An uncontrolled open label trial of a nutritional supplement to reduce measures of biological and immune ageing and improve physical function and quality of life in healthy older people

Research reference numbers

IRAS Number	308774
Sponsor reference number	RG_22-026
ISRCTN number	
REC reference number	

This protocol has regard for the HRA guidance

Signature page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to adhere to the signed University of Birmingham's Sponsorship CI declaration.

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 investigation without the prior written consent of the Sponsor

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

Chief Investigator: Dr Thomas Jackson

Signature: ..... Date: 6/4/22

Name: (please print):.....Thomas Jackson.....

Sponsor statement:

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

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Study protocol

Study sponsor

University of Birmingham

Study site

Institute of Inflammation and Ageing, University of Birmingham, UK

Principal Investigators

Professor Janet M Lord FMedSci, Institute of Inflammation and Ageing, Professor of Immune Cell Biology, Director of the MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research

Dr Thomas Jackson BSc MBBS MRCP(Geriatrics) PhD, Institute of Inflammation and Ageing, Clinician Scientist and Visiting Consultant in Geriatric Medicine (acting as Clinical Lead)

Title

An uncontrolled open label trial of a nutritional supplement to reduce biological and immune ageing in healthy older people

Background

Ageing of the immune system has long been held as a key mechanism in organisms as they age (Lopez-Otin et al. 2013). Age associated dysregulation of the immune system contributes towards an increased susceptibility of older adults to infectious diseases, reduced ability to respond to vaccinations and increased risk of autoimmune inflammatory diseases such as Rheumatoid Arthritis (Franceschi and Campisi 2014). Immune ageing also results in an increase in another key hallmark of ageing, cellular senescence, resulting in senescent cells accumulating across different organs (Yousefzadeh et al. 2021). Both immune ageing and cellular senescence contribute to an increase in circulating levels of pro-inflammatory cytokines, and reduction in the circulating levels of anti-inflammatory cytokines, termed “Inflammaging” (Franceschi and Campisi 2014). The degree of inflammaging is associated with increased risk or a wide range of age-related diseases as well as physical frailty (Wilson et al, 2017).

Therefore, immune ageing is a correlate of global biological ageing and indeed may be a key driver of age-related morbidity. Studies in mice in which senescence was induced only in T cells resulted in mice developing and aged phenotype and multimorbidity (Desdin-Mico et al. 2019). Immune ageing can now be reliably quantified using a combination of biological parameters to describe the so-called inflammatory immune age (i-AGE) of a person and compared to that person’s chronological age (Sayed et al. 2021). Global biological ageing can also be measured through specific epigenetic markers, namely DNA methylation at specific sites, termed an epigenetic clock (Horvath 2013).

This trial will test the hypothesis that a combination of vitamins and nutraceuticals (“Essentials”), all known to have an effect on immune ageing or systemic inflammation will improve both biological markers of immune and biological age, and markers of physical function and general well-being (Studenski et al. 2011).

Research question

Does a 12-week course of a nutraceutical product containing a combination of vitamin D3, vitamin B3 (niacinamide), vitamin C, omega 3 fatty acids (DHA/EPA), olive polyphenols, resveratrol and astaxanthin improve measures of biological and immune ageing, quality of life, and physical function (Short Physical Performance Battery, SPPB) in healthy older people?

Objectives

To test the hypothesis that a 12-week treatment of Essentials, a nutritional supplement developed by Bayer, will improve biological ageing, immune ageing, quality of life (QoL), and physical function (SPPB) in healthy older adults, by conducting an uncontrolled open label trial in 76 participants.

Essentials composition:

- Vitamin D (20 µg)
- Vitamin C (85 mg)
- Vitamin B3, as niacinamide (50 mg)
- Omega 3 PUFA, EPA + DHA (250 mg)
- Resveratrol (30 mg)
- Olive fruit extract (50 mg), delivering hydroxytyrosol (10 mg)
- Astaxanthin (3.2 mg)

Outcome

An uncontrolled open-label trial, of a 12-week intervention of the Essentials supplement.

85 participants will be recruited to allow for 9 drop outs.

Data will be collected pre-intervention to test:

- Biological ageing by DNA methylation in peripheral blood and saliva;
- Immune ageing by measuring 51 different serum cytokines, chemokines and growth factors to give an iAGE score (Sayed et al 2021), hs-CRP will also be measured;
- QoL (SF36) and diet (7 day food diary, to confirm there was no significant change in the diet of the participants) from questionnaires;
- Physical function assessed by the SPPB (Guralink et al. 1994).

Data collection will be repeated after the 12-week intervention, with the exception of QoL questionnaires which will additionally also be taken at 4 and 8 weeks in to the intervention.

Data analysis will compare differences within groups between pre-intervention and 12 week measures.

Venous blood will be collected to process serum and will also be collected in to a PAX tube for isolation of DNA. The serum will be used for measurement of the inflammatory immune clock of ageing (iAGE) which is based upon the levels of 51 different cytokines, chemokines and growth factors (Sayed et al. 2021). The measurement will be carried out using a multiplex based methodology. The DNA isolated from the PAX tube will be analysed for DNA methylation using the Illumina EPIC array and the data analysed using both the Horvath (Horvath 2013) and Hannum (Hannum et al. 2013) epigenetic clocks of biological ageing. A second assessment of epigenetic age will be conducted on saliva samples by the company Chronomics. The difference between

chronological age and iAGE will then be calculated as a measure of immune ageing and the epigenetic clock age will establish the degree of overall biological ageing. High sensitivity C-reactive protein from blood will be measured by ELISA.

Study setting

Participants will be invited to attend the Clinical Research Facility at the University Hospital Birmingham where all trial procedures will take place.

Participant recruitment

Eligibility Criteria
The study population is healthy older people.

Inclusion criteria

- Age ≥60 years.
- Ability to provide informed consent.
- Willing to stop taking multivitamins, and dietary supplements containing vitamins C and D, B3, DHA/EPA, Olive polyphenols, resveratrol and astaxanthin or food/beverage products supplemented with the above ingredients 2 weeks prior to and throughout the study.
- Able to travel to the clinic for initial and subsequent evaluations.

Exclusion criteria

- Current smokers, or ex smokers who have stopped within the last 12 months, or are using nicotine replacement products.
- History of diabetes, myocardial infarction, congestive heart failure, kidney failure, liver disease or stroke.
- Untreated thyroid disorder, cancer, active neoplasms.
- Untreated gastrointestinal, or pulmonary diseases.
- Surgery or trauma in last 60 days.
- Inflammatory diseases, auto immune diseases or recent infection in last 60 days.
- Allergies to any of the ingredients to be studied.
- Currently taking or using multivitamins or dietary supplements containing vitamins C and D, B3, DHA/EPA, Olive polyphenols, resveratrol and astaxanthin or food/beverage products supplemented with the above ingredients.
- Currently taking medication known to be metabolised by CYP3A.
- Currently taking any of the following medications, which confound the effects of inflammation: Tamoxifen, Cyclosporine A, immunosuppressants or Anti TNF inhibitors, NSAIDs.
- Currently taking any of the following anticoagulant drugs (blood thinners): Warfarin, Direct oral anticoagulant drugs (DOACs), and antiplatelet drugs including aspirin.
- Individuals at risk of bleeding complications, including those with inherited bleeding disorders (such as haemophilia), or increased risk hemorrhagic stroke or events.
- Unable or unwilling to maintain current lifestyle throughout study such as eating habits, exercise habits, etc.
- Assessed as being frail by Fried phenotype criteria.

Recruitment target
76 healthy older adults required, so we will aim for 85 recruited to allow for drop outs.

Size of recruitment target
Our sample size calculation is based on iAGE measurements from a previous study of a nutraceutical intervention in adults by Bayer. The number of participants required at 80% power and a significance level of 0.05 to detect a mean difference of 1.5 years in the iAGE score would be 76. To allow for an approximate 10% drop out we will recruit 85 participants.

Recruitment technique

Participants will be invited by contact through the 1000 Elders database. The 1000 Elders is a database of research active healthy older people who have consented to be contacted about studies run through the University of Birmingham. Names, addresses and contact details (telephone and email) are stored complying fully with DPA and CPRD legislation. The invitation will be in the form of an email. This will include an attached PIS, summary of the study, and an invitation to contact the research team if they would be interested in taking part.

Recruitment
Potential participants will contact the study team via email or telephone to express an interest to join the study. At this point eligibility screening will take place, either by telephone or through email communication

Participant identification
Eligible people will then be invited to the Clinical Research Facility at Queen Elizabeth Hospital Birmingham for the initial visit. Reasonable travel expenses will be paid to participants.

Consent

At the initial visit eligibility will be confirmed and informed consent will be gained for all activities in the trial. The nature and objectives of the study will be explained, and potential risks discussed. This information will be presented in a written patient information sheet (PIS) sent to potential participants prior to the in person visit, giving participants time to consider their participation. The process for withdrawal, including the subsequent impact on data and sample storage will be described.

At this point capacity to consent to be in the trial will be assessed. Participants will then sign a written consent form, and a copy of the information sheet and consent form given to them, and other copies stored in the trial file.

	Assessment periods	
Visits	**1 (Baseline, in person)**	**V2 (Week 12, in person)**
Time per session	**Max 2 hr**	**Max 1 hr**
Day	**0**	**84**
Medical history, incl. Concomitant medication	X	
Assessment of inclusion/exclusion criteria	X	
Informed consent	**X**	
SPPB	**X**	**X**

Qol questionnaire (also to be filled in at wk 4, 8)	**X**	**X**
7 day food intake questionnaire*	**X**	**X**
Blood	**X**	**X**
Saliva	**X**	**X**
Vital signs/bmi	**X**	**X**
Adverse event reporting		**X**
Capsule distribution	**X**	
Compliancy pill count		**X**

*Food diary to be returned at V2

Data Protection and Confidentiality

All laboratory specimens and case record forms will be identified in a manner designed to maintain participant confidentiality. Laboratory specimens will arrive at the lab in a pseudo anonymised form. The anonymisation will be done by the person taking the sample. All records will be kept in a secure storage area in the Institute of Inflammation and Ageing with limited access to study staff only. Information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee or Regulatory Authorities. The CI and study staff involved with this study will not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study.

The CI and study staff involved with this study will comply with the requirements of the Data Protection Act 2018 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to the CI and appropriate study staff.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

GP Notification

We will inform he GP of the participants involvement in the study if the participant consents to this

Safety reporting

An adverse event (AE) is any untoward medical event affecting a clinical trial participant. Each initial AE will be considered for severity, causality or expectedness and may be reclassified as a serious event or reaction based on prevailing circumstances.

An adverse reaction (AR) is where it is suspected that an AE has been caused by a reaction to the trial supplement

A serious adverse event (SAE), serious adverse reaction (SAR) or suspected unexpected serious adverse reaction (SUSAR) is any AE, AR or UAR that at any dose:

- results in death
- is life threatening
- requires hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- Or is otherwise considered serious

Note: Hospitalisations for treatment planned prior to consent and hospitalisation for elective treatment of a pre-existing condition will not be considered as an SAE. However any adverse events occurring during such hospitalisation will be recorded. Adverse events, adverse reactions and serious adverse events will be reported by participants or other health care professionals.

A telephone contact will be made at 4 and 8 weeks to ensure no adverse events are missed.

All AEs and SAEs will be recorded from the time a participant consents to join the study until the last study visit. Participants with unresolved AEs at the last study visit will be followed up until resolution or 30 days after last visit of that participant, whichever is sooner. SUSARS will be followed until resolution.

The CI, or delegate will ask about the occurrence of AEs and hospitalisations at every telephone check (week 4 and 8) and at the second/final study visit (week 12). AEs will be recorded on the AE Log in the CRF. SAEs will be submitted on an SAE form to the Sponsor within 24 hours of becoming aware of the SAE. SAEs will be assessed for expectedness and causality by the Investigator. The evaluation of expectedness will be made based on the knowledge of the reaction and the relevant product information.

The Sponsor, together with the CI, is responsible for reporting SUSARs to the competent authority, the Research Ethics Committee (REC) and any other competent authorities. Fatal or life threatening SUSARs will be reported within 7 days and non-fatal and non-life threatening SUSARs within 15 days.

Ethical and regulatory considerations

The trial will fit within existing ethical and regulatory frameworks. Participants are healthy volunteers, and not recruited as NHS patients. However, as the recruitment and assessments will take place within an NHS institution we will secure approval through the Health Research Authority (HRA) and an NHS Research Ethics Committee (REC).

The trial product is a nutraceutical, and all components are safe. As such we do not expect the product to present any risks to participants. There are potential benefits to the participants from the product. Application will be made for ethical approval through the HRA, NHS REC, and Hospital trust approval through R+D and Clinical Research Facility governance processes. The study will be sponsored by the University of Birmingham.

Assessment and management of risk

Resveratrol	Minor or none, GI adverse effects in high doses. Safe up to 450mg/day, does in trial is 30mg/day. Resveratrol has been shown to inhibit metabolism of drugs through the CY3PA enzyme. There is a risk of interaction with drugs metabolised by CYP3A if doses >1000mg/day, and long term. Studies up to 12 months
Astaxanthin	Minor or none serious adverse effects reported (GI – increased bowel movements, red stool colouration). Safe up to 8mg/day, dose in trial 4mg/day No recognised drug interactions. Studies up to 24 months
Vitamin C, D and B3	No concerns about safety at trial doses
Olive derived polyphenols	No concerns about safety
DHA and EPA	Theoretic risk if >6g/day of interaction with warfarin, but no evidence found in reviews. Dose in trial 250mg/day

Full safety details in Appendix 1

To mitigate any potential risks of drug interactions we will exclude people taking anticoagulants, antiplatelets, or drugs metabolised by the CYP3A system

Research Ethics Committee (REC) and other Regulatory review & reports

Before the start of the study, a favourable opinion will be sought from UK NHS Health Research Authority and NHS REC for the study protocol, informed consent forms, patient information sheets, and initial invitation.

Substantial amendments that require review by NHS HRA and REC will not be implemented until that review is in place and other mechanisms are in place to implement at site. All correspondence with the HRA will be retained. It will be the Chief Investigator's (TJ) responsibility to produce the annual reports as required. The Chief Investigator (TJ) will notify the HRA of the end of the study. If the study is ended prematurely, the Chief Investigator (TJ) will notify the HRA, including the reasons for the premature termination. Within one year after the end of the study, the Chief Investigator (TJ) will submit a final report with the results, including any publications/abstracts, to the HRA.

Regulatory Review & Compliance

Before we enrol participants into the study, the Chief Investigator (TJ) will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance. Different arrangements for NHS and non NHS sites are described as relevant.

Amendments

Any amendments will be the responsibility of the Chief Investigator (TJ). The CI will seek Sponsor approval for any amendments to the Protocol or other study documents. The Sponsor will decide if the amendment is substantial, or non-substantial. These will then be notified to the HRA in line with current guidance. http://www.hra.nhs.uk/resources/after-you-apply/amendments/

Amendments to the protocol or other study docs will not be implemented without approval from the Sponsor and subsequent approval from the appropriate REC and/or Regulatory Authority, as appropriate, and NHS R&D Office.

Conflict of interests

Professor Lord as key investigator/collaborator of this study declares her role as a Consultant for Bayer Pharmaceuticals. She reports receiving consultancy fees from Bayer Pharmaceuticals during the preparation and upcoming conduct of the proposed study. Professor Lord will not be involved in Adverse Event assessment in deciding causality. The Chief Investigator Dr Thomas Jackson will be assessing causality. Bayer Pharmaceuticals shall provide the nutritional supplement but will have no role in conduct, analysis and write-up of the results. As per Good Clinical Practice conflicts of interest will be reported at all levels of dissemination (including publications and University of Birmingham communications) relating to this study.

Funding arrangements

This study is funded by a research grant from Bayer Healthcare Pharmaceuticals

Peer review

This protocol has undergone both internal (within the Institute of Inflammation and Ageing) and external peer review

Protocol Deviations and Breaches

The CI will not implement any deviation from the protocol without agreement from the Sponsor, except where necessary to eliminate an immediate risk to trial participants.

In the event that the CI needs to deviate from the protocol, the nature of and reasons for the deviation will be recorded in the CRF, documented and notified to the Sponsor. If this necessitates a subsequent protocol amendment, this will be submitted to the Sponsor for approval and then to the appropriate REC, Regulatory Authority and local NHS R&D for review and approvals as appropriate.

In the event that a serious breach of the protocol or GCP is suspected, this will be reported to the Sponsor immediately.

End of Study and Archiving

The end of study is defined as last patient last visit. The Sponsor and CI have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the Sponsor, REC, Regulatory Authority and NHS R&D Office(s) within 90 days, or 15 days if the study is terminated prematurely.

Archiving of study documents will be carried out as specified in UoB-ARC-SOP-001 Standard operating Procedure: Archiving . All study documentation will be kept for 110years.

Access to the final data set

The final data set will be available on request to the CI

Dissemination plans

The clinical study report will be used for publication and presentation at scientific meetings.

Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion).

Authorship Eligibility Guidelines

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared.

Authorship eligibility for each manuscript arising from this study will be determined according to the criteria laid out in the Working Practice Document on Authorship filed in the Study Operations Manual.

We have no plans to use professional writers

References

Franceschi, C. and J. Campisi (2014). "Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases." J Gerontol A Biol Sci Med Sci **69 Suppl 1**: S4-9.DOI: 10.1093/gerona/glu057.

Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. (1994). "A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission". J Gerontol. **49(2)**:M85–94.

Hannum, G., J. Guinney, L Zhao, L Zhang, G Hughes, S Sadda, B Klotzle, M Bibikova, J-B Fan, Y Gao, R Deconde, M Chen, I Rajapakse, S Friend, T Ideker, K Zhang (2013). "Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates". Mol Cell, **49**:359-367.

Horvath S (2013) " DNA methylation age of human tissues and cell types" Genome Biol 14:R115.

Lopez-Otin, C., M. A. Blasco, L. Partridge, M. Serrano and G. Kroemer (2013). "The hallmarks of aging." Cell **153**(6): 1194-1217.DOI: 10.1016/j.cell.2013.05.039.^PMC3836174.

Sayed, N., Y. Huang, K. Nguyen, Z. Krejciova-Rajaniemi, A. P. Grawe, T. Gao, R. Tibshirani, T. Hastie, A. Alpert, L. Cui, T. Kuznetsova, Y. Rosenberg-Hasson, R. Ostan, D. Monti, B. Lehallier, S. S. Shen-Orr, H. T. Maecker, C. L. Dekker, T. Wyss-Coray, C. Franceschi, V. Jojic, F. Haddad, J. G. Montoya, J. C. Wu, M. M. Davis and D. Furman (2021). "An inflammatory aging clock (iAge) based on deep learning tracks multimorbidity, immunosenescence, frailty and cardiovascular aging." Nature Aging **1**(7): 598-615.DOI: 10.1038/s43587-021-00082-y.

Studenski, S., S. Perera, K. Patel, C. Rosano, K. Faulkner, M. Inzitari, J. Brach, J. Chandler, P. Cawthon, E. B. Connor, M. Nevitt, M. Visser, S. Kritchevsky, S. Badinelli, T. Harris, A. B. Newman, J. Cauley, L. Ferrucci and J. Guralnik (2011). "Gait Speed and Survival in Older Adults." JAMA **305**(1): 50-58.DOI: 10.1001/jama.2010.1923.

Wilson D, Jackson T, Sapey E, Lord JM (2017). "Frailty and Sarcopenia: The potential role of an aged immune system". Ageing Res Rev **36**:1-10.

Yousefzadeh, M. J., R. R. Flores, Y. Zhu, Z. C. Schmiechen, R. W. Brooks, C. E. Trussoni, Y. Cui, L. Angelini, K.-A. Lee, S. J. McGowan, A. L. Burrack, D. Wang, Q. Dong, A. Lu, T. Sano, R. D. O'Kelly, C. A. McGuckian, J. I. Kato, M. P. Bank, E. A. Wade, S. P. S. Pillai, J. Klug, W. C. Ladiges, C. E. Burd, S. E. Lewis, N. F. LaRusso, N. V. Vo, Y. Wang, E. E. Kelley, J. Huard, I. M. Stromnes, P. D. Robbins and L. J. Niedernhofer (2021). "An aged immune system drives senescence and ageing of solid organs." Nature **594**(7861): 100-105.DOI: 10.1038/s41586-021-03547-7