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Shedding light on the direct vs indirect mechanisms of action of dietary flavonoids in the human brain

Submission date 09/09/2024	**Recruitment status** No longer recruiting	[X] Prospectively registered [_] Protocol [_] Statistical analysis plan [_] Results [_] Individual participant data [X] Record updated in last year
Registration date 16/09/2024	**Overall study status** Completed	
Last Edited 24/11/2026	**Condition category** Other	

Plain English summary of protocol

Background and study aims

Age-related cognitive decline is a leading cause of disability in the UK, with the number of cases projected to triple by 2060. Diets rich in flavonoids - small molecules naturally present in vegetables and fruits (e.g., cocoa, blueberries, apples) that often contribute to their vibrant colours - have been shown to delay age-related cognitive decline. Previous research has demonstrated that consuming cocoa can lead to immediate improvements in cognitive function; however, the mechanisms behind this effect remain unclear.

It is generally suggested that flavonoids in cocoa (i.e., cocoa flavanols) improve blood flow and oxygenation in the brain, which in turn enhances cognitive function. However, cocoa flavanols might also directly affect brain cells by improving the brain's ability to convert nutrients (e.g., glucose and oxygen) into the energy needed to support cognitive processes, and thus leading to cognitive improvements.

Therefore, this study aims to determine whether cocoa flavanols exert their positive impact on cognition either indirectly by boosting blood flow and oxygenation to the brain and/or directly by enhancing the energy processes within brain cells. The findings of this study will, for the first time, provide a direct comparison of the effects of acute and chronic intake of dietary flavonoids, their impact on healthy young versus older adults, and the underlying mechanisms of action in the brain and skeletal muscle in humans.

Who can participate?

Healthy young adults (18 to 40 years old) and older adults (65 years old and over) without a history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease.

What does the study involve?

The study involves one screening/familiarisation visit to assess eligibility (about 45 minutes). Participants who meet the inclusion criteria will be assigned to receive either low- or high-flavanol cocoa beverages daily for 8 weeks. During this period, they will be invited to attend three trial visits (totalling about 10.5 hours, each separated by 4 weeks) in the mornings whilst in a fasted state. Baseline measures of heart rate, blood pressure, executive cognitive function, and skeletal muscle/brain oxygenation and mitochondrial activity will be recorded continuously

during rest and physiological stresses (muscle occlusion of the biceps and hypoxia simulated by breathing 11% oxygen). After these baseline measurements, participants will consume either a low- or high-flavanol cocoa beverage (as assigned) and rest for 90 minutes. After the resting period, the previous measures will be repeated.

What are the possible benefits and risks of participating?
Participants will receive monetary compensation upon completion of the study. Those assigned to the high-flavanol cocoa group may benefit from improved blood flow and oxygenation in both the body and brain, which could promote cardiovascular health and cognitive function. However, those who benefit from this will not be known due to the double-blinded nature of the study. Moreover, data from this study will offer valuable insights for designing and testing effective dietary strategies to mitigate age-associated cognitive decline, a leading cause of disability in the UK.
There is minimal risk associated with this study. The primary risks include potential adverse reactions to the ingestion of low- or high-flavanol cocoa supplements and temporary lightheadedness during the acute hypoxia phase. Routine screening for lifestyle, health, and allergies will be conducted before participation, and all procedures will be supervised by fully trained investigators.

Where is the study run from?
The Vascular Function Laboratory at the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham (UK)

When is the study starting and how long is it expected to run for?
January 2024 to September 2026

Who is funding the study?
This study is supported by the Biotechnology and Biological Sciences Research Council (BBSRC) and University of Birmingham (School of Sport, Science, and Rehabilitation Sciences) funded Midlands Integrative Biosciences Training Partnership (MIBTP; grant number: BB/T00746X/1), and Barry Callebaut.

Who is the main contact?
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Contact information

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Additional identifiers

Protocol serial number
2147295

Study information

Scientific Title
Comparing the effects of acute versus chronic, flavanol-rich cocoa intake on cerebral haemodynamics, metabolism, and cognitive function in healthy young (18 to 40 year-old) and older (65 + year-old) adults

Acronym
CocoaClarity

Study objectives
The main aim of this study is to determine whether dietary flavonoids exert their effects on the brain - and subsequently on cognition - by indirectly improving cerebral blood flow and oxygenation and/or by directly enhancing neuronal mitochondrial activity, as indicated by cytochrome c oxidase activity.

The researchers will test the following hypotheses:
1. Acute supplementation of high-flavanol cocoa, but not low-flavanol cocoa, will improve cerebral oxygenation and blood flow, but not neuronal mitochondrial metabolism, leading to improved cognitive function in healthy young (18 to 40 years old) and older adults (≥ 65 years old).
2. Chronic supplementation of high-flavanol cocoa, but not low-flavanol cocoa, will improve cerebral oxygenation, blood flow, and neuronal mitochondrial activity, leading to improved cognitive function in healthy young (18 to 40 years old) and older adults (≥ 65 years old).

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 26/07/2024, Science, Technology, Engineering and Mathematics Ethical Review Committee of the University of Birmingham (Edgbaston, Birmingham, B15 2TT, United Kingdom; -; aer-ethics@contacts.bham.ac.uk), ref: ERN_1644-Jul2024

Primary study design
Interventional

Study design
Acute within chronic interventional double-blinded randomized placebo-controlled parallel human study

Study type(s)
Other

Health condition(s) or problem(s) studied
Healthy volunteers

Interventions
Participants (n = 100; 50 young and 50 older adults) will take part in a chronic, double-blind, randomized, placebo-controlled, parallel-group trial. This study aims to assess whether dietary flavonoids affect the brain - and, subsequently, cognition - by indirectly improving blood flow and oxygenation and/or directly enhancing neuronal mitochondrial activity.

Participants who meet the inclusion criteria will be required to visit the Vascular Function Laboratory at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham on four occasions (including a screening/familiarisation visit). Interested participants will receive a participant information sheet outlining the study before the initial screening session, during which they will be asked to provide written consent and complete a general health and lifestyle questionnaire. The study will be fully explained during this session, and participants will have the opportunity to ask any questions. If participants meet the inclusion criteria and are willing to take part in the study, this initial session will also be used to assess the quality of their broadband near-infrared spectroscopy (bNIRS) signals. If their bNIRS signals are poor, they will not continue in the study. During the same visit, those with good bNIRS signals will be familiarised with the study procedures through a brief exposure (~5 min) to a hypoxic gas mixture (11% O2) and practice with cognitive tasks.

Participants will be randomly assigned using covariate adaptive randomisation to ensure balance in key variables (e.g., age, gender). Upon enrolment, participants will be categorised by covariates (e.g., 18 y/o, male), and ‘Randomizer.org’ will be used to assign them to two intervention groups. If an imbalance occurs within a category, the randomisation will be biased towards the under-represented group by adjusting the probability of assignment, ensuring a balanced distribution across groups.

Participants will be randomly assigned to either the low-flavanol (~36 mg of flavanols) or high-flavanol (~660 mg of flavanols) cocoa group as part of a double-blind, placebo-controlled, sex-balanced, parallel-arm study. Each participant will consume 15.5 g of cocoa powder mixed in 250 ml of cold water or milk (their choice) daily for 8 weeks.

The first (day 1) and third experimental visits (approximately day 56) will take place in the morning, starting around 8/9 a.m. These visits will be separated by 8 weeks, which is the duration of the dietary intervention. Participants will be asked to arrive in a fasted (no food for at least 12 hr prior) and a rested state (no vigorous exercise for at least 24 hr prior). Upon arrival at the laboratory, participants' body weight and height will be recorded, and they will complete a 24-hour dietary recall questionnaire. Following this, they will undergo three cognitive assessments to measure executive function accuracy and reaction time. Immediately after, participants will rest in a supine position for 10 min, and baseline measures will be collected for: 1) heart rate, 2) blood pressure, 3) skeletal muscle (brachioradialis) tissue oxygenation and mitochondrial activity during rest and vascular occlusion, 4) brain oxygenation and mitochondrial activity during rest and hypoxia (stimulated by 11% oxygen), and 5) blood flow in the common and internal carotid arteries during rest and hypoxia (stimulated by 11% oxygen).

Once baseline measures are complete, participants will be asked to consume 15.5 g of either low-flavanol or high-flavanol cocoa powder (dependent on the assigned group), mixed in 250 ml of cold water, within 10 min. Participants will then rest for 90 min. After this period, participants will rest in a supine position for 10 min before repeating the aforementioned outcome measures. During the 90-minute rest in the first study visit, participants will be asked to complete a food frequency questionnaire, which provides an overview of their habitual diet over the past year.

The second study visit will take place 4 weeks (approximately day 28) following the first study visit. This experimental visit will begin at around 8/9 a.m. Prior to this visit, participants will be asked to consume the cocoa beverage from the previous day, approximately 24 hr before the study visit (i.e., around 8 a.m. the previous day). Participants will be asked to arrive in a fasted (no food for at least 12 hours prior) and a rested state (no vigorous exercise for at least 24 hours prior). Upon arrival at the laboratory, participants' body weight and height will be recorded, and they will complete a 24-hour dietary recall questionnaire. Following this, participants will rest in a supine position for 10 min, and measures will be collected for: 1) heart rate, 2) blood pressure, 3) skeletal muscle (brachioradialis) tissue oxygenation and mitochondrial activity during rest and vascular occlusion, 4) brain oxygenation and mitochondrial activity during rest and hypoxia (stimulated by 11% oxygen), and 5) blood flow in the common and internal carotid arteries during rest and hypoxia (stimulated by 11% oxygen).

Intervention Type
Supplement

Primary outcome(s)
Prefrontal cortical concentrations of oxygenated and deoxygenated haemoglobin will be measured using broadband near-infrared spectroscopy (bNIRS) during rest and physiological stress (i.e., hypoxia simulated with 11% oxygen delivery) at the following time points: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 28 and day 56, before (0 hours) cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).

Key secondary outcome(s)
1. Prefrontal cortical neuronal mitochondrial activity (i.e., cytochrome c oxidase activity) measured using bNIRS during rest and physiological stress (i.e., hypoxia simulated with 11% oxygen delivery) at the following time points: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 28 and day 56,

before (0 hours) cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).
2. Prefrontal cortical levels of oxygenated and deoxygenated haemoglobin concentrations during cognitive performance measured using bNIRS at the following time points: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 56, before cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).
3. Prefrontal cortical neuronal mitochondrial activity (i.e., cytochrome c oxidase activity) during cognitive performance measured using bNIRS at the following time points: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 56, before cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).
4. Executive function accuracy and reaction time (indicator of cognitive performance) will be measured using the tasks listed below at the following time points: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 56, before cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).
4.1. Modified Attention Network Task measures response to cognitive load
4.2. Switch Task considers cognitive flexibility with participants responding to stimuli according to two different paradigms (or rule) shifts
4.3. Modified Stroop Task assesses selective attention and prepotent response inhibition during decision making
5. Common and internal carotid artery blood flow measured using duplex ultrasound during rest and physiological stress (i.e., hypoxia simulated with 11% oxygen delivery) at the following timepoints: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 28 and day 56, before (0 hours) cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).
6. Skeletal muscle (brachioradialis) levels of oxygenated and deoxygenated haemoglobin concentrations measured using bNIRS during rest and physiological stress (i.e., vascular occlusion) at the following timepoints: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 28 and day 56, before (0 hours) cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).
7. Skeletal muscle (brachioradialis) mitochondrial activity (i.e., cytochrome c oxidase activity) measured using bNIRS during rest and physiological stress (i.e., vascular occlusion) at the following timepoints: i) on day 1, before (0 hours) and 2 hours after supplementation with either low- or high-flavanol cocoa (acute effect); ii) on day 28 and day 56, before (0 hours) cocoa supplementation (chronic effect); and iii) on day 56, 2 hours after cocoa supplementation (acute within chronic effect).

Completion date
10/09/2026

Eligibility

Key inclusion criteria
Current inclusion criteria as of 24/11/2026:
1. Healthy male or female
2. Aged between 18 to 40 years old OR above 60 years old

Previous inclusion criteria:
1. Healthy male or female
2. Aged between 18 to 40 years old OR above 65 years old

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Mixed

Lower age limit
18 Years

Upper age limit
90 Years

Sex
All

Total final enrolment
107

Key exclusion criteria
1. Consumption of more than 21 units of alcohol per week
2. A history of cardiovascular, respiratory, metabolic, liver, inflammatory, or neurological diseases, including but not limited to hypertension (blood pressure >140/90 mmHg), diabetes mellitus, asthma, elevated cholesterol, anemia, immune conditions, and smoking
3. Use of prescribed or over-the-counter medications (except for oral contraception)
4. Allergies or intolerances to any ingredients in the dietary supplement
5. Currently following a weight-reducing dietary regimen
6. Use of any dietary supplements, including fatty acids and vitamins
7. Long-term medication use or use of antibiotics in the past 3 months
8. Current infection (e.g., cold)
9. Pregnancy or plans to become pregnant (for females of reproductive age)

Date of first enrolment
30/10/2024

Date of final enrolment
16/07/2026

Locations

Countries of recruitment
United Kingdom

England

Study participating centre
School of Sport, Exercise and Rehabilitation Sciences
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Sponsor information

Organisation
University of Birmingham

ROR
https://ror.org/03angcq70

Funder(s)

Funder type
Government

Funder Name
Biotechnology and Biological Sciences Research Council

Alternative Name(s)
UKRI - Biotechnology And Biological Sciences Research Council, Agricultural and Food Research Council, Biotechnology & Biological Sciences Research Council, BBSRC, BBSRC UK, AFRC

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location
United Kingdom

Funder Name
Barry Callebaut

Funder Name
University of Birmingham

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study will be available upon request from Dr Catarina Rendeiro (c.rendeiro@bham.ac.uk). Pseudo-anonymised raw data of primary and secondary outcome measures will be available to the scientific community on completion of the overall study end date, for up to 10 years in accordance with the University of Birmingham policies, for specific secondary analyses of data that have not been performed as part of our original study objectives. Material containing potentially identifying information will be non-publicly available. The anonymisation and confidentiality of data and data processing are addressed in the participant's information sheet and informed consent form for the study.

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Other files			10/09/2024	No	No
Participant information sheet	version 3	19/07/2024	10/09/2024	No	Yes