Research Protocol

Title of project: 'Running blue': The effects of blueberry intake on cerebrovascular and cognitive responses to exercise in low and high-fit young adults

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Lay synopsis of project

Dementia and cognitive decline are major public health issues and leading causes of older adult disability. Decline in 'our ability to think' is part of natural aging and is partly due to deficits in blood flow delivering oxygen and nutrients to the brain. Evidence shows that being physically active improves blood flow to the brain and results in better cognition later in life. The long-term cognitive benefits of being active are linked to blood vessels being repeatedly exposed to rises in blood flow, which causes better vessel functioning when delivering nutrients/oxygen to the brain. We have shown that better cognition can be seen after a single bout of exercise. As such, enhancing exercise-induced changes in brain blood flow has the potential to result in better cognition, and over the longer-term optimizing the benefits of physical activity can be a way to improve resilience against cognitive decline later in life. Critically, there is evidence showing that individuals with low levels of fitness have lower increases in brain blood flow when engaging in moderate/high intensity physical activity, when compared to high fit individuals, and this is also linked to poorer cognition. Therefore, dietary strategies, such as intake of blueberries, prior to exercise may help to optimize the adaptive stimulus to exercise for the poor responders (low-fit), whilst still being able to maximize or accelerate benefits in good responders (high-fit). Indeed, we have previously shown that blueberries can result in immediate increases in blood flow and oxygenation in areas of the brain important for cognition, with such improvements resulting in measurable benefits in cognitive function. Here, our key objectives are to investigate whether blueberry intake (equivalent to 1 cup of fresh) prior to low, moderate, and high intensity exercise results in better brain blood flow and blood oxygenation, which then leads to better cognitive performance.

These data will establish whether blueberries might be effective in optimizing cognitive health in young healthy adults in the context of physical activity and will further identify which groups of individuals are most likely to benefit from blueberry intake. This work will be important to inform long-term preventive measures for aging-associated cognitive decline, whilst providing more specific recommendations for those physically active and looking to maximize the health benefits of exercise, as well as more sedentary individuals.

Scientific rationale & background information

Effective regulation of blood flow to and within the brain is vital for optimal brain function across the lifespan. Cerebral blood flow declines by approximately half across healthy adulthood and is associated with deterioration in cognitive performance[1]. Regular physical activity has the capacity to delay the longitudinal decline in cerebral blood perfusion during sedentary aging[2] and reduce the risk of cognitive decline and dementia[3,4]. In particular, executive function, which refers to the ability for flexible thinking and goal-directed behaviours, has been extensively reported to be protected in aging populations that are physically active[5,6].

The mechanical force of blood flow through the vessels that occurs during exercise (termed shear rate/stress) is a central mechanism for improving vascular function[7]: Shear rate/stress stimulates the release of nitric oxide (NO) from the vessel wall, increasing its bioavailability and thus exerting a positive effect on vascular function[8]. Indeed, single bouts of exercise-induced increase in cerebral blood flow are typically accompanied by transient increases in cerebral cortical oxygenation[9]. In turn, this produces acute improvements in cognition, such as executive function[10-12]. This suggests that exercise can effectively result in immediate and measurable benefits to brain performance. More importantly, repeated increases in blood flow/oxygenation during bouts of exercise are believed to contribute to the cognitive benefits associated with regular physical activity[13-15] and are likely to predict the long-term benefits of exercise on brain function[7]. In agreement with this, our group has shown previously that acute exercise-induced increases in cerebral blood flow/ oxygenation can translate into benefits on cognitive performance, likely via better coupling of blood flow to the brain to meet neuronal metabolic demands[16,17].

As such, maximizing acute cerebrovascular responses to exercise may hold the potential to improve resilience to later life decline in brain blood flow and cognitive function. Notably, our recent work indicates that environmental factors (e.g., water immersion) and dietary factors (e.g., nitrates) can modulate exercise-induced cerebral blood flow patterns and enhance cerebrovascular responses during exercise[18-21]. Therefore, strategies that optimize exercise recommendations, by targeting key underlying mechanisms of exercise-induced adaptation, may provide opportunities for enhanced cognitive and cerebrovascular resilience in aging.

In that regard, mounting evidence shows that regular consumption of flavonoid-rich fruits, such as blueberries, improve vascular health and protect against cognitive decline in aging[22-25]. These effects have been partially attributed to up-regulation of circulating vasodilators such as NO[26], sharing a common underlying mechanism with exercise. Indeed, anthocyanins and flavanols, found in high concentrations in blueberries, induce clinically relevant improvements in NO-dependent endothelial function in healthy adults (as previously shown by our group)[27,28]. Further, we have demonstrated that one single acute dose of blueberries (equivalent to 1-1.5 cups of fresh blueberries) can improve resting cerebral blood flow[29] as well as episodic memory and executive function (e.g. Flanker Task) domains of cognitive function[30-33] in young healthy adults. We have also recently shown that flavanols, also present in blueberries, increase cortical oxygenation by ~3-fold during a hypercapnia challenge (5% CO_2), with these improvements linked to better executive function (Stroop Task) in young healthy adults[34]. This suggests that cerebrovascular benefits of blueberry intake, detectable both at rest and during a physiological challenge, can translate into better executive function even in young healthy individuals. Therefore, blueberry intake prior to exercise is likely to be an efficacious strategy to enhance brain blood flow and the cognitive benefits of exercise (both short and long term).

Importantly, blueberry intake prior to exercise may be particularly useful in populations with lower levels of cardiorespiratory fitness, by augmenting acute vascular responses of physical activity to levels that otherwise may not be achievable. It is well established that better cardiorespiratory fitness later in life is associated with better cerebral blood flow and cognition[35,36]. Recent observations show that levels of cardiorespiratory fitness in young adults also influence cerebrovascular responses during exercise, with individuals of low fitness experiencing smaller rises in cerebral blood flow and cortical oxygenation levels in comparison to fit individuals[37,38]. For example, during the transition from moderate to high intensity exercise, cortical oxygenation is reduced in low-fit individuals, whilst in

high-fit individuals it continues to rise indicating a superior capacity to deliver and extract oxygen[37]. Notably, such differences in exercise-induced cerebral blood flow responses have been linked to differences in cognitive function between high and low fit individuals[15,39,40]. Indeed, we have preliminary data from young healthy individuals, suggesting a lower resting blood flow in the common carotid, as well as lower cortical oxygenation, in low-fit individuals compared to high-fit. Overall, these data indicate that fitness levels can influence cerebrovascular and cognitive benefits of acute exercise, likely to affect long-term adaptation. Therefore, blueberry intake prior to exercise has the potential to enhance and optimize the benefits of exercise, particularly in individuals with low cardiorespiratory fitness so that they may reach levels of cerebrovascular responses more in line with what high-fit individuals experience. Ultimately, these processes may accelerate cerebrovascular adaptation and, over the long-term, the targeted combination of exercise and dietary strategies (such as blueberries) may boost the benefits of exercise, leading to overall improved cognitive and vascular resilience in late adulthood.

In this project we will investigate the impact of flavonoid-rich whole blueberries, in combination with exercise, in both high-fit and low-fit individuals. We hypothesize that the intake of blueberries prior to exercise (low, moderate and high intensity) will be an effective dietary strategy to: **1)** enhance exercise-induced increases in cortical oxygenation, cerebral blood flow/velocity and shear rate in healthy young adults; **2)** improve post-exercise cognitive performance (executive function), by enhancing local cortical oxygenation levels; and we further predict that **3)** cardiorespiratory fitness will affect blueberry-induced physiological and cognitive responses to exercise, with low-fit individuals benefiting more from blueberry intake compared to high-fit individuals.

Study goals and objectives

The overall aim of this project is to investigate whether intake of flavonoid-rich whole blueberries can improve cerebrovascular and cognitive benefits of exercise in high and low-fit healthy individuals. Specifically, we aim to:

1) **Determine the effect of acute blueberry intake on cortical oxygenation, cerebral blood flow/velocity and shear rate during low, moderate, and high-intensity exercise in young healthy adults.**

   Rationale: Blood flow/ shear rate rises during moderate/high intensity exercise are known to contribute to the long-term cognitive and cerebrovascular benefits of habitual exercise. We have further shown that intake of a single dose of blueberry flavonoids results in acute improvements in brain blood flow and cortical oxygenation. As such, blueberry intake prior to exercise is likely to optimize vascular responses to exercise and potentially open up a new avenue to explore dietary strategies to accelerate long-term cerebrovascular adaptations to exercise.

2) **Examine the impact of acute blueberry intake on post-exercise cognitive performance (executive function) and cerebral oxygenation in healthy young adults.**

   Rationale: We have shown previously that exercise–induced increases in blood flow/ shear rate have been linked to better cognitive performance, likely due to improved efficiency in blood flow/oxygen delivery during neuronal activity. On the other hand, we have also shown that blueberry flavonoids result in improvements in executive function in young healthy adults. As such, we predict that blueberry intake will result in better levels of oxygenation and blood flow to the brain during cognitive performance, effectively resulting in improved performance.

3) **Evaluate the impact of cardiorespiratory fitness on cognitive and cerebrovascular benefits of blueberry intake prior to exercise in young healthy adults.**

Rationale: Exercise-induced cortical oxygenation is reduced in low-fit individuals in comparison to high-fit individuals, where levels of oxygenation continue to rise, indicating a superior capacity to deliver and extract oxygen. Such differences are believed to contribute to poorer cognitive function in low-fit individuals. As such, we anticipate that blueberry intake prior to exercise may particularly help low-fit individuals reach levels of vascular and cognitive responses that are closer to those of high-fit individuals.

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Study design

This study will use a randomized placebo-controlled double-blinded acute human study in healthy young adults, aged 18-40 yrs, to investigate the impact of flavonoid-rich whole blueberries, in combination with exercise, in both low-fit and high-fit individuals. Those that meet the inclusion criteria for fitness will be invited to individually attend two laboratory-based face-to-face intervention trials (approx. 5 h each) at the School of Sport, Exercise and Rehabilitation Sciences at least 2 weeks apart. Participants will have completed a 12 h overnight fast, and refrain from exercising, alcohol, caffeine, and polyphenol-rich foods for 24 h prior to the visits.

Baseline cardiorespiratory, peripheral vascular, and cerebrovascular haemodynamics will be measured continuously at rest and cardiorespiratory and cerebral haemodynamics will be measured during an incremental cycling test at low, moderate, and high-intensity exercise. After exercise, baseline measures of executive cognitive function will be assessed whilst continuously monitoring cognitive performance-induced changes in cerebral haemodynamics. Measurements will be collected by a research associate (doctorate in training) trained in non-invasive monitoring of cerebrovascular physiology, specifically duplex and transcranial Doppler Ultrasound, cardiovascular physiology, and cardiopulmonary exercise testing.

Following these measures, participants will consume a single dose of either freeze-dried highbush blueberry powder (approximately 30g/equivalent to 1 cup of daily recommended fruit or 190 g of fresh whole blueberry, delivering approximately 960 mg of total polyphenols) or a low-flavonoid control (isocaloric and matched for carbohydrate profile, minerals, and vitamins). This blueberry dose has been shown to be effective at modulating vascular function and cognitive performance in healthy subjects. The order of trials will be decided by simple randomisation using Randomizer.org.

Post-intervention measurements at rest, and of cognitive and exercise-induced cerebral haemodynamics will be repeated at 1-2 h post blueberry/control intake, coinciding with the peak of blueberry polyphenols in circulation and informed by our previous work showing improvements in vascular function and cognitive performance 1 h post-blueberry intake.

Eligibility criteria

We will recruit those who:

- Are a healthy male or female
- Are aged 18-40 years old
- Do not smoke and do not consume more than 21 units of alcohol per week
- Have no history of cardiopulmonary, cerebrovascular, musculoskeletal affecting the limbs, respiratory, metabolic, metabolic, liver, inflammatory diseases, or neurological illness
- This may include but not limited to; blood-clotting disorders, hypertension (BP > 140/90 mmHg), diabetes mellitus, anaemia, asthma (only if you take regular/daily medication or require medication before or after exercise), immune conditions, elevated cholesterol, smokers, or have recently had prolonged bed rest.
- Do not have a known allergy to berries.
- 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 or have been on antibiotics for the last 3 months
- Do not have an infection at present (e.g., cold)
- Do not have a VO_{2max} between Female: >34 and <41 and Male: >40 and <50 ml/kg/min

Methodology:

Maximal aerobic exercise capacity (VO_{2max}) protocol: Participants will use a cycle ergometer with initial workload set to ~40 Watts (W) and increased by 30 W/2 min until volitional exhaustion (VO_{2max}) as described previously[37] to determine their aerobic fitness.

Nutritional supplementation: Participants will be asked to take a supplement of 30 g of polyphenol-rich freeze-dried highbush blueberry powder (equivalent to 1 cup of daily recommended fruit or 190 g of fresh whole-blueberry, delivering approximately 960 mg of total polyphenols) or a placebo supplement that will consist of a low-flavonoid control (isocaloric and matched for carbohydrate profile, minerals, and vitamins). High-flavanol blueberry powder and low-flavanol placebo powder products are provided by US Highbush Blueberry Council (California, United States).

The placebo powder primarily constitutes of maltodextrin and subsequently, may be supplemented by the investigators to best match the micro and macronutrients, except for the levels of polyphenols (i.e., phenolics and anthocyanins) present.

Detailed product specification of all micro and macronutrients data sheets in high-flavanol blueberry powder and low-flavanol placebo powder will be provided to the participants during recruitment if requested. Participants with a food allergy to any berries will be automatically excluded to ensure safety. Participants with any other food allergies will be considered on an individual basis and authorization to take part in the study will be determined by the Principal Investigator (CR).

Blueberry powders will be packaged in individual doses by a researcher that is Food Safety trained (doses of approximately 30 g). Packages will be clearly labelled by a 3-digit number for each condition (to ensure double-blindness) and kept at –20°C. Details of the code will only be revealed once the study has finished and all data analysed. Blueberry/placebo drinks will be prepared by the researcher 5 minutes before delivery to the volunteer, by dissolving the powder in approx. 200 mL of Buxton water. The drink will be presented to the volunteer in an opaque container with a black opaque straw to ensure double-blindness.

Individual doses of total polyphenols delivered will be approx. 966 mg per person (12.88 mg/kg of body weight [75kg standard body weight]), with approximately 339 mg of anthocyanins (4.52 mg/kg of body weight). This mimics previous doses that have been shown in previous studies to be safe and effective at modifying cardiovascular outcomes (e.g., Rodriguez-Mateos et al., 2013 found benefit to FMD between 1 – 2 hrs of consuming 310 mg anthocyanins and 766 mg total polyphenols from 34g blueberry powder).

Incremental exercise trial: Participants will complete an incremental exercise trial that will consist of three 6.5-minute increments at 30%, 60% and 80% of VO_{2max}. The rate/intensity of these sessions will be fixed based on the results of each participant VO_{2max} test, as a percentage of the maximum oxygen consumption (VO_2), heart rate, or power output. The first 90 seconds of each exercise increment will enable the participant to reach steady state, whilst the final 5 minutes the participant will be asked to complete a cognitive task (Modified Stroop Task).

Blood pressure: Brachial artery blood pressure will be assessed via a blood pressure cuff wrapped around the arm to obtain systolic, diastolic, and mean blood pressure using an oscillometric blood pressure monitor. Beat-by-beat blood pressure and heart rate will also be measured from a small cuff placed around the middle finger of the left hand (finometer system).

End-tidal blood gases: Breath-by-breath expired oxygen and carbon dioxide will be measured by a respiratory gas analyser to derive end-tidal values.

Tissue haemodynamics and oxygenation***:*** Near-infrared spectroscopy (NIRS) will be used to measure tissue haemodynamics and oxygenation of the brain at the prefrontal lobes. This method uses near-infrared probes placed on the skin of the target tissue to measure haemoglobin content and calculate ratios of oxygenated and deoxygenated haemoglobin (i.e., tissue saturation) at the local level (NIRO 200NX system).

Transcranial Doppler Ultrasound (TCD): Transcranial Doppler ultrasonography will be used to measure blood velocity in intracranial cerebral arteries (i.e., middle/posterior arteries). An ultrasound probe will be placed in the area above the cheekbone and held in place via an adjustable head piece. Rest and exercising blood velocities will be measured, along with functional responses to standardised cerebrovascular responsiveness testing (e.g., cognitive testing).

Carotid (CCA) and Internal Carotid Artery (ICA) and Vertebral Artery (VA) blood flow: Doppler ultrasonography (uSmart 3300, Terason) will be used to assess changes in flow within larger conduit arteries supplying the brain. Duplex Doppler is a non-invasive ultrasound technique that measures the diameter of the carotid and internal carotid arteries as well as flow velocity measures. Thus, providing an index of changes in blood flow to the brain.

Flow Mediated Dilation (brachial artery): Endothelial function of the target artery will be assessed using the flow-mediated dilatation (FMD) technique (Thijssen et al. 2011). Briefly, a blood pressure cuff is placed around the forearm/calf of the participants, and inflated to 50 mmHg above systolic pressure for 5 minutes to obstruct blood flow, followed by a rapid deflation allowing reactive hyperaemia to occur. Brachial artery blood flow velocity and diameter will be non-invasively monitored prior, during and post cuff inflation/deflation using Doppler ultrasonography (uSmart 3300, Terason) interfaced with industry-leading Quipu FMD analysis software.

Cognitive assessments: Executive function accuracy and reaction time will be measured using three tasks: (1) Modified Attention Network Task[33] which measures response to cognitive load. Measures of accuracy and reaction time are taken for this task with participants typically responding more slowly and making more errors on un-cued, incongruent, high load trials; (2) Switch Task considers cognitive flexibility with participants responding to stimuli according to two different paradigm (or rule) shifts. Measures of accuracy and reaction time are taken for this task with participants typically responding more slowly and making more errors on the first trial following a change (switch) from one paradigm to the other; (3) Modified Stroop Task assesses selective attention and prepotent response inhibition during decision making. It involves the presentation of colour words (e.g., RED) displayed in either congruent (e.g., red) or incongruent (e.g., blue) colours, with the modified version of this task including an additional level of conflict at response selection. Measures of accuracy and reaction time will be taken. These tasks have all been previously shown by our group to be sensitive to blueberry flavonoids[34,42], and different versions of the tasks, matched for difficulty, will be pseudo-randomised in their presentation to participants across the test sessions and visit days.

Safety considerations

The named researchers on this project have extensive experience in designing, conducting, and participating in research that involves the aforementioned measurements and procedures. All the procedures included within this project are well established and widely used internationally, are established at the institute, and have been included in previous published works by the researchers. A Hazard and Risk assessment form was completed approved by the School Health and Safety Officer (Dr. James Watson) and the University of Birmingham STEM Ethics Committee prior to the commencement of the study (March 2023). Please see 'Ethics' section for ethical considerations and detail of risks associated with this project.

Follow-up

No follow-up will be provided to the research participants as there is no risk of adverse effects from blueberry interventions. The participants that consented will be contacted for the purposes of sharing the main study results.

Data management and statistical analysis

Participant recruitment: Participant recruitment will take place in the UK. The study will predominately be advertised via word-of-mouth, noticeboard advertisements (physical and electronic), lecture shout-outs or emails to various community groups in the Birmingham area. Potential participants expressing an interest in a study will be contacted by the study investigators. Some participants may have an existing relationship with one or more of the investigators; however, this will not be used to coerce participation in the study.

Data Management: Participant details and data will be kept strictly confidential. All paper records will be kept in a locked filing cabinet that only the named research personnel have access to. Computerized records of experimental data will be coded and will be maintained on a password secure system. Participants will be assigned a unique ID code under which their individual data will be recorded and stored. The research team will maintain a written record that links the identity of the participants to the assigned ID codes, which will be stored in a safe and secure location.

Data generated throughout the duration of a study will be managed in accordance with the University's Code of research Practice and the terms and conditions of the Data Protection Act 2018. Data will be collated and stored in a locked file cabinet and a password-protected electronic database and only those directly involved with the study will have access to the data. Following publication, data will be maintained in an easily understandable and accessible format so that it can be made available to academic researchers upon request. Data will be stored by the Principal Investigators of the study for a minimum of 10 years, in accordance with the University of Birmingham's data storage policy. If after this time the data is deemed to be of no further use to the academic world it will be permanently deleted.

Participant withdrawal: Participants will be informed of their right to withdraw from the study both verbally and in writing. Participants may withdraw without giving a reason at any time during the study and up to two weeks after their last laboratory visit. No attempts will be made by any member of the research team to dissuade any participant from withdrawing if they wish to do so. In addition to self-withdrawal, participants may be withdrawn from the study by the Principal Investigators if the health and safety of the participant is deemed to be at risk at any point throughout the duration of the study. There will be no consequence to any participant that withdraws from a study under this project. In the event of a withdrawal, data collected will be retained for analysis with consent from the participant at the time of withdrawal. If consent is not given, the participant will be removed from the study and their details and data permanently deleted/destroyed.

Statistical Methods: All outcome measures will be analysed by a 3-way repeated measures ANOVA, with 2-within subject factors: Time (Baseline; 1 h) and Diet (High/ Low-Anthocyanin Blueberry) and 1-between subject factor: Fitness (High/Low).

Quality assurance

The study followed all the guidelines for Good Clinical Practice. A Standard Operations Procedure was developed for quality control checks for preparations of blueberry interventions, and this has been revised and approved by University of Birmingham STEM Ethics Committee (March 2023).

Expected outcomes of the study

This project addresses the USHBC research focus of the impact of blueberry on cognitive health, applying this in the context of physical exercise and fitness. Although consumption of blueberries improves cognition and vascular function, this will be the first time that this knowledge is applied strategically during physical exercise to enhance exercise-related cerebrovascular and cognitive function. This is important because it is believed that acute responses to exercise can influence long-term benefits of physical activity, particularly in regards to cognitive resilience later in life. From a mechanistic point of view, this work will enable the physiological impact (e.g., cerebral blood flow/oxygenation) of blueberries to be characterized in the context of exercise, whilst establishing linkages between these changes and cognitive function. Ultimately, this work will open an avenue to use blueberries to enhance the benefits of physical activity. Therefore, one long-term anticipated outcome from this research is informing future dietary recommendations for intake of fruits/vegetables (i.e. 1 cup equalling 190 g fresh blueberries) to target periods of physical activity.

Secondly, this work will help identify groups of individuals most likely to benefit from blueberry intake, by quantifying and comparing the benefits across high and low-fit individuals. In practical terms, this will translate into more specific recommendations for these different fitness grouping and will allow for more targeted marketing strategies.

Taken together, this work has strong relevance to the blueberry industry as it can target populations that are physically active and looking to maximize the health benefits of exercise. Pre-exercise nutritional products are a fast-growing market in the US, estimated at $12.6 billion (2019) and predicted to grow by 8.3%/year.

This work will also be highly relevant to the increasing sedentary portion of the population, which is also at higher risk of cardiovascular disease and dementia later in life. It is estimated that ~31% of the US population is physically inactive, so finding simple and practical ways to optimize the benefits of any physical activity that takes place will likely help improve future cognitive outcomes. For example, if the data from this work indicates that blueberry intake prior to low intensity exercise is beneficial, this could inform future recommendations of blueberries to be used as ‘a healthy snack prior to a walk/hike’.

Dissemination of results and publication policy

The project aims to produce a publication examining the effects of blueberry intake on cerebrovascular and cognitive responses to exercise in low and high-fit young adults. The publication will be a high quality and internationally recognised paper and will target a high impact factor peer-reviewed journals in the field of Clinical Nutrition (e.g., American Journal of Clinical Nutrition (IF): 6.77). Results will be disseminated to the community through public lectures and via press-release when the manuscript is published. In the publication, the PI, Dr. Catarina Rendeiro will be leader author, with Alexander Friend as first author and Dr. Sam Lucas, Dr. Claire Williams and Dr. Lynne Bell as co-authors. US Highbush Blueberry Council will be acknowledged.

Duration of the project

Overall study; start date: 01/08/2021, end date: 31/12/2024

Recruitment; start date: 15/06/2023, end date: 31/12/2024

Project management

Mr. Alexander Friend is conducting the research under the management of Dr. Catarina Rendeiro, Dr. Sam Lucas, and Prof. Claire Williams.

Ethics
A complete risk assessment was conducted as part of this project. Below is a summary of the range of ethical considerations relating to this project:

Exercise Testing: Performing moderate and vigorous exercise carries the following risks that the participant will be made aware of prior to enrolment through the Participant Information Sheet:

- Sensations of fatigue and physical exhaustion – this will be short lived and will subside shortly after exercise is ceased.
- Fainting – often related to physical exhaustion and sudden stopping of exercise, this risk will be mitigated by the inclusion of low intensity recovery exercise following all high intensity or exhaustive exercise bouts.
- Cardiovascular event (e.g. myocardial infarction or 'heart attack') – this is a small risk, particularly considering the mostly healthy and young cohorts to be included in this project. The risk will be further mitigated by full screening of participants and exclusion of those with a history of heart problems of cardiovascular disease. Participants aged 50 and over, will have a 12-lead electrocardiogram as part of the screening process. This will be assessed by a cardiologist before the participant can take part in an exercise-based study.

Nutraceutical Ingestion: Information sheets for this study will contain a section outlying allergies that will allow the research team to screen participants and see if they meet inclusion/exclusion criteria. During familiarisation participants will fill out a health questionnaire that will allow the research team to see if they are able to take the supplements. Although this should avoid any issues with allergic reactions, participants will be closely monitored during and immediately after taking the supplements. Nutritional-based studies will abide by the University of Birmingham's standard operating procedures related to Food and Nutritional components, which describes the procedures for sourcing, storage, preparation, and administration of food and/or nutritional components to participants.

Cuff inflation: The inflation of blood pressure cuffs to pressures greater than systolic blood pressure can result in a tingling sensation distal to the point of inflation, similar to 'pins and needles'. These symptoms will quickly subside in deflation of the cuff. Participants will be informed that they can have the cuff deflated prematurely in the eventuality that the discomfort becomes excessive.

Transcranial Doppler and Duplex Doppler are non-invasive and painless procedures with which to assess blood flow velocity within the brain and systemic vasculature. Best practice guidelines will be followed to minimise participant exposure to the ultrasound by stopping the ultrasound transmission and recording between each monitoring period.

NIRS: NIRS is a non-invasive and painless imaging technique with which to assess tissue haemodynamics and that are no known adverse effects. Equipment can be removed quickly by unclipping chin strap and removing the helmet (in case of emergency such as a fire alarm and evacuation).

If any tests show incidental findings related to the participants health (e.g. high blood pressure), the participant will be advised to visit their GP. In the event of such a finding, the PI will send a letter to the participant's GP outlining this finding (provided the participant gives their consent for this to occur)

Informed consent forms

Prospective participants will be provided with an information sheet outlining all the study details and will be given sufficient time (information provided at least 48 hours prior to the first visit) to consider this information before making their decision whether or not participate (please see file attachment). The study investigators will in no way attempt to pressure or coerce any individual into taking part. Upon arrival, participants will have the study explained to them in full and given the opportunity to ask any questions before being asked to sign the participant consent form and complete a health and background questionnaire (attached). Participants must be able and willing to comply with all research requirements but will be informed of their right to withdraw from the study at any point without giving a reason.