LEEDS BECKETT UNIVERSITY
CARNEGIE SCHOOL OF SPORT

HUMAN BEHAVIOURAL NEUROSCIENCE LAB

Pro-COG Trial Protocol

Long Title: A double-blind placebo-controlled exploratory trial to assess the impact of daily Lab4P probiotic supplementation on Cognitive Performance and Metabolic regulation in Overweight Young Adults with Impaired Glucose Regulation

Short Title: Probiotic impact on **Cog**nitive performance, and metabolic outcomes in overweight young adults with impaired glucose regulation, shortened to **Pro-COG**

LREC Reference: 152291
Clinicaltrials.gov Number: NCT07073781
Protocol Version: 1.4 23/09/2026

This protocol has regard for the HRA guidance

Leeds Beckett University

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

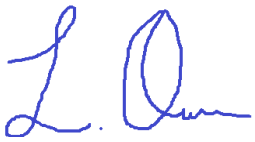
I also confirm that I will make the findings of the trial publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

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Position: Project Supervisor and Director of the Behavioural Neuroscience Lab, Leeds Beckett University

Date: 23/09/2026

Signature: 

Chief Investigator:

Date: 23/09/2026

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Signature: 

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Ii. LIST OF ABBREVIATIONS

AE	Adverse Event
AUC	Area Under the Curve
BA	Brachial Artery
BBB	Blood Brain Barrier
BG	Blood Glucose
BMI	Body Mass Index
CANTAB	Cambridge Neuropsychological Test Automated Battery
CFU	Colony Forming Units
CPM	Clinical Project Manager
CRA	Clinical Research Associate (synonym: trial monitor)
CRE	Clinical Radiation Expert
CRF	Case Report Form
CSS	Carnegie School of Sport
CV	Coefficient of Variation
CVR	Cerebrovascular reactivity
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDQF	Electronic Data Query Forms
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
eTMF	Electronic Trial Master File
FMD	Flow-Mediated Dilation
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
HbA1c	Glycated Haemoglobin A1c
HDL-C	High Density Lipoprotein Cholersterol
HRA	Health Research Authority
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
iDXA	Dual-energy X-ray Absorptiometry
IEC	Independent Ethics Committee
IED	Intra-Extra Dimensional Set Shift Task
IGM	Impaired Glucose Metabolism
IGT	Impaired Glucose Tolerance

IR	Insulin Resistance
IRMER	Ionising Radiation Medical Exposure Regulations
IL-6	interleukin-6
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intent-To-Treat
LBU	Leeds Beckett University
LDL-C	Low Density Lipoprotein Cholersterol
LSAF	Life Science Analytics Framework
MCA	Middle Cerebral Artery
MD	Medical Doctor
MOT	Motor Screening Task
MPE	Medical Physics Expert
OGTT	Oral Glucose Tolerance Test
PM	Project Manager
PAL	Paired Associates Learning Task
PI	Principal Investigator
PIS	Participant Information Sheet
PP	Per-protocol
PSQI	Pittsburgh Sleep Quality Index
REC	Research Ethics Committee
RNV	Recommended Nutrient Value
RTI	Five Choice Reaction Time task
RTSM	Randomisation Trial Supply Management
SAE	Serious Adverse Event
SOP	Standard Operating Procedures
SST	Stop Signal Task
SUSAR	Serious Unexpected Suspected Adverse Reaction
SWM	Spatial Working Memory Task
TFM	Total Fat Mass
TLM	Total Lean Mass
TMF	Trial Master File
TNF-α	Tumor necrosis factor-alpha
T2D	Type 2 Diabetes
UK	United Kingdom

iii. TRIAL SUMMARY

TRIAL TITLE	A double-blind placebo-controlled exploratory trial to assess the impact of Lab4P probiotic supplementation on Cognitive Performance and Metabolic

	regulation in Overweight Young Adults with Impaired Glucose Regulation
TRIAL N°	LBU-Lab4P-ProCogTrial-2025
TRIAL OBJECTIVES AND ENDPOINTS	**Primary objective** Evaluate changes in verbal recall memory task performance from baseline to 3 months in individuals with impaired glucose tolerance receiving Lab4P probiotic vs. placebo. **Co-primary endpoints** – Change in verbal episodic memory task accuracy from baseline to 3 months. **Secondary objectives** – To evaluate the effect of Lab4P supplementation on performance in cognitive tasks assessing memory, executive function and psychomotor ability. – To evaluate the effect of Lab4P supplementation on subjective sleep quality. – To evaluate the impact of Lab4P on cardiometabolic health, inflammatory cytokine levels, body composition, and gut microbiome composition. **Secondary endpoints** Change in the following measures from baseline to 3 months: – Performance score on 5 cognitive tasks. – Cardiometabolic markers. – Peripheral and central vascular function. – Inflammatory cytokines. – Markers of platelet activity. – Anthropometric measures. – DXA scan-assessed body composition. – Gut microbiota community composition, dispersion and abundance of individual microbial taxa. – Sleep quality scores. **Secondary Safety objectives** To assess the tolerability and safety profile of Lab4P over 3 months. **Secondary Safety endpoints** – Adherence to investigational product. – Frequency and severity of gastrointestinal side effects.
TRIAL DESIGN	Double-blind, placebo-controlled, randomized parallel-group design. Participants will be randomly assigned to receive either the investigational treatment or a placebo.
TRIAL POPULATION	Healthy adults between 18-40 with Impaired Glucose Tolerance, defined by a glucose area under the curve (AUC), of 290 mg h/dl during an oral glucose tolerance test (OGTT; Sakaguchi et al., 2015).
INCLUSION CRITERIA	Must provide informed consent, have a BMI of 25.0 kg/m² or higher, be in good general health, report regular sleep patterns, and be able to complete study procedures, including fasting and an oral glucose tolerance test (OGTT).
EXCLUSION CRITERIA	Exclusion applies to those with diabetes, diagnosed sleep disorders, fasting glucose >6.9 mmol/L, bariatric surgery, recent major surgery or illness,

	pregnancy, or recent night shift work, taking medications that may interfere with probiotic activity or glucose metabolism at time of screening.
INVESTIGATIONAL PRODUCT	**Test product**: Lab4P probiotic consortium, contains a blend of the following probiotic strains; Lactobacillus acidophilus CUL60 (NCIMB 30157), Lactobacillus acidophilus CUL21 (NCIMB 30156), Lactobacillus plantarum CUL66 (NCIMB 30280), Bifidobacterium bifidum CUL20 (NCIMB 30153), and Bifidobacterium animalis subsp. lactis CUL34 (NCIMB 30172). The consortium will be delivered on a microcrystalline cellulose base at a total concentration of 5×10^{10} colony-forming units (CFU) per capsule. The intervention also contains Vitamin C, Vitamin D, and Zinc. **Control product**: Placebo capsules composed of microcrystalline cellulose, identical in appearance to the test product.
MODE OF ADMINISTRATION	Participants will take one capsule orally per day at any time of day. Capsules should be swallowed whole and not opened or chewed. Participants will be encouraged to take the capsule at the same time each day to support consistency.
TREATMENT DURATION	Participants will receive either Lab4P or placebo for a total of 12 weeks (84 days). There is no run-in or washout period. The treatment period concludes with a follow-up visit at the end of week 12.
ADMINISTRATION SCHEME	Capsules will be dispensed in sealed, pre-labelled containers at the baseline visit. Unused capsules will be returned at the week 12 follow-up visit to assess compliance via capsule count. Participants will be instructed not to double up on missed doses.
STATISTICAL METHODS	**Sample size:** Based on prior probiotic studies reporting effect sizes of 0.60–0.67 for change in verbal memory score, a conservative average effect size of 0.63 was used. Power analysis (G*Power v3.1.9.7) with one-tailed $\alpha = 0.05$ and 80% power yielded a required sample size of 64 participants (32 per group). To allow for an estimated 10% attrition rate, up to 70 participants will be enrolled. **Endpoint Analysis:** The primary analysis will evaluate the effect of Lab4P probiotic supplementation versus placebo on verbal memory performance, measured by accuracy on the VRM task, from baseline to Week 12. A linear mixed-effects model will assess change over time, with fixed effects for time, treatment group, and their interaction, and a random intercept for participants. Secondary analyses, using data collected at three timepoints (baseline, Week 6, and Week 12), will be conducted using the same approach. For secondary outcomes measured only at baseline and 12 weeks, between-group differences in change will be assessed at both 6- and 12-week timepoints using delta scores, adjusting for baseline values. These analyses are exploratory and hypothesis-generating; no formal statistical adjustments will be made for multiple testing. Analyses will follow an intention-to-treat approach, with sensitivity analyses for protocol adherence and missing data. No adjustment for multiple comparisons

	is required as there is a single primary outcome.

iv. FUNDING AND SUPPORT IN KIND

FUNDER	FINANCIAL AND NON FINANCIALSUPPORT GIVEN
Cultech Limited Unit 2 Christchurch Road, Baglan Industrial Estate, Port Talbot, W Glam, SA12 7BZ	**Cash:** £ 9,097.32 **Staff time:** £ 5,000 **Materials provided:** £ 10,000
BBSRC i-NutriLife Hub Biotechnology and Biological Sciences Research Council (BBSRC), part of UKRI i-NutriLife Hub PoC_R3_012	**Cash:** £24,097.32

V. ROLE OF TRIAL SPONSOR AND FUNDER

Sponsor:

Leeds Beckett University will act as the Sponsor for this study. The Sponsor holds overall responsibility for the initiation, management, and financing (or arrangement of financing) of the research. This includes ensuring that appropriate systems are in place for the management, monitoring, and reporting of the study. While the Sponsor may delegate specific duties to individuals or organisations as appropriate, it retains overall accountability.

Funder:

Cultech LTD is providing financial support for the study and will contribute expert input specifically related to the handling of the probiotic intervention, as well as the design, logistics, and analysis of gut microbiome outcomes. They will have no involvement in the overall study design, data collection (outside of microbiome analysis), outcome measurement, interpretation of findings, or dissemination. The academic research team will lead all other aspects of the study independently and will comply fully with the funder's Terms and Conditions.

The BBSRC i-NutriLife grant supports academic research infrastructure and activities. Funding includes directly incurred costs (staff time, travel/subsistence, consumables, other non-equipment costs), as well as directly allocated contributions for project team members. The BBSRC has no involvement in the study's design, conduct, analysis, or dissemination.

Vi. PROTOCOL CONTRIBUTORS

This protocol was developed as part of a doctoral research programme and reflects input from a multidisciplinary team with specialist expertise in clinical trial design, cognitive neuroscience, cardiovascular physiology, and microbiome science. The study design has been shaped by academic supervisors with internationally recognised expertise in human intervention research,

particularly in the neurocognitive and physiological effects of dietary and bioactive compounds. Contributions from an industry collaborator have supported the development and implementation of the probiotic intervention, microbiome analyses and randomisation process.

Lewis Hepburn - *PhD student, Principal Investigator*

Lead author of the protocol and leader of the overall design of this trial. Responsible for the development and coordination of the study, drafting of all protocol sections, and integration of feedback from collaborators.

Professor Lauren Owen - *Primary Academic Supervisor*

Provided strategic oversight the entire trial design and development process. Professor Owen's contributions included critical expert input on the formulation of research questions, study methodology, participant recruitment strategy, and outcome selection. Professor Owen also ensured that the protocol meets both academic standards and ethical requirements, and her supervision has ensured the scientific robustness and feasibility of the project.

Dr Steve Trangmar: - *Secondary Academic Supervisor*

Reviewed the protocol and contributed to the conceptual and practical aspects of trial planning alongside the primary academic supervisor. Dr Trangmar provided expert input on the vascular and haemodynamic components of the trial, including the selection of relevant outcome measures and data acquisition protocols.

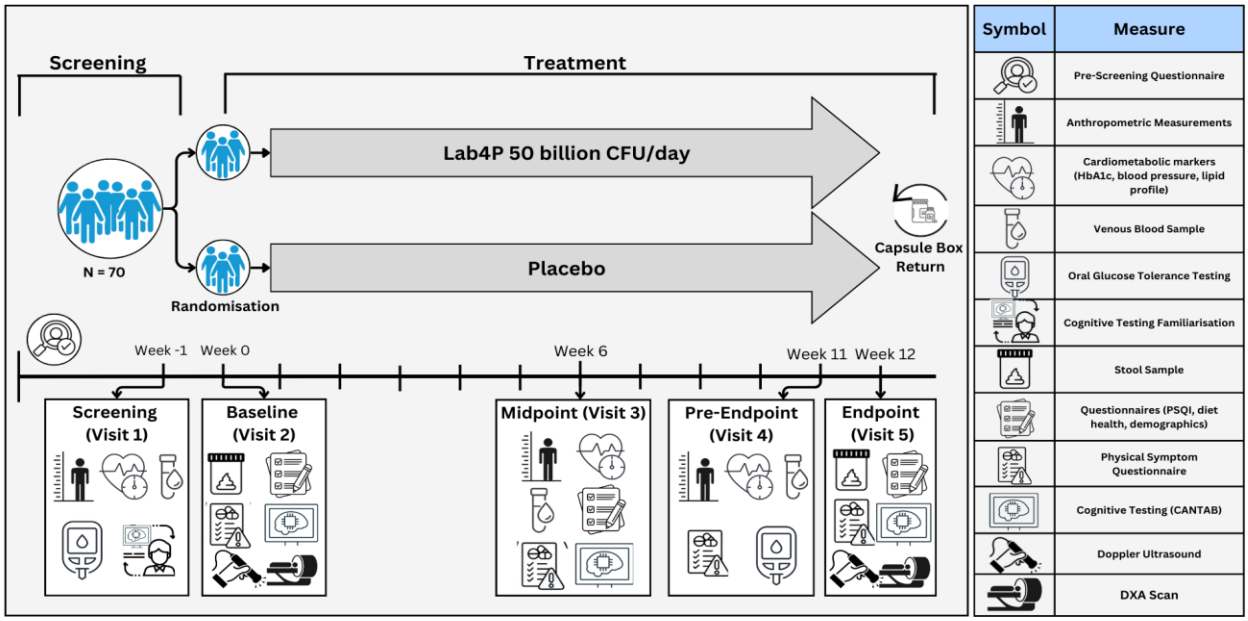
Dr Daryn Michael: - *Industry Collaborator Primary Contact, Senior Research Manager*

Contributed specialist input on the interventional product and placebo, including formulation, supply logistics, and safety profile. Also advised on the design and analytical approach to gut microbiome sampling and data analysis. As the industry partner will be responsible for managing randomisation, Dr Michael provided guidance on the randomisation strategy to ensure the process is operationally feasible, blinded as appropriate and compliant with trial integrity standards.

viii. KEY WORDS:

Probiotic supplementation
Psychobiotic
Cognitive performance
Memory improvement
Metabolic function
Impaired glucose tolerance

Ix. DIAGRAMMATIC SCHEDULE OF VISITS AND ASSESSMENTS



1. BACKGROUND

An estimated 2.11 billion adults are affected by overweight and obesity (Ng et al., 2025). The metabolic health consequences linked to excess body weight are well established. Overweight and obesity are consistently associated with a higher prevalence of impaired glucose metabolism (Power & Thomas, 2011). Disruptions in glucose regulation are both characteristic of and contributory to the pathogenesis of metabolic disorders, including type 2 diabetes (T2D), metabolic syndrome. Clinical perturbations in glucose homeostasis are strongly associated with sleep disturbances, cognitive impairments, and an increased risk of neurodegenerative diseases (Marseglia et al., 2019; Procaccini et al., 2016). Increasing evidence suggests that even slightly impaired glucose metabolism (IGM) may contribute to cognitive decline (Kirvalidze et al., 2022). Under normal physiological conditions, glucose serves as the primary energy substrate for nearly all brain cells, particularly neurons, which have a high demand for continuous energy supply, but limited capacity for fuel storage (Faria-Pereira & Morais, 2022; Ritter, 2017). The regulation of brain glucose metabolism is tightly controlled by the neurovascular unit (NVU), a coordinated system comprising neurons, astrocytes, endothelial cells, pericytes, and microglia (Takahashi, 2020). Neuronal activation prompts the NVU to increase cerebral blood flow (CBF) through coordinated vasodilation, ensuring adequate energy supply (Kaplan et al., 2020; Takahashi, 2022). Thus, the NVU solves the brains paradoxical need for sustained energy supply despite low storage capacity by regulating the precise delivery of glucose, and other metabolic substrates.

Cerebral glucose metabolism changes throughout the lifespan, alongside brain development. In healthy individuals, cognitive ability undergoes rapid development throughout childhood, reaches peak efficiency in early adulthood, and declines gradually across most domains from mid to late adulthood (Nichols et al., 2021). Central and peripheral glucose metabolism follows a similar trajectory; with cerebral metabolic rate and overall glucose requirements peaking in early

childhood to support development, gradually declining throughout childhood and adolescence, before stabilizing in late adolescence (Iozzo & Guzzardi, 2019). This stability persists until middle age, when metabolism begins to deteriorate (Assuncao et al., 2018; Tan et al., 2011). Given the brain's reliance on glucose for energy, it is unsurprising that midlife represents a critical period in brain aging, demonstrating a slow decline in cognition and neurostructural alterations (Aalst et al., 2021; Carmichael et al., 2019). Older adults with prediabetes have been shown to exhibit significantly reduced memory retention and executive functioning compared to normoglycemic individuals (Hoyos et al., 2022). IGM in older adults is associated with deficits in global cognition, executive function, processing speed, and working memory (Lamport et al., 2009; Mortby et al., 2013). In addition to reductions in cognitive performance, glycaemic dysregulation is also associated with alterations in both structural and functional brain integrity. For example, neuroimaging studies in adults above the age of 50 have associated IGM with reductions in hippocampal and temporal lobe volume, increased neurovascular burden, and decreased total brain volume (Jang et al., 2024; Oh et al., 2021). Some research has suggested that the hippocampus, associated with spatial awareness, and both long- and short-term memory consolidation is particularly susceptible to glycaemic dysregulation in older adults (Sevim et al., 2024; Soleymani et al., 2024; Zhong et al., 2020). Thus, the brain appears to exhibit region-specific structural and functional vulnerability in response to declining metabolic homeostasis, changes that become increasingly pronounced by midlife.

While most evidenced in mid to older adulthood, neurocognitive deficits are also observed alongside IGM in adults below the age of 40 (Lamport et al., 2009; Soleymani et al., 2024). The prevalence of metabolic dysregulation in this age group has risen markedly in recent decades and is projected to continue its upward trajectory (Chong et al., 2023; Rooney et al., 2023). Indeed, epidemiological data indicate that markers of IGM may be detectable in as high as 24% of individuals aged 18 to 37 (Andes et al., 2019; Madeira et al., 2013; Magliano & Boyko, 2021). Research is increasingly demonstrating that dysregulation impacts cognition across multiple domains. Elevated fasting BGL, and HbA1c in young, overweight and obese individuals have been associated with lower working memory, reading ability, and executive function (Hawkins et al., 2016; Hawkins et al., 2021). Longitudinal evidence reinforces this pattern, with higher HbA1c in non-diabetic younger men associated with poorer processing speed and both immediate and delayed recall ability (Anstey et al., 2015). Similarly, higher-normal fasting BGL appears to predict impaired inhibitory control, spatial perception, and selective attention (Gluck et al., 2013; Hawkins et al., 2016; Razzak et al., 2018). In addition to static measures of IGM, dynamic indices of glycaemic regulation, such as Oral Glucose Tolerance Testing (OGTT) are also associated with cognition in young adults. For example, IGM assessed via postprandial glucose handling has been associated with poorer verbal memory performance, and impaired behavioral flexibility, a subdomain of executive function in young, nondiabetic adults (Messier et al., 2011; Riby et al., 2017). Alongside direct measures of glucose, subclinical levels of IR in young adults are associated with lower visuomotor ability and reductions in both working and episodic memory performance (Abbasi et al., 2023; Larsen et al., 2023). Amid the ongoing global obesity epidemic, early manifestations of metabolic dysregulation, once primarily observed in midlife, are increasingly prevalent in early adulthood. This indicates a possible downward trend in the age at which cognitive vulnerability emerges, raising concern that cognitive decline may now begin earlier in the lifespan than historically observed.

Few neuroimaging studies have focused on the consequences of IGM in early adulthood.

However, the limited research available suggests that the brain regions affected in this demographic resemble those observed in diabetes, though typically to a lesser degree. For instance, studies including young to midlife adults have reported age-independent associations between IGM and moderate structural and functional alterations in both grey and white matter, particularly in brain regions involved in visual processing, memory, and semantic integration (Grundmann et al., 2020; Shan et al., 2019; Weinstein et al., 2015). In studies involving exclusively young adults aged 22-35, Diffusion Tensor Imaging has revealed an association between elevated, within-normal HbA1c levels, and decreased markers of white matter integrity in areas involved in executive function, attention, motor coordination, and emotional regulation (Repple et al., 2019; Ribeiro et al., 2023). Indeed, Repple and colleagues (2019) found these decrements to mediate a relationship between HbA1c and performance in global cognition, working memory, and fluid intelligence tasks. These microstructural alterations in white matter may underlie functional connectivity weaknesses observed in similar demographics. In prediabetic adults aged 22-35, reduced functional connectivity in white-matter associated networks supporting self-control and attentional regulation have been observed (Sadler et al., 2019; Santillo et al., 2024; Shearrer, 2023). White matter is particularly susceptible to cerebrovascular damage, owing to its cellular metabolic demand and relatively limited vascular supply (Levit et al., 2020). As functional connectivity depends on the structural integrity of white matter tracts, and is independently sensitive to NVU function, disruptions in both structure and function are likely manifestations of early NVU impairment (Guilbert et al., 2022; Nozais et al., 2023). Collectively, these findings support the hypothesis that the cognitive impairments observed in young adults with IGM are, at least in part, attributable to NVU dysfunction.

One proposed pathway by which IGM disrupts NVU function involves endothelial dysfunction at the blood-brain barrier (BBB; Lamport et al., 2009). Exaggerated glucose excursions, even within the subclinical IGM range, promotes excessive glucose uptake into endothelial cells, activating biochemical pathways that increase the generation of reactive oxygen species (ROS) and promote the formation of advanced glycation end products (AGEs; Di Pino et al., 2019; Korkmaz et al., 2013). Both ROS and AGEs contribute to oxidative stress and endothelial damage through mitochondrial dysfunction, redox imbalance and pro-apoptic signaling (An et al., 2023; Clyne, 2021). Further, this oxidative stress elicits a pro-inflammatory cellular response that contributes to endothelial damage, and independently disrupts neurophysiological processes (Collier et al., 2008; De Carvalho Vidigal et al., 2013; Theofilis et al., 2021). In addition to contributing to hyperglycaemia, IR further impairs BBB endothelial integrity and selective permeability (Cui et al., 2022; Kullmann et al., 2016). At the BBB, endothelial dysfunction compromises vasoactive signaling and barrier homeostasis, disrupting the NVU's capacity to regulate CBF in response to neuronal activity (Feng & Gao, 2024; Toth et al., 2017). It has also been suggested that both IR and chronic hyperglycaemia may impair the expression and function of glucose transporter proteins at the BBB, further disrupting the brain's energy supply (Blázquez et al., 2014; Leão et al., 2020). Together, these processes may compromise the ability of the NVU to maintain cerebral homeostasis, leading to subtle but functionally meaningful disruptions in brain perfusion and energy metabolism, both of which are essential for supporting cognition.

While metabolic dysfunction has most consistently been linked to impaired cerebrovascular function in T2D cohorts, importantly, similar impairments have been observed in individuals at earlier stages of dysregulation (Hardigan et al., 2016; Vidyashree et al., 2022). For instance,

reduced internal carotid artery reactivity has been reported in mid-life adults with IGT compared to normoglycemic controls (Selvarajah et al., 2016). Peripheral endothelial dysfunction, a proxy measure for central vascular health, has been reported in individuals with IGT during both early and midlife stages (Salmon et al., 2024; Smirnova et al., 2013; Su et al., 2008). Indeed, NVU dysfunction has been observed directly in young adults with IGM. Under typical conditions, CBF and regional cerebral glucose metabolism (CMRGlc) are tightly coupled, whereby a reduction in one prompts an increase in the other to maintain cerebral metabolic homeostasis (Paulson et al., 2010). However, Deery et al. (2024b) reported altered coupling between CBF and CMRGlc in young, insulin-resistant adults without overt diabetes. In a similar population, lower rates of regional cerebral glucose metabolism were observed in prefrontal, parietal and temporal regions, and was associated with lower working memory, slower reaction time and psychomotor speed (Deery et al., 2024a). These findings suggest that early cerebrovascular alterations in IGM may disrupt the NVU's capacity to regulate and utilise glucose efficiently, with IR potentially contributing to these impairments. Thus, current evidence suggests that metabolically mediated brain microvascular dysfunction may present in young and middle-aged adults prior to the onset of overt metabolic pathology.

The observation of a detectable decoupling between CBF and brain glucose metabolism in young adults with poor peripheral glucose regulation aligns with evidence that insulin administration significantly increases brain glucose metabolism in individuals with IGT, but not in healthy controls (Hirvonen et al., 2011). This indicates that cerebral insulin action is not saturated at fasting levels in those with IGT, potentially limiting glucose availability and insulin-mediated neuromodulatory processes, thereby contributing to cognitive impairment (Malin et al., 2022). Further evidence of altered cerebral glucose autoregulation in young adults with IGM comes from glucose administration studies, wherein cognitive performance is enhanced to a greater degree than normoglycaemic peers (Meikle et al., 2004; Owen et al., 2013). In these individuals, acute increases in glucose availability following administration likely elevate extracellular glucose concentrations in the brain, temporarily restoring cerebral energy supply, compensating for deficits in glucose transport and improving cognitive function (Duarte, 2015; Gold, 2005). Memory is the cognitive domain most consistently enhanced by glucose administration in young adults, possibly due to its reliance on medial temporal lobe regions that are especially sensitive to changes in glucose availability (Smith et al., 2011). This sensitivity is attributed to the high density of both insulin receptors and glucose transporters in these areas (Koepsell, 2020; Pomytkin et al., 2018). Overall, this evidence suggests that even in the early stages of glycaemic dysregulation, impairments in vascular-metabolic coupling are evident and may offer a mechanism underlying the cognitive impairment associated with IGM.

Whether interventions targeting metabolic health can mitigate observed cognitive deficits is a question of clear importance. Current therapeutic strategies for metabolic dysfunction primarily involve either lifestyle modification or pharmacological treatment (Blüher et al., 2023). Growing interest in the gut-brain axis and its role in neurocognitive processes has led to exploration of probiotics as a potential alternative or adjunctive strategy to improve cognition. Systematic reviews and meta-analyses indicate that probiotics may offer cognitive benefits (Irwin et al., 2020; Marx et al., 2020). Probiotic supplementation has been associated with improved mental flexibility in healthy older adults and to significant gains in immediate memory, delayed recall, and visuospatial abilities in those with mild cognitive impairment (Kim et al., 2020; Xiao et al., 2020). Additionally, working memory and verbal learning improvements have been observed in

young adults under stress, following supplementation (Lew et al., 2019; Oluwagbemigun et al., 2022). While the underlying mechanisms of these cognitive effects remain to be fully elucidated, some evidence suggests that they may be mediated, at least in part, by probiotic-induced modulation of metabolic function. Several meta-analyses of randomised controlled trials have reported that probiotic supplementation significantly improves glycaemic control in both clinical and sub-clinical populations (Nikbakht et al., 2016; Ruan et al., 2015). These effects are thought to arise from multiple mechanisms, including modulation of the gut-microbiome, strengthening of intestinal and immune barriers, reduction of IR and oxidative stress, and regulation of metabolic enzyme activity (Shen et al., 2024). Taken together, these findings suggest that probiotics may exert cognitive effects through their capacity to restore glucose-specific aspects of metabolic function.

Typically, research examining the effects of probiotics on both glucose metabolism and cognition has focused on the genera Lactobacillus and Bifidobacterium (McCarthy & Martirosyan, 2025; Pintarič & Langerholc, 2022). Assessing the therapeutic efficacy of probiotics is challenging however, due to strain and dose-specific variations in effects (Torres-Maravilla et al., 2022). Among these, Lab4P, a probiotic consortium consisting of five Bifidobacterium and Lactobacillus bacterial strains, has been investigated for its potential to exert favourable metabolic effects. Randomized controlled trials have found Lab4P supplementation in overweight and obese adults significantly reduces body weight, waist circumference, and hip circumference compared to placebo (Michael et al., 2020; Michael et al., 2021). The first of these studies also reported improvements in participants' overall wellness scores. Animal studies further support this, with rats given Lab4 probiotics showing lower systemic inflammation and improved lipid profiles, potentially via altered gut bile acid metabolism (Joyce et al., 2014; Webberley et al., 2021). Moreover, probiotic strains belonging to the same genera as those comprising Lab4P have been shown to improve glycaemic regulation in prediabetic adults by enhancing lipid metabolism, reducing inflammation, alleviating oxidative stress and stimulating the secretion of GLP-1 in the body (Abolbaghaei et al., 2018; Li et al., 2022). Given the established links between metabolic dysfunction, inflammation, and cognitive decline, these findings suggest that Lab4P may offer a strategy not only for improving metabolic health but also for supporting cognitive function.

Indeed, preclinical studies suggest that Lab4P may exert direct neuroprotective effects. In animal models, Lab4P supplementation preserved hippocampal dendritic spine density, and prevented learning and memory deficits induced by a high-fat diet, indicating protection against synaptic degeneration linked to metabolic dysfunction (Webberley et al., 2021). These structural benefits were accompanied by suppressed expression of pro-inflammatory cytokines, and preservation of anti-inflammatory signalling, within the hippocampus. Further, in vitro, Lab4P-derived metabolites protected human cholinergic neuronal cells from β-amyloid–induced cytotoxicity and significantly reduced IL-6 expression (Webberley et al., 2021). These findings suggest that Lab4P may mitigate cognitive decline by preserving synaptic integrity and dampening neuroinflammation in metabolically vulnerable states. The precise mechanisms underlying these effects remain to be fully elucidated, however, Lab4P harbours genes involved in the production of short-chain fatty acids (SCFAs), which are known to modulate systemic inflammation and may exert anti-inflammatory effects within the brain (Li et al., 2018; Vinolo et al., 2011). Further, microbiota-derived SCFAs are thought to mitigate hyperglycaemia-mediated oxidative stress and inflammatory response and appear to preserve the integrity of

cerebrovascular endothelial cells in the presence of ROS and AGEs (Huang et al., 2017; Kassan et al., 2023; Yan et al., 2022). This is particularly relevant in the context of IGM, where such mechanisms are associated with impaired NVU coupling and cognitive decline. Together, the evidence suggests that Lab4P may exert protective effects on cerebral function in metabolically vulnerable states by modulating key pathways implicated in cognitive impairment. While direct evidence of Lab4P's effects on cognition in humans is limited, its demonstrated ability to reduce systemic inflammation and improve metabolic parameters indicates potential relevance.

2. RATIONALE

IGM is a precursor to T2DM and other metabolic diseases. Increasing evidence also links IGM to early cognitive decline, likely mediated by insulin resistance, chronic inflammation, and endothelial dysfunction. Young adults with IGM represent a critical intervention group, as early metabolic dysregulation significantly increases the risk of progression to more severe cardiometabolic and neurological disorders. Intervening at this early stage may offer a valuable opportunity to prevent or even reverse these effects before they become irreversible.

Current therapeutic strategies for metabolic dysfunction primarily involve either lifestyle modification or pharmacological treatment (Blüher et al., 2023). These strategies have found concurrent enhancements in glycaemic regulation and cognitive performance (Fontana et al., 2021; Komleva et al., 2021). Lifestyle interventions, such as dietary modification and increased physical activity, can be effective; however, long-term adherence remains a significant challenge (Perone et al., 2024). Pharmacological interventions that improve glucose metabolism parameters have also been associated with improving weight management, glycaemic control, and, in some cases, cognitive function (Hui et al., 2025; Vosoughi et al., 2022). For example, a recent study examining liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist that enhances insulin secretion and improves glycaemic control, also improved associative learning in young obese individuals (Hanssen et al., 2023). Nonetheless, the broader use of GLP-1 receptor agonist, and similar medication is constrained by high cost, potential adverse effects, and the frequent recurrence of symptoms upon treatment discontinuation (Sikirica et al., 2017). These limitations highlight the need for alternative approaches that are effective, better tolerated, and more sustainable for long-term management of metabolic dysfunction.

Probiotics offer a promising alternative or adjunctive approach. They are generally well tolerated, with fewer side effects than pharmacological agents, and may promote lasting metabolic improvements through modulation of the gut microbiome (Torres et al., 2024). The Lab4P probiotic consortium has shown encouraging results in improving metabolic markers and reducing inflammation in overweight adults, and preclinical studies suggest additional neuroprotective effects. However, its potential to improve cognitive function in humans with IGM has not yet been evaluated. Clinical research is needed to establish whether Lab4P can mitigate the cognitive and behavioural impairments associated with metabolic dysfunction.

This trial will evaluate whether Lab4P supplementation, compared to placebo, can enhance cognitive performance, and metabolic health in overweight young adults with IGM, aiming to establish it as a potential non-pharmacological strategy to mitigate long-term neurocognitive and metabolic decline. The inclusion of a placebo control is scientifically and ethically justified in this context. Given the absence of an established probiotic intervention for IGM, a placebo provides

an appropriate comparator, enabling a rigorous evaluation of efficacy and reducing the risk of bias due to expectancy effects or non-specific changes.

2.1 Assessment and management of risk

This trial involves the use of the probiotic Lab4P or a matching placebo. The safety profile of Lab4P is well established, with its constituent strains used in commercially available products. The anticipated risks are minimal and consistent with those seen in everyday probiotic use. No procedures in this study exceed the level of standard care. Stool and blood sample collection, DEXA scans, and blood pressure measurements are routine clinical and research assessments and will be performed by trained professionals using approved SOPs. Participants will be fully informed of potential side effects and provided with access to the research team should concerns arise.

The table below outlines the potential risks associated with each intervention component and the corresponding risk mitigation strategies:

Intervention	Potential Risk	Risk Management
Oral dose of Lab4P probiotic	Allergic reaction	Individuals with a known allergy to probiotics or any component of the supplement will be excluded from participation. Participants will also be monitored for any adverse events throughout the trial.
Oral dose of Lab4P probiotic	Gastrointestinal symptoms	Participants will be informed of the possibility of temporary gastrointestinal symptoms. They will have access to the PI to report symptoms or seek advice at any time. Ongoing symptoms will be monitored and managed according to clinical guidelines.
Oral dose of placebo containing microcrystalline cellulose	Allergic reaction	Microcrystalline cellulose is considered to have a very low allergenic potential. Nonetheless, participants will be informed of this minimal risk. Any participant with a known sensitivity to excipients will be excluded.

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Baseline is defined as the assessments conducted prior to randomisation (Weeks –1 and 0). Follow-up assessments will take place at Weeks 6, 11, and 12 following randomisation.

3.1 Primary objective

The primary objective of this trial is to evaluate the effect of once-daily Lab4P probiotic supplementation (5×10^{10} CFU) versus placebo on memory performance over a 12-week intervention period in overweight adults with IGT.

3.2 Secondary objectives

To evaluate the effect of Lab4P versus placebo on the following secondary outcomes in overweight adults with IGT:

- Performance across five computerized cognitive tasks measuring memory, executive function, and psychomotor speed
- Cardiometabolic function
- Peripheral and central vascular function
- Inflammatory status
- Anthropometry
- Body composition
- Fecal microbiota community composition, dispersion and abundance of individual microbial taxa
- Subjective sleep quality

3.2.1 Secondary Safety Objectives

To assess the tolerability and safety profile of Lab4P over 3 months.

3.3 Primary endpoint/outcome

The primary endpoint is the change from baseline to week 12 in mean verbal memory performance, assessed by accuracy on the Cambridge Neuropsychological Test Automated Battery (CANTAB) verbal recognition memory (VRM) task.

3.4 Secondary endpoints/outcomes

3.4.1 Cognitive Performance Endpoints

Changes from baseline to Week 6 and Week 12 in performance on the following computerized CANTAB cognitive tasks:

- Paired Associates Learning (PAL) task, assessing visual episodic memory and associative learning.
- Spatial Working Memory (SWM) task, assessing visuospatial working memory and strategy use.
- Stop Signal Task (SST), assessing response inhibition and impulse control.
- Intra-Extra Dimensional Set Shift (IED) task, assessing cognitive flexibility and attentional set-shifting.
- Five Choice Reaction Time (RTI) task, assessing processing speed, psychomotor response, and attention.

3.4.2 Sleep Quality Endpoints

Change from baseline to Week 6 and Week 12 in subjective sleep quality, assessed using Pittsburgh Sleep Quality Index (PSQI) component scores. PSQI components include:

- Global Sleep Quality Score
- Sleep Latency
- Sleep Duration
- Habitual Sleep Efficiency
- Sleep Disturbances
- Use of Sleep Medication
- Daytime Dysfunction

3.4.3 Cardiometabolic Endpoints

Change from baseline to Week 12 in:

- Capillary blood glucose (BG) area under the curve (AUC) during a 2-hour OGTT.
- HbA1c
- Fasting BG Level
- Systolic blood pressure
- Diastolic blood pressure
- Total cholesterol
- LDL-C
- HDL-C
- Triglycerides

3.4.4 Peripheral and Central Vascular Endpoints

Change from baseline to Week 12 in:

- Cerebrovascular reactivity (CVR) of the middle cerebral artery
- Flow-mediated dilation (FMD) of the brachial artery

3.4.5 Inflammatory Endpoints

Change from baseline to Week 12 in plasma concentrations of inflammatory cytokines, including:

- Interleukin-6 (IL-6)
- Tumor necrosis factor-alpha (TNF-α)

3.4.6 Platelet Activity Endpoints

Change from baseline to Week 12 in circulating markers of platelet activity, assessed using validated laboratory methods.

3.4.7 Anthropometric Endpoints

Change from baseline to week 12 in:

- Body Mass Index (BMI)
- Waist-to-hip ratio.

3.4.8 Body Composition Endpoints

Change from baseline to week 12 in:

- Total lean mass (TLM)

- Total fat mass (TFM)
- Percent body fat.

3.4.9. Gut Microbiome Diversity Endpoints

Change from baseline to week 12 in:

- Gut microbiota community composition and diversity
- Gut microbiota community dispersion
- The relative abundance of individual microbial taxa

3.4.10 Secondary Safety Endpoints

- Proportion of participants with ≥80% adherence to Lab4P, as determined by pill count at the Week 12 visit.
- Number and proportion of participants reporting gastrointestinal (GI)-related adverse events (AEs), including but not limited to nausea, diarrhea, bloating, constipation, and abdominal discomfort, as captured through a recall diary.

3.5 Table of endpoints/outcomes

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure
	Primary Objective	
To evaluate the effect of once-daily Lab4P probiotic supplementation (5×10^{10} CFU) versus placebo on memory performance over a 12-week intervention period in overweight adults with IGT	Accuracy score on the CANTAB Verbal Recognition Memory (VRM) Task.	Week 0, week 6 and week 12.
	Secondary Objectives	
Evaluate the effect of Lab4P versus placebo on memory, executive function, and psychomotor speed performance.	Paired Associates Learning (PAL) task performance. Spatial Working Memory (SWM) task performance. Stop Signal Task (SST) performance. Intra-Extra Dimensional Set Shift (IED) task performance. Five Choice Reaction Time (RTI) task performance.	Week 0, week 6 and week 12.
Evaluate the effect of Lab4P versus placebo on Subjective sleep quality	The following measures of the PSQI: Global Sleep Quality Score Sleep Latency Sleep Duration Habitual Sleep Efficiency	Week 0, week 6 and week 12.

	Sleep Disturbances Use of Sleep Medication Daytime Dysfunction	
Evaluate the effect of Lab4P versus placebo on cardiometabolic function	Capillary BG AUC during a 2-hour oral glucose tolerance test (OGTT). HbA1c Fasting BP Level Systolic blood pressure Diastolic blood pressure Total cholesterol LDL-C HDL-C Triglycerides	Week –1, week 6 and week 12.
Evaluate the effect of Lab4P versus placebo on inflammatory status	Circulating plasma Interleukin-6 (IL-6) Circulating plasma Tumor necrosis factor-alpha (TNF-α)	Week -1 and week 12.
Evaluate the effect of Lab4P versus placebo on platelet activity	Circulating markers of platelet activity	Baseline, Week 6 and Week 12
Evaluate the effect of Lab4P versus placebo on anthropometry	BMI Waist-to-hip ratio.	Week –1, Week 6 and week 12.
Evaluate the effect of Lab4P versus placebo on body composition	Total lean mass (TLM) Total fat mass (TFM) Percent body fat.	Week 0 and week 12.
Evaluate the effect of Lab4P versus placebo on peripheral and central vascular function	Cerebrovascular reactivity (CVR) of the middle cerebral artery Flow-mediated dilation (FMD) of the brachial artery	Week 0 and week 12.
Evaluate the effect of Lab4P versus placebo on fecal microbiota community composition and community dispersion	The relative abundance of individual microbial taxa Gut microbiota community composition Gut microbiota community dispersion	Week 0 and week 12.
	Secondary Safety Objectives	
Assess the tolerability and safety profile of Lab4P over 3 months.	Proportion of participants with ≥80% adherence to Lab4P, as determined by pill count at the Week 12 visit. Number and proportion of participants reporting physical symptoms and GI-related AEs, including but not limited to nausea,	Weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12

	diarrhea, bloating, constipation, and abdominal discomfort, as captured through a weekly recall diary.

4. TRIAL DESIGN

This study is a 12-week, randomized, double-blind, placebo-controlled, two-arm, parallel-group, single-centre superiority trial. It is designed to evaluate the effect of once-daily Lab4P probiotic supplementation (5×10^{10} CFU) compared to placebo on cognitive performance and sleep quality in overweight adults with IGT.

Participants will be randomly assigned in a 1:1 ratio to receive either the probiotic or a matching placebo for the duration of the intervention. Study visits and assessments will occur at five time points: Screening (Week –1), Baseline (Week 0), Mid-Intervention (Week 6), Pre-Final Assessment (Week 11), and End-of-Intervention (Week 12). GI-related adverse events will be recorded weekly throughout the intervention using participant diaries. Outcome measures will be collected at each visit as appropriate, with primary endpoints assessed at Week 12. A summary of the visit schedule is provided in Section 4: Diagrammatic Schedule of Visits and Assessments.

5 TRIAL SETTING

This is a single-centre trial, and no additional sites are involved in recruitment, treatment, or follow-up. All experimental trial activities will be conducted at a single site: the Carnegie School of Sport at the Leeds Beckett University (LBU) Headingley Campus. The Carnegie School of Sport provides the necessary facilities and specialist staff required to run the intervention and data collection activities. All trial interventions will be administered by appropriately qualified personnel, meeting any professional eligibility criteria relevant to the intervention. Participants will be recruited from the local population, primarily through community and university channels, as the trial is not targeting a clinical patient population.

6 PARTICIPANT ELIGIBILITY CRITERIA

The study population will consist of adults aged 18 to 40 years with a body mass index (BMI) of 25.0 kg/m² or higher who self-report good general health. This population was selected to examine early signs of metabolic dysfunction in a relatively similar group and to support the applicability of the findings to other adults with a BMI in this range.

6.1 Inclusion criteria

Participants are eligible for enrolment in the study if **all** the following criteria are met:

- Provision of written informed consent prior to any study-related procedures.
- Aged 18 to 40 years, inclusive, at the time of consent.
- BMI above 25.0 kg/m².
- Self-reported good general health with no chronic medical conditions requiring ongoing treatment.
- Self-reported normal sleep patterns with no history of diagnosed sleep disorders.

- Willing and able to comply with study procedures, including fasting and undergoing the OGTT.
- Non-smoker at time of screening, with no regular tobacco use in the last 6 months.
- Proficient in English equivalent to a native speaker.

6.2 Exclusion criteria

Participants will be excluded from the study if any of the following criteria apply:

- Current or past diagnosis of any form of diabetes mellitus.
- Fasting plasma glucose ≤3.9 and ≥7.0 mmol/L at screening.
- History of bariatric surgery (e.g., gastric bypass, sleeve gastrectomy) or other gastrointestinal procedures that may influence glucose absorption or metabolism.
- Major surgery, significant trauma, systemic infection, or myocardial infarction within six weeks prior to screening.
- History of kidney or liver disease.
- History of Low/High Blood Pressure or heart disease
- History of any health conditions that would effect food metabolism including the following: food allergies, kidney disease, liver disease and/or gastrointestinal diseases (e.g. irritable bowel syndrome, peptic ulcers)?
- Current or recent (within 4 weeks) use of medications known to affect glucose metabolism or agents known to alter gut microbiota composition or probiotic efficacy (as defined in Section 8.6).
- History of psychiatric illness, including anxiety or depression.
- Current use of prescription or over-the-counter medications, excluding hormonal contraception.
- Participation in night shift work within 30 days prior to screening or planned night shift work at any time during the study.
- Pregnancy, breastfeeding, or planning to become pregnant during the study period.
- Known allergy or hypersensitivity to any component of the investigational product or placebo.

7 TRIAL PROCEDURES

All procedures and assessments outlined below are aligned with the study objectives defined in Section 3 and are scheduled as per the Schedule of Activities (Appendix A).

7.1 Recruitment

Participants will be recruited from the general population within the local community, including both university-affiliated and non-university-affiliated individuals. Recruitment will target adults aged 18 to 40 years who meet the eligibility criteria defined in Section 6. While the student body at LBU represents a key recruitment pool, the study is open to all eligible individuals within the local area.

Recruitment will be conducted using a combination of digital and in-person strategies. These will include:

- Email invitations circulated via university mailing lists

- Physical posters placed in high-traffic locations across university campuses and community venues
- Social media advertisements posted through institutional and local accounts
- In-class announcements (with prior approval from academic staff)
- Online postings on research participation websites and community bulletin boards

Recruitment materials requiring gatekeeper access (e.g., private social media groups) will only be shared after obtaining permission from the relevant gatekeepers. All recruitment materials will clearly communicate the study's purpose, voluntary nature, participant requirements, and the key inclusion and exclusion criteria. Contact information for the research team will be provided. No covert or deceptive recruitment methods will be used.
Where members of the research team hold teaching or supervisory responsibilities, recruitment will be conducted entirely outside any academic assessment context to mitigate perceived coercion. Recruitment will continue on a rolling basis until the required sample size is achieved, as outlined in Section 10.2.

7.1.1 Participant identification
Potential participants will be recruited through self-referral in response to study advertisements. Recruitment materials will direct interested individuals to a secure Qualtrics link, accessible via QR code or by contacting the PI, where they can view the Participant Information Sheet (PIS) and begin the pre-screening process.

Pre-screening will be conducted using an approved study pre-screening procedure and may involve an online questionnaire, telephone assessment with a member of the research team, or a combination of these methods. The pre-screening process will assess relevant eligibility criteria, including age, general and medical history, pregnancy status, height and weight for BMI assessment, sleep and lifestyle factors, medication and supplement use, and willingness to comply with study requirements. Where appropriate, researchers may seek further information or clarification from potential participants to determine whether an eligibility criterion is met.

Information collected during pre-screening will be reviewed against the predefined study eligibility criteria. Potential participants who appear eligible will be invited to attend a formal in-person screening visit. Where eligibility cannot be determined from the information provided, further clarification or review by the research team may be undertaken before an individual is invited to screening. Individuals who do not meet the pre-screening eligibility criteria will not proceed to the formal screening visit.

Pre-screening will be completed no more than one month prior to the scheduled screening visit to ensure that the information used to assess potential eligibility remains current.

To support transparent reporting in line with CONSORT guidelines, anonymised data will be collected on all individuals who complete pre-screening but are not enrolled. This will include age, gender, ethnicity, eligibility status (eligible/ineligible), and, where applicable, the reason for ineligibility or reason for declining participation. These data will be used solely for reporting recruitment flow and assessing generalisability and will not be linked to personally identifiable information.

7.1.2 Screening Procedures

Participants who meet the initial pre-screening criteria will be invited to attend a screening visit (Visit 1), which must occur within 14 days prior to the baseline visit (Visit 2). The purpose of this visit is to confirm eligibility through defined clinical, anthropometric, and laboratory assessments, and to obtain written informed consent before initiating any study procedures.

Following consent, the study team will verify adherence to pre-trial requirements (see Table 2). Anthropometric measurements will be taken, including height, weight, and waist and hip circumference, for calculation of BMI. Participants will then have their fasting BG tested using a validated CE-marked glucometer.

Table 2: Pre-trial requirements.

Requirement	Details	Timeframe Before visit
Alcohol restriction	No consumption of alcoholic beverages	24 hours
Fasting and beverage restriction	No food or caloric beverages. Water is permitted.	10 hours
Smoking restriction	No smoking or use of nicotine products	10 hours
Vigorous physical activity restriction	No vigorous exercise (e.g., running, high-intensity cycling, competitive sports).	10 hours

To be eligible for inclusion, participants must meet all of the following criteria at screening:

- BMI above 25.0 kg/m²
- Fasting capillary glucose < 7.0 mmol/L
- Confirm compliance with all pre-trial requirements

Eligibility will be reviewed and confirmed prior to baseline assessments and randomisation. Participants who do not meet the eligibility criteria will be classified as screen failures. However, rescreening may be allowed once, at the discretion of the study team, if the ineligibility is due to a temporary or correctable factor (e.g. recent supplement use or minor BMI deviation). All rescreening decisions and rationale will be documented in the case report form (CRF).

Participants who complete the screening visit will be issued a Fe-Col® stool collection kit with written instructions. They will be asked to collect the sample at home within 24 hours prior to the baseline visit and return it in the provided container.

7.1.3 Cognitive Task Familiarization

During the Screening visit (week –1) participants will complete a cognitive task familiarization session involving the Motor Screening Task (MOT) from the Cambridge Neuropsychological Test Automated Battery (CANTAB; Robbins et al., 1998). This serves to ensure that participants are comfortable with the CANTAB system prior to the main cognitive battery. The MOT provides a baseline measure of sensorimotor coordination, visual comprehension, and motor responsiveness, and helps identify impairments that may compromise the validity of subsequent cognitive data (Soares et al., 2014). Although not a direct measure of cognition, it ensures participants possess the necessary motor and perceptual skills to engage reliably with tasks. This task is expected to take around 2 minutes to complete. Task outcomes are outlined in table 3.

Table 3: Motor Screening Task Outcomes

Outcome Measures	Description	Unit of Measurement
Mean Latency	The average time (in milliseconds) between stimulus onset and participant response across ten trials.	Milliseconds (ms)
Mean Error	The average spatial deviation between the center of the target and the point of contact registered on the screen.	Millimeters (mm)

7.1.3 Payment

Participants will receive a voucher for £150 upon completion of the study to recognize their time and contribution. This amount is intended to compensate for the inconvenience of attending five in-person visits, completing assessments, and adhering to study procedures over the 12-week period. The payment will be made in full at the final study visit (Week 12), contingent on completion of all required visits and procedures.

This reimbursement is not intended as an incentive to participate but rather as fair compensation for time and effort, in accordance with HRA guidance on payments in research (HRA, 2020). No additional travel expenses will be reimbursed, as all study visits take place within a single site easily accessible to participants through public and university transport links.

7.2 Consent

Participation in this study is entirely voluntary. Informed consent will be obtained in accordance with the principles of Good Clinical Practice (GCP), the Declaration of Helsinki, and applicable regulatory and institutional standards. The PI retains overall responsibility for ensuring that the informed consent process is conducted appropriately at the study site. Any member of the research team delegated to take consent will be trained, competent, and authorised to do so.

All prospective participants will be provided with a LREC approved PIS at least 24 hours before the screening visit. The PIS will clearly outline the purpose, procedures, duration, potential risks and benefits of the study, the voluntary nature of participation, the right to withdraw at any time without penalty, and data confidentiality protections. It will also state how participants may contact the research team for additional information.

At the screening visit (Visit 1), individuals will be given the opportunity to ask questions and discuss the study with a member of the research team before giving consent. Written informed consent will be obtained prior to any study-specific procedures, including the collection of identifiable data or the initiation of screening assessments.

The process of obtaining consent will be documented in the study records. A signed copy of the informed consent form (ICF) will be given to the participant, and the original will be retained securely with the study documentation. Where appropriate, additional consent will be obtained to allow for the use of data and samples collected prior to withdrawal, as outlined in the consent materials.

Participants will be assumed to have capacity to consent unless evidence indicates otherwise. The research team will ensure that individuals are capable of understanding the study and making an informed decision at the time of consent. Capacity will be assessed informally by the consenting researcher in line with ethical guidelines. The study will exclude individuals who are unable to provide informed consent for themselves. This trial does not involve the enrolment of children, young people under 18 years of age, or adults lacking mental capacity.

The study does not involve any procedures requiring translation services; however, if a participant requests written materials in another language, the research team will make reasonable efforts to provide translated versions of key documents (e.g. PIS and ICF, subject to availability. Due to funding limitations, the hiring of professional verbal interpreters is not included in the study budget, and verbal translation services cannot be provided by the research team.

Should any new information arise during the study that may influence a participant's willingness to continue, the PI will ensure that this is communicated promptly and that re-consent is obtained using updated, REC-approved materials.

7.2.1 Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies

Participants will be asked to provide additional, optional consent for the storage and future use of their blood and stool samples beyond the main trial. These samples may be used in ancillary studies related to metabolic health, probiotic interventions, or gut microbiome research, including projects conducted by universities, NHS or non-profit research institutions, or commercial research partners, both in the UK and internationally.

Participation in ancillary research is entirely optional and not a requirement for inclusion in the main trial. Participants may choose whether to allow the use of their biological samples for:

- Specified ancillary studies related to the current trial
- Future ethically approved research that may be unrelated to the primary study condition
- Submission to an approved research tissue bank for long-term storage and broader research use

Participants will indicate their preferences on the ICF. All samples used for ancillary research will be anonymised (labelled only with a unique participant ID) and handled in accordance with the Human Tissue Act (HTA) 2004 and UK GDPR. If a participant consents to future use but later decides to withdraw from ancillary research, they may do so at any time by contacting the study team. Upon withdrawal, any stored identifiable samples that have not yet been used will be securely destroyed. However, data or results already generated from those samples may not be retractable due to anonymisation.

Participants will not receive individual results from ancillary research, as these analyses are exploratory and not designed for clinical interpretation. Participants will also not be contacted regarding findings unless they have given separate consent to be re-contacted for future studies.

7.3 The randomisation scheme

Participants will be randomly assigned to either the probiotic intervention group or the placebo group with equal allocation (1:1). Randomisation will be performed at the point of manufacture by Cultech, the UK manufacturer of Lab4P, using a web based randomisation platform (www.sealedenvelope.com), employing permuted block sizes of size 2 to maintain allocation balance.
Each product container will be labelled with a unique investigational product (IP) number corresponding to the randomisation sequence.

7.3.1 Method of implementing the randomisation/allocation sequence

Each participant will be assigned a unique IP number at the point of intervention dispensing. These IP numbers correspond to the pre-generated randomisation list and are used to track product assignment throughout the trial.

Study personal will allocate participants the next sequential IP number from the pre-labelled containers, without access to the underlying treatment group. The randomisation sequence will be stored in a password protected excel file, permitting individual level unblinding if clinically required. The password will be held by the primary trial supervisor, Professor Lauren Owen and provided in a security sealed envelope labelled with the trial identifier. This envelope will be retained securely and returned unopened at the end of the study to confirm that blinding was maintained. Professor Owen is not involved in participant-facing procedures or site-based trial activities.

The allocation sequence itself will not be accessible to site staff at any point. IP numbers will be recorded in the trial master file and case report forms to ensure traceability.

There is no participant registration or randomisation procedure at the study site, as allocation is fully managed in advance of product delivery. All trial documentation and data capture systems will reference participants by their IP number, ensuring treatment concealment is maintained at every stage.

7.4 Blinding

This trial will implement a blinded design to minimise bias in the delivery, assessment, and analysis of the intervention. The investigational product and the placebo will be identical in appearance, packaging, and flavour to ensure full comparability and maintain blinding integrity. Both products will be provided in matching containers and administered using the same instructions for use. The following individuals will be blinded to participant allocation throughout the trial:

- Trial participants
- Research personnel involved in data collection, intervention delivery, and outcome assessment

The project supervisor, who holds the password to the randomisation file, will remain unblinded but will not be involved in any site-based procedures, participant interaction, or outcome assessment. If operational interaction between blinded and unblinded personnel is required, strict separation of roles will be maintained and communication limited to non-allocation-related matters.

Unblinding of individual participants will occur only if necessary for clinical safety and only following documented approval by the project supervisor. In such cases, the unblinding process will be clearly documented and reported. Final unblinding of all participants and treatment groups will take place only after the analysis dataset has been locked and signed off by the project supervisor. The trial does not involve any procedures, tests, or equipment that could inadvertently reveal group allocation to participants or blinded researchers.

7.5 Emergency Unblinding

Unblinding may be undertaken only in the case of a valid medical emergency, such as a serious or unexpected adverse event, where it is deemed essential to know the participant's treatment allocation in order to provide appropriate clinical care. The decision to unblind must always prioritise participant safety, in line with the Declaration of Helsinki and ICH-GCP (Section 4.3). Wherever possible, the remainder of the research team will remain blinded.

7.5.1 Code Break Access and Control

The randomisation code is held securely by Professor Lauren Owen, and access is restricted to designated personnel not involved in trial assessments or analysis. The code break mechanism will be managed through a secure password-protected database, which permit individual-level unblinding only. The unblinding mechanism will be designed to ensure that only the treatment allocation of the individual concerned is revealed. Under no circumstances will unblinding of the entire trial or access to the full randomisation list be permitted. Unblinding will only be allowed in the case of medical emergency.

7.5.2 Emergency Unblinding Procedure

In the event of a medical emergency, a formal request for unblinding will be made by the PI. The code will be accessed by the authorised holder, and the allocation disclosed only to the clinician responsible for the participant's care. The participant will not be informed of their allocation unless medically necessary. The code break procedure will allow rapid unblinding, as required under ICH GCP 5.13.4. The reason for unblinding, along with the date, time, and signature of the individual who performed the unblinding, must be documented in the CRF and the trial master file.

7.5.3 Documentation and Notification

The following documentation and notifications are required following any unblinding:

- The date, time, and reason for unblinding will be recorded in the participant's individual CRF, and the site file.
- The PI will notify the project supervisor in writing as soon as possible, including a summary of the event and justification for unblinding.
- The event will be included in the final trial report and, if applicable, in the statistical analysis documentation.
- The PI will notify the LREC and other relevant regulatory bodies if the unblinding is associated with a serious adverse reaction or significant protocol deviation.
- SUSARs are required to be reported unblinded, as per regulatory requirements.

7.6 Baseline Data

All baseline data will be collected during the screening visit (week –1) following screening confirmation, and the baseline visit (week 0), prior to randomisation. These measures are essential for characterising the study population and for evaluating primary and secondary outcomes. Descriptions of all measures are presented in section 8.

7.6.1 Participant Reported Data

The following categories of participant reported data will be collected:

- Subjective sleep Quality (Week 0)
- Demographics and lifestyle (Week 0)
- Dietary Habits (Week 0)
- Physical symptoms and adverse events (Week 0)

7.6.2 Cognitive Performance Data

The following Cognitive performance data categories will be collected at the baseline visit (week 0):

- Memory task performance (3 tasks)
- Executive function task performance (2 tasks)
- Attention and psychomotor speed task performance (1 task)

7.6.3 Clinical Assessment Data

The following baseline clinical assessments will be conducted at the screening visit (Week –1):

- Cardiometabolic data (OGTT BG, HbA1c, Lipid profile, Resting blood pressure)
- Venous blood draw
- Anthropometric data (Height, Weight, BMI calculation)
- Body Composition data (Fat mass, lean mass, percentage fat mass)
- Vascular function (Cerebrovascular reactivity (CVR) of the middle cerebral artery, Flow-mediated dilation (FMD) of the brachial artery)

7.7 Trial assessments

This section outlines the timing and content of all trial assessments after baseline. Assessments are organized by visit, with visit windows, methods of data collection, and device/protocol references provided. A summary schedule of assessments is included in Table 2. Descriptions of all measures are presented in section 8.

7.7.1 Testing Visit 2 (Week 6) ±3 days

The following self-reported and cognitive measures will be collected via Qualtrics:

- Cognitive performance (CANTAB tasks as above)
- Venous blood draw
- Resting blood pressure
- Fasting BG
- HbA1c
- Lipid profile
- Weight
- Height

- Waist and hip circumference
- Subjective sleep quality
- Health events and physical experiences

7.7.2 Testing Visit 3 (Week 11) ±3 days

Cardiometabolic and anthropometric assessments from the baseline section of the screening visit will be repeated to assess intervention effects:

- Fasting BG
- OGTT BG (0, 30, 60, 120 minutes)
- HbA1c
- Lipid profile
- Venous blood draw
- Resting blood pressure
- Weight
- Height
- Waist and hip circumference

7.7.3 Testing Visit 4 (End of Intervention, Week 12) ±3 days

Final outcome assessments will be conducted, repeating baseline measures:

- Subjective sleep quality
- Physical symptoms and adverse events
- CANTAB cognitive battery
- Peripheral and central Vascular function (Cerebrovascular reactivity (CVR) of the middle cerebral artery, Flow-mediated dilation (FMD) of the brachial artery)
- Body composition (Fat mass, lean mass, percentage fat mass)

These data will serve as endpoint measures for all primary and secondary outcomes.

7.8 Withdrawal criteria

A participant may be withdrawn from the trial or from treatment with the investigational product (IP) under the following circumstances:

7.8.1 Participant-Initiated Withdrawal

Participants have the right to withdraw consent and discontinue participation in the trial at any time, without reprisal or loss of benefits, in accordance with the Declaration of Helsinki (World Medical Association, 2014). Upon withdrawal of consent, no further trial-specific assessments will be conducted, and no additional data will be collected. However, data collected up to the point of withdrawal may be retained and used by the PI unless the participant explicitly withdraws consent for data processing.

7.8.2 Investigator-Initiated Withdrawal

The Investigator may withdraw a participant from the trial or the IP if, in their clinical judgment, continued participation poses a risk to the participant's wellbeing. If this decision is based on an AE or serious adverse event (SAE), it must be reported in accordance with the protocol's safety

reporting procedures.

7.8.3 Scope of Withdrawal

It will be clearly recorded whether the withdrawal pertains to:

- The entire trial (i.e. no further participation or data collection),
- Withdrawal from treatment with the IP but continuation in trial assessments (e.g. follow-up visits), or
- Withdrawal from a specific component of the trial, such as translational research.

7.8.4 Documentation and Follow-Up

All instances of withdrawal will be documented in the case report form (CRF), including the reason for withdrawal if known. The Investigator will make every effort to conduct appropriate follow-up, particularly in relation to unresolved AEs, and to ensure the return of any unused investigational product. Follow-up procedures will be carried out as per protocol unless consent for follow-up has also been withdrawn.

7.8.5 Investigational Product Discontinuation

Participants may be discontinued from receiving the IP under the following circumstances:

- Participant chooses to stop treatment with the IP.
- Investigator determines that continued IP exposure is not in the participant's best interest (e.g. due to AEs or SAEs). In this case, the event will be reported appropriately.

Participants who discontinue the IP will not continue with the trial and will be withdrawn from further scheduled visits and assessments. Temporary disruptions to IP administration do not constitute formal discontinuation and will be handled according to specific guidance in the protocol.

7.8.6 Replacement of Withdrawn Participants

Participants who discontinue prior to trial completion will not be replaced.

7.8.7 Trial Termination Criteria

The trial may be prematurely terminated for reasons including, but not limited to:

- Decision by a regulatory authority, LREC, or supervisory team due to safety concerns or feasibility issues,
- Project supervisor decision based on emerging data or other significant considerations.

In the event of early termination or suspension, the Investigator will promptly inform all trial participants and study staff. All trial materials will be collected, and the case report forms will be completed to the extent possible.

7.9 End of trial

The end of the trial will occur within 28 days (±7 days) of the 64th participant completing their final follow-up visit at Week 12. This period allows for any final data collection, safety monitoring, and administrative closure in line with GCP requirements.

8 MATERIALS AND MEASURES

8.1 Participant reported data

All participant-reported data will be collected electronically using the Qualtrics survey platform. This method ensures secure, standardized administration and allows for real-time data capture, minimization of missing responses through enforced validation rules, and improved data quality through automated logic and branching. The platform also facilitates remote completion when applicable.

8.1.1 Health Events and Physical Experiences

To evaluate the impact of Lab4P on commonly occurring gastrointestinal and systemic symptoms, participants will be asked to complete a structured, self-administered questionnaire, adapted from the Gastrointestinal Symptom Rating Scale (GSRS; Kulich et al., 2008). This questionnaire is designed to capture the frequency and severity of commonly reported gastrointestinal symptoms, as well as other physical health experiences.

Participants will be asked to review a list of health-related experiences and, for each, report:

The number of days they experienced the symptom or event in the past 14 days (0–14), and

The typical severity of the experience during that time, using a 7-point scale ranging from “No discomfort at all” to “Very severe discomfort.”

The questionnaire includes a comprehensive list of gastrointestinal symptoms, such as abdominal pain or discomfort, heartburn, acid reflux, nausea, bloating, belching, bowel changes (e.g., constipation, diarrhoea, urgency), and sensations of incomplete emptying.

It also includes other physical health experiences, including sore throat, headache, muscle ache, symptoms of common cold, and recent use of oral antibiotics. For participants assigned female at birth, additional items include the use of vaginal pessaries or creams, and the occurrence of menstruation.

8.1.2 Sleep Quality

Sleep quality will be evaluated using the Pittsburgh Sleep Quality Index (PSQI), a validated, self-administered instrument designed to measure subjective sleep quality and disturbances over a 1-month interval (Buysse et al., 1989). The PSQI consists of 19 self-rated items grouped into seven component domains:

- Subjective sleep quality
- Sleep latency
- Sleep duration
- Habitual sleep efficiency
- Sleep disturbances
- Use of sleep medication
- Daytime dysfunction

Each component is scored on a 0–3 scale, with higher scores indicating greater impairment. The sum of these component scores yields a global PSQI score ranging from 0 to 21, where scores >5 typically indicate poor sleep quality.

8.2.3 Demographics and Lifestyle

The following demographic and lifestyle variables will be collected using a single self-report questionnaire composed of validated instruments:

- Sex assigned at birth: Male or Female (self-reported)
- Ethnicity: Categorised according to the most recent UK census classifications (Office for National Statistics, 2023)
- Socioeconomic status (SES): Assessed using the National Statistics Socio-economic Classification (NS-SEC; Rose & Pevalin, 2003), derived from occupation and education data
- Alcohol use: Measured using the Alcohol Use Disorders Identification Test (AUDIT; Horváth et al., 2023)
- Physical activity: Measured using the International Physical Activity Questionnaire – Short Form (IPAQ-SF; Lee et al., 2011)

8.1.4 Dietary Habits

Dietary habits will be captured using the EPIC-Norfolk 7-Day Diet Diary (7dDD), a validated, structured food diary originally developed from the British Birth Cohort tool (Lentjes et al., 2013). Participants will be instructed to record all food and drink consumed over seven consecutive days, including weekdays and weekend days, with details on portion sizes, preparation methods, and time of intake. Each day is divided into eight sections: seven meal time slots covering intake from midnight to midnight, and a final section for recording snacks, additional items, and contextual information.

8.2 Cognitive Performance

Cognitive performance testing will be administered using the Cambridge Neuropsychological Test Automated Battery (CANTAB) software, a computerized tool designed to assess several cognitive domains (Robbins et al., 1998). CANTAB has been extensively validated and used in the assessment of cognitive function across a broad range of neurological and psychiatric disorders (Aslam et al., 2018; Janssen et al., 2013). Further, CANTAB tasks have demonstrated sensitivity to detecting both cognitive deficits linked to clinical and subclinical metabolic dysfunction, as well as the cognitive effects of probiotic interventions (Ahmed et al., 2025; Allen et al., 2016; Wild & Musser, 2014). The CANTAB Connect Research software is validated for use on all iPad Air devices and is fully compliant with GCP guidelines. The full battery of tasks is outlined below.

8.2.1 Memory Tasks

Task	Outcome Measures	Description	Unit of Measurement
Verbal Recognition Memory (VRM)	Free Recall Score	Number of words recalled correctly during the free recall phase.	Count (n)
	Immediate	Number of correctly identified	Count (n)

	Recognition Correct	target words in the immediate recognition phase	
	Immediate Recognition Errors	Number of incorrect (distractor) selections in the immediate recognition phase	Count (n)
	Delayed Recognition Correct	Number of correctly identified target words in the delayed recognition phase	Count (n)
	Delayed Recognition Errors	Number of incorrect (distractor) selections in the delayed recognition phase	Count (n)
Paired Associates Learning (PAL)	Total Errors Adjusted	Sum of location errors across stages, adjusted for unattempted stages due to task termination.	Count (n)
	Stages Completed	Number of successfully completed stages as task difficulty escalates.	Count (n)
	Trials to Success	Number of trials required to correctly learn all pattern-location pairings within a stage.	Count (n)
Spatial Working Memory (SWM)	Between-Search Errors	Number of times a previously successful box is revisited in a new search.	Count (n)
	Within-Search Errors (count):	Number of successfully completed stages as task difficulty escalates.	Count (n)
	Strategy Score	Computed based on first box selection consistency in 6–8 box trials.	(unitless index)

8.2.2 Executive Function Tasks

Task	Outcome Measures	Description	Unit of Measurement
Stop Signal Task (SST)	Stop Signal Reaction Time (SSRT)	Estimated latency of stopping an already started action	Milliseconds (ms)
	Go Reaction Time	Time from cue onset to response in go trials.	Milliseconds (ms)
	Proportion of Successful Stops	Percentage of trials where the participant successfully inhibits an already started response.	Percentage (%)
Intra-Extra Dimensional Set Shift (IED)	Total Errors	Sum of all incorrect responses across the task.	Count (n)
	Stages Completed	Number of correctly completed	Count (n)

		discrimination stages.	
	Extra-Dimensional Shift Errors	Errors specifically made during the extra-dimensional shift phase.	Count (n)

8.2.3 Attention and psychomotor speed Tasks

Task	Outcome Measures	Description	Unit of Measurement
Five Choice Reaction Time (RTI)	Reaction Time	Time from stimulus onset to button release.	Milliseconds (ms)
	Movement Time	Time from button release to screen touch.	Milliseconds (ms)
	Accuracy	Proportion of correct responses across trials.	Percentage (%)

8.3 Laboratory assessments

8.3.1 Cardiometabolic data

All of the following data that includes capillary sampling will be performed by trained study personnel under the supervision of the Project Lead, following standardised SOPs. Results will be recorded directly into the eCRF.

8.3.1.1 Blood Glucose

Capillary blood will be collected via fingertip lancet (Fisher Scientific). BG measurements will be conducted via direct application to single-use glucose test strips inserted into a validated point-of-care glucometer. Fasting glucose and OGTT responses will be measured using a validated, CE-marked glucometer. BG will be assessed at four timepoints during the OGTT: fasting (0 min), and at 30, 60, and 120 minutes post-glucose ingestion.

8.3.1.2 Glucose Tolerance

Glucose tolerance will be assessed via the incremental area under the curve (AUC) for capillary blood glucose during a standardized 2-hour OGTT. The primary metabolic endpoint will be the total AUC for capillary glucose, calculated using a weighted approximation method adapted from Sakaguchi et al. (2015). The AUC will be expressed in units of mmol·h/L, with glucose concentrations in mmol/L and time in hours. The formula is defined as:

Capillary Glucose AUC= $\frac{CG(0)+CG(30)\times 2+CG(60)\times 3+CG(120)\times 4}{4}$

where CG(t) represents the capillary glucose concentration at time t minutes post-glucose load.

8.3.1.3 Glycated Hemoglobin (HbA1c)

Capillary blood will be collected via fingertip lancet (Fisher Scientific) into micro-collection tubes (Abbott Laboratories). HbA1c will be measured from using the Afinion 2 (Abbott).

8.3.1.4 Lipid Profile

Capillary blood will be collected via fingertip lancet (Fisher Scientific) into micro-collection tubes (Abbott Laboratories). Lipid profile will be analyzed using the Cholestech LDX analyzer (Abbott). The following data will be recorded in the eCRF:

- Total cholesterol
- LDL-C
- HDL-C
- Triglycerides

8.3.1.5 Blood Pressure

Blood pressure will be measured using a clinically validated, automated oscillometric device (Omron). Measurements will be taken after the participant has been seated at rest for 5 minutes. The following data will be recorded in the eCRF:

- Resting systolic blood pressure (mmHg)
- Resting diastolic blood pressure (mmHg)

8.3.2 Anthropometric data

Anthropometric measurements will be assessed following ISAK-compliant procedures (Norton, 2018). The following data will be measured:

- Height (cm)
- Weight (kg)
- Waist circumference (cm)
- Hip circumference (cm)

Waist circumference is measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, while hip circumference is taken at the widest portion of the buttocks. Two measurements will be taken at each site, and a third will be performed if the first two differ by more than 1 cm; the mean of the closest two values will be recorded.

From these measurements, BMI will be calculated as weight (kg) divided by height squared (m^2), and waist-to-hip ratio (WHR) will be calculated as waist circumference divided by hip circumference. All anthropometric assessments will be performed by trained research personnel, using calibrated equipment to ensure accuracy and reproducibility.

8.3.3 Body Composition data

Body composition will be assessed using a GE Lunar iDXA scanner (GE HealthCare). Scans will follow manufacturer protocols, with daily calibration and quality checks. Certified radiographers will conduct all scans. Procedures comply with IRMER (British Institute of Radiology, 2015) and University safety guidelines, with radiation exposure kept as low as reasonably practicable (ALARP) and considered negligible.

The following data will be measured:

- Resting Metabolic Rate (Kcal/day)
- Relative Skeletal Muscle Index (kg/m2)
- Total Body Water (L)
- Intracellular Water (L)
- Extracellular Water (L)

- Total Fat Mass (kg)
- Total Lean Mass (kg)
- Percentage body fat (%)

Percentage body fat will be calculated using the following formula:

Percentage Body Fat= $\left(\frac{Total\ fat\ mass\ (kg)}{Total\ body\ mass\ (kg)}\right) \times 100$

Where:
Total Body Mass (kg) is the sum of Total Fat Mass (kg) and Total Lean Mass (kg).

8.3.4 Peripheral and central vascular function

The following vascular measures will be assessed at baseline to characterize cerebrovascular and peripheral endothelial function:

- Cerebrovascular reactivity (CVR) of the middle cerebral artery
- Flow-mediated dilation (FMD) of the brachial artery

8.3.4.1 Cerebrovascular Reactivity (CVR)

CVR will be assessed using transcranial Doppler ultrasonography (Multidop T, DWL Compumedics Germany GmbH) to measure cerebral blood flow (CBF) velocity in the middle cerebral artery (MCA; Seigworth et al., 2025). Participants will be positioned either supine or seated in a quiet, temperature-controlled environment. A 2-MHz probe will be placed at the temporal window to insonate the MCA. Both left and right MCAs will be insonated, and the side of head with the highest quality spectral waveform will be selected for analysis.
Baseline MCA velocity will be recorded for 2–3 minutes under resting conditions. CVR will be elicited using a standardized breath-hold protocol. Participants will first breathe normally through the nose for one minute to establish normocapnia, after which they will pinch their nose and perform a 30-second breath hold. At the end of the maneuver, participants will be instructed to exhale through the nose to allow capture of peak end-tidal CO_2 ($EtCO_2$), before resuming normal breathing. Continuous recording of CBF velocity and $EtCO_2$ will be obtained throughout. Baseline MCA velocity will be defined as the average during the normocapnic period, and peak velocity will be defined as the average of the highest values at peak $EtCO_2$. CVR will be calculated as the percent change in MCA velocity from baseline to peak divided by the absolute change in $EtCO_2$, expressed as %/mmHg (Liu et al., 2013).

8.3.4.2 Flow-Mediated Dilation (FMD)

Endothelial function will be evaluated via ultrasound-based measurement (Vivid IQ, GE Healthcare, Chalfont St Giles, UK) of brachial artery FMD, following established guidelines (Corretti et al., 2002). Participants will rest supine in a quiet, temperature-controlled room for at least 10 minutes prior to imaging.
Baseline brachial artery diameter and flow velocity will be recorded above the antecubital fossa. A pneumatic cuff placed on the forearm will be inflated to at least 50 mmHg above systolic blood pressure (minimum 200 mmHg) for 5 minutes to induce arterial occlusion. Upon rapid cuff release, artery diameter will be recorded continuously for 2–3 minutes to determine the peak post-occlusion response. FMD will be calculated as the percentage change from baseline to peak diameter (Thijssen et al., 2008).

8.4 Clinical sample collection procedures

8.4.1 Venous blood collection

The researcher will clean the venipuncture site thoroughly using an alcohol-based antiseptic and allow it to dry completely to prevent contamination. A tourniquet will be applied to aid vein visualization. Once the vein is located, a sterile needle will be inserted using standard phlebotomy technique at a shallow angle (approximately 15–30 degrees) to ensure smooth entry and minimize discomfort.
One 2 mL blood sample will be drawn into a purple topped, Ethylenediaminetetraacetic acid (EDTA)-coated tube (Uk Medi). The tube will be labeled with the participant's unique identification number and time of collection.

8.4.2 Stool sample collection

Participants will be provided with FTA faecal sample collection cards (Botnen et al., 2023) at the screening visit (week –1) and again at visit 3 (week 11). Each kit will include the FTA card, disposable gloves, and clear written instructions for proper at-home sample collection. Participants will collect the stool sample by applying a small amount to the card, allow it to dry thoroughly, and return it to the study site in the pre-supplied protective envelope. Due to the preservative properties of the FTA card, refrigeration or ice packs are not required during transport.

8.5 Storage and analysis of clinical samples

It is the responsibility of the trial site to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with the 1998 Data Protection Act. Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 HTA.

Table 4: Biological Sample Collection Details

Purpose	Visit 1 (Week –1)	Visit 2 (Week 0)	Midpoint (Week 6)	Visit 3 (Week 11)	Visit 4 (Week 12)
Venous Blood Samples					
Cytokine Concentration Platelet Activity	2 x 2mL		2 x 2mL	2 x 2mL	
Fingertip Capillary Blood Samples					
Oral Glucose Tolerance Testing	3 x 1 µL			3 x 1 µL	
Lipid Assessment	1 x 40 µL		1 x 40 µL	1 x 40 µL	
HbA1c Assessment	1 x 1.5 µL		1 x 1.5 µL	1 x 1.5 µL	
Fasting Blood Glucose	1 x 1 µL		1 x 1 µL	1 x 1 µL	
Stool Samples					

Gut Microbial Analyses		1 x5g		1x5g	

8.5.1 Venous Blood Samples

Venous blood samples will be collected at Visit 1 (Week –1), Visit 3 (Week 6) and Visit 4 (Week 11) for the assessment of inflammatory cytokine concentration and markers of platelet activity. Each participant will provide one venous blood sample per visit (total: 3 samples per participant).

8.5.1.1 Processing and storage

Samples will be processed within 2 hours of collection: centrifuged at 1,500 × g for 10 minutes at 4°C. Plasma aliquots will then be immediately transferred to pre-labeled cryotubes and stored at −80°C until batch analysis to minimize freeze-thaw cycles. All pre-analytical handling will follow the LBU CSS Biospecimen Handling SOP.

8.5.1.2 Analysis

Cytokine concentrations will be from plasma derived from venous blood samples, using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Thermo Fisher Scientific). The following pro-inflammatory and anti-inflammatory cytokines will be quantified:

- IL-6
- TNF-α

Markers of platelet activity will also be assessed from venous blood samples using validated and appropriate laboratory methods.

All assays will be performed using validated high-sensitivity ELISA kits, where applicable, with known lower limits of detection and quantification appropriate for low circulating levels of biomarkers in plasma. Each sample will be run in duplicate, and mean concentrations will be used for analysis. Absorbance will be read using a Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific) at the wavelength specified by the kit protocol. Raw absorbance data will be analyzed using SkanIt™ Software, applying a 4-parameter logistic (4PL) regression model to generate standard curves and interpolate sample concentrations. Standard curves must achieve $R^2 \geq 0.99$ for data to be considered valid. Internal controls and reference standards provided with the kits will be included on each plate to ensure inter-assay comparability. Inter-assay and intra-assay coefficients of variation (CVs) will be calculated. CVs exceeding 15% will trigger a review and potential re-analysis of affected samples.

8.5.1.3 Documentation and Destruction

Sample tracking will include collection time, storage events, and analysis outcomes. Samples will be retained for up to 1 year's post-analysis, unless otherwise stated in the ICF. Samples will then be destroyed securely under institutional biosafety policy. No shipping is planned unless deemed necessary, in which case samples will be sent on dry ice with appropriate documentation.

8.5.2 Capillary Blood Samples

Capillary blood samples will be collected at Visit 1 (Week –1), Visit 3 (Week 6) and Visit 4 (Week 11) for the assessment of glucose metabolism, glycated haemoglobin (HbA1c), and lipid profile. Each participant will provide seven capillary blood samples per visit (total: 14 samples

per participant).

8.5.2.2 Processing and storage
All capillary samples will be analysed immediately at the point of care using integrated analyser systems. No biological material will be stored, and samples will be disposed of in accordance with institutional biosafety and clinical waste protocols immediately after analysis. No samples will be retained, transported, or biobanked.

8.5.2.3 Analysis
All capillary blood analyses will be performed using CE-marked point-of-care diagnostic devices to ensure standardisation and accuracy. These methods are outlined in section 8.3.2.

8.5.3 Stool Samples
Stool samples will be collected at Week 0 and Week 12 for gut microbiome analysis.

8.5.3.2 Processing and storage
Upon return to the study site, samples will be stored at room temperature. Samples will be temporarily stored under these conditions for up to 1 week, after which they will be shipped to Cultech Ltd. (Port Talbot, UK) for microbiome analysis. All shipments will be tracked and include full chain-of-custody documentation.

8.5.3.2.1 Shotgun metagenomic sequencing
Genomic DNA will be recovered from faecal samples preserved on FTA cards. Sections of the cards containing stool will be excised, and DNA will be extracted using an appropriate protocol in accordance with the manufacturer's instructions. DNA concentration and purity will be assessed fluorometrically, and minimum thresholds for DNA yield and fragment quality will be applied prior to sequencing.

Shotgun metagenomic sequencing will be conducted on a high-throughput Illumina platform, generating paired-end reads. Raw reads will undergo quality control, including trimming of adapters and low-quality bases, and filtering of short reads. Host-derived sequences will be removed by alignment to the human genome reference (GRCh38; Langmead & Salzberg, 2012), and only non-host reads will be retained for downstream analysis.

8.5.3.3 Analysis

8.5.3.4 Taxonomic profiling
Taxonomic classification of microbial reads (bacteria, archaea, viruses, fungi) will be performed using a k-mer–based approach (e.g., Kraken2 or equivalent; Wood et al., 2019), using a curated reference database. Species-level abundance estimates will be refined using an appropriate algorithm (e.g., Bracken; Lu et al., 2017), with filtering thresholds applied to exclude rare taxa from downstream analysis.
Presence of probiotic strains included in the investigational product will be evaluated using strain-resolved genomic methods, comparing mapped reads to available reference genomes. A predefined coverage threshold will be used to determine strain-level detection.
Specific analytical tools and pipelines may be updated at the time of analysis to reflect current best practice and advancements in metagenomic analysis.

8.5.3.4 Documentation and Destruction
Each sample will be assigned a unique identifier and logged in the study's sample tracking system. Any residual stool material will be destroyed by Cultech according to their biosafety disposal protocols following analysis, unless otherwise specified in the ICF. Participants will be asked to provide informed consent for the use and transfer of stool samples for microbiome research.

9. TRIAL TREATMENTS

9.1 Name and description of product under investigation

Test product name: Lab4P Probiotic Consortium, Cultech LTD.
The investigational product used in this study is the Lab4P Probiotic Consortium, a multi-strain live microbial product supplied in capsule form. The test product contains per serving:
A total of 5×10^{10} colony forming units (CFU), comprising the following five bacterial strains:

- Lactobacillus acidophilus CUL60 (NCIMB 30157)
- Lactobacillus acidophilus CUL21 (NCIMB 30156)
- Lactobacillus plantarum CUL66 (NCIMB 30280)
- Bifidobacterium bifidum CUL20 (NCIMB 30153)
- Bifidobacterium animalis subsp. lactis CUL34 (NCIMB 30172)

In addition to probiotic strains, each capsule also contains the following micronutrients at 100–200% of the recommended nutrient reference value (NRV):

- Vitamin D – 10 µg (200% NRV)
- Vitamin C – 80 mg (100% NRV)
- Zinc – 10 mg (100% NRV)

Lab4P is a patented and marketed product it has been approved for use in humans. It is categorised as a food supplement and is available to purchase in the marketplace without prescription. Capsules are produced by Cultech LTD, Baglan Industrial Park, Unit 34 Aberavon Rd, Port Talbot SA12 7DJ. Both the investigational product and the placebo will be packaged in an identical manner.

9.2 Storage and supply

9.2.1 Storage and shipment to sites
The investigational product and matching placebo will be shipped under controlled conditions to the trial site. Upon delivery, research personnel must verify the contents of the shipment against the packing list and document receipt using trial-specific accountability forms. These forms will serve as source documents and must be completed in real time by the investigator or a delegated and trained staff member. While controlled conditions are not required for short-distance transport, any visible damage to the packaging or concerns regarding product integrity must be recorded and reported to the supervisory team immediately.

9.2.2 Accountability

The research team must maintain accurate records of the investigational product and placebo received from Cultech. These records will include receipt and dispensing dates, and dates of return and/or destruction of unused supplies. During the course of the trial and at the termination, clinical supplies must be accounted for and a written clarification provided in case of discrepancies. All dispensing, use, returns, and other dispositions of the investigational and placebo products must be recorded in the accountability log. Trial monitors will review accountability records during routine monitoring visits to ensure compliance with protocol requirements.

The investigational and placebo products must only be used as specified in the trial protocol. Under no circumstances may site staff distribute the trial medication to external investigators, healthcare services, or use it for purposes outside of this study.

9.2.3 Storage at trial site

All trial products must be stored under refrigeration at 4–8°C to maintain product stability, as indicated by the manufacturer. All products must be stored in a dedicated, secure, temperature-monitored refrigerator with restricted access. Storage conditions, including daily temperature logs, must be recorded and maintained in the investigator site file.

9.2.4 Transportation and storage at subject home

At the baseline visit, participants will receive both verbal and written instructions on proper storage and administration of the investigational product. Capsules must be stored in a household refrigerator at a temperature between 4–8°C. Participants will be advised not to freeze the capsules and to avoid storing them in warm or humid environments. If refrigeration is temporarily unavailable (e.g., during travel), participants will be instructed to store the capsules in a cool, dry location and return them to refrigeration as soon as possible.
Study staff will reinforce these instructions at all contact points and will discuss any barriers to proper storage, daily adherence, or timing of administration. All deviations from recommended storage or administration will be documented and reviewed by the research team.

9.2.5 Return and destruction of product

Returned medication will be checked for compliance and documented accordingly. Any remaining investigational product or placebo will be collected by the PI, repackaged in a tamper-evident container, and stored securely at the trial center until the conclusion of the study. All unused supplies will be properly destroyed at the trial site or sent back when indicated by Cultech. Written authorization must be obtained from Cultech prior to the destruction which must be documented.

9.3 Preparation and labelling of Investigational Product

Both the investigational product and the placebo are packaged in induction-sealed, opaque plastic pots to prevent exposure to light and moisture and to maintain product stability throughout the study period.
Minimum information to be included on the trial product label will be:

- **Study Title:** ProCOG

- **Participant Number:** *To be entered at the time of dispensing*
- **Contents:** 90 capsules
- **Investigator:** Lewis Hepburn, Principal Investigator
- **Investigator Contact:** L.Hepburn3449@student.leedsbeckett.ac.uk
- **The statements:** “For study purposes only.” “Keep out of reach of children.”
- **Expiry date:** *To be added according to the product lot supplied*
- **Batch number:** *To be added according to the product lot supplied*
- **Manufacturer:** Cultech Ltd, United Kingdom.
- **Statements pertaining to dosage, administration and storage information, including:** “Take one capsule per day with food (with or without a drink).” “Avoid hot drinks at the time of ingestion.” “Take with any meal during the day.” And “Store capsules in a refrigerator.”

9.4 Dosage schedules

Participants will consume one capsule per day for 3 months (90 days). Participants will be instructed to take one capsule, orally once daily, with food, at a consistent time of day. No specific preparation is required prior to administration.

The selected dose, route, and duration of administration for the Lab4P Probiotic Consortium are supported by existing clinical data on its safety, tolerability, and product stability (Michael et al., 2020; Michael et al., 2021). Oral administration was selected as it is the most established and effective route for probiotic delivery in human studies (Baral et al., 2021).

The 12-week treatment duration was informed by previous research demonstrating the efficacy of Lab4P in reducing body weight and improving metabolic parameters within this timeframe (Michael et al., 2021). In addition, the 12-week duration is consistent with studies involving other probiotic formulations that have reported measurable changes in metabolic and cognitive outcomes over similar periods (Mo et al., 2024; Salles et al., 2020; Zhang et al., 2020). This duration is also considered a practical compromise to support participant retention and overall study feasibility.

9.4.1 Missed Doses

If a participant forgets to take a dose, they will be advised to take it as soon as they remember. If they do not remember until the following day, the missed dose should be skipped and they should continue with their usual schedule. Participants should not take two doses on the same day to make up for a missed dose.

9.4.2 Vomiting Post-Dose

If vomiting occurs within 30 minutes of dosing, participants may take a replacement capsule. Vomiting after 30 minutes will not require redosing, as sufficient absorption of probiotic organisms is likely to have occurred.

9.4.3 Treatment Resumption After Temporary Suspension

In the event of temporary discontinuation (e.g. due to illness or travel), participants may resume daily dosing without dosage adjustment once able to comply with the protocol. Missed days will be recorded, but no dose compensation will be applied.

9.5 Known reactions and interaction with other therapies

The use of medications or treatments that may interfere with the IP, confound key efficacy endpoints, or pose safety concerns will be carefully monitored during the study. While participants will not be prohibited from using medically necessary treatments, they will be instructed to avoid consciously starting new probiotics, prebiotics, synbiotics, or related supplements during the study period. All concomitant medications and supplements will be recorded and considered during data analysis.

To minimize the risk of interference, individuals with recent or current use of specific agents known to impact the gut microbiota, glucose metabolism, or probiotic viability will be excluded at screening. Specifically, participants will be excluded if they have used any of the following within 4 weeks prior to baseline:

- **Antibiotics** (oral, intravenous, or topical with potential for systemic absorption), due to their impact on gut microbiota composition and potential to reduce probiotic efficacy (Éliás et al., 2023).
- **Proton pump inhibitors** (PPIs), H2-receptor antagonists, and other agents that alter gastric pH, as they may impair probiotic survival (Garg & Mohajeri, 2024; Vila et al., 2020).
- **Immunosuppressive agents**, which may affect probiotic-host interactions (Gibson et al., 2020).
- **Systemic corticosteroids or medications known to significantly alter glucose metabolism**, such as insulin, metformin, sulfonylureas, GLP-1 receptor agonists, β-blockers, and thiazide diuretics, due to their influence on OGTT outcomes and metabolic endpoints (Eyth et al., 2023).

Participants will be advised to report any new medications or supplements taken during the trial. Use of agents listed above during the study will be reviewed on a case-by-case basis by the research team, recorded in the eCRF and flagged for consideration during analysis. These events may be recorded as protocol deviations only if they substantially affect outcome interpretation, in line with the monitoring and statistical analysis plans.

9.6 Concomitant medication

Study subjects may receive any needed medically prescribed treatment/therapy/medication. Information regarding medications, treatments or therapies administered, including the name of the active ingredient and duration of treatment, will be collected throughout the trial. The start date, stop date and indication for concomitant treatments/medications received from ICF signature (screening visit) until the last visit (including follow-up).

All concomitant medications, prescription, over-the-counter, herbal, and nutritional supplements, must be documented in the relevent eCRF, including the drug name, indication, route of administration, dose, frequency, start and stop dates.

9.7 Trial restrictions

To minimize confounding factors and preserve data integrity, the following pre-visit requirements must be followed prior to each in-person assessment:

Table 5: Pre-laboratory assessment requirements

Requirement	Details	Timeframe Before visit
Alcohol restriction	No consumption of alcoholic beverages	24 hours
Fasting and beverage restriction	No food or caloric beverages. Water is permitted.	10 hours
Vigorous physical activity restriction	No vigorous exercise (e.g., running, high-intensity cycling, competitive sports).	10 hours

Restrictions will be clearly communicated to participants through the PIS, reinforced via the pre-screening questionnaire, and compliance will be checked verbally at each in-person visit.

In addition, to reduce potential confounding effects on gut microbiota composition, participants will be asked to refrain from starting any new probiotic supplements or regularly consuming probiotic-rich foods (e.g., yogurt with live cultures, kefir, kombucha, kimchi, sauerkraut, miso, or other fermented products containing live bacteria) throughout the study period (from recruitment to Week 12 final visit). Participants who already consume these products regularly will be eligible to take part but will be asked to discontinue them if they choose to participate, so that the study intervention is the sole source of probiotic exposure. All dietary patterns, including probiotic food intake, will be recorded at baseline and monitored throughout the study to allow for consideration during data analysis.

9.8 Assessment of compliance with treatment

The research team will record the number of capsules dispensed; the number returned and calculate adherence as the proportion of capsules consumed relative to the number expected for the treatment period. These data will be entered into the eCRF.
Product compliance will be assessed at Visit 4 (week 12). To be considered compliant, participants must have consumed a minimum of 80% of the assigned study product.
Participants not meeting this threshold will be included in the ITT population for primary analysis and excluded from the PP population unless otherwise justified. Compliance data may also be used in secondary or sensitivity analyses, as defined in the Statistical Analysis Plan.

10 PHARMACOVIGILANCE

10.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

	All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. It is important to note that this is entirely separate to the known side effects listed in the SmPC. It is specifically a temporal relationship between taking the drug, the half-life, and the time of the event or any valid alternative etiology that would explain the event.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the reference safety information: • in the case of a product with a marketing authorisation, this could be in the summary of product characteristics (SmPC) for that product, so long as it is being used within it's licence. If it is being used off label an assessment of the SmPCs suitability will need to be undertaken. • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question

10.2 Operational definitions for (S)AEs

10.2.1 Identification of (S)AEs

Adverse events will be identified through:

- Participant self-report
- Investigator questioning during study visits
- Physical examinations and laboratory assessments
- Other clinically relevant observations

10.2.2 Evaluation of AEs and SAEs

All AEs, SAEs, SARs, and SUSARs will be documented and assessed for severity, causality,

and expectedness in accordance with ICH-GCP, the UK Clinical Trials Regulations, and the requirements of the approving REC. These will be assessed using the criteria outlined below.

10.2.2.1 Severity

Category	Definition
Mild	Symptoms hardly perceived, only slight impairment of general well-being.
Moderate	Clearly noticeable symptom, but tolerable without immediate relief.
Severe	Overwhelming discomfort.

10.2.2.2 Causality

Investigators will make causality assessments as though the participant received the active investigational product, to preserve blinding integrity.

Category	Definition
Unrelated	An adverse event, which is clearly not related to the product(s) and/or study procedure(s).
Unlikely	The event is unlikely related to the product(s) and/or procedures if it occurs within a temporal relationship to product(s) administration and/or study procedure(s) that makes a causal relationship improbable and in which drugs, chemicals or underlying disease provide more plausible explanations.
Probable	The event is probably related to the product(s) and/or procedures if it occurs in a reasonable time sequence to administration of the product(s) and/or study procedure(s), and if the event is unlikely to be attributed to concurrent disease, drugs or chemicals. The event should follow a clinically plausible response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
Related	The event is related to the product(s) and/or study procedure(s) if it occurs in a plausible time relationship to product(s) administration and/or study procedure(s), and if it cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the product(s) and/or study procedure(s) (dechallenge) should be clinically plausible. The event must be plausible pharmacologically or phenomenologically. Confirmation must include a satisfactory rechallenge procedure.

10.2.2.3 Expectedness

Category	Definition
Expected	An adverse event which is consistent with the information about the intervention clearly defined in this protocol.
unexpected	An adverse event which is not consistent with the information about the intervention clearly defined in this protocol.

10.2.3 Events to Be Reported to the Sponsor

The following events will be reported to the Sponsor:

- All SAEs, regardless of causality, within 24 hours of the site becoming aware
- All SARs, including any unexpected worsening of known adverse reactions
- Unexpected ARs not consistent with the known safety profile of the investigational product

These events will be reported using the Serious Adverse Event Case Report Form (SAE CRF) provided in the Investigator Site File and submitted via secure electronic or fax transmission to the Sponsor or designee.

10.2.4 Events Not Requiring Reporting to the Sponsor

The following will be recorded but not routinely reported to the Sponsor, unless considered clinically significant or unexpected in severity, frequency, or duration:

- Mild gastrointestinal symptoms such as bloating or flatulence
- Expected minor adverse reactions consistent with the known safety profile of probiotic supplements
- Elective or pre-planned hospital admissions not associated with clinical deterioration
- Admissions for routine treatment or monitoring of the studied indication
- Outpatient treatment not fulfilling criteria for seriousness

All non-serious AEs will be recorded in the eCRF. These records will be available to the Sponsor and regulatory authorities upon request.

10.3 Recording and reporting of SAEs, SARs AND SUSARs

AEs and SAEs will be recorded from the time of written informed consent until the final study visit at Week 12. ARs, SARs, SUSARs will be recorded and reported from the first administration of the investigational product until the final dose is taken, with all relevent events captured at the final visit. Upon becoming aware of any SAE, the investigator must enter the event into the eCRF within 24 hours, regardless of whether the event is considered related to the investigational product. All SAEs occurring up to and including the final trial visit and within 30 days following the last dose of study product must be reported in this way. Initial reports should be submitted even if causality and expectedness cannot yet be determined.

Upon entry of an SAE in the eCRF, an automatic notification will be sent to the PI, who must also be directly notified within 24 hours of the investigator becoming aware of the event. The PI will review all SAEs and determine the appropriate course of action in collaboration with the research supervisory team. The supervisory team, acting on behalf of the Sponsor, will be responsible for notifying the LREC and any other relevant regulatory bodies, in line with applicable reporting timelines and procedures

Each SAE must be electronically signed by the reporting investigator within the eCRF. Expedited reporting requirements apply to SUSARs. Any SUSAR that is fatal or life-threatening must be reported to the LREC and MHRA (if applicable) within 7 calendar days of the PI or project supervisor becoming aware of the event, with any follow-up information submitted within an additional 8 days. All other SUSARs must be reported to the LREC and MHRA within 15 calendar days of awareness.

For each SAE and/or SUSAR, the following information will be collected:

- Subject and date
- Description of event
- Reporting source
- Suspect product
- Duration
- Frequency
- Intensity
- Seriousness
- Action taken
- Outcome and sequelae if any
- Relationship to test product

If a participant withdraws consent for further data collection, this does not remove the requirement to report SARs or SUSARs that occur or become known to the investigator following withdrawal, as these are reportable for regulatory and safety purposes. This will be clearly explained to participants in the Participant Information Sheet (PIS).

10.4 Responsibilities

PI:

- Checking for AEs and ARs when participants attend for treatment / follow-up.
- Using medical judgement in assigning seriousness, causality and whether the event/reaction was anticipated.
- Ensuring that all SAEs are recorded and reported to the project supervisor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
- Ensuring that SAEs are chased with the project supervisor if a record of receipt is not received within 2 working days of initial reporting.
- Ensuring that AEs and ARs are recorded and reported to the project supervisor in line with the requirements of the protocol.

Project Supervisor:

- Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
- Using medical judgement in assigning the SAEs seriousness, causality and whether the event was anticipated (in line with the Reference Safety Information) where it has not been possible to obtain local medical assessment.
- Immediate review of all SUSARs.
- Expedited reporting of SUSARs to the LREC or Competent Authority (MHRA in UK) as necessary and within required timelines.
- The unblinding of a participant for the purpose of expedited SUSAR reporting.

10.5 Notification of deaths

In the event that a participant dies during the trial period (from informed consent until the final study visit at Week 12), the project supervisor will assess whether the death is related to study

participation. Only deaths deemed related to the investigational product will be reported to the LREC, and in no case later than 24 hours from awareness.
Deaths unrelated to the IMP are not routinely reportable but will be documented in the participant's medical records, trial documentation, and reported in the final study report, where applicable. Any unexpected deaths, regardless of relatedness, will be flagged for review by the project supervisor and reported to the LREC if deemed relevant to participant safety.

10.6 Pregnancy reporting

Although pregnancy should not be considered as an AE/SAE, any pregnancies will be captured using the participants CRF and followed up as if they were SAEs to determine the outcome, especially in the case of the pregnancy going to term to determine the outcome with respect to the baby.

10.7 Reporting urgent safety measures

If any urgent safety measures are taken the PI shall immediately and, in any event, no later than 3 days from the date the measures are taken, give written notice to the supervisory team and the relevant LREC of the measures taken and the circumstances giving rise to those measures.

10.8 The type and duration of the follow-up of participants after adverse reactions.

All SAEs must be followed up until the outcome is resolved or stable. In case of SAE(s) persisting beyond trial termination, a follow-up visit may be required. If further analyses are required for the evaluation of a potential cause-effect relationship between the investigational product and/or procedures and the AE, all examinations and laboratory analyses will be documented in the (e)CRF or in an attached file.

11 STATISTICS AND DATA ANALYSIS

11.1 Sample size calculation

The sample size calculation is based on the study's primary outcome: performance on a verbal memory task. Given the exploratory nature of the trial, the sample size was informed by effect sizes reported in comparable studies. Two previous trials investigating probiotic interventions versus placebo on verbal memory performance tasks reported moderate effect sizes of 0.60 and 0.67, respectively (Önning et al., 2023; Schneider et al., 2023). While the study populations in those trials were not directly comparable to the population targeted in the present study, they provide a reference point for estimating effect size in the absence of directly relevant prior data. Based on this, a conservative average effect size of 0.63 was selected for power analysis. Using G*Power software (version 3.1.9.7), a sample size calculation was conducted based on an independent samples t-test, with equal group allocation, a one-tailed alpha of 0.05, and 80% power. The resulting required sample size was 64 participants (32 per group) to detect the specified effect size. To account for an estimated attrition rate of 10%, this study will aim to enroll 70 participants.

11.2 Planned recruitment rate

This study aims to recruit a total of 70 participants over two recruitment waves, each lasting

approximately 3 months, for a total recruitment period of 6 months. As no formal feasibility study has been conducted, these estimates are based on anticipated levels of interest and response to similar recruitment strategies used in health research.

It is anticipated that each recruitment wave will generate 20–30 expressions of interest per week. Based on the expected inclusion/exclusion profile, we estimate a screening eligibility rate of approximately 50–60%. Of those eligible, a consent rate of 70–80% is expected. This corresponds to an expected recruitment rate of 3–5 participants per week, with a maximum of 10 per week, per wave.

Assuming a consistent response across both waves, we anticipate completing recruitment within the 6-month timeline. This staggered approach allows for recruitment monitoring and, if needed, protocol refinements or additional outreach strategies between waves. Participant flow, eligibility rates, and retention will be reviewed regularly. Adjustments to recruitment materials or procedures will be made in consultation with the research team if accrual falls below target.

11.3 Statistical analysis plan

11.3.1 General

All statistical tests will be two-sided, with a significance level of $p < 0.05$, unless otherwise specified. Where appropriate, results will be accompanied by 95% confidence intervals and effect size estimates (e.g., Cohen's d, partial η^2). All statistical analyses will be conducted using SPSS (Version 30.0.0).

11.3.2 Summary of baseline data and flow of patients

Baseline data will be summarised descriptively to assess the comparability of treatment groups at randomisation. No formal statistical tests will be conducted on baseline variables. Summary statistics will be presented by treatment group, with an additional "Total" column to reflect overall sample characteristics.

Data will be summarised according to variable type: means and standard deviations (SD) for normally distributed continuous variables, medians and interquartile ranges (IQR) for skewed distributions, and frequencies and percentages for categorical variables. Analyses will be conducted on the treated set (participants who received at least one dose of the investigational product), with additional analysis sets used as appropriate, depending on context.

A CONSORT-compliant participant flow diagram will be provided, detailing the number of participants screened, eligible, consented, randomised, treated, completing follow-up, and included in the final analysis.

11.3.3 Primary outcome analysis

The primary outcome will be accuracy on the CANTAB VRM task at Week 12. The primary analysis will compare the Lab4P and placebo groups at Week 12, adjusting for baseline VRM accuracy.

VRM accuracy at Week 6 and Week 12 will be analysed using a linear mixed-effects model. The

model will include treatment group, time, the treatment group × time interaction, and baseline VRM accuracy as fixed effects, with a participant-level random intercept to account for repeated measurements. The prespecified primary comparison will be the baseline-adjusted between-group difference at Week 12. The corresponding Week 6 comparison will be exploratory.

The Week 12 difference will be reported with a 95% confidence interval and a two-sided p-value. Model assumptions will be assessed using appropriate diagnostic procedures. The significance threshold for the single primary comparison will be $p < 0.05$.

11.3.4 Secondary outcome analysis

Secondary outcome analyses will explore the effect of Lab4P probiotic supplementation compared to placebo on a range of cognitive, sleep, cardiometabolic, vascular, inflammatory, anthropometric, body composition (outlined in section 3.4). These analyses are considered exploratory and hypothesis-generating, as the trial is not powered to detect statistically significant effects in these outcomes.

For secondary outcomes measured at three time points (e.g., cognitive and sleep measures, cardiometabolic data, anthropometric data), change over time will be assessed using linear mixed-effects models with fixed effects for treatment group, time, and their interaction (time × group), and a random intercept for participant to account for repeated measures. Where appropriate, baseline values of the outcome variable will be included as covariates. Non-normally distributed outcomes may be transformed or modelled using alternative techniques (e.g., generalized linear models).

For outcomes measured only at baseline and week 12 (e.g., glucose tolerance, cytokines, DXA metrics), between-group differences in change from baseline will be assessed using delta scores, adjusting for baseline values. Dietary intake data from the food diaries will be summarised and analysed using suitable statistical techniques, selected based on the characteristics of the data and distributional assumptions. For gastrointestinal and systemic symptom occurrence, as measured by the 14-day recall symptom questionnaire, outcomes will be treated as count data (i.e., number of days with each symptom out of 14). Analyses will use generalized linear models with an appropriate distribution (e.g., negative binomial or Poisson, depending on dispersion) to compare symptom frequency between groups at week 12, adjusting for baseline symptom frequency.

Estimates of between-group differences in change over time will be reported as mean differences with 95% confidence intervals, along with associated two-sided p-values. However, formal hypothesis testing will not be emphasised, and no adjustment for multiple comparisons will be applied given the exploratory nature of these analyses.

11.3.5 Microbial Diversity analysis

11.3.5.1 Alpha Diversity

Microbial diversity will be assessed to explore differences in gut microbiota composition between the probiotic and placebo groups. Both alpha diversity (within-sample diversity) and beta diversity (between-sample diversity) metrics will be calculated.

Analyses will focus on species-level profiles and may include standard ecological measures such as Shannon diversity, Bray–Curtis dissimilarity, and ordination techniques (e.g., NMDS). Statistical comparisons between groups will account for relevant covariates including age, BMI, and menstruation status. Multivariate models may be used to examine group-level differences in community structure and dispersion, incorporating appropriate correction for multiple comparisons.

Differential abundance testing will be conducted where appropriate to identify microbial taxa with significantly different relative abundance between groups.

11.4 Subgroup analyses

Exploratory analyses will examine whether the effect of Lab4P compared with placebo varies by sex, ethnicity, or baseline BMI. Effect modification will be assessed using treatment group × time × subgroup interaction terms in mixed-effects models, where the available data support these analyses. Baseline BMI will be analysed as a continuous variable. Participants may also be described by BMI category (25.0–29.9 kg/m² and ≥30.0 kg/m²), but any comparisons within these categories will be exploratory.

These analyses are hypothesis-generating and will be interpreted cautiously given the limited statistical power, particularly where subgroup sizes are small. No adjustment for multiple comparisons is planned.

11.5 Additional exploratory analyses

Additional analyses may be undertaken after examination of the trial data to investigate unexpected findings, assess the robustness of observed patterns, or generate hypotheses for future research. These may include alternative ways of modelling outcomes, associations between changes in microbiota and clinical outcomes, and analyses of participant characteristics that may influence treatment response.

For each additional analysis, the research question, rationale, outcome, population, covariates, and statistical method will be documented. Reports and publications will clearly distinguish analyses specified in this plan from those developed while treatment allocation remained blinded and those undertaken after unblinding or inspection of treatment results. Post hoc findings will be presented with effect estimates and measures of uncertainty where feasible, and interpreted cautiously in light of the number of analyses performed, sample size, and potential for chance findings. They will not replace or alter the prespecified primary analysis or be presented as confirmatory evidence.

11.6 Adjusted analysis

As specified in section 11.3.3, the primary analysis will adjust for baseline VRM accuracy. It will not include other participant-level covariates.

Additional analyses adjusting for factors such as age, sex, or BMI may be undertaken to assess the robustness and precision of the primary result. The covariates and rationale for these analyses will be specified in the statistical analysis plan before treatment allocation is unblinded. Any analyses chosen after unblinding or inspection of treatment results will be identified as post

hoc and interpreted as exploratory.

11.7 Interim analysis and criteria for the premature termination of the trial

No formal interim analysis is planned for this study. However, interim review of recruitment, retention, adherence, and safety data may be conducted by the PI in collaboration with the academic supervisors, where appropriate. These reviews will focus on operational aspects of the study (e.g. participant flow, data completeness, protocol adherence) and will not involve formal statistical comparisons between treatment groups. All reviews will be conducted using blinded unless unblinding is specifically justified and approved by the project supervisor.
There are no predefined statistical stopping rules for efficacy, harm, or futility. However, the PI, in consultation with the project supervisor may recommend pausing or terminating the trial early in the event of:

- Emerging safety concerns or unexpected adverse events
- Significant protocol violations or operational challenges compromising trial integrity
- Poor recruitment or retention making it unlikely the trial can meet its objectives

Any such decisions will be made on the basis of judgment, documented review of available data, and oversight from the supervisory team. In the absence of a formal DMC, ethical oversight will be maintained through ongoing supervisory review and required reporting to the LREC.

11.8 Participant Population

11.8.1 Full analysis dataset

The full analysis dataset (FAS) will include all the randomized subjects, except those that never started the trial, or subjects who failed to satisfy study entry eligibility criteria or had no post-randomization data. The FAS will be analyzed according to the assigned group per randomization. The Full Analysis Set (FAS) will be used for primary and secondary analyses, following the intention-to-treat (ITT) principle.

11.8.2 Per-Protocol analysis dataset

The Per-protocol (PP) analysis dataset will consist of subjects included in the FAS, without any departures from the protocol which are believed to impact the analyses of interest. The subjects with major protocol deviations related to GCP (e.g. ICF process not followed) will not be removed from PP by default. A separate listing of these subjects will be provided in the statistical report. A list of protocol violations that would exclude subjects from inclusion in the PP analysis dataset will be specified during the blind data review meeting. A Per-Protocol (PP) analysis will be conducted as a sensitivity analysis, including only participants who complete the study without major protocol deviations.

11.8.3 Safety analysis set

Safety population includes all randomized subjects that started the trial, classified according to the product received. The Safety Set will be used for safety and tolerability assessments.

11.9 Procedure(s) to account for missing or spurious data

Missing values for the primary endpoint may be imputed or completely handled using maximum

likelihood approaches. Unless another pattern of missingness is demonstrated during the review of the data, missing data will be assumed to be missing at random. Handling of missing values will be refined and finalized during the blind review of the data. Exploration of outliers will be carried out at the blind review of the data. If outliers are detected on any of the endpoints, robust approaches or sensitivity analysis would be used.

11.10 Other statistical considerations.

A detailed SAP will be developed prior to the final analysis. The SAP will be reviewed and, if necessary, revised following a blinded review of the data to address any outstanding analytical considerations. The final version of the SAP will be signed off **prior to unblinding** to ensure the integrity of the analysis.

12. DATA MANAGEMENT

12.1 Data collection tools and source document identification

12.1.1 Case Report Forms

All study data will be captured using structured Case Report Forms (CRFs), which function as the primary tools for recording protocol-specified variables across both on-site and remote visits. CRFs in paper or in electronic (eCRF) format via a web-based application and must be signed and dated by the PI, handwritten or electronic signature as appropriate. Paper CRFs are printed in duplicate and are filled out in black ink. If an entry on a paper CRF requires a change, the correction must be made so that the corrected item remains legible. When eCRFs are used, corrections are recorded in an audit trail and the data are extracted from the eCRF database after final check and signature.

CRFs will be developed in accordance with the visit schedule and outcome measures defined in this protocol. The Investigator and/or designee will accurately, completely, and in a timely manner record data resulting from the execution of the protocol on paper CRFs or eCRFs, respectively). One CRF will be completed for each participant. Data will be entered into CRFs either by direct transcription from source documents or imported from validated electronic systems where applicable. Each data point collected for this study will have a verifiable source.

12.1.2 Source documents

For this study, the following data sources are identified:

12.1.2.1 Investigator's site data

Site-collected data will include anthropometric measurements, anthropometric measurements, point of care cardiometabolic markers, OGTT timepoints and glucose values, and the outcomes of FMD and CVR assessments. Such data will be recorded directly into the eCRF by authorised site staff at the time of collection. In these cases, the eCRF will serve as the source document (see section 12.1.2.5).

Other site assessments, such as sample collection logs and blood sampling details, may initially be recorded on paper or site worksheets and subsequently transcribed into the eCRF. In such cases, the original paper documentation will constitute the source document and must be retained in the participant file for monitoring and audit purposes.

12.1.2.2 Patient reported outcomes

Self-completed questionnaires including the PSQI, demographic and lifestyle questionnaires, and any symptom or adverse event reports, will be collected electronically using the Qualtrics platform. All responses will be linked to pseudonymised participant ID numbers and exported in coded format. The Qualtrics export files will serve as the source documents for these data.

12.1.2.3 External data and laboratory data

Body composition, biochemical and stool samples will be analysed at LBU, Cultech LTD or by an accredited third-party laboratory. All results will be transferred securely in coded format and merged into the clinical dataset. Laboratory reports will serve as the source documents for these data.

12.1.2.4 Cognitive Assessment Data (CANTAB Platform)
All CANTAB task performance data will be automatically recorded by the software and exported in pseudonymised, coded format. These electronic output files will constitute the source data for cognitive outcomes and will be imported into the study database for analysis.

12.1.2.5 Case Report Forms as source Documents
The eCRFs will serve as source documents in specific instances where data are recorded directly by study personnel during the visit, including anthropometric measurements (height, weight, waist and hip circumference), point of care cardiometabolic markers (HbA1c, blood pressure, lipid profile) the OGTT timepoints and glucose values, and the outcomes of FMD and CVR assessments. In these cases, the eCRF will constitute the first and only recording of the data, and thus will be considered the source document. For all other data, the eCRF will not be considered a source document, and the original record (e.g., paper forms, electronic platform exports, or laboratory reports) will serve as the source and must be retained accordingly.

12.2 Data handling and record keeping

12.2.1 Audit trail and eSignature
All electronic data entry systems used in this trial will include audit trail functionality to ensure full traceability of data entry, edits, and corrections. The audit trail will capture the date, time, user ID, and reason for changes made. Where electronic signatures are used (e.g., for consent or data verification), they will comply with HRA standards for eSignatures and data integrity.

12.2.2 Data validation
Data will undergo routine validation checks at the point of entry and during scheduled monitoring visits. Validation procedures include range checks, logic checks, and cross-verification against source documents. Any data queries will be resolved and documented through the data query management system prior to database lock.

12.2.3 Coding

Type of data	Dataset	Dictionary
Adverse Events	AE	MedDRA version 24
Serious Adverse Events	SAE	MedDRA version 24

12.2.4 Database Lock

The database will be locked following completion of data cleaning, resolution of all queries, and final study visits. Once locked, no further changes will be made to the dataset. The research team will only receive access to unblinded treatment codes after database lock to maintain data integrity.

12.3 Access to Data

Access to study data will be limited to authorised personnel involved in the conduct, monitoring, and oversight of the trial. These include the PI, designated research staff, approved monitors, and auditors from LBU or relevant regulatory authorities. All individuals accessing the data must be trained in data protection and trial-specific confidentiality protocols. Access will be managed via user-specific logins with role-based permissions.

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections- in line with participant consent.

12.4 Archiving

Archiving will be authorised by the project supervisor following submission of the End of Trial Report. The PI will retain responsibility for archiving the trial master file, final protocol, participant-facing documents, regulatory correspondence, and the final locked database. The PI will be responsible for archiving the Investigator Site File, signed ICFs, source documents, and completed CRFs.

All essential documents, including the trial database and associated metadata, will be retained for a minimum of five (5) years after completion of the trial, in accordance with GCP and applicable regulations. Records will be stored securely in an access-controlled environment with appropriate conditions for document preservation. No essential documents may be destroyed without prior written authorisation from the project supervisor. The project supervisor will ensure that archiving responsibilities are fulfilled and documented.

13. MONITORING, AUDIT & INSPECTION

Throughout the trial and following its completion, the PI will permit oversight activities by the Sponsor or its designated representatives. This may include trial monitoring, audits, and regulatory inspections.

Monitoring may be conducted periodically by the project supervisor and/or university-appointed personnel to review the progress of the trial, verify the accuracy and completeness of (e)CRFs, and ensure compliance with the protocol, GCP guidelines, and applicable regulations. The PI is responsible for ensuring (e)CRFs are completed regularly and are up to date before each monitoring activity.

Audits may be conducted by internal university representatives or external auditors acting on behalf of the Sponsor. The aim of an audit is to evaluate trial conduct, compliance with GCP and Sponsor procedures, and the integrity of the trial data. The PI and study team are expected to be available during audits, cooperate fully, and provide access to source documents, (e)CRFs,

and other relevant trial documentation. The PI will be contacted in advance to schedule any audit visit.

Regulatory Inspections may be carried out by government authorities, research ethics committees, or institutional bodies (e.g., MHRA, LREC). These inspections serve to independently assess trial conduct, documentation, and facilities. The PI and relevant staff must be available and provide full access to all necessary trial records. In the event of prior notice of an inspection, the PI must notify the Sponsor immediately.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Research Ethics Committee (REC) review & reports

Prior to the commencement of the trial, ethical approval will be sought from the appropriate Local Research Ethics Council (LREC) at LBU. This review will cover the trial protocol, PIS, ICF, and all other relevant documents, such as recruitment advertisements. No substantial amendments to the protocol or supporting documentation will be implemented until the LREC has granted a favourable opinion.

All correspondence with the LREC will be retained in the Trial Master File (TMF), in accordance with LBU's research governance procedures. The PI will notify the LREC of the end of the trial. If the trial is ended prematurely, the PI will provide notification to the REC, including a statement outlining the reasons for early termination. Within one year of trial completion, a final report will be submitted to the REC, detailing the study results and including any relevant publications or abstracts.

14.2 Regulatory Compliance

This trial will be conducted in compliance with the International Conference on Harmonization (ICH) guidelines and the ethical principles that have their origin in the Declaration of Helsinki and its subsequent amendments (World Health Organization, 2005; World Medical Association, 2014). The study will also adhere to the UK Policy Framework for Health and Social Care Research (Health Research Authority, 2025), the Data Protection Act 2018, and the UK GDPR. Initiation of the trial is contingent upon receipt of all required regulatory approvals, including a favourable opinion from the LREC.

As the study involves exposure to ionising radiation through iDXA, it falls under the scope of the Ionising Radiation Medical Exposure Regulations 2017 (British Institute of Radiology, 2015). All DEXA procedures will be conducted in accordance with IRMER standards. The protocol has been reviewed by a Medical Physics Expert (MPE) and a Clinical Radiation Expert (CRE), who have confirmed the justification, optimisation, and safety of the proposed radiation exposure. Supporting documentation has been submitted as part of the LREC application.

14.3 Protocol compliance

The trial will be conducted in full compliance with the approved protocol. No deviations will be made without prior approval, unless necessary to eliminate an immediate hazard to a participant. In the event of a medical emergency requiring a protocol deviation, the PI will use

clinical judgment to protect the participant's safety and will promptly inform the supervisory team of the incident. If, in their judgment, the deviation warrants further action, the matter will be escalated to the LREC in accordance with institutional reporting requirements. All such decisions and actions will be documented and retained in the TMF.

14.4 Notification of Serious Breaches to GCP and/or the protocol

Serious breaches will be managed in accordance with regulatory requirements and LBU policy. A “serious breach” is defined as a breach likely to significantly affect either the safety or physical/mental integrity of trial participants, or the scientific value of the trial. In the event of a suspected serious breach during the conduct of the study, the academic supervisory team will be notified immediately. The supervisory team will assess the breach and, where applicable, notify the LREC in writing within seven calendar days of becoming aware of the incident. Notification will be made if the breach concerns either the conditions and principles of GCP or the approved trial protocol, including any amendments. Documentation of the breach, assessment, and subsequent actions will be maintained in the TMF.

14.5 Data protection and patient confidentiality

The PI and all designated research staff will comply with all applicable data protection laws, including the UK GDPR and the Data Protection Act 2018. The research team will implement and maintain appropriate technical and organisational measures to protect personal data against unauthorised or unlawful processing, and against accidental loss, destruction, damage, alteration, or disclosure.

All research team members involved in the processing of personal data will have completed mandatory data protection training as required by LBU. They will be bound by institutional confidentiality agreements or statutory obligations of confidentiality and will only process personal data as necessary for the conduct of the study.

Personal data will be stored securely on LBU's password-protected servers or encrypted drives, with access limited to authorised members of the research team. Any physical documents (e.g. signed ICFs) will be stored in locked cabinets in secure locations on university premises.

14.5.1 Subject data’ rights

Participants will be informed of their data protection rights in the PIS, including their right to access, rectify, or request deletion of their data. No personal data will be transferred outside the UK without prior approval from the University and in full compliance with data protection legislation. The investigator or designee will implement and maintain appropriate technical and organizational measures to ensure accessibility to subjects of their personal data and their results of biological analysis collected during the study. Upon request, the investigator or designee will ensure to provide extracts of personal data to subjects. The investigator or designee will implement appropriate procedures to immediately inform the project supervisor about any subject’s request to rectify or delete their personal data or biological samples during the course of the trial.

14.5.2 Confidentiality

The PI will ensure protection of subject’s personal data and that all reports, publications, subject

samples and any other disclosures, except where required by laws. Subjects are identified only by a subject identification number to maintain subject confidentiality. All subject trial records will be kept safely in an access-controlled area. Identification code lists linking subject names to subject identification numbers will be stored separate from subject records. In case of data transfer, the study team will maintain high standards of confidentiality and protection of subject personal data. Clinical information will not be released without the written permission of the subject, except for monitoring by Regulatory Authorities or the trial Sponsor.

The PI will not engage any external third party to process personal data on behalf of the project without formal approval from LBU and without ensuring that appropriate data processing agreements are in place.

14.5.3 Data Breaches reporting

In the event of an actual or suspected data breach, including any unauthorised access to, or loss, disclosure, or destruction of personal data, the PI must report the incident to the University's Data Protection Officer within 24 hours by calling 0113 812 7542 or emailing DPO@leedsbeckett.ac.uk. The research team will cooperate fully with the University's breach response protocol, including any required actions to mitigate harm, restore data, and prevent recurrence.

Where the PI or research team receives any request, complaint, or inquiry related to data protection, such as subject access requests or concerns about data handling, this must be referred immediately to the University's Data Protection Officer.

14.5.4 Retention of Data

The PI will maintain adequate trial records including (e)CRFs, laboratory reports, original signed ICFs, investigational product disposition records, safety reports, information regarding subjects who discontinued, and other pertinent data such as correspondence with the LREC and administrative documents exchanged with the research team. Records may be stored in either paper or electronic format. The PI must retain all trial records for five years, the maximum period required by LBU, unless otherwise notified.

Biological samples collected during the trial will be retained for one year unless participants have agreed to a longer storage period.

To avoid any possible errors, the PI must contact the project supervisor prior to the destruction of any trial records. The PI must also notify the project supervisor immediately in the event of accidental loss or destruction of any trial records.

14.6 Financial and other competing interests

None

14.7 Indemnity

This trial is sponsored by LBU, which holds appropriate insurance to cover liabilities arising from the design and management of the study, provided the protocol is followed. The PI is covered under the institution's professional indemnity and clinical trials insurance. This includes cover for negligent harm arising from the conduct of the trial, where standard procedures are followed.

14.8 Amendments

Any permanent changes to the protocol will be formalized in an amended protocol which must be approved by the Research supervisor and, if substantial as defined in directive 2001/20/EC, submitted for approval to the LREC, prior to implementation. If the amendment results in a modification of trial treatment or subject assessments, a new version of the informed consent must be prepared and submitted for approval to the IRB. All protocol amendments will be documented and tracked in the protocol amendment log (Appendix B).

14.9 Post trial care

As the investigational product is already commercially available, participants will not receive continued access to the product from the research team after the trial concludes. However, participants will be informed, both during the informed consent process and at the end of the study, about how and where they can purchase the product should they wish to continue its use, based on discussions with their healthcare provider.

No additional funding arrangements are in place for post-trial provision of the investigational product. All participants will return to standard NHS care following trial participation. Should any clinically relevant findings emerge during or after the trial that may affect participants' ongoing care, they and their healthcare providers will be appropriately informed.

14.10 Access to the final trial dataset

Access to the full trial dataset will be restricted to the PI and their academic supervisors at LBU, who are responsible for the design, conduct, and analysis of the study. These individuals will oversee data integrity, statistical analysis, and reporting.

As this is a single-centre academic study, no formal restrictions apply to site-specific data access. However, should any additional investigators or collaborators be identified later in the trial, access to the full dataset will be granted only with documented approval from the Chief Investigator and academic supervisory team, following submission of a formal request outlining the intended use of the data.

15 DISSEMINIATION POLICY

15.1 Dissemination policy

The data arising from this trial will be owned by the Sponsor, LBU. Upon completion of the trial, the data will be cleaned, analysed, and tabulated. A Final Trial Report will be prepared by the PI under the supervision of the academic supervisory team. This report will form part of the PI's PhD thesis and may also contribute to peer-reviewed publications and conference presentations.

Cultech, the commercial partner in this study, will have publication rights and will be acknowledged in all outputs arising from the trial. All publications and public dissemination of trial data will be shared with Cultech prior to submission, to allow for review and comment, but not to restrict or unduly delay publication. LBU and Cultech will collaborate to ensure timely and accurate communication of results.

Participating investigators will not have independent rights to publish trial data without the written consent of the Chief Investigator and academic supervisory team. Any publication

requests will be reviewed to ensure they do not compromise the integrity or timing of the main study results. There are no embargo periods or restrictions currently in place, though the primary publication will precede any secondary analyses.

Publications will adhere to the CONSORT guidelines and checklist to ensure that reporting meets the standards required for submission to high-quality peer-reviewed journals (http://www.consort-statement.org/).

Participants will be informed of the overall results of the trial after the main findings have been published or the Final Trial Report completed. This may be achieved through a lay summary disseminated via a newsletter, email, or study website, depending on participant preferences. Participants will also have the option to request a copy of the results from the PI. Individual-level results will not be disclosed, but summaries of study findings will be available on request after final analysis.

Subject to ethical and data protection considerations, the trial protocol, anonymised participant-level dataset, and statistical analysis code may be made publicly available following publication of the main findings. These materials will be hosted in the open-access LBU Research Data Repository, in line with institutional and funder policies. Access may require a formal data-sharing agreement, and details will be included in the published article.

15.2 Authorship eligibility guidelines and any intended use of professional writers

Authorship of the final trial report and any resulting publications will be determined in accordance with the criteria established by the International Committee of Medical Journal Editors (ICMJE). Individuals will be granted authorship if they have made substantial contributions to: (1) the conception or design of the study, or the acquisition, analysis, or interpretation of data; (2) drafting the manuscript or revising it critically for important intellectual content; (3) final approval of the version to be published; and (4) agreement to be accountable for all aspects of the work and its integrity.

The PI will serve as lead author on the final trial report and primary publications, under the supervision of academic supervisors at LBU, who will also qualify for authorship based on their involvement in study design, oversight, and analysis. Representatives from Cultech may be included as co-authors where they meet the ICMJE criteria. All contributors who do not meet these criteria will be acknowledged appropriately in line with journal policies. Group authorship (e.g., on behalf of the Trial Team) may be used where appropriate and will be determined in advance of manuscript submission, particularly if additional collaborators contribute meaningfully to the conduct of the trial.

No professional medical writers will be employed for this trial. Should this change, the involvement and funding source for any professional writing support will be transparently disclosed in the relevant publications.

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17 APPENDICIES

17.1 Appendix A: Schedule of Assessments

		Timepoint	-1	0	1	2	3	4
		Week	-4 to-1	-1	0	6	11	12
		Window (days)	≤30 before screening	0	±7	±7	±7	±7
Activity	**Estimated duration (minutes)**							
Pre-screen	10		X					
Informed Consent	10			X				
Eligibility Screen	5			X				
Randomisation	N/A				X			

Cognitive Testing Familiarisation	20			X				
Anthropometric Measurements	5			X		X	X	
Blood Pressure Assessment	2			X		X	X	
Fasting Blood Glucose Test	2			X		X	X	
HbA1c Testing	2			X		X	X	
Lipid Assessment	2			X		X	X	
Fasting Venous Blood Collection	4			X		X	X	
Oral Glucose Tolerance Test	120			X			X	
PSQI	3							X
Demographics and lifestyle Questionnaire	6				X			
Dietary Habit Diary	N/A				X			X
Cognitive Testing (CANTAB)	40				X	X		X
Doppler Ultrasound FMD and CVR	35				X			X
DXA Scanning	15				X			X
Stool Sample Collection	N/A				X			X
Health events and physical experiences questionnaire	3					X	X	X
Adherence check	N/A					X	X	X
Participant Compensation	N/A							X

17.2 Appendix B: Amendment History

Amendment No.	Protocol version No.	Date issued	Author(s) of changes	Details of changes made
1.	1.1	28/08/ 2025	Lewis Hepburn	Midpoint assessment revised from an online virtual visit to an in-person laboratory visit. Additional procedures introduced, including anthropometric measurements (height, weight, waist/hip circumference), cardiometabolic assessments (resting blood pressure, fasting capillary blood samples for glucose, HbA1c, and lipid profile), and venous blood collection. All relevant sections updates. Details of stool sample processing updated to reflect use of FTA card sampling.
2.	1.2	20/03/ 2026	Lewis Hepburn	The eligibility criteria have been amended to expand the upper age limit for participant inclusion from 35 years to 40 years. The revised age range is therefore 18–40 years. This amendment was introduced to improve recruitment feasibility while maintaining a study population that is appropriate for the research objectives. Individuals within this age range are generally considered to represent a metabolically healthy young adult population with relatively low prevalence of age-related metabolic disease, thereby preserving the scientific rationale for studying mechanisms in healthy adults. Expanding the upper age limit by five years is not expected to introduce meaningful differences in cognitive or metabolic physiology that would compromise interpretation of the study outcomes. The amendment therefore supports improved recruitment while maintaining alignment

				with the study's scientific aims and target population.
2.	1.2	20/03/2026	Lewis Hepburn	The CVR protocol has been updated to use a standardized breath-hold paradigm in place of externally administered CO_2. Participants will complete a 1-minute normocapnic baseline followed by a 30-second breath hold with nasal occlusion, with exhalation at the end of the maneuver to capture peak $EtCO_2$. Continuous MCA velocity and $EtCO_2$ will be recorded throughout. CVR will be calculated as the percent change in MCA velocity from baseline to peak divided by the change in $EtCO_2$ (%/mmHg). This modification improves feasibility and tolerability while maintaining a physiologically valid hypercapnic stimulus and aligning the analysis approach with the stimulus.
2.	1.2	20/03/2026	Lewis Hepburn	Addition of platelet activity as an exploratory outcome, assessed using venous blood samples already being collected. No changes to participant procedures, burden, or risk. For participants already enrolled, only those who have provided explicit consent for additional analyses of their blood samples will be included; those who have not will be excluded from this component. All newly recruited participants will be consented for this analysis as part of standard study procedures.
3.	1.3	18/08/2026	Lewis Hepburn	Pre-screening procedures revised to allow eligibility assessment to be conducted via an online questionnaire, telephone assessment with a member of the research

				team, or a combination of these methods. The online pre-screening questionnaire has been shortened to collect initial eligibility information and participant contact details, with more detailed eligibility questions able to be completed by telephone. This change allows participants to seek clarification from the researcher where required and supports more accurate assessment of eligibility. Relevant protocol and participant-facing documents updated accordingly.
4.	1.4	23/09/2026	Lewis Hepburn	The upper BMI eligibility limit of 29.9 kg/m² has been removed. Adults with a BMI above 25 kg/m², including those with obesity, may now participate if they meet the remaining eligibility criteria. This amendment is intended to improve recruitment while retaining a population relevant to the study's investigation of metabolic dysregulation. Baseline BMI has also been added to the exploratory subgroup analyses to assess whether treatment effects vary across the expanded BMI range.
4.	1.4	23/09/2026	Lewis Hepburn	Impaired glucose tolerance has been removed as an eligibility requirement. Participants who meet the remaining criteria will no longer be excluded on the basis of their glucose tolerance status. This amendment is intended to improve recruitment and will broaden the range of baseline metabolic characteristics represented in the study.
4.	1.4	23/09/2026	Lewis Hepburn	The participant incentive has been increased from £100 to £150 to better

				reflect the time and effort required to complete study assessments and to support recruitment and retention. The revised amount has been incorporated into the Participant Information Sheet and recruitment materials. The increase can be accommodated within the existing study budget.
4.	1.4	23/09/2026	Lewis Hepburn	Ambiguity in the original description of the primary analysis prompted revisions to sections 11.3.3 and 11.6. The revised sections specify that the primary comparison is the difference in CANTAB VRM accuracy between the Lab4P and placebo groups at Week 12, adjusted for baseline VRM accuracy. In the mixed-effects model, Week 6 and Week 12 accuracy are repeated outcome measurements, while baseline accuracy is a covariate. The Week 6 group comparison is exploratory. Section 11.6 also clarifies that analyses adjusting for additional participant characteristics will be reported separately.