PROTOCOL

Version 3: 10 February 2023

Title of study: Soluble corn fibre and markers of immunity and inflammation in older adults: a randomised controlled trial

Study identifiers

IRAS ID: 317212

LREC number:

University of Southampton RGO number: 76736

Funder of the study: Tate & Lyle

Sponsor of the study: University of Southampton

Principal investigator: Professor Philip C. Calder, School of Human Development & Health, Faculty of Medicine, University of Southampton, IDS Building, MP887 University Hospital Southampton, Tremona Road, Southampton SO16 6YD

Email: pcc@soton.ac.uk

Phone: 02381 205250

Researchers: Dr Ella Baker, University of Southampton; and Research Nurses in the NIHR Clinical Research Facility at University Hospital Southampton

1. Background & rationale

The number and proportion of older people is increasing in the UK and many other countries as people are living longer. However, while lifespan is increasing, healthspan is not keeping pace and many older people live with significant illness. Ageing ultimately leads to the loss of functional capacity in many body systems including the cardiovascular, musculoskeletal, osteoarticular and neuroendocrine. A hallmark feature of ageing is immune decline, termed immunosenescence, with the functional decline of the innate and adaptive immune systems resulting in compromised immunity [1-3]. Paradoxically, in parallel there is an elevation in systemic inflammation with ageing, a phenomenon termed inflammageing [4, 5]. Inflammageing and its associated conditions including cardiovascular disease, metabolic diseases, sarcopenia and osteoporosis, some cancers and possibly dementia, make significant contributions to morbidity, poor quality of life, mortality and increased social and health care costs in older people. Furthermore, immunosenescence increases susceptibility to infections contributing to illness and mortality [6]. Importantly, these immune changes also limit responses to public health measures like influenza vaccination [7, 8]. The coronavirus pandemic has highlighted the vulnerability of older people to infections; this vulnerability is likely to be at least in part due to age-related immune changes. Thus, there is an urgent need to understand and overcome the drivers of age-related immune changes that cause ill health.

The intestinal microbiota has an influence on host health by, for example, acting through direct and indirect effects on the host immune system and inflammatory response [9, 10]. The structural complexity and functional capability of the intestinal microbiota decline with ageing [11-14]. How this occurs and the nature of the relationship between changes in the microbiota, the onset of low-grade inflammation, declining gastrointestinal tract function, and immunosenescence in older people are not well described. The loss of intestinal barrier function in older people which can result in translocation of bacterial endotoxins and whole bacteria into the bloodstream is a plausible link between intestinal dysbiosis and inflammageing, and is consistent with the concept that manipulation of the intestinal microbiota may be of therapeutic benefit in older people [15]. Nutritional approaches may be used to modify the intestinal microbiota [16, 17] and to support the immune system including regulating inflammation [18].

Fibre is considered essential for the gut to work optimally, and dietary fibre intake is an important determinant of the gut microbiota. Hence fibre affects immunity and inflammation. A high fibre diet seems to reduce the risk of chronic diseases such as cardiovascular disease, type 2 diabetes and bowel cancer. In the UK the recommended dietary intake of fibre is 30 g/day. However, many people do not achieve this. The National Diet and Nutrition Survey reports that, on average, UK adults aged 19 to 64 years consume 19.7 g fibre/day (i.e. 66% of the recommended intake), adults over the age of 65 years consume, on average, 18.7 g fibre/day (62% of the recommendation), and women aged 65 years and above consume, on average, 17.6 g fibre/day (59% of recommendation). It is estimated that only 9% of all adults meet the recommended intake for fibre; this is even lower in older adults where it is estimated that only 4% of women over the age of 65 years achieve the recommended 30 g fibre per day. Thus, there is a shortfall in dietary fibre intake amongst adults, including older people, living in the UK. Soluble corn fibre is used in the food industry. It resists digestion and absorption in the human small intestine and passes into the large intestine (colon) where it can be fermented by intestinal bacteria. In contrast to most available fermentable prebiotic fibres which are fermented in the proximal colon, soluble corn fibre is fermented in the distal colon [19]. Human studies have used

soluble corn fibre at intakes of 10 to 20 g/day in both adolescents and adults [20-24]. Soluble corn fibre has been shown to modify faecal microbiota in adults [23] and adolescents [20,21], including an increase in bifidobacteria [23,25]. However, the effect of soluble corn fibre on markers of the immune response is not known, although probiotic bifidobacteria themselves [26] and bifidogenic prebiotics [27,28] have been shown to improve markers of the immune response in older people. The effect of soluble corn fibre on markers of immunity and inflammation will be investigated in this study. Older adults are the target population because immune decline, low grade inflammation and intestinal dysbiosis occur with ageing (these may be linked) and interventions to prevent, slow or reverse such age-related changes are of health relevance. The study will use 20 g soluble corn fibre/day since this amount will bridge the gap between the recommended and average intakes of fibre for the older population and may also positively impact health status of this population. The most recent WHO commissioned systematic review and meta-analysis concluded that additional fibre intake beyond the recommended values of 25-29 g may accrue further health benefits [29]. The proposed dose of soluble corn fibre (20 g/day) is unlikely to result in any gastrointestinal discomfort (doses up to 65 g per day have been shown to be tolerable [30]).

2. Objective

The objective of this study is to identify the effects of soluble corn fibre, in the form of PROMITOR® a Tate and Lyle product used in the food industry, on markers of immunity and inflammation and on faecal microbiota in older adults.

3. Study hypotheses

Soluble corn fibre will:

a) increase markers of innate immunity (phagocytosis of bacteria (E. coli) by neutrophils and monocytes; natural killer cell activity; monocyte responses to LPS);
b) increase markers of acquired immunity (T cell responses to stimulation);
c) decrease markers of intestinal inflammation (faecal calprotectin and iFABP);
d) decrease systemic markers of inflammation (plasma cytokines, chemokines and CRP);
e) modify faecal microbiota with an increase in bifidobacteria;
f) modify the urinary metabolome signalling changes in intestinal microbiota;
g) increase faecal and circulating short chain fatty acids;
j) will be well (digestively) tolerated by participants.

4. Inclusion and exclusion criteria

Inclusion criteria

1. Community dwelling males and females aged 60 years and older
2. Body mass index 18.5-30 kg/m^2
3. Have regular bowel movements
4. Willing to adhere to the study protocol
5. Able to provide written informed consent

Exclusion criteria

1. Living in a care or nursing home
2. Diagnosed with diabetes or other metabolic and endocrine disorders
3. Presence of active gastrointestinal disease (coeliac disease, Crohn's disease, diagnosed IBD etc.), autoimmune disease, or inflammatory disease (lupus, rheumatoid arthritis, multiple sclerosis)
4. Use of prescribed medicine to control inflammation (e.g. non-steroidal anti-inflammatory drugs; NSAIDs) or regular use of over-the-counter NSAIDs
5. Use of dietary supplements (will allow a 4-week washout period)
6. Use of probiotic drinks or yoghurts (will allow a 4-week washout period)
7. Have extreme habitual fibre intake (lower than 10 g per day or higher than 30 g per day) based on a validated fibre screening tool Fruit/Vegetable/Fiber Screener – Free Assessment Tools For Individuals – Wellness – NutritionQuest [31]
8. Blood donation in the previous 3 months.
9. Participation in any other clinical trial in the previous 3 months

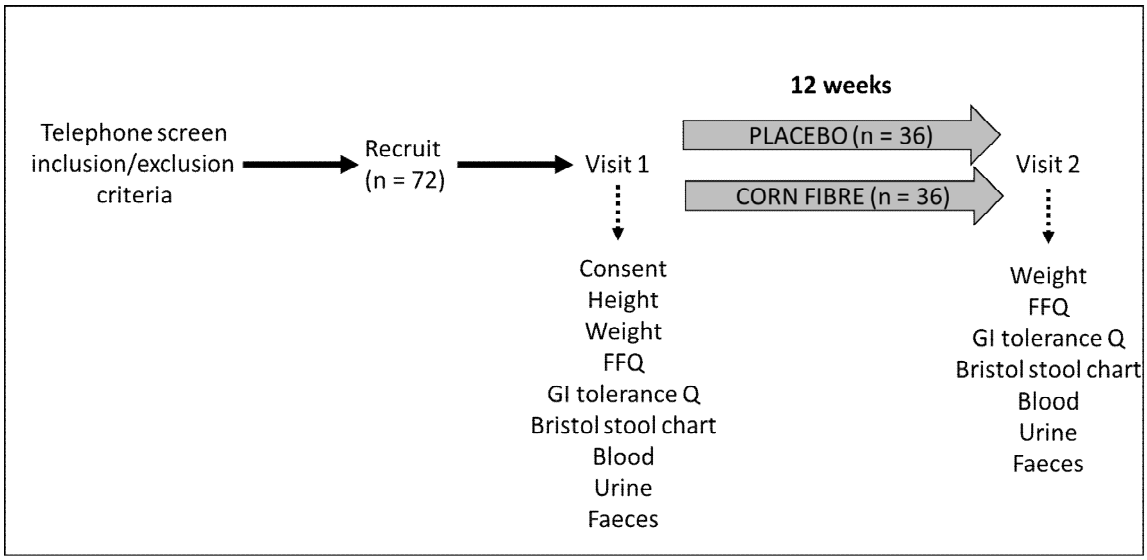
5. Study design and participant schedule

This will be a randomised, controlled, parallel trial in healthy adults aged 60 years and over with BMI 18.5 to 30 kg/m^2. Volunteers will be screened for eligibility to be enrolled into the study according to standard inclusion/exclusion criteria. 72 participants will be recruited into the study.

All procedures involving human participants will be approved by a relevant NHS Research Ethics Committee. This study will be conducted according to the guidelines established in the Declaration of Helsinki. The study will be registered at www-controlled-trials.com.

Participants will be assessed at baseline, take daily supplements for 3 months, then return for further assessment and conclude the study (see Figure 1).

Figure 1. Overview of the study plan and of participant flow through the study



Recruitment

Participants will be sought through poster advertisements; articles in the media (newsletters, newspapers, radio, and university project specific social media pages); posters and email within the University of Southampton and University Hospital Southampton NHS Foundation Trust; and by contacting those on a GDPR compliant database held by the University Hospital Southampton. Individuals who are interested will contact the research team by telephone. They will then be sent the Participant Information Sheet. They will be able to contact the researcher to confirm their interest. If they do not contact the researcher within 7 days, they will be contacted by the researcher to see if they remain interested in the study. Those individuals who indicate interest in the study will answer a small number of questions to ascertain whether they are likely to meet the inclusion/exclusion criteria (screening questionnaire). If so, an appointment will be made for them to attend visit 1 (V1) at the NIHR Clinical Research Facility at Southampton General Hospital.

Visit 1

Participants will attend the NIHR Clinical Research Facility at Southampton General Hospital in the morning (between 8 and 10 am) in the fasted state (no food or drink apart from water from 9 pm the night before). Participants will be given the opportunity to discuss the study and have any questions answered. If they are happy to be enrolled, they will be asked to sign an Informed Consent Form.

At this visit, participant's height and weight will be measured. ~30 mL of blood will be collected into heparinised tubes to provide whole blood and plasma. After blood collection, participants will be given breakfast (orange juice, toast and jam, tea or coffee) and their habitual diet will be assessed using a Food Frequency Questionnaire. They will complete a gastrointestinal tolerance questionnaire and the Bristol stool chart. During the visit they will provide a urine sample if possible; otherwise they will take a urine collection kit away with them to collect their sample at home. Participants will be provided with a kit to collect a faecal sample to be done prior to using any study products (e.g. later that same day as the clinic visit or the next day) and instructions on how to collect and store the sample. Participants will complete a log recording collection of the sample. A courier service will be used to collect faecal samples from participant's homes.

Participants will then be randomised to one of two groups (corn fibre (20 g/day) or placebo (maltodextrin (calorie-matched dose)) and will receive 4 months supply along with a diary to record that they have taken their supplement each day. The supply of study products will last the 12 week intervention with some to spare in case of a delay in returning for V2. The study materials will be provided in sachets to be stirred into cold water or fruit juice and drunk with meals twice per day. Both corn fibre and maltodextrin are tasteless. Blinding, randomisation, and supplement packaging will be completed by individuals independent of the researchers involved in the study. Participants will be randomised stratified by sex to ensure equal numbers of males and females in each treatment group.

Between visits 1 and 2

Participants will consume soluble corn fibre or maltodextrin daily. They will record this in a daily diary.

Participants will be contacted by the researcher at week 4 and week 8 and will be requested to bring unused supplements to their end of study clinic visit (visit 2; week 12). These will be used to assess compliance.

Visit 2

Three months (12 ± 1 weeks) after Visit 1, participants will return to the NIHR Clinical Research Facility at University Hospital Southampton for visit 2 (V2). Again, they will attend in the fasted state. Participants will be weighed and a ~30 mL blood sample collected into heparinised tubes. After blood collection, participants will be given breakfast (orange juice, toast and jam, tea or coffee) and their habitual diet will again be assessed using a Food Frequency Questionnaire. During the visit participant's will provide a urine sample. One again they will complete the gastrointestinal tolerance questionnaire and the Bristol stool chart. During the visit they will provide a urine sample if possible; otherwise they will take a urine collection kit away with them to collect their sample at home. Participants will be provided with a kit to collect a faecal sample to be done as soon as possible after the study visit and instructions on how to collect and store the sample. Participants will complete a log recording collection of the sample. A courier service will be used to collect faecal samples from participant's homes.

Overview of participant involvement:

	Visit 1	Between visits 1 and 2	Visit 2
Informed consent	X		
Height	X		
Weight	X		X
FFQ	X		X
GI tolerance Q	X		X
Faecal log and Bristol stool chart	X		X
Blood sample	X		X
Urine sample	X		X
Faecal sample	X		X
Sachet use diary		Daily	

6. The intervention

Participants will consume 20 g of soluble corn fibre or calorie-matched maltodextrin daily for 3 months. These materials will be supplied by Tate & Lyle as identical sachets to be stirred into cold water or fruit juice and drunk with meals two times per day. Tate & Lyle will be responsible for preparation, labelling and transportation of materials to the site. Study materials will be stored in a locked, limited access room on Level D of the IDS Building (University of Southampton, Southampton General Hospital campus) dedicated to this purpose. The soluble corn fibre will be PROMITOR® Soluble Fibre as used in the food industry. PROMITOR® Soluble Fibre has been shown to be well-tolerated even at high intake levels (40 g/day bolus or to a total of 65 g/day in multiple doses) [30]. The comparator will be MALTOSWEET™ G 120 (Maltodextrin Non-GMO) given at 2 g/day to match the calorie intake of the soluble corn fibre.

7. Summary of product characteristics

Investigational product

- Product name: PROMITOR® Soluble Fibre
- Active compound: 85A powder (PCR-negative)
- Manufactured by: Tate & Lyle
- Sachets packaged by Calleva Ltd
- Sachet: 12 g compound per Sachet (10 g fibre), consumed twice per day.

PROMITOR® Soluble Corn Fibre 85A is a glucose polymer obtained from partially hydrolysed corn starch and contains a mixture of α 1-6, α 1-4, and α 1-2 glucosidic linkages. In appearance it is an agglomerated powder, white and soluble in water.

It complies with US FDA Regulation 21CFR184.1444 - generally recognised as safe.

A sudden increase in dietary fibre may cause mild gastrointestinal disturbances, but these are generally transient and improve with adaptation to the dietary fibre source. PROMITOR® Soluble Fibre has been shown to be well-tolerated even at high intake levels (40 g/day bolus or to a total of 65 g/day in multiple doses) (30).

Placebo product

- Active compound: MALTOSWEET™ G 120 (Maltodextrin Non-GMO)
- Manufactured by: Tate & Lyle
- Sachets produced by Calleva Ltd
- Sachets: 2 g compound per sachet (0 g fibre), composed of: Maltodextrin

The placebo will be an isocaloric match using Maltodextrin (MALTOSWEET™ G 120). It is a white, practically odourless powder which is soluble in water.

MALTOSWEET™ G 120 complies with the requirements of EU Directives and Regulations in force on foods and food ingredients.

8. Labelling and packaging

The label will contain the following information:
University of Southampton Study code – xxxxx
Ingredient manufactured by Tate & Lyle
Participant Randomisation Number/Code
Investigator name
For clinical trial use only
Batch/Lot number
Storage conditions / keep away from children (if applicable)
Product is packaged in an area with other allergens
Directions for use
Emergency Contact Number

Expiry date

Packaging and labelling will be performed by Calleva Ltd. Labelling of individual boxes with participant randomisation number will be performed by an independent to the study personnel at University of Southampton (keeping study personnel blinded).

Each box will contain 225 sachets allowing for 2 sachets per day, for 84 days (12 weeks) and an extra 57-day supply, in case of delay of visit or loss of investigational product.

9. Compliance

Participants will keep a daily diary recording the use of sachets. They will be asked to return all used and unused sachets at visit 2. The diary and the returned unused sachet count will be used to estimate compliance.

Compliance will be calculated as follows:

$$Compliance\ \% = \frac{Number\ of\ sachets\ dispensed - number\ of\ sachets\ returned}{number\ of\ days\ in\ intervention\ period\ x\ 2\ doses\ per\ day}$$

In the statistical analysis, overall compliance will be considered.

If a participant states that they have not returned all unused sachets (e.g. forgot to return, loss), these details will be recorded. Participants must have consumed between 80 and 120 % of the investigational product, in order to be deemed compliant. Non-compliant participants will be withdrawn from the per-protocol analysis.

10. Variables and analyses

Markers of immunity and inflammation will be measured in blood samples collected at visits 1 and 2. There is no single marker of "immunity" and therefore a range of static and dynamic markers of innate and acquired immune responses should be measured, as recommended by Albers et al. [32]. Neutrophil phagocytosis of E. coli (median fluorescence intensity reflecting the number of bacteria taken up per neutrophil) will be the primary outcome. All other markers are secondary outcomes.

The following will be measured:

- Immune cell phenotypes in blood (T cells, B cells, monocytes, T helper cells, cytotoxic T cells, regulatory T cells, natural killer cells): flow cytometry [real-time measurement]
- A panel of cytokines and chemokines in plasma: multiplex immunoassay [measurement in stored samples]
- C reactive protein in plasma: immunoassay [measurement in stored samples]
- Neutrophil and monocyte phagocytosis of E. coli: flow cytometry [real-time measurement]
- Natural killer cell activity: flow cytometry [real-time measurement]
- T cell response to stimulation with Con A: CD69 expression (activation marker) and immunoregulatory cytokine production: flow cytometry for CD69; multiplex immunoassay

for cytokines [real-time culture and flow cytometry; cytokine measurement in stored samples]
- Monocyte response to stimulation with LPS: immunoregulatory cytokine production: multiplex immunoassay [real-time culture and cytokine measurement in stored samples]

Other markers to be assessed (all are secondary outcomes)
- Faecal microbiota – to be done in collaboration with Catholic University of Louvain, Brussels
- Faecal short chain fatty acids – chromatography [stored samples]
- Faecal calprotectin and intestinal fatty acid binding protein – immunoassay [stored samples]
- Plasma short chain fatty acids – chromatography [stored samples]
- Urinary metabolome – NMR [stored samples]
- GI questionnaire score and Bristol Stool Chart score
- Energy and macronutrient intake from FFQ

Samples will be used as follows:

Sample	Measurement	Analysed by	Reason
Blood plasma	Multiple cytokines and chemokines	Luminex	Inflammatory markers
Blood plasma	hs-C-reactive protein	ELISA	Inflammatory marker
Blood	Multiple immune cell subsets	Flow cytometry	Identifies blood immune cell phenotypes
Blood	Phagocytosis of E. coli by neutrophils and monocytes	Commercial kit + flow cytometry	Indicator of ability to kill bacteria
Blood	Natural killer cell activity	Commercial kit + flow cytometry	Indicator of ability to kill tumour cells
Blood	T cell activation and cytokines	Culture with stimulant + flow cytometry (CD69) or Luminex (cytokines)	Markers of T cell response
Blood	Monocyte cytokines	Culture with stimulant + Luminex (cytokines)	Marker of monocyte response
Faeces	Microbiota profiling	Metagenomic sequencing	Identify change in gut microbiota
Faeces	Short chain fatty acids	Chromatography	Link with change in gut microbiota
Blood plasma	Short chain fatty acids	Chromatography	Link with change in gut microbiota
Faeces	Calprotectin; iFABP	ELISA	Markers of intestinal inflammation
Urine	Metabolome	NMR	Link with change in gut microbiota

11. Sample size and statistical analysis

Neutrophil phagocytosis of E. coli will be the primary outcome. Wenisch et al. [33] reported that the number of E. coli taken up by neutrophils was lower by 25% in people aged 62 to 83 years compared to those aged 38 to 56 years. This difference is considered to be relevant to age-related susceptibility to bacterial infection. Some studies of probiotics and prebiotics have reported

enhanced phagocytosis including in older people. The sample size of the current study is based on a 20% increase in neutrophil phagocytosis of E. coli, an effect size based upon the prior studies of probiotics and prebiotics. Using in-house data on mean and SD phagocytosis (median fluorescence intensity using a flow cytometry based commercial kit) 27 participants per group are required to give 80% power to detect a 20% increase as significant with a type-1 error of 5%. To account for 25% drop-out 36 participants will be recruited into each group (i.e. 72 participants in total). All other measurements are secondary outcomes.

12. Data handling and statistical analysis

Data will be entered onto password protected Excel files. Changes between visits 1 and 2 for all outcomes will be compared between groups using unpaired tests. The exact tests used will depend upon the distribution of the data. Where data are not normally distributed, they will be log transformed to attempt to achieve a normal distribution. Regression analyses considering variables such as age, sex and BMI will also be conducted. Statistical analysis will be conducted using the current version of SPSS.

13. Study setting

Clinic visits will take place in the NIHR Clinical Research Facility at Southampton General Hospital. This is an MHRA compliant research facility.

Sample processing and storage and laboratory analysis will take place in laboratories within the Faculty of Medicine, University of Southampton [apart from analysis of faecal bacteria which will be conducted at Catholic University of Louvain, Brussels].

All activities will be according to GCP and follow GDPR protocols.

All staff will be fully trained, including in GCP, with training records.

All laboratory processes will follow SOPs.

14. Adverse events

14.1 What is an adverse event?

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical study subject administered an investigational product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

An adverse reaction is defined as all untoward and unintended responses to an investigational product related to any dose administered, i.e. where a causal relationship between the investigational product and an adverse event is at least a reasonable possibility.

An unexpected adverse reaction is an adverse reaction, the nature or severity of which is not consistent with the information about the investigational product or intervention in question set out in the Summary of Product Characteristics (see section 7).

An adverse event, adverse reaction, or unexpected adverse reaction, is defined as serious if it:

a) results in death;

b) is life-threatening;

Life threatening in the definition of a serious adverse event (SAE)/serious adverse reaction (SAR) refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

c) requires hospitalisation or prolongation of existing hospitalisation;

In general, hospitalisation signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment which would not have been appropriate at the investigator site. When in doubt as to whether hospitalisation occurred or was necessary, the adverse event should be considered as serious. Hospitalisation for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AE and should be recorded on a Clinical Assessment form and added to the study file. If something untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'non-serious' attributed according to the usual criteria.

d) results in persistent or significant disability or incapacity;

e) consists of a congenital anomaly or birth defect.

Medical judgement should be exercised in deciding whether an SAE/SAR is serious in other situations. Important SAE/SARs that are not immediately life-threatening or do not result in death or prolonged hospitalisation but may jeopardise the subject or may require intervention to prevent one or the other outcomes listed in the definition above, should also be considered serious.

A suspected serious adverse reaction (SSAR), is any serious adverse reaction that is suspected (possibly or probably) to be related to the investigational product.

A suspected unexpected serious adverse reaction (SUSAR) is an SSAR which is also "unexpected", meaning that its nature and severity are not consistent with the information about the investigational product in question set out in the IB.

14.2 Intensity

The assessment of intensity will be based on the chief investigator's clinical judgement using the following definitions:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities.

The term severity is often used to describe the intensity (severity) of a specific event. This is not the same as 'seriousness', which is based on participant/event outcome or action criteria.

14.3 Causality

The relationship between the investigational product/procedure and the occurrence of each AE will be assessed and categorised as below by the chief investigator. The chief investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors etc. will be considered.

- Not related: Temporal relationship of the onset of the event, relative to administration of the product, is not reasonable or another cause can by itself explain the occurrence of the event.
- Unlikely: Temporal relationship of the onset of the event, relative to administration of the product, is likely to have another cause which can by itself explain the occurrence of the event.
- Possibly related: Temporal relationship of the onset of the event, relative to administration of the product, is reasonable but the event could have been due to another, equally likely cause.
- Probably related: Temporal relationship of the onset of the event, relative to administration of the product, is reasonable and the event is more likely explained by the product than any other cause.
- Definitely related: Temporal relationship of the onset of the event, relative to administration of the product, is reasonable and there is no other cause to explain the event, or a re-challenge (if feasible) is positive.
- Where an event is assessed as possibly related, probably related, definitely related the event is an adverse reaction.

14.4 Expectedness

Adverse reactions must be considered as unexpected if they add significant information on the specificity or severity of an expected adverse reaction. The expectedness of an adverse reaction shall be determined according to the reference documents.

- Expected: Reaction previously identified and described in protocol and/or reference documents.
- Unexpected: Reaction not previously described in the protocol or reference documents.

All AEs occurring during the period from screening visit to the trial completion will be registered and reported if applicable.

For all adverse event/reactions the chief investigator will make an assessment of intensity, causality, expectedness and seriousness.

14.5 Expedited reporting of serious adverse events

In the event of a SUSAR, the chief investigator (or delegated person) will make an initial report, preferably using the UHS SAE/SUSAR report form. If the initial report is made orally, the written report must be made with 24 hours of the oral report. The written report will include as much information as is available at the time and will immediately be sent to.
- the chief investigator
- the University of Southampton as sponsor (scanned form emailed to rgoinfo@soton.ac.uk)
- UHS R&D department

- UHS patient safety team (using Trust incident Reporting form)

The University of Southampton as sponsor will be responsible to further expedite the reporting of the SUSAR to the REC that gave approval as soon as possible but within 7 days.

After the initial report the chief investigator will actively follow up the subject. The chief investigator (or delegated person) will provide information missing from the initial report within five working days of the initial report. This updated report will be set to:
- the chief investigator
- the University of Southampton as sponsor (scanned form emailed to rgoinfo@soton.ac.uk)
- UHS R&D Department

In the event of an SAE, the chief investigator (or delegated person) will make an initial report, using the UHS SAE/SUSAR report form. The initial report will include as much information as is available at the time and will sent within 24 hours to.
- the chief investigator
- the University of Southampton as sponsor (scanned form emailed to rgoinfo@soton.ac.uk)
- UHS R&D Department

After the initial report the chief investigator will actively follow up the subject. The chief investigator (or delegated person) will provide information missing from the initial report within five working days of the initial report. This updated report will be set to:
- the chief investigator
- the University of Southampton as sponsor (scanned form emailed to rgoinfo@soton.ac.uk)
- UHS R&D Department

SAEs that are related to the study and unexpected will be reported to the REC that approved the study by email using the non-CTIMP Safety Report to REC form and will be sent within 15 days of the chief investigator becoming aware of the event. The chief investigator will be responsible for sending such reports. The sponsor (University of Southampton) will be responsible for ensuring SAEs are reported to the REC.

The study team will maintain a log of all SAEs. This will be kept in the site file.

At the end of the study all AEs and SAEs recorded during the study will be subject to analysis and subsequent conclusions will be included in the final study report.

Urgent Safety Measures/ Temporary Halt of the Trial must be reported immediately to the University of Southampton as sponsor and to UHS R&D Department. As soon as possible and within 3 days this must be reported to the REC that granted approval in the form of a substantial amendment. The report must include the reasons for the urgent safety measure and the plan for further action.

15. Protocol deviations and serious breaches of GCP

All serious protocol deviations/violations and serious breaches of Good Clinical Practice and/or the trial protocol will immediately be reported to the sponsor at rgoinfo@soton.ac.uk. The Sponsor will notify the REC which provided the original approval for the study of any serious

breaches within 7 days of becoming aware. This reporting requirement may be delegated by the Sponsor to the Chief Investigator.

16. Ethical and governance considerations

The study will be approved by an NHS Ethics Committee; such approval will be sought as soon as the protocol is finalised.

The study will be approved by the University of Southampton Research Governance Office.

The study sponsor will be University of Southampton.

The study will be registered at a relevant clinical trial registration site.

The study will be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 as revised and recognized by governing laws and EU Directives; and the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

The PI will submit a final report at conclusion of the trial to the REC within the timelines defined in the Regulations.

17. References

1. Shaw AC, et al., Aging of the innate immune system. Curr Opin Immunol, 2010; 22: 507-13.
2. Pawelec G, et al., Senescence of the human immune system. J Comp Pathol, 2010; 142(Suppl. 1): S39-44.
3. Agarwal S & Busse PJ, Innate and adaptive immunosenescence. Ann Allergy Asthma Immunol, 2010; 104: 183-190.
4. Franceschi C & Campisi J, Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci, 2014; 69 Suppl 1: S4-S9.
5. Calder PC, et al., Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition. Ageing Res Rev, 2017; 40: 95-119.
6. Pera A, et al., Immunosenescence: implications for response to infection and vaccination in older people. Maturitas 2015; 82: 50-55.
7. Goodwin K, et al., Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine, 2006; 24: 1159-1169.
8. Fulop T, et al., Immunosenescence and vaccination in nursing home residents. Clin Infect Dis, 2009; 48: 443-448.
9. Thomas C & Versalovic J. Probiotics-host communication: Modulation of signaling pathways in the intestine. Gut Microbes, 2010; 1: 148-63.
10. Kau AL, et al., Human nutrition, the gut microbiome and the immune system. Nature, 2011; 474: 327-336.

11. Hopkins MJ, et al., Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. Gut, 2001; 48: 198–205.
12. Arboleya S, et al., Gut bifidobacteria populations in human health and aging. Front Microbiol, 2016; 7: 1204.
13. Claesson M, et al., Gut microbiota composition correlates with diet and health in the elderly. Nature, 2012; 488: 178–184.
14. An R, et al., Age-dependent changes in GI physiology and microbiota: time to reconsider? Gut Microbes, 2018; 67: 2213-2222.
15. Nicoletti C, Age-associated changes of the intestinal epithelial barrier: local and systemic implications. Expert Rev Gastroenterol Hepatol, 2015; 9: 1467-1469.
16. Singh RK, et al., Influence of diet on the gut microbiome and implications for human health. J Transl Med, 2017; 15: 73.
17. Valdes A, et al., Role of the gut microbiota in nutrition and health. BMJ, 2018; 361: k2179.
18. Pae M & Wu D, Nutritional modulation of age-related changes in the immune system and risk of infection. Nutr Res, 2017; 41: 14-35.
19. Marzorati M, et al., Soluble corn fiber is fermented in the distal colon, leading to positive modulation of the microbiota and the gut wall. Paper presented at 6th International Dietary Fibre Conference; June 1–3, 2015; Paris.
20. Whisner CM, et al., Soluble maize fibre affects short-term calcium absorption in adolescent boys and girls: a randomised controlled trial using dual stable isotopic tracers. Brit J Nutr, 2014; 112: 446-456.
21. Whisner CM, et al., Soluble corn fiber increases calcium absorption associated with shifts in the gut microbiome: a randomized dose-response trial in free-living pubertal females. J Nutr, 2016; 146: 1298-1306.
22. Weaver CM, ert al., Novel fibers increase bone calcium content and strength beyond efficiency of large intestine fermentation. J Agric Food Chem, 2010; 58: 8952-8957.
23. Costabile A, et al., Prebiotic potential of a maize-based soluble fibre and impact of dose on the human gut microbiota. PLoS One, 2016; 11: e0144457.
24. Canene-Adams K, et al., A randomized, double-blind, crossover study to determine the available energy from soluble fiber. J Am Coll Nutr, 2021; 40: 412-418.
25. Boler BM, et al., Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men. Brit J Nutr, 2011; 106: 1864-1871.
26. Arunachalam K, et al., Enhancement of natural immune function by dietary consumption of Bifidobacterium lactis (HN019). Eur J Clin Nutr, 2000; 54: 263-267.
27. Vulevic J, et al., Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. Am J Clin Nutr, 2008; 88: 1438-1446.
28. Vulevic J, et al., Influence of galacto-oligosaccharide mixture (B-GOS) on gut microbiota, immune parameters and metabonomics in elderly persons. Brit J Nutr, 2015; 114: 586-595.
29. Reynolds A, et al., Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet, 2019; 393: 434-445.
30. Housez B, et al., Evaluation of digestive tolerance of a soluble corn fibre. J Hum Nutr Diet, 2012; 25: 488-496.
31. Block G., et al., A rapid food screener to assess fat and fruit and vegetable intake. Am J Prev Med, 2000; 18: 284-288.
32. Albers R, et al., Markers to measure immunomodulation in human nutrition intervention studies. Brit J Nutr, 2005; 94: 452-481.

33. Wenisch C, et al., Effect of age on human neutrophil function. J Leukoc Biol, 2000; 67: 40-45.