UNIVERSITY OF BIRMINGHAM

Participant Information Sheet

Study Title: The Cocoa Clarity Study – Shedding Light on the Direct vs. Indirect Mechanisms of Action of Dietary Flavanols in the Human Brain

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An Invitation to take part:

Thank you for taking the time to read this leaflet. We would like to invite you to take part in this study at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham.

Before you decide if you want to participate or not, it is important that you understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

1. What is the purpose of the study?

Age-related cognitive decline is a leading cause of disability in the UK and the number of cases is projected to triple by 2060. Diets rich in flavonoids, a small molecule naturally present vegetables and fruits (e.g. cocoa, blueberries, apples) that typically give these foods their strong colours, have been shown to delay age-related cognitive decline. We have previously shown that consuming cocoa can result in immediate improvements in cognitive function, however, how this occur remains unclear. It is generally suggested flavonoids present in cocoa (i.e. cocoa flavanols) improve blood flow and blood oxygenation in the brain, leading to improvements in cognition. However, it is possible that cocoa flavanols also act directly on brain cells, improving the brain's ability to convert nutrients (e.g. glucose and oxygen) into energy needed to support cognitive processes, leading to improvements in cognitive function.

Therefore, this study aims to determine whether cocoa flavanols make their positive impact on cognition either *indirectly* by boosting blood flow and oxygenation to the brain and/or by *directly* enhancing the energy processes inside brain cells.

2. Am I suitable to participate in this study?

<u>You are suitable for this study if you:</u>

- Are healthy,
- Are aged between 18 to 40 years old *OR* above 65 years old,
- Do not smoke,
- Do not consume more than 21 units of alcohol per week,
- Have no history of cardiovascular, respiratory, metabolic, liver, inflammatory or neurological diseases,
- Do not have allergies or intolerances to any foods,
- Are not on a weight reducing dietary regiment,
- Are not taking any dietary supplements, including fatty acids and vitamins,
- Are not taking any long-term medication (expect for oral contraceptives) or have been on antibiotics for the last 3 months,
- Do not have an infection at present (e.g. cold, flu...).

Additional requirements for <u>females only</u>, you are suitable for this study if you:

- Are not pregnant,
- Are willing to provide information on or confirm the phase of your menstrual cycle during each study visit. *

* For females aged 18 to 40 years old, the 1st experimental session will be scheduled for during the first 5 days of your menstrual cycle. For those using oral contraceptive, the 1st experimental session will be scheduled for during the pill-free days of your prescription. It is important female participants are tested in the same phase of their menstrual cycle or oral contraceptive pill cycle to reduce the effects of fluctuating sex hormones on blood vessel function. A female investigator will confirm with you at the screening visit if you are comfortable with providing this information.

3. What will happen to me if I agree to take part?

If you agree to take part, you will be invited to a screening/familiarisation visit to assess your eligibility for the study (~ 45 min). After the screening/familiarisation visit, you will then be booked in for the first (~ 4.5 hr), second (~ 45 min), and third (~ 4.5 hr) experimental visits, all separated by 4 weeks.

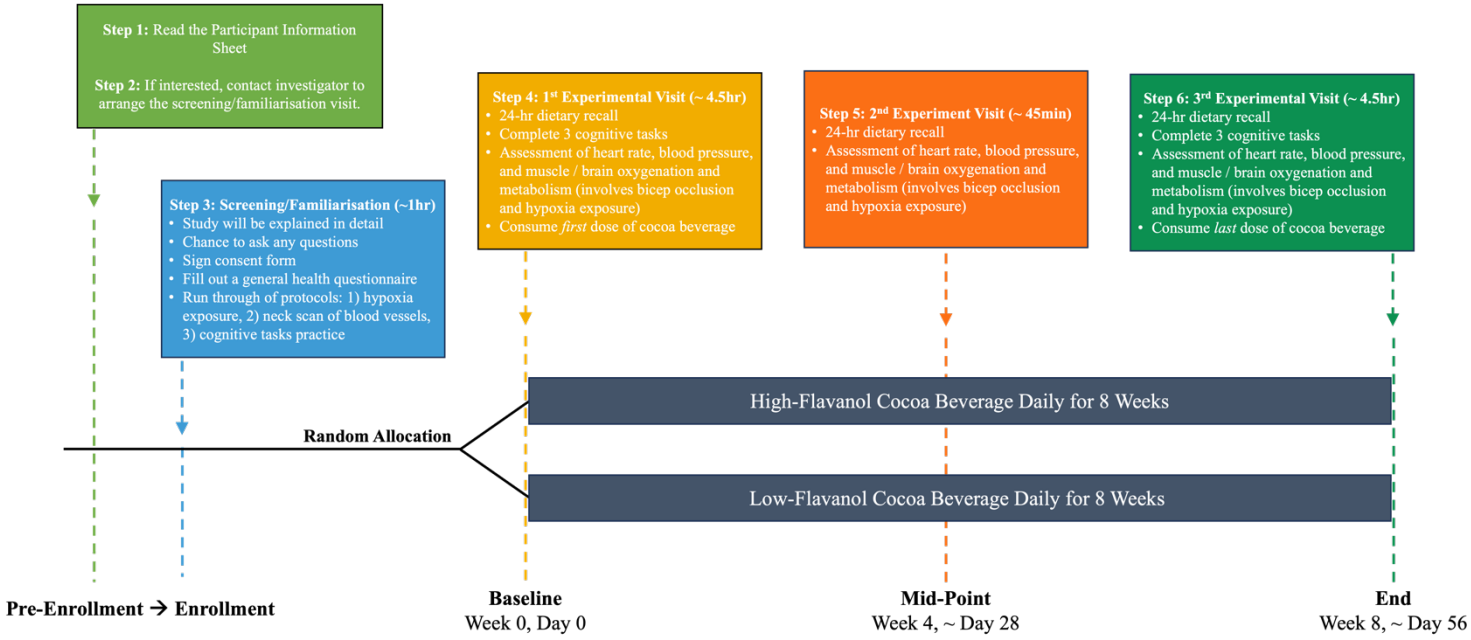
4. What will I be required of me if I take part?

Firstly, you will be asked to spend some time reading through this information sheet (~ 30 min). If you are interested in participating, you will be invited to attend 4 total study visits (1 screening/familiarisation and 3 experimental visits, a total of 10.5 hr) at the at School of Sports, Exercise and Rehabilitation Sciences building at the University of Birmingham.

Briefly, you will be asked to:

1) consume a cocoa beverage daily for 8-weeks (provided by us),
2) complete a general health and lifestyle questionnaire, a 24-hr dietary recall questionnaire, a food frequency questionnaire, and a 3-day food diary,
3) refrain from drinking alcohol or caffeinated drinks, vigorous exercise, and eating certain foods (a detailed list of foods to avoid will be provided to you) for 24 hr prior to the experimental visits,
4) fast for 12 hr (excluding water) before the morning of the experimental visits,
5) have a regular night sleep the day before any laboratory visit,
6) maintain your regular diet and physical activity levels to the best of your ability throughout the course of the study.

Overview of Study Timeline



Visit 1: Screening/Familiarisation Visit (~ 45 min)

During this visit an investigator will explain the nature of the study procedures to you in detail and you will have the opportunity to ask any questions you may have. If you remain interested in, you will be asked to sign a consent form and fill out a general lifestyle and health questionnaire.

If you are eligible to take part in the study, you will undergo a general health check and be familiarised with some of the procedures involved in the study. The general health check will include having your height, body weight, blood pressure, and resting heart rate measured. Following this, we will go through the cognitive tasks used in the study and you will be given the opportunity to practice them. Lastly, you will be briefly exposed (~ 15 min) to a hypoxic gas mixture (11% oxygen) that will be used in the study. These are done for you to become accustomed with the assessments that will take place in the experimental sessions.

Before All Experimental Days

The 24 hr before each experimental day, you will be asked to refrain from eating certain foods that are rich in the same class of compounds that we are testing. This includes fruits, vegetables, coffee, tea, chocolate – a detailed list of foods to avoid will be provided to you. You will also be asked to refrain from drinking alcohol and caffeinated drinks, as well as vigorous exercise for the 24 hr prior to the visits. Lastly, you will be asked to fast for 12 hr before your morning visits to the laboratory. This means that you must not eat or drink anything other than water, for example, from 8 p.m. the night before until your arrival at the laboratory at 8 a.m. the next morning (or 9 p.m. to 9 a.m etc.).

Before the 3rd study visit only, you will be asked to complete a 3-day food diary, where you will need to keep track of everything you are eating and drinking for 3 days (2 weekdays and 1 weekend), excluding the 24 hr before the study visit when you are asked to refrain from certain foods.

Visit 2 & 4: During Experimental Session 1 & 3 (~ 4.5 hr)

The 1st and 3rd experimental sessions will be separated by 8 weeks. For the 1st and 3rd experimental sessions, we will ask you arrive at 9 a.m. in a fasted (no food for ≥ 12 hr) and rested (no vigorous exercise for ≥ 24 hr) state. When you arrive, your body weight and height will be taken, and you will be asked to complete a 24-hr dietary recall questionnaire where you will have to describe everything you ate and drank the day before. You will then complete 3 cognitive tasks (more details below).

Once complete, you will be asked to sit in a comfortable chair and fitted with equipment to measure your heart rate, blood pressure, and muscle oxygenation and metabolism. For heart rate and blood pressure measure, you will be fitted with an inflatable cuff on your right arm (above the elbow). For muscle oxygenation and metabolism measures, in addition to the inflatable cuff on your right arm, you will be fitted with 2 probes on your forearm (secured by a band). Once these measures are taken, we will remove the inflatable cuff, move the probes from your forearm to your forehead (secured by a headband), and you will breathe a gas mixture that has a lower oxygen concentration than at sea level (equivalent to an altitude of approximately 5000 m). This will be done via a mouthpiece connected to a large bag of premixed gas, and we will measure brain oxygenation and metabolism whilst your brain is being challenge by this low oxygen levels (more detail below). During this time, your common carotid and internal carotid artery blood flow will also be measured. This will conclude the baseline measurements.

Immediately following baseline measurements, you will be asked to consume either a low- or high-flavanol cocoa beverage (depending on which group you are assigned to) within 10 mins and rest for an hour and a half. During the 1st experimental session's rest period, you will be asked to complete a food frequency questionnaire, which provides an overview of your habitual diet within the last year. Otherwise, you can engage in any activity of your choice (e.g. reading, writing, compute work etc.). For each experimental visit, you are advised to bring some form

of entertainment with you, such as a book or laptop with headphones. Following this rest period, the measurements and protocols outlined above will be repeated. Once all the measurements are taken, we will remove the equipment and you will be given coffee or tea and a snack. At the end of the 1st experimental session, you will be given 4-week's supply of cocoa powders to take home with you to have every day – instructions will be provided.

Visit 3: During Experimental Visits 2 (~ 45 min)

The 2nd experimental session will take place 4 weeks following the first study visit. For this experimental session, we will ask you to arrive at 8 a.m. in a fasted (no food for ≥ 12 hr) and rested state (no vigorous exercise for ≥ 24 hr). **Importantly, we will request that you consume the cocoa beverage from the day before approximately 24 hr prior to the study visit** (i.e. ~ 8 a.m. the day). When you arrive, we will ask you to complete a 24-hr dietary recall questionnaire where you will have to describe everything you ate and drank the day before.

Next, you will be fitted with equipment to measure your heart rate, blood pressure and muscle and brain oxygenation/metabolism, and we will repeat the protocol outlined above. Once complete, we will remove the equipment and you will be given coffee or tea and a snack. At the end of this session, you will be given the next 4-week's supply of cocoa powders to take home with you to have every day – instructions will be provided.

Experimental Techniques in Detail

- **Heart Rate & Blood Pressure** – These will be monitored simultaneously using an automated blood pressure cuff, which consists of an inflatable cuff placed around your upper arm. This cuff will gently tighten on your arm but should not be uncomfortable.

- **Artery Blood Flow** – This will be measured non-invasively using ultrasonography. A trained sonographer will place a scanning probe on your neck area. We will be imaging the common and internal carotid artery to record the rate of blood flow in these vessels. Some gel on the probe may feel cold against your skin initially, and you will feel the probe pressing gently on your neck for a short 2 min measurement; but this should not cause you discomfort.

- **Cognitive Function** – This will be assessed using 3 computer-based tasks: 1) Modified Attention Network Task, 2) Switch Task, and 3) Modified Stroop Task. Each of these tasks presents a sequence of visual stimuli, which you will interact with to determine your executive function accuracy and reaction time. We will explain these in detail during the familiarisation visit, where you will also have the opportunity to practice each task.

- **Muscle Tissue Oxygenation & Metabolism** – These will be monitored simultaneously using broadband near-infrared spectroscopy (bNIRS). bNIRS detects the state of oxygenation of haemoglobin (i.e. oxygenated or deoxygenated) and an important energy-

producing molecule (cytochrome c oxidase), which gives us an index of tissue oxygenation and rate of metabolism, respectively. This device consists of 2 non-invasive probes. This test will be performed whilst you are in a seated position. You will be fitted with an inflation cuff placed above your elbow and the probes will be attached to your forearm and secured by a band. bNIRS signal will be acquired continuously during a 5-min resting period, followed by a 5-min occlusion period, and another 5-min following release of the cuff occlusion. Occlusion will be achieved by rapidly inflating the cuff to obstruct blood flow. Once the 5 min occlusion period is finished, it will be rapidly deflated to allow blood flow to return to your forearm. It is very important that you remain as still as possible so the probes on your forearm do not move during the procedure. You should wear a short-sleeve t-shirt to allow for accessibility during this procedure.

- **Brain Tissue Oxygenation & Metabolism** – This will also be monitored using bNIRS. In this case, the probes will be attached to your forehead and secured by a headband. bNIRS signal will be acquired continuously during a 5 min resting period whilst you breathe in

  normal room air (with 21% oxygen), followed by a 15 min period where we will be challenging your brain by having you breathe a lower amount of oxygen (11%, which is equivalent to an altitude of ~ 5000m) from a premixed Douglas bag, and after that another 5 min resting period whilst you breathe normal room air again. When you are breathing in the gas containing less oxygen (11%), you may feel dizzy or lightheaded whilst increasing your breathing rate. The gas mixture will be administrated via a mouthpiece, which can be easily removed if there are any issues, and the hypoxic-induced symptoms will quickly resolve within a few seconds of breathing normal room air. We will be closely monitoring you to ensure you tissue saturation does not fall below what is acceptable and safe.

5. Do I have to take part?

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any point without giving a reason for up to two weeks after your last study visit.

You should feel under no pressure to participate and if at any time you are asked questions that you are not comfortable answering (e.g. those asked in the general and health lifestyle questionnaire) you are free to not disclose this information. Please note that not answering some questions may mean you cannot participate. Please also bear in mind that all information collected will be kept strictly confidential.

6. Will I be paid for participating?

You will be reimbursed for your time and commitment. For the completion of the full 8-week study period, you will receive £80. You will not be reimbursed for any travel expenses.

7. What are the possible benefits of taking part?

Long-term high-flavanol cocoa beverage consumption, similar to the ones supplied in this study, has been shown to improve body and brain blood flow and oxygenation, which promotes cardiovascular health and cognitive function. Also, at your request, you can find out about your blood pressure and resting heart rate.

8. What are the possible disadvantages and risks of taking part?

There is little risk associated with this study. Whilst we assess your brain oxygenation and metabolism during reduced-oxygen exposure, you may feel you may feel dizzy or lightheaded whilst increasing your breathing rate; however, this is a temporary sensation that will go away within a few seconds of breathing normal room air. During this test, we will monitor your closely, and if you express extreme discomfort, we will immediately remove the mouthpiece, after which any discomforting sensations will go away. In extremely rare cases, you may experience a 'hypoxia hangover,' which refers to any lingering effects from the hypoxic exposure. Symptoms such as fatigue, headache, dizziness, nausea, and cognitive impairment may persist for a short period (i.e. 1 – 2 mins typically, maximum 15 mins) after returning to normal oxygen levels. If this occurs, we will continue to monitor you and provide advice on alleviating the symptoms and promoting recovery.

Prolonged experiments may cause physical discomfort for some people. However, you will sit in a comfortable chair during all study visits. The investigators will regularly check on your comfort levels, and at any point if you express discomfort, we will adjust and/or remove any equipment. All investigators are experienced in performing all the procedures with hundreds of similar sessions completed safely in the recent past. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above.

There are no known risks associated with consuming cocoa drinks provided within this study. All cocoa sachets included in this study are produced for human consumption by a food company (Barry Callebaut). The cocoa drinks do not contain any known allergenic compounds – detailed ingredient composition of the cocoa powders can be provided upon request. These drinks are low in sugar and fat and may have a slightly bitter taste to some people. If you, for any reason cannot continue drinking one cocoa beverage a day, please contact an investigator immediately. A phone number will be provided to you for you to ask any questions or voice any concerns you may have in between study visits.

9. Will my taking part in this study be kept confidential?

Yes, your participation in this study will be kept confidential. Any information that we obtain from this study about you, including your name, will be confidential. This information will be stored in locked filing cabinets or password-protected files only accessible to the researchers of the study, according to the University of Birmingham regulations. At the start of the study,

you will be given a unique ID number, all your data will be stored and analysed using that unique ID number.

10. What will happen to the results of the study?

The results of this study are expected to be published anonymously in scientific journals and within a PhD thesis. Confidentiality will be ensured at all times, and you will not be identified in any publication.

11. Who is organising and funding the research?

This study is supported by the Biotechnology and Biological Sciences Research Council, University of Birmingham (School of Sport, Science and Rehabilitation Sciences), and Barry Callebaut. Dr Catarina Rendeiro, Dr Sam Lucas, and PhD Candidate Jasmine Yeh are organising the research.

12. What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis.

Additionally, you can withdraw your data from the study for up to two weeks following completion of the data collection by notifying us via email or telephone. If you withdraw, the data collected to date cannot be erased but it will not be used in any data analysis or publications.

If you took park in this study for research hours, you will still receive the credit for the hours you completed up till withdrawal. Similarly, you will still receive compensation for your time and commitment. The amount received will be based on the numbers of days completed, for example for the completion of 4 weeks of the study you will receive £30.

13. Can I obtain feedback from the study?

Yes. If you wish to know the results of the study, a summary of the results can be provided once the study has concluded. On the consent form, there is a space to indicate if you would like to receive a study summary.

14. Do I have to sign anything?

Yes. If you agree to participate, we will ask you to sign a consent form. This is to show that you have understood what is involved and that you have read this information sheet. You can still withdraw at any time without having to give us an explanation.

15. Do you have any further questions or concerns?

If you have any further questions about the study, please feel free to contact:

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