FULL/LONG TITLE OF THE STUDY

*Investigation of short and intermediate term effects of a **s**upplement mix designed to **t**arget **a**geing **m**echani**s**ms o**n** v**a**scular function in healthy middle-aged participants (STAMINA Study)*

SHORT STUDY TITLE / ACRONYM

*Effects of a **s**upplement to **t**arget **a**geing **m**echani**s**ms o**n** v**a**scular function (**STAMINA** Study)*

PROTOCOL VERSION NUMBER AND DATE

V5.0 23/02/2024

NCT number

NCT06145087

STUDY SUMMARY

Study Title	*Investigation of short and intermediate term effects of a **s**upplement mix designed to **t**arget **a**geing **m**echani**s**ms o**n** v**a**scular function in healthy middle-aged participants (STAMINA Study)*
Internal ref. no. (or short title)	STAMINA
Planned Size of Sample (if applicable)	60
Planned Study Period	01/12/2022 – 01/11/2024

FUNDING AND SUPPORT IN KIND (Optional)

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this study)	**FINANCIAL AND NON FINANCIAL SUPPORT GIVEN**
AgeLessSciences, a Public Benefit Corporation, Kris Verburgh, PhD CEO email: kris@novoslabs.com	Charitable donation to Prof. Heiss plus provision of supplement and placebo

INVESTIGATORS

NAME	**Position**	**Signature (optional)**
Prof. Christian Heiss	**Professor of Cardiovascular Medicine**	
Dr. Danuta Sampson	**Visiting research fellow**	
~~**Mr. Abdullah Durrani**~~	~~**PhD student**~~	
Dr. Mariam Bapir	~~**PhD student/**~~**post-doctoral researcher**	
Mr. Charles Piercy	**PhD student**	
Ms. Kenza Sackho	**PhD student**	

STUDY PROTOCOL

1. Abstract

In our ageing society, extending the healthy lifespan is a major challenge and a healthy diet may play an import role in maintaining health throughout life. With increasing age cardiovascular function declines and large and small blood vessels change in various and complex ways and these changes may lead to many age-related diseases. On a molecular level, there are many mechanisms that are associated with ageing including cellular senescence, loss of proteostasis, altered cellular communication, genomic instability, epigenetic alterations, telomere shortening, deregulated nutrient sensing, stem cell exhaustion and protein crosslinking. Animal and human studies suggest that dietary supplements may be able to affect these mechanisms. What the effect of the NOVOS supplement is on cardiovascular functions is not known.

The aim of the present study is to investigate the short and intermediate term effects of a **s**upplement mix designed to **t**arget ageing **m**echan**i**sm o**n** v**a**scular function in healthy middle-aged subjects. (STAMINA Study) We hypothesise that the supplement will lead to acute and sustained changes in biomarkers of vascular function and health.

We will recruit 60 healthy middle-aged people and randomly assign them to either daily intake of the NOVOS supplement (n=30) or placebo (n=30) for 6 months. The supplement (NOVOS) is a commercially available product and has 13 components. It was developed and is provided together with the placebo by AgeLess Sciences LLC, a Public Benefit Corporation. The study will require 2 visits by participants during which non-invasive vascular exams will be performed, venous blood taken and spot urine sample collected (Visit 1: 3 h, visit 2: 60 min). The primary endpoint is change in flow-mediated dilation, secondary endpoints are change in blood pressure, cholesterol, arterial stiffness, microvascular function cardiovascular risk SCORE and daily walking distance. Tertiary endpoints are changes in biomarkers of ageing as assessed in blood samples including DNA damage. In addition, we will assess anxiety, depressive feelings, happiness, well-being and the diet with several questionnaires. After consent, measurements will be taken on the first day before and 2 hours after ingestion of the first supplement or placebo. Participants will consume the supplement or placebo for 6 months and vascular exams and one blood draw will be repeated during the final visit. During the time, participants will receive 2 phone calls to improve compliance.

2. Background or rationale of the project

In our ageing society, extending the healthy lifespan is a major challenge and a healthy diet may play an import role in maintaining health throughout life. We know that with increasing age cardiovascular function declines and that large and small blood vessels change in various and complex ways and that these changes are also associated with the development of many age-related diseases. While vascular ageing is a key determinant of healthy ageing overall, a reason for the complexity of vascular ageing is that there are many molecular mechanisms involved. Animal and human studies suggest that dietary supplements may be able to affect these mechanisms.

According to the classical free radical theory of ageing, production of endogenous oxygen radicals results in cumulative damage to the genetic information (DNA) in cells that line the blood vessels, endothelial cells, which is not efficiently repaired. Accumulation of unrepaired DNA damage may then lead to accumulation of not properly functioning cells that cannot divide anymore (senescent cells) but cause inflammation (‘inflamageing’). More recently it is appreciated that ageing is more complex, and the hallmarks of ageing include genomic instability, telomere attrition, epigenetic alterations, loss of

proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.[1,2]

A large body of research indicates that many of these processes can be modulated by various substances, including natural ingredients, micro-nutrients, and substances present in the human body and of which concentrations decline during aging.[2] For instance novel evidence suggests that molecules that are present in fruits and vegetables – polyphenols – can reduce the risk or slow down the progress of aging-related diseases such as cardiovascular disease,[3] Alzheimer's disease, macular degeneration, and cancer.[4] Other substances with proven effects individually include alpha-ketoglutarate,[5-9] glucosamines, glycine,[10-15] nicotinamide mononucleotide,[16-18] theanine,[19-21] magnesium[22-24] and ascorbic acid. Overall the results for most of these isolated nature-derived and nutritional bioactives have shown both in humans and mice that vascular function and genetic stress response pathways can be improved in both young and old over hours to weeks.[25,26] However, we do not know if consumed over longer periods of time or even throughout life, this would affect the gradual ageing of blood vessels over months and years. While these studies with individual components are promising, combining components that promise to act on different mechanisms implicated in the ageing process and have synergistic effects seems like a more effective way to target ageing than focusing on one. How this combined approach affects vascular function and health parameters is not known.

The aim of the study is to assess aging molecular pathways and biomarkers while consuming a supplement with a combination of components to address multiple molecular pathways of aging simultaneously.

3. Patient/Participant involvement

We have discussed our vascular ageing research including development of monitoring devices, research into potential interventions including dietary supplements and planned studies in the Surrey Healthy Ageing Research Partnership (SHARP). Most of the (lay) participants have indicated that they would be keen to participate in a future study and provided contact information. We are planning another PPI event soon to explain and promote the study.

4. Aims and objectives

The aim of the present study is to investigate the short and intermediate term effects of a ***s****upplement mix designed to* ***t****arget ageing* ***m****echani****s****m o****n*** *v****a****scular function in healthy middle-aged subjects (STAMINA Study).* We hypothesise that the supplement will lead to acute and sustained increases in vascular function.

5. Benefits of the study

The benefits to the participants will include better understanding of their current stage of vascular health and biological vascular age. The comprehensive information on the participants vascular function and health is not generally available in the NHS and quite expensive, if at all available, in the private sector and includes endothelial function, arterial stiffness/age, cholesterol, glucose and inflammatory markers. In addition, it will provide information on how these progress over half a year. Half of the participants will benefit from being provided with high quality supplement mix worth each approximately £500. In terms of knowledge generation, it will provide first data on what the combined effect of a supplement mix will be on vascular biomarkers that are associated with vascular ageing. The data will help to evaluate the potential of the supplement mix to modulate vascular function and plan future studies.

To contribute to participant's travel expenses and time we offer £50 after completion of the study. For this, we will collect bank details which will not be share outside the study team. The bank details do not need to be provided this if the participant opts out and prefers to not receive it.

The expenses will be submitted by Mariam Bapir to visitorexpenses@surrey.ac.uk and bank transfers processed by Faculty Finance. The bank details (recipient's name, sort code, account number) will only be used to be filled into the expense form. As soon as visitorexpenses@surrey.ac.uk confirm the expense have been processed, the study files with bank details and emails/sources with the bank details will be deleted.

6. Recruitment Methods

Recruitment will be via word to mouth, posters/advertisements at the university, social media posts and PPI event organised together with the Surrey Healthy Ageing Research Partnership. See attached posters and social media posts.

7. Adverse Publicity

We would not expect any adverse publicity. Rather the opposite as this study will help to understand if and how supplements affect vascular ageing and health.

8. Informed Consent and Withdrawal of Consent

Potential participants will be contacted, or they will contact us by telephone or email in response to word to mouth, posters or social media posts. We will explain the study briefly and provide the participant information material and consent form by mail or email. On the first visit, we will provide further information and answer questions the participants may have. After written informed consent is obtained, the participants will be screened for inclusion and exclusion criteria. If they fit with the criteria, they will be included, and we will then perform baseline measurements.

Inclusion criteria:

- Generally healthy. (Note: those with stable conditions that do not interfere with the study objectives/procedures will be included at the PI discretion).
- >40 years
- BMI >20 kg/m^2
- Systolic blood pressure >=120 mmHg
- Smartphone with step counter

Exclusion criteria:

- Symptoms of acute infection
- Cardiac arrhythmias
- Active malignancy
- Clinical signs or symptoms of unstable cardiovascular disease (coronary artery disease, lower extremity artery disease, cerebrovascular disease). These include angina pectoris, dyspnoea, palpitation, syncope, claudication, active vasoactive medication.
- Women who have been pregnant in the last three months, currently pregnant, preparing to become pregnant during the study, or breastfeeding.

- Those that have undergone a change in hormone-based therapies such as, but not limited to, oral contraceptive pills or progesterone pills within the last 2 months. Progesterone releasing IUDs are considered hormone-based therapy. Spironolactone is not considered a hormone-based therapy.
- Subjects who are unwilling or unable to comply with the requirements of the protocol.
- Subjects who have a history of or a current psychological illness or condition that would interfere with their ability to understand and follow the requirements of the study in the opinion of the principal investigator.
- Subjects with or who have recently experienced traumatic injury, infections, or major surgery at the discretion of the qualified investigator.
- Subjects who are likely to start taking drugs/medication on a continuous basis or that will undergo surgery during the trial.
- Subjects engaged in donation or who were recipients of blood products within 90 days before the start of the study.
- Subjects with alcohol use of more than 2 alcoholic beverages per day within the past month.
- Subjects participated in a clinical trial with a medicinal, supplemental, nutraceutical or drug within the past two months prior to the first dose in the current study.
- . If participants take fish oil supplements, they will be asked to stop taking these supplements 21 days before the start of trial and during the trial.
- Subjects using anti-inflammatory drugs on a chronic basis (e.g. aspirin, ibuprofen, diclofenac, celecoxib, etc).
- Subjects using supplements or substances present in formulation (malate, fisetin, glucosamine, alpha ketoglutaric acid, glycine, theanine, Rhodiola rosea, hyaluronic acid, ginger extract, pterostilbene, lithium). Participants will be asked to stop taking these supplements 21 days before the start of trial and during the trial.
- Subjects using supplements or substances similar as those used in the formulation (e.g. resveratrol (similar to pterostilbene) or quercetin (similar to fisetin). Participants will be asked to stop taking these supplements 21 days before the start of trial and during the trial.
- Subjects taking NAD boosters, like NMN, nicotinamide riboside (NR), high-dose niacin (nicotinic acid), high-dose vitamin B3, high-dose nicotinamide (niacinamide) within 21 days before the start of the trial. Participants will be asked to stop taking these supplements 21 days before the start of trial and during the trial. .
- Subjects with clinically significant abnormal laboratory results at screening.
- Known allergy/intolerance to any of the components in the supplement product.

Consent can be withdrawn at any time. In this case, all personal and research data will be deleted. After data have been anonymised, only personal data will be deleted and it will not be possible to delete research data after this point.

9. Experimental design, data collection and methods (including data analysis)

In order to assess the vascular effects of the supplement mix, we will perform a 2 armed parallel group, randomised, controlled, double blind clinical trial. Healthy subjects will be recruited and after reviewing a participant information sheet and signing the informed consent document randomised to either the supplement or placebo and consume it daily over 6 months (3-6 months ~~±1month~~ to ensure completion of study and allow flexibility for participants). The randomisation will be based on a sequential list with equal numbers of treatments A and B, with one being placebo and one verum. Recruited participants

will also be numbered sequentially and receive the respective treatment (A or B). The containers of treatments will be labelled with either A or B. The identity of what was A and what was B will be stored in a sealed envelope in a locked cabinet in Prof. Heiss's office. The measurements will take place in the morning, after an overnight fast and in the fasting stage, before and at 2 hours after the first dose and once in the morning fasted at 6 months. After measurements are completed, participants will be offered a breakfast snack and drink. Venous blood samples (<50 ml) will only be taken once on each study day.

The **primary endpoint** will be a change endothelial function as assessed by flow-mediated dilation (FMD) with ultrasound on the brachial artery.

Secondary endpoints will be change in blood pressure and arterial stiffness as determined by an upper arm cuff and optical sensors and ECG. Additional secondary endpoints are carotid artery stiffness, intima-media thickness as assessed by carotid artery ultrasound and microvascular structure and function with optical coherence tomography angiography (OCTA) as assessed in parallel with FMD measurements on the arm. As additional secondary endpoints, we will assess blood biomarkers of cardiovascular health including blood lipids (fasting total cholesterol, fasting LDL, fasting HDL, ApoB, fasting triglycerides, and fasting insulin) and glucose control (fasting glucose and HbA1c). A change in cardiovascular risk score will be another secondary endpoint. We will used the Heart SCORE2 estimate 10-year risk of a myocardial infarction, stroke or cardiovascular death, based on age, sex, smoking habits, systolic blood pressure and non-HDL cholesterol (https://www.escardio.org/Education/Practice-Tools/CVD-prevention-toolbox/HeartScore). A pre-specified subgroup analysis will be performed in people with intermediate-high (<1% estimated 10-year cardiovascular risk) and high (>5% estimated 10 year cardiovascular risk) baseline CV risk. We expect more pronounced effects of the supplement in people with higher risk i.e. higher blood pressure or cholesterol at baseline. **Exploratory tertiary endpoints** will include blood biomarkers of inflammation (high sensitivity C-reactive protein, TNF-alpha receptor II, Interleukin 6), platelet reactivity, DNA damage (measured via γH2AX in blood and 8-hydroxydeoxyguanosine in urine), ageing (growth differentiation factor 15, adiponectin, leptin), liver (transaminases) and kidney function (creatinine, cystatin C). We also want to explore novel biomarkers of aging, including epigenetic and transcriptomic aging clocks (on DNA samples), RNA and protein expression. In this regard, we will ask participants to allow us to store surplus blood, serum, plasma, RNA and DNA samples for future studies. To explore potential effects on dental health, we will take a digital photo of the teeth. Funding for the analyses needs to be obtained separately in the future. Sample processing and storage will comply with HTA regulations (see attached STAMINA_Human Tissue Governance Application Form V1.0.docx). Most blood and urine biomarkers will be analysed by Affinity Biomarker Lab. Governance including MTA is currently being prepared. The measurements (except for venous blood draws) will be entirely non-invasive, pain-free and require a single session of approximately 3 hours on the first day and 1 hour at 6 (3-6) months. Additional exploratory endpoints will be physical activity based on step/distance covered software on participants cell phone if they have this, dietary habits as assessed by food frequency questionnaire and 24 food recall and quality of life based on standard SQL questionnaire, and the completion at the start and end of the study of 8 questionnaires that assess anxiety, depressive feelings, happiness and well-being, given various substances in the formulation could also impact brain health and could contribute to improved cognitive and mental functioning, such as magnesium, glycine and Rhodiola rosea. All questionnaires are in the appendix. Pre-specified subgroup analyses will be performed in people with healthier diet, estimated high polyphenol intake at baseline and very high physical activity. These people may exhibit a smaller effect of the supplement.

Composition of supplement and placebo

The supplement mix is supplied as a powder to be mixed in water or other beverages like tea or coffee. The powder contains 12 ingredients, and it is commercially available (see also https://novoslabs.com/). All components are also part of a natural diet or are found naturally in the body. The supplement and matched placebo will be provided by AgeLess Sciences.

The composition is detailed in the table below. The placebo looks and tastes similar but does not contain the 12 supplements ingredients, only excipients and flavours.

Participants will receive a jar containing powder (supply of 6 months) with a spoon. Participants add one spoon of powder to 1 glass of water (240 ml), mix it and immediately drink it in the morning with breakfast. The participants will receive a paper diary to record at what time the supplement was consumed and if alone or with breakfast. If a dose is missed in the morning, they should just take it once they remember during the same day. If on a day the dose is missed entirely, we ask the participants to document this and carry on with the same amount on the next days but not take more than usual.

Table 1: Composition of one serving size (15 g) of supplement mix and placebo.

	VERUM Supplement mix	PLACEBO
Vitamin C (ascorbic acid)	100 mg	0
Magnesium (as magnesium malate)	304 mg	0
Calcium (as calcium alpha-ketoglutarate)	231 mg	0
Glycine	2,000 mg	0
Malate (as magnesium malate)	1,700 mg	0
Calcium alpha-ketoglutarate	1,100 mg	0
GreenGrown® glucosamine sulfate (vegan)	1,000 mg	0
Rhodiola rosea root extract (3% rosavins and 1% salidrosides)	300 mg	0
L-Theanine	150 mg	0
Hyaluronic acid	100 mg	0
Fisetin	100 mg	0
Organic ginger root extract (2% gingerols)	100 mg	0
Pterostilbene	50 mg	0
Lithium aspartate (providing 1 mg lithium)	20 mg	0
Other ingredients: Erythritol, malic acid, natural flavours, sodium bicarbonate, silicon dioxide, calcium silicate, stevia	X	X

“Overdosing” and adverse events

The recommended dose of the study supplement is 15 g which contains for example 100 mg Vit. C (approx. recommended daily intake 100 mg) and Lithium 1 mg (average daily intake). It is unlikely that participants will consume above the recommended dose as the supplement is insoluble in water if added in amounts above 15 g. However, to mitigate the unlikely occurrence of overconsumption, the following mitigation plan will be followed:

- A diary will be completed to document the ingested amount every day and advise on not to take more than recommended.

- Training on preparing the solution will be conducted by the investigator staff on the first study day

Participants will be advised to call the investigator if they have consumed above the recommended dose or if any adverse event independent of dose occurs. The investigator will carefully assess any adverse events and advice accordingly and perform unblinding if required.

Sample size justification

The sample size is based on the primary endpoint FMD and the key secondary endpoints pulse wave velocity (PWV) and microvascular diameter.

Our previous experiments have shown average **FMD** values of 6% with standard deviation of 1%. While the supplement has not been tested in terms of effects on FMD, we expect a change in 1%. To detect this, we will need to study 17 verum subjects and 17 placebo subjects to be able to reject the null hypothesis that the population means of the verum and placebo groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

Our previous experiments have shown average **PWV** values of 9.3 m/s with the standard deviation of 1.3 m/s. While the supplement has not been tested in terms of effects on PWV, we expect a change in PWV of 1 m/s. To detect this, we will need to study 28 verum subjects and 28 placebo subjects to be able to reject the null hypothesis that the population means of the placebo and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

Our previous experiments have shown on average of **microvascular diameter using OCTA** of 48 μm with standard deviation is 6.4 μm. While the supplement has not been tested in terms of effects on microvascular diameter, we expect a change in diameter of 5 μm. To detect this, we will need to study 27 verum subjects and 27 placebo subjects to be able to reject the null hypothesis that the population means of the verum and placebo groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

Taken together, and taking 5-10% attrition into account we plan to recruit a total sample size of 60 (30 verum and 30 placebo).

Table 2: Schedule of events

Procedures	**Visits (insert visit numbers as appropriate)**

	Screening (Visit 1)	**Baseline (Visit 1)**	**Month 2 (Phone)**	**Month 4 (Phone)**	**6 (3-6~~±1~~) Months (visit 2)**
Informed consent	x				
Screening/medical history	x				
Randomisation		x			
Demographics		x			
Questionnaires		x	x	x	x
Vascular exam		x x			x
Blood sampling		x			x
Spot urine		x			x
Treatment (supplement or placebo)		x			

10. **Risk Assessment**

1. Identified Risks	**2. Likelihood**	**3. Potential Impact/ Outcome**	**4. Potential Severity of Outcome**	**5. Risk Management/Mitigating Factors**	
Identify risks/hazards present	*Identify how likely the event is i.e.* *Very likely/ Likely/ Possible/ Unlikely*	*Who might be harmed and how?* *Ensure you have considered the research team, participants and anyone not directly involved in the research.*	*Classify the severity of outcomes identified in 3.* *i.e. High/ Medium/ Low*	*Evaluate the risks and decide on the precautions.*	*Standard Operating Procedures*/ risk assessments* *Enter Ref no/ title/ expiry date*
Data loss	Unlikely	The investigators and funders. Bad reputation for University and research team.	High	Data will be backed up and paper printout of CRFs stored.	
Data breach	Unlikely	The participants and investigators. Bad reputation for University and research team.	High	Data will be store only with anonymous ID and only with “age” but not DOB in password protected file on university computers and drives. Paper copies stored in locked cabinet.	

1. Identified Risks	2. Likelihood	3. Potential Impact/ Outcome	4. Potential Severity of Outcome	5. Risk Management/Mitigating Factors	
Incidental findings of medical condition	Possible	High blood pressure, cholesterol or glucose or other unexpected abnormal biochemical measurements could possibly be detected. Participant may require treatment.	Low (this may actually be a benefit for the participant as this may otherwise not have been detected)	PI will inform participant and offer to personally inform the GP to initiate treatment.	
"Overdosing"	Unlikely as supplement is not soluble in water in higher amounts.	Upset stomach	Low (no toxicity for components reported even in 10xamounts)	- Diary with instruction - Training by investigators how to prepare drinks	

11. Data Management

A paper list linking study ID with study subjects will be kept in a locked cabinet in Professor Heiss's office (14AY04). Personal data including name, age, sex and contact details will be stored to allow participation in future studies. In addition, personal data will be stored in accordance with HTA regulations. All data will only be stored on secure university servers. Research data consisting of study measurements and observations will be anonymised. Research data will be shared among the participating researchers named above to allow analyses for publication. The anonymised data and blood samples may also be used for future research by the participating researchers. While we do currently not plan to make the anonymised and non-identifiable research data publicly available, we will ask participants for consent to allow us to do so in the future.

12. Ethical considerations

In case any medically relevant findings are incidentally discovered, these findings will in the first instance presented to Prof. Heiss, who is a consultant in the NHS, to evaluate the validity and potential meaning. For instance, in healthy people elevated blood pressure, cholesterol or glucose could be discovered that would potentially require treatment. He will then personally discuss the findings with the participant and offer to inform the GP if the participant wishes.

13. Dissemination

We are planning on presenting the findings in seminars at the university including SHARP network, national and international conferences. In addition, the results will be published in scientific journals.

14. References

1 Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194-1217, doi:10.1016/j.cell.2013.05.039 (2013).

2 Heiss, C., Spyridopoulos, I. & Haendeler, J. Interventions to slow cardiovascular aging: Dietary restriction, drugs and novel molecules. *Exp Gerontol* **109**, 108-118, doi:10.1016/j.exger.2017.06.015 (2017).

3 Sesso, H. D. *et al.* Effect of cocoa flavanol supplementation for prevention of cardiovascular disease events: The COSMOS randomized clinical trial. *Am J Clin Nutr*, doi:10.1093/ajcn/nqac055 (2022).

4 Bondonno, N. P. *et al.* Flavonoid intake is associated with lower mortality in the Danish Diet Cancer and Health Cohort. *Nat Commun* **10**, 3651, doi:10.1038/s41467-019-11622-x (2019).

5 Bell, G. A., Kantor, E. D., Lampe, J. W., Shen, D. D. & White, E. Use of glucosamine and chondroitin in relation to mortality. *Eur J Epidemiol* **27**, 593-603, doi:10.1007/s10654-012-9714-6 (2012).

6 Pocobelli, G. *et al.* Total mortality risk in relation to use of less-common dietary supplements. *Am J Clin Nutr* **91**, 1791-1800, doi:10.3945/ajcn.2009.28639 (2010).

7 Ma, H. *et al.* Association of habitual glucosamine use with risk of cardiovascular disease: prospective study in UK Biobank. *BMJ* **365**, l1628, doi:10.1136/bmj.l1628 (2019).

8 Duan, R., Xie, H. & Liu, Z. Z. The Role of Autophagy in Osteoarthritis. *Front Cell Dev Biol* **8**, 608388, doi:10.3389/fcell.2020.608388 (2020).

9 Weimer, S. *et al.* D-Glucosamine supplementation extends life span of nematodes and of ageing mice. *Nat Commun* **5**, 3563, doi:10.1038/ncomms4563 (2014).

10 Miller, R. A. *et al.* Glycine supplementation extends lifespan of male and female mice. *Aging Cell* **18**, e12953, doi:10.1111/acel.12953 (2019).

11 Obata, F. & Miura, M. Enhancing S-adenosyl-methionine catabolism extends Drosophila lifespan. *Nat Commun* **6**, 8332, doi:10.1038/ncomms9332 (2015).

12 Ding, Y. *et al.* Plasma Glycine and Risk of Acute Myocardial Infarction in Patients With Suspected Stable Angina Pectoris. *J Am Heart Assoc* **5**, doi:10.1161/JAHA.115.002621 (2015).
13 Guasch-Ferre, M. *et al.* Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis. *Diabetes Care* **39**, 833-846, doi:10.2337/dc15-2251 (2016).
14 Cruz, M. *et al.* Glycine treatment decreases proinflammatory cytokines and increases interferon-gamma in patients with type 2 diabetes. *J Endocrinol Invest* **31**, 694-699, doi:10.1007/BF03346417 (2008).
15 Gannon, M. C., Nuttall, J. A. & Nuttall, F. Q. The metabolic response to ingested glycine. *Am J Clin Nutr* **76**, 1302-1307, doi:10.1093/ajcn/76.6.1302 (2002).
16 de Picciotto, N. E. *et al.* Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* **15**, 522-530, doi:10.1111/acel.12461 (2016).
17 Das, A. *et al.* Impairment of an Endothelial NAD(+)-H2S Signaling Network Is a Reversible Cause of Vascular Aging. *Cell* **173**, 74-89 e20, doi:10.1016/j.cell.2018.02.008 (2018).
18 Liao, B. *et al.* Nicotinamide mononucleotide supplementation enhances aerobic capacity in amateur runners: a randomized, double-blind study. *J Int Soc Sports Nutr* **18**, 54, doi:10.1186/s12970-021-00442-4 (2021).
19 Zarse, K., Jabin, S. & Ristow, M. L-Theanine extends lifespan of adult Caenorhabditis elegans. *Eur J Nutr* **51**, 765-768, doi:10.1007/s00394-012-0341-5 (2012).
20 Unno, K. *et al.* Theanine intake improves the shortened lifespan, cognitive dysfunction and behavioural depression that are induced by chronic psychosocial stress in mice. *Free Radic Res* **45**, 966-974, doi:10.3109/10715762.2011.566869 (2011).
21 Zeng, L. *et al.* l-Theanine attenuates liver aging by inhibiting advanced glycation end products in d-galactose-induced rats and reversing an imbalance of oxidative stress and inflammation. *Exp Gerontol* **131**, 110823, doi:10.1016/j.exger.2019.110823 (2020).
22 Petrovic, J. *et al.* Magnesium Supplementation Diminishes Peripheral Blood Lymphocyte DNA Oxidative Damage in Athletes and Sedentary Young Man. *Oxid Med Cell Longev* **2016**, 2019643, doi:10.1155/2016/2019643 (2016).
23 Fuentes, J. C., Salmon, A. A. & Silver, M. A. Acute and chronic oral magnesium supplementation: effects on endothelial function, exercise capacity, and quality of life in patients with symptomatic heart failure. *Congest Heart Fail* **12**, 9-13, doi:10.1111/j.1527-5299.2006.04692.x (2006).
24 Veronese, N. *et al.* Effect of magnesium supplementation on glucose metabolism in people with or at risk of diabetes: a systematic review and meta-analysis of double-blind randomized controlled trials. *Eur J Clin Nutr* **70**, 1354-1359, doi:10.1038/ejcn.2016.154 (2016).
25 Heiss, C. *et al.* Impact of cocoa flavanol intake on age-dependent vascular stiffness in healthy men: a randomized, controlled, double-masked trial. *Age (Dordr)* **37**, 9794, doi:10.1007/s11357-015-9794-9 (2015).
26 Schroeter, H. *et al.* (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc. Natl. Acad. Sci. USA* **103**, 1024-1029 (2006).

Amendment History *(to be completed for subsequent versions after initial authorisation)*

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	3	14/11/2022	C. Heiss	Changed 6 (±1) month to 6 (3-6) months Changed that nicotinamide component of supplement is supplied as capsules and not as part of the powder

2	4	17/12/2022	C. Heiss	Deleted nicotinamide mononucleotide from protocol
3	5	23/02/2024	C. Heiss	Added reimbursement for travel and time