suggested to influence brain functioning through complex connections between the gut and the brain. In addition to protection against cognitive decline, flavonoids have been reported to affect the function and composition of gut bacteria.

There have been no previous studies looking at the interactions of cranberry flavonoids and gut bacteria, and their impact on cognitive function. We aim with the current study to identify how flavonoids from cranberries could influence gut bacteria and brain function.

Who can take part in the study?

We are looking for married couples who are/have:

- Aged between 65 and 75 years old.
- Fluent in written and spoken English.
- Normal or corrected to normal vision and hearing.
- Are living together.

You and your partner will not be eligible for the study if you have any of the following:

- Diagnosis of any form of dementia or significant neurological condition.
- Significant memory complaints.
- Past 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 I or II, or gall bladder abnormalities.
- History of alcohol or drug dependency.
- Clinically diagnosed psychiatric disorder.
- Existing diagnosed gastrointestinal disorders likely to impact on absorption of flavonoids.
- Known allergy to cranberry or any of the ingredients of our study food.
- 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.
- Absence of a spouse to be a study partner with whom you are also currently living.
- Uncontrolled hypertension (systolic blood pressure >140mmHg, diastolic blood pressure >90mmHg).
- Major cardiovascular event, such as myocardial infarction, within the last 12 months.

- Prescribed blood pressure lowering medication, anti-depressants, anti-coagulants, anti-psychotics, cholinesterase inhibitors, anti-convulsants, non-steroidal anti-inflammatory drugs (NSAIDs).
- Taking flavonoid containing supplements with 4 weeks preceding trial, or taking more than 15 portions of flavonoid-containing foods per day.

Participants will also not be eligible for the MRI component of this study if they have any implants which preclude them from the scanner (eg. pacemaker) or if they experience claustrophobia.

If you are unsure whether you meet the criteria for our study, or have any questions, please get in touch with our study team (contact details provided below) and we can contact you via telephone to screen you for your suitability for the study.

What will the study involve?

Once we have established you as a potentially suitable participant you will be asked to come to the clinical trials unit at Norwich Medical School, UEA or the Norfolk and Norwich University Hospital on 3 occasions over approximately 13 weeks. Furthermore, you will be asked to take our cranberry study food twice daily for 12 weeks, provide us with 3 blood, 3 urine and 2 stool samples, and complete a number of questionnaires and cognitive tests.

The study involves the following stages:

1. Telephone screening
2. Consent and screening visit at UEA
3. Baseline study day at UEA and NNUH
4. Follow-up study day at UEA and NNUH

<u>Telephone Screening</u>
After you have had a chance to read over this information and have expressed an interest in participating in the study, we will screen you over the telephone and ask you a series of questions to establish your general health status and eligibility for the study, and you will have the opportunity to ask any questions you may have about the study. We will also ask you about how often you consume certain flavonoid rich foods, including fruit and vegetables, tea, red wine, etc. If you eat more than 15 portions of flavonoid rich foods per day you will be ineligible to participate as we are unlikely to detect changes due to the study food. If you are eligible for the study at this stage we will invite you to UEA for the full screening visit. This telephone screening stage will take approximately 20 minutes.

<u>Consent and Screening Visit</u>
You will be asked to attend the clinical trials unit located at UEA. Before any research activities are conducted, a member of the study team will go through all the details of the study, and you will be provided the opportunity to ask any questions you might have about the study. At the end of this meeting, when you are satisfied with the information provided,

you will be asked to complete a consent form regarding your participation in the study if you are interested in taking part.

Your height, weight, and blood pressure will be measured, and a fasted sample of your blood (less than 2 tablespoons) will be collected to ensure your suitability for the study. Fasting will take place from the evening before and will involve you having no food or beverages after 10pm, except for water and any medication. We will also collect a pre-study urine sample. We will then provide you with breakfast.

We will then conduct some brief cognitive testing with you to measure your overall cognition. We will also give you some questionnaires for you to complete at home and bring with you to your next visit, which will assess your background, lifestyle, overall health and mood.

This screening visit will begin in the morning take approximately 3 hours to complete.

Baseline and Follow-up Study Days

Following the screening visit there will be 2 study visit: a baseline visit before you start taking the study food and then a follow-up visit after 12 weeks of taking the study food.

We will ask you to collect a stool sample in the 48-hours before each study day and a sample of the first urine you produce on the study day. We will provide you with collection pots and kits, and instructions as to how to collect your samples. We will also provide you with a blood pressure monitor which we will ask you to wear for the 24hours prior to your visit. Before each study visit we will also provide you with a special monitor to wear for a week, which will measure your heart rate, activity and energy expenditure. This small device is adhered to the chest to be worn under clothing, and is waterproof so that you will not need to remove it.

At each visit we will also collect a fasted blood sample upon arrival (approximately 2 tablespoons each time) and take your height, weight and blood pressure, after which we will collect another urine sample. We will again then provide you with breakfast.

For each of these visits we will also conduct some additional cognitive testing which looks at specific domains such as attention, memory, and spatial navigation. These tests will be conducted using pen and paper, computers, or tablets. However, these tests do not require you to be a confident computer or table user. At the follow-up visit we will also provide you again with health and lifestyle questionnaires to detect whether there have been any changes from when you completed these at the beginning of the study. These can be completed at home, and we will provide you with a reply-paid envelope to send these back to us.

Participants will also be invited to undergo a 45-minute MRI scan of their brain at NNUH. This scan will allow us to look at the structure of your brain, as well as blood flow within the brain. Before you enter the scanner you will be asked to complete a safety questionnaire regarding any metal you may have in your body, such as metal plates or pacemakers. As the

scanner contains a strong magnet, anyone with metal in their body will be unable to do this part of the study, but can complete the other components of the study.

Each of these study visits will begin in the morning and will take approximately 3-4 hours.

During the Study

Over the study period you will be asked to consume the cranberry study food twice daily: once in the morning and once at night. You will be provided with recipes for how to prepare the cranberry, so there will be a variety of ways you can take it. You will be given either the active cranberry powder or an equivalent placebo powder that does not contain flavonoids. Neither you nor the study team will know which powder you have, and you will be assigned to the test or placebo group at random by a computer, with group assignment kept secure until the study is complete. The cranberry powder will contain approximately 500mg flavonoids per day, which is a dose of flavonoids determined to be both safe and effective.

We will ask you to maintain your regular diet and exercise patterns, and take any drugs or supplements as normal. However, if you are taking flavonoid-containing supplements (such as berry extracts or herbal supplements) we ask that you cease taking them at least 1 month prior to the beginning of the study and for the 12-weeks during the study.

During the study we will also provide you with sleep and food diaries which we will ask you to complete for each day of the study.

What will be measured in the samples provided?

Your screening blood sample will be analysed for measures of liver and kidney function, and may be used to identify variations in a number of genes known to be associated with cognitive decline. These analyses are for research purposes only. However, any incidental abnormal or genetic findings from your samples will not be communicated to you directly by the research team, but rather we will notify your GP, with your consent, who will then discuss these results with you and take any necessary steps.

The blood samples collected during the baseline and follow-up study days will be analysed for blood lipids, fatty acids, markers of brain cell function, vascular health, inflammation and levels of antioxidants.

We will analyse your urine samples for flavonoid levels in order to relate changes in their concentrations to cognition, gut bacteria, and brain function, and as an indication that you have been taking the study food.

The stool samples will be used to quantify different types of gut bacteria and their activity, which will then be related to cognition, brain function, flavonoid intake and overall health.

These samples may also undergo DNA analysis for genes related to age-related neurodegenerative conditions and brain functioning in order to identify relationships between these genes and the impact of the cranberry intervention. However, this is not a

diagnostic test, and any abnormal genetic findings will be communicated to you via your GP with your permission. If you would rather not find out about any genetic results from your samples you can indicate this on the consent forms, and your GP will not be informed of these findings.

What will happen to the study results?

Any personal information (eg. name, address, contact details) supplied by you during the study will be handled by research staff trained in information governance and will be treated as strictly confidential. Personal information will be kept securely in locked filing cabinets separately to study data within a room with restricted access, and on password-protected databases to which only the research team have access. Your name, date of birth, hospital number and GP details will also be sent to NNUH radiology staff prior to your MRI scan, and your details and neuroimaging data will be kept securely on servers located within NNUH and UEA.

In order to protect anonymity, individual data will be anonymised and indexed by an alphanumeric reference code that is kept separately from personally identifiable data, which will be kept only by the study team. Physical de-identified data will be stored within locked filing cabinets accessible only to approved personnel for this study, within an office that requires security key-card access. This de-identified data will also be electronically stored on University servers on an encrypted database requiring a password known only by study personnel, and data may be accessed on University computers and private computers of study personnel in this de-identified state for analysis.

All electronic and hard copies of cognitive, clinical, and imaging data will be kept securely by the University of East Anglia, Faculty of Medicine and Health Sciences for a minimum of 10 years and possibly indefinitely in accordance with good research practice, even after the study is completed. We will publish the results in an anonymous way, for example, as brain pictures in a medical or scientific journal. The anonymised findings might be presented to neurodegenerative and dementia support groups, including carer support groups as well as local carer groups.

Any blood, urine and stool samples collected for this study will be stored securely at the Norwich Research Park Biorepository in partnership with the University of East Anglia. At the end of the study, these samples will be retained within the tissue bank of the Norwich Biorepository in accordance with Human Tissue Authority guidelines and the Human Tissue Act 2004. Samples will not be labelled with your name or personal information, but instead will be identified by a code which is only able to be linked to you by the study team.

For those also consenting to the future use of their samples and / or data, appropriate ethics committee approval will be sought prior to the use of these resources.

Who will be able to access or see my data?

All results and documents associated with this research are strictly confidential and stored securely in compliance with the Data Protection Act 1998. Information from your medical notes will be looked at and likely used in the research, however only by the approved research team. The individual results will remain anonymous, as participants' names will not be attached to their results, and the papers will be identified only by a numerical code. The names or identifiable information of individual participants will not be published anywhere. We will only share your results between the research team and ethically approved collaborators. For more information, please refer to Appendix 1. We will ask your permission to share your anonymous data for other collaborating researchers to use in similar ethically approved studies, including collaborations that may be based outside the EU and United Kingdom.

Will my GP or specialists be informed?

Your GP will be informed of your participation in this study. Occasionally, research studies identify things that might be relevant to your individual treatment, and if this were the case, you would be informed. We will also ask your permission to share any relevant findings with your GP and your clinical team. With your permission, we can also feedback a summary of our findings to your clinical team.

If any abnormal results emerge when analysing your blood samples or brain scans (extremely rare), with your consent, we will contact your GP immediately who may request you come to see him/her to further investigate, and perhaps do a retest. Any genetic findings will be sent to your GP only if you give permission to be informed of these results.

Are there any risks or side effects associated with taking part?

The cranberry supplement used in our study provide a safe amount of 500mg active nutrients per day, which is a safe amount as determined by earlier research. The supplement is produced by a reputable company (The Cranberry Institute) and is fit for human consumption. In this respect, we do not anticipate any side effects from the cranberry supplement.

Some people may feel tired when completing the psychology tasks but we will give you as many breaks as you need to complete them. There are no known risks, lasting effects or anticipated discomfort from taking part in the cognitive testing.

Only people trained and experienced in taking blood samples will take blood samples, and you will be provided with all necessary equipment for the urine and stool sample collection which will minimise contamination. It is normal to feel slight discomfort when giving a blood sample, and there is a slight risk of bruising. There are no other risks associated with the collection of these samples beyond what would be expected when you supply similar samples as part of your usual treatment or check-ups.

The MRI scanner can be loud and you will be given earplugs and a selection of music to listen to, to block out some of the noise. The MRI environment is quite confined and shaped like a tunnel, and people are who uncomfortable in small or confined spaces may not be

able to participate. If this could be you, remember that you may withdraw from this component of the study at any time without explaining why and without it affecting the other study components. Generally, MRI is considered a safe, non-invasive imaging technique.

What about my medication?

If your medication is not listed in our exclusion criteria (see 'Who will not be able to volunteer' above), we would ask that you continue to take your medication as you normally would.

What are the benefits of taking part in this study?

Participating in this study is on a voluntary basis. However, we do recognise that taking part will require a generous contribution of your time and effort, and as such, you will receive £25 for your involvement in the study.

There will also be reimbursement of transportation costs to and from the UEA. Participants travelling by car will be reimbursed at the UEA's current mileage rate of 45p/mile (up to a 30-mile radius). Free parking will also be available on site. Those participants travelling by public transport will be reimbursed costs on production of a ticket or receipt.

What if I require further information about the study or my involvement?

When you have read this information, a member of the study team will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, contact details for the study team are provided at the end of this information sheet.

What if I have a complaint, or if something goes wrong?
In the unlikely event of anything untoward happening, you may complain either directly to the Chief Investigator or the Head of Norwich Medical School at the University of East Anglia (Prof Michael Frennaux, Ph: 01603 593959). Compensation arrangements for negligent harm are covered by the normal NHS and University indemnity.

Can I withdraw from the study?

It is up to you whether you decide to take part in this study. An expression of interest in the study does not commit you to participation. If you decide to participate, you may withdraw from the study at any time without needing a reason. Withdrawal would not affect your medical care, legal rights, or participation in other projects being conducted by the University of East Anglia. Data collected up until the point of withdrawal will continue to be used in the study.

If you would like more information, or need to contact our research team regarding taking part in the study, please call or write to us at:

Ms. Emma Flanagan
Research Assistant
Norwich Medical School
Bob Champion Research and Education Building
Norwich NR4 7UQ
Phone: +44 (0) 1603 591623
Email: e.flanagan@uea.ac.uk

Dr David Vauzour
Chief Investigator
Norwich Medical School
Faculty of Medicine and Health Sciences
Norwich NR4 7UY
Phone: +44 (0)1603 59
Email: d.vauzour@uea.ac.uk

This study is funded by The Cranberry Institute, Carver MA, USA, and has been approved by the Local Research Ethics Committee.

Appendix 1 – Data Confidentiality

The Faculty of Medicine and Health Sciences, University of East Anglia comply with the requirements of the Data Protection Act (1998) with regard to the collection, storage, processing and disclosure of personal information, and are committed to upholding the Act's core Data Protection Principles. Your brain scans may include personal data, including your name, date of birth and/or a reference number. The brain scans will be reviewed by a radiologist, who will give a report of the findings on the scan to your doctors. Copies of the scan will be kept on the University of East Anglia computer system. Members of the Faculty of Medicine and Health Sciences may have access to relevant data. It is possible that researchers working with the University of East Anglia for other similar ethically approved research protocols, where the same standards of confidentiality will apply, will also use relevant data. The Norfolk and Norwich University Hospitals Trust is the Data Protection Controller for disclosed medical information and MRI data acquired at the hospital. Enquiries concerning these issues should be addressed to the local Caldicott Guardian. The University of East Anglia is the Data Protection Controller for all other information including MRI scans at the Norfolk and Norwich University Hospital.