


BMJ Open Effects of 3 months of multi-nutrient supplementation on the immune system and muscle and respiratory function of older adults in aged care (The Pomerium Study): protocol for a randomised controlled trial

Ahmed Al Saedi,^{1,2} Ben Kirk,^{1,2} Sandra Iuliano,^{1,2} Jesse Zanker,^{1,2} Sara Vogrin,^{1,2} Lata Jayaram,¹ Shane Thomas,² Christine Golding,³ Diana Navarro-Perez,^{1,2} Petra Marusic,² Sean Leng,⁴ Ralph Nanan,⁵ Gustavo Duque ^{1,2}

To cite: Al Saedi A, Kirk B, Iuliano S, *et al.* Effects of 3 months of multi-nutrient supplementation on the immune system and muscle and respiratory function of older adults in aged care (The Pomerium Study): protocol for a randomised controlled trial. *BMJ Open* 2022;**12**:e059075. doi:10.1136/bmjopen-2021-059075

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-059075>).

Received 16 November 2021
Accepted 20 April 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Professor Gustavo Duque;
gustavo.duque@unimelb.edu.au

ABSTRACT

Introduction Immunosenescence leads to increased morbidity and mortality associated with viral infections and weaker vaccine responses. This has been well documented for seasonal influenza and the current pandemic with SARS-CoV-2 (COVID-19), which disproportionately impact older adults, particularly those in residential aged care facilities. Inadequate nutrient intakes associated with impaired immunity, respiratory and muscle function are likely to augment the effects of immunosenescence. In this study, we test whether the impact of inadequate nutrition can be reversed using multi-nutrient supplementation, consequently enhancing vaccine responses, reducing the risk of viral infections and improving respiratory and muscle function.

Methods and analysis The Pomerium Study is a 3-month, single-blind, randomised, controlled trial testing the effects of two daily servings of an oral multi-nutrient supplement (330 kcal, 20 g protein, 1.5 g calcium 3-hydroxy-3-methylbutyrate monohydrate (CaHMB), 449 mg calcium, 500 IU vitamin D₃ and 25 vitamins and minerals) on the immune system and muscle and respiratory function of older adults in aged care in Melbourne, Australia. 160 older adults (≥75 years old) will be recruited from aged care facilities and randomised to treatment (multi-nutrient supplement) or control (usual care). The primary outcome is a change in T-cell subsets CD8 + and CD28null counts at months 1 and 3. Secondary outcomes measured at baseline and month 3 are multiple markers of immunosenescence (also at 1 month), body composition (bioimpedance), handgrip strength (dynamometer), physical function (short physical performance battery), respiratory function (spirometry) and quality of life (EQ-5D-5L). Incidence and complications of COVID-19 and/or viral infections (ie, hospitalisation, complications or death) will be recorded throughout the trial, including 3 months after supplementation is ceased.

Ethics and dissemination This study was approved by Melbourne Health Human Research Ethics Committee (Ref No. HREC/73985/MH-2021, ERM Ref No. RMH73985,

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study performs comprehensive immune, respiratory and functional assessments in aged care residents after consuming a commercially available multi-nutrient supplement.
- ⇒ The method of intervention enables rapid wide-scale implementation into practice.
- ⇒ This study is randomised, and assessors will be blinded to treatment allocation.
- ⇒ Any biological effect observed cannot be attributed to one component of the multi-nutrient supplement.
- ⇒ If group differences in energy intake occur, they can only be monitored by regular assessment of dietary intake and weight changes during the study period.

Melbourne Health Site Ref No. 2021.115). Written informed consent will be obtained from participants. Results will be published in peer-reviewed journals and made available to key aged care stakeholders, including providers, residents, and government bodies.

Trial registration number ACTRN12621000420842.

INTRODUCTION

Ageing is characterised by a decline in immune function known as immunosenescence.¹ This process reduces resistance to infectious diseases (eg, pneumonia, influenza, meningitis and urinary tract infections).¹ During a pandemic, the concept of immunosenescence is of relevance as older adults, particularly those living in residential aged care facilities (RACFs), are at high risk of acquiring infectious diseases and experiencing more adverse outcomes.^{2 3} Both intrinsic and extrinsic factors contribute to the predisposition of institutionalised

older adults to respiratory viral infections. Intrinsic risk factors include immunosenescence, malnutrition, low serum vitamin D levels, limited mobility, poor muscular and respiratory function, and comorbidities.⁴⁻⁶ Extrinsic factors include lack of appropriate infection control procedures and limited access to personal protective equipment.⁷ Vaccination to viral assaults is the primary preventative strategy to reduce both onset and severity of viral infection.⁷

Malnutrition is common in aged care residents⁸ and is associated with a compromised immune profile (ie, lower levels of T-cell subsets (CD8 + CD28null), high NK:CD4 +T-cell ratio and low serum levels of interleukin (IL)-7).¹⁻¹⁰ Dietary protein contains immunoglobins that protect against antigens,¹¹ and vitamin D modulates immune function by stimulating the differentiation of regulatory T and B cells.¹² Inadequate protein intakes and vitamin D deficiency are common in older adults in aged care.¹³⁻¹⁵ On average, daily protein intakes of 0.8 g/kg bodyweight have been observed in older adults living in Australian residential aged care⁸; an amount considered insufficient to support optimal immune and musculoskeletal function.¹⁶ Furthermore, up to 77.5% of older adults in aged care are vitamin D deficient (serum 25(OH)D levels <50 nmol/L),^{17 18} and adequate vitamin D levels are associated with reduced severity and mortality from COVID-19.^{19 20}

Sarcopenia is a progressive and generalised skeletal muscle disorder characterised by decreased muscle quality, quantity and function.^{21 22} This process is particularly evident in respiratory muscles,²³ where it impairs the ability to produce appropriate tidal volume²⁴ and perform high force expulsive airway clearance manoeuvres.²⁵ Multiple studies have shown that area of the pectoralis, psoas and paravertebral muscles on cross-sectional CT images is associated with increase in lean muscle mass, handgrip strength, sarcopenia and health.^{22 26 27}

Sarcopenia was also evident in patients with COVID-19.^{28 29} Baseline sarcopenia was independently associated with prolonged hospital stay in patients with COVID-19.³⁰ Higher paraspinal muscle radiodensity, a proxy measure of lower muscle fat deposition, was associated with a reduced risk of disease deterioration and decreased likelihood of prolonged viral shedding among female patients with severe COVID-19.³¹ In addition to the well-known independent risk factors (ie, age, obesity, chronic obstructive pulmonary disease and C reactive protein level), low grip strength is independently associated with increased severity of COVID-19.³² Moreover, decreased muscle strength is an independent risk factor for COVID-19 severity in adults 50 years of age or older.³³ Therefore, low muscle mass and strength are considered risk factors for COVID-19 severity.^{34 35}

Of the nutrients purported to support immune and muscle function, whey protein contains bioactive immunoglobins (such as lactoferrin) with immunostimulatory properties,¹¹ as well as essential amino acids (in particular leucine) required for muscle protein synthesis.^{36 37}

Another potent stimulator of muscle protein synthesis is calcium β -hydroxy- β -methyl-butyrate (CaHMB),³⁸ a leucine metabolite, which appears to offer complementary benefits to leucine by simultaneously dampening muscle proteolysis.³⁹ This may be important for older adults with compromised immune function and/or sarcopenia, where chronic low-grade inflammation may drive muscle loss.^{40 41} Vitamin D also interacts with protein to support this anabolic signalling network, and sufficient vitamin D levels (>50 nmol/L) may be required for protein to increase muscle mass as observed in animal models and older adults with sarcopenia.^{42 43}

Several other vitamins and minerals, such as calcium and iron that act as cofactors in metabolism,^{40 44} may help support the immune, respiratory and muscular systems and reduce the risk of adverse events (AEs) in this population, such as falls, fractures and respiratory infections.⁴⁴ A randomised controlled trial (RCT) involving 157 older adults (>65 years) living in long-term care supplemented with a nutrition formula that contained triacylglycerol, protein, antioxidants, selenium, zinc and 28 vitamins and minerals for 1 month demonstrated enhanced immune function as indicated by increased influenza vaccine response and lymphocyte activation, less fever and fewer days of symptoms of upper respiratory tract infections.⁴⁵ Similar improvements to response to influenza and pneumococcal vaccination have been observed in older adults living in the community who were provided with similar nutritional supplements.^{46 47} Therefore, correcting nutritional inadequacy is a viable option to support immune, muscle and respiratory function in older adults living in aged care.

The aim of this 3-month single-blind, randomised controlled study (Protocol V.6.0) is to test the effects of two daily servings of a multi-nutrient supplement (containing whey protein, leucine, CaHMB, vitamin D₃, calcium plus 25 vitamins and minerals) on the immune system and muscle and respiratory function of older adults in aged care. We hypothesise that provision of this multi-nutrient supplement will improve multiple immune and functional variables and reduce the number and severity of cases of respiratory viral infections. To test this hypothesis, we propose measuring T-cell subsets (CD8+ CD28null) as our primary outcome. Our secondary outcome measures include a comprehensive immune profile (cell counts and serum cytokines), body composition (bioimpedance), handgrip strength (dynamometer), physical function (short physical performance battery (SPPB)), respiratory function (spirometry) and quality of life (QoL) (EQ-5D-5L).

METHODS AND ANALYSIS

Trial design and population

This is a 3-month single-blinded (assessors are blinded) randomised controlled trial involving 160 older adults living in aged care, who fulfil the inclusion criteria (box 1) and are randomised to either two daily doses of

Box 1 Inclusion and exclusion criteria

Inclusion criteria

- ⇒ Men and women aged 75 years or older.
- ⇒ Sarcopenia diagnosed using Sarcopenia Definition and Outcomes Consortium criteria.
- ⇒ Participants must weigh at least 40.0 kg at the time of screening and have a body mass index within the range of 18.0–30.0 kg/m².

Exclusion criteria

- ⇒ Less than 2 points in the Eating Validation Scheme.
- ⇒ Bedbound residents.
- ⇒ Not able to give informed consent.
- ⇒ Conditions that affect swallowing or administration of the multi-nutrient supplement.
- ⇒ Participants on immunomodulator or corticosteroid medication.

a multi-nutrient supplement (treatment) (online supplemental appendix 1) or usual care (control) (figure 1). Assessments will be performed at baseline (immune, serum, respiratory and muscle-related measures and QoL), month 1 (immune and serum only), month 3 (immune, serum and other measures) and 3 months following cessation of supplementation (COVID-19 incidence and other respiratory viral infections). The trial will follow the CONSORT guidelines for reporting randomised trials.⁴⁸

Patient and public involvement

The research question and methodology were based on previous experience by the investigators testing nutritional interventions in aged care residents, including surveying their preferences and asking for their feedback and acceptance of the multi-nutrient supplement.⁸ Our consumer representative at the Australian Institute for Musculoskeletal Science (AIMSS) participated in the design of this protocol and is also an associate investigator in the grant application. Results will be disseminated via regular reports submitted to the participating aged care facilities and distributed among the participants and their families.

Recruitment

The researchers will contact aged care facility managers and provide information about the trial including the purpose, duration and possible benefits and side effects. For those expressing an interest, a written agreement will be finalised between the aged care facility manager and the University of Melbourne (UOM) who is the sponsor of the trial. Recruitment of participants will involve detailing the trial in aged care facility newsletters and presenting the trial to residents and their families at resident and relative meetings. This recruitment method has been successfully used in the past.^{49 50}

Trial population and randomisation

The target population is sarcopenic aged care residents aged 75 years and older that may or may not have received a COVID-19 or seasonal influenza vaccination. Sarcopenia will be diagnosed using the Sarcopenia Definition and Outcomes Consortium criteria.⁵¹ The trial statistician will generate the randomisation sequence using a permuted block design stratified by aged care facility and uploaded to research electronic data capture (REDCap). Treatment allocation will be concealed until the time of randomisation.

Testing procedures

The visit schedule is illustrated in figure 1, and assessments are presented in table 1. The visit schedule consists of screening (visit 1), baseline/randomisation (visit 2), immune function (visit 3), immune function and functional assessments (visit 4), and a review of events 3 months after the final assessment (follow-up; visit 5). It is anticipated that the time commitment for participants will be between 30 and 60 min per visit.

Immune and nutritional profile

Non-fasting blood (25 mL) will be collected by venepuncture by trained personnel. Twenty millilitres will be analysed by flow cytometry (Aurora) to quantify full blood counts, T and B cell counts and their subsets (particularly

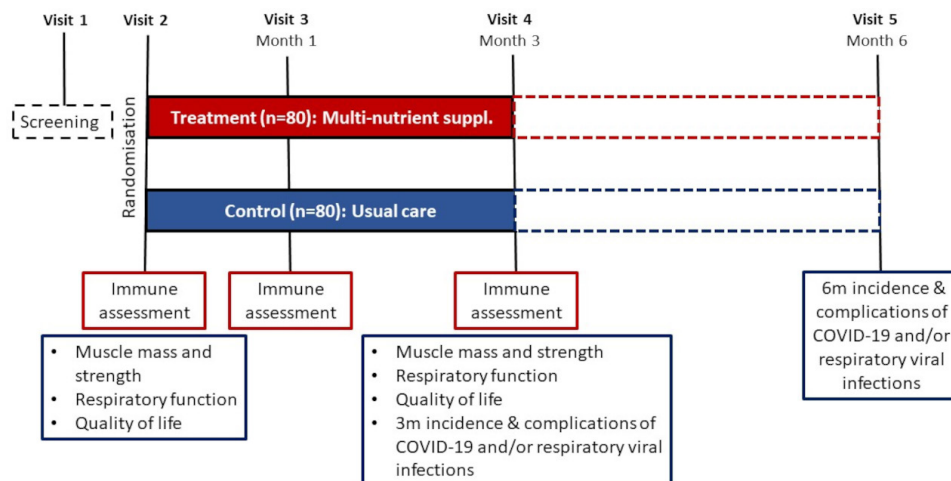


Figure 1 The Pomerium study design.

Table 1 Assessment and visit schedule

| Enrolment | | Screening | Treatment | | | | Follow-up |
|--|----------|-------------------------|-------------------------|---------|-----------------|-------------------------------|-----------|
| Intervention | Duration | 30 days | 3 months | | | 3 months | |
| Assessment | Name | Screening | Randomisation | Month 1 | Month 3/ EOT | Unscheduled visit | 6 months |
| Assessment schedule | Time | Within 30 days of day 1 | ≥9 days after screening | ±7 days | ±7 days) | Any day between day 1 and EOT | ±7 days |
| Informed consent | | X* | | | | | |
| Medical history | | X | | | X† | | X† |
| Concomitant therapy | | X | X | X | X | X | |
| Vital signs (seated) (BP, pulse, tympanic temperature) | | X | X | | X | X | |
| Height | | X | | | | | |
| Weight‡ | | X | X | | X | X | |
| Dietary intake | | | X | | X | | |
| Gait speed | | X | X | | X | | |
| Handgrip strength | | X | | | X | X | |
| SPPB (includes gait speed) | | | X | | X | X | |
| Body comp. (impedance) | | | X | | X | X | |
| Nutritional profile (blood) | | | X§ | X§ | X§ | X§ | |
| Nursing Home Life-Space Diameter instrument | | | X | | X | X | |
| Nutritional assessment: MNA | | | X | | X | | |
| Patient-reported QoL EQ-5D | | | X | | X | | |
| Immune profile | | | X¶ | X¶ | X¶ | X¶ | |
| Respiratory function (spirometry) | | | X | | X | X | |
| Study treatment dispensing/return | | | X | X | X | X | |
| SAE/AEs | | | X | X | X | X | |

*Informed Consent must be obtained prior to study specific assessments being completed.

†Documented cases of COVID-19 and/or respiratory viral infections will be collected from the participants' charts, including details of adverse outcomes (hospitalisation, complications, or death).

‡Additional monthly data on weight will be collected as part of usual practice at participating RACFs.

§Serum albumin, vitamin D and calcium.

¶Full blood count, T and B cell sub-types, cytokine arrays.

AEs, adverse events; EOT, end of treatment; MNA, mini-nutritional assessment; QoL, quality of life; SPPB, Short Physical Performance Battery.

T-cell subsets CD8 + and CD28null) and other surface phenotypes of immunosenescence (table 2). Serum concentrations of 40 interleukins (online supplemental appendix 2) will be quantified at AIMSS using a MILLI-PLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore). Serum vitamin D, calcium and albumin will be assayed at Dorevitch Pathology (Melbourne, Australia) using validated techniques.⁵²

Physical function

Handgrip strength

Will be assessed using a Jamar hydraulic dynamometer (Sammons Preston, Bolingbrook, Illinois, USA). Participants will be seated with their arm resting (at 90 degrees) on the chair arm and instructed to squeeze the dynamometer at maximal effort (test is performed three times on each side with 30 seconds rest between each test). The highest results of three attempts will be recorded.

Gait speed

Will be determined as the time to walk 6 m at normal speed using a stopwatch. The use of walking aids (eg, cane, walker) will be recorded. Three tests (3 min rest between) are performed with the best speed recorded.⁵³

Short Physical Performance Battery (SPPB)

Will be used to assess lower extremity function using tasks that mimic daily activities. The SPPB examines static balance, gait speed and lower body strength. Balance assessments are composed of three tasks that become progressively more challenging, that is, standing unaided for 10 s with feet together, feet in semi-tandem (one foot in front of the other foot, with the big toe of the back foot in the groove of the front foot) and full tandem position.⁵⁴ The five times sit-to-stand test is performed with the participant starting in the seated position. After confirming the ability to perform one sit-to-stand action,

Table 2 Markers of immunosenescence included in the flow cytometry analyses

| No | Marker | Cell type | Gating |
|----|---------------|---------------------------|--------------------------|
| | Viability dye | | |
| 1 | CD3 | T cells | |
| 2 | CD4 | Monocytes, helper T cells | |
| 3 | CD8 | Cytotoxic T cells | |
| 4 | CD19 | B cells | |
| 5 | CD56 | NK cells | CD8+ |
| 6 | CD57 | Senescent and NK subsets | CD27+ CD57+ CD127+ |
| 8 | CD45RA | Treg cells | |
| 9 | CD25 | Naïve T reg | |
| 10 | CD27 | | |
| 11 | CD127 | | |
| 12 | CD28 | Senescent and NK subsets | |
| 13 | CCR7 | Effector memory cells | CD45+ RA |
| 14 | CD16 | Monocytes/dendritic cells | |
| 15 | CD11c | | |
| 16 | HLA DR | | |
| 17 | CD14 | | |
| 18 | CD31 | Thymic migration | CD4+ |
| 19 | CD38 | | |

participants are instructed to stand and sit five times as quickly as possible, ensuring feet are flat on the floor. Scores are allocated according to performance, with an overall maximum score of 12 (0–6 is low performance, 7–9 moderate and 10–12 high performance).⁵⁵

Body composition

Bioelectrical Impedance (BC-545, Tanita, Wedderburn, Australia) will be used to calculate body composition after calibrating the device for the following variables: weight, height, age and sex.

Vital signs

Blood pressure (BP) will be measured by trained personnel in a seated position using an automated electronic BP monitor. Heart rate and temperature will also be recorded.

Respiratory function

Spirometry (forced expiratory vital capacity in 1 s (FEV₁), forced vital capacity (FVC) and FEV₁/FVC ratio) pre-salbutamol and post-salbutamol will be assessed using a calibrated portable hand-held Micro spirometer (CONTEC Medical Systems SP10BT, Hebei, China) and performed to American Thoracic Society (ATS) standards with predicted values from NHANES III.⁵⁶

Anthropometry

Height (digital stadiometer (SECA20)) and weight (homologated electronic balance (SECA20)) will be

measured with the participant barefoot (or wearing socks or stockings) and wearing light clothing. Body mass index (BMI) (weight in kilograms divided by height in metres squared) will be calculated. Nutritional status will be assessed using the long Mini-Nutritional Assessment (MNA) tool,⁵⁷ a validated instrument that contains 18 items and evaluates four different aspects of nutritional status: anthropometric assessment (BMI, weight loss, and arm and calf circumferences); general assessment (lifestyle, medication, mobility and presence of signs of depression or dementia); short dietary assessment (number of meals, food and fluid intake and autonomy of feeding); and subjective assessment (self-perception of health and nutrition).

Quality of life

Assessed using the EQ-5D-5L questionnaire.⁵⁸ Briefly, the questionnaire enables comparisons of QoL across different diseases or health states using a single score and has five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression)

Nursing Home Life-Space Diameter instrument

Used to identify social isolation and self-restricted life-space mobility.⁵⁹ This instrument, which is measured over 2 weeks, separates a resident's living area into four spaces: their room, outside the room but within the unit, outside the unit but within the facility, and outside the facility.

Dietary intake

Determined on two random days using the validated method of visual estimation of plate waste.⁶⁰ Standard serves will be weighed on a digital food scale (± 1 g) (Soehnle Page Profi), and foods and beverages served and wasted will be compared against the standard serve using a 7-point scale. The 7-point scale represents portions of each food consumed (or remaining): 0=no food remaining, +M=1 mouthful remaining, 1/4=25% remaining, 1/2=50% remaining, 3/4=75% remaining, -M=1 mouthful consumed (90% remaining), 1=no food eaten. Meals served will be rated against the standard meal (medium given the value of 100%); small serving=75%, large serving=125%, extra-large serving=150%. Consumption will be calculated as the difference between amounts served and wasted.

Medical record review

Medical history, documented cases of COVID-19 and/or respiratory viral infections will be collected from the participant's medical records, including details of serious adverse outcomes (hospitalisation, complications, or death).

Adverse events

All compulsory incident reports documented at each facility will be reviewed, and any AEs reported for study participants will be recorded. AEs are defined as any untoward medical occurrence(s) in a study participant that may or may not be temporally or causally associated

with the use of the multi-nutrient supplement and is considered serious if it results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, or results in persistent or significant disability.

Study visits

Study visits are outlined in [figure 1](#).

Visit 1—screening

- ▶ Informed consent (online supplemental appendix 3) is obtained before study assessments commence
- ▶ Medication use (existing therapies or therapies changed or ceased in the last 3 months) will be documented
- ▶ Vital signs, weight, height, gait speed and handgrip strength

Visit 2—randomisation

- ▶ MNA.
- ▶ SPPB (includes gait speed)
- ▶ Body composition
- ▶ Nursing Home Life-Space Diameter instrument
- ▶ QoL
- ▶ Respiratory function
- ▶ Non-fasting blood sample (immune and nutritional profile)

Visit 3 (1 month)

- ▶ Non-fasting blood sample (immune and nutritional profile)
- ▶ Concomitant medication and AE record

Visit 4 (3 months) (similar assessment to visit 2; baseline)

- ▶ Document cases, severity and outcomes of COVID-19 and/or respiratory viral infections
- ▶ Non-fasting blood sample (immune and nutritional profile)
- ▶ Concomitant medication and AE record

Visit 5 (6 months; 3 months after cessation of supplementation)

- ▶ Document cases, severity and outcomes of COVID-19 and/or respiratory viral infections

Unscheduled visits

If deemed by the investigators that additional assessments are required for medical or safety reasons.

Treatment

Multi-nutrient supplementation and storage

After screening, eligible participants ([box 1](#)) will be randomised (as described earlier) to receive two daily 220 mL bottles of the multi-nutrient supplement (intervention) (online supplemental appendix 1) or usual care (control). The multi-nutrient supplement will be supplied to the facility by an assigned investigator, handled and stored safely and properly, and kept in a secure location that only the assigned investigator and designated staff at the facility have access to. On receipt, the multi-nutrient supplement will be stored according to the instructions

specified by the manufacturer. Where possible, the multi-nutrient supplement will be refrigerated at 5 degrees at the aged care facility prior to administration. Documentation of the dispensing process will be maintained.

Potential side effects and monitoring

The multi-nutrient supplement doses are recommended by the manufacturer (Abbott Australasia). The potential risks from consuming the multi-nutrient supplement are considered similar to other commercially available supplements.

Two daily doses of the multi-nutrient supplement will provide 1000 IU of vitamin D. The Institute of Medicine has set the dose of 4000 IU per day as the tolerable upper limit, so total vitamin D intake, including that from other supplements, will be recorded. Participants will be monitored for signs of toxicity (nausea, vomiting, diarrhoea or frequent urination) and blood 25(OH)D and calcium levels evaluated at visits 3 and 4. A clinical trials monitoring committee is established at the research institute. Any AEs will be communicated to this committee, and action taken based on the severity of the event.

Treatment compliance

Compliance will be monitored weekly with all bottles returned to the research institute and unused product measured and recorded.

Treatment blinding

Staff involved in assessments will be blinded to treatment allocation. Randomisation data will be kept confidential and only accessible by designated authorised, unblinded study personnel. Unblinding will only occur in the case of participant emergencies and at the conclusion of the study and statistical analyses.

Participant withdrawal

Participation will be discontinued if the investigator or the monitoring committee deems that continuing study treatment would be detrimental to a person's well-being. This may include but is not limited to: (1) emergence of one or more AEs or laboratory abnormalities that, in the judgement of the investigator, prevents the person from safely continuing in the study; (2) intentional loss of adherence for 7 consecutive days (including hospitalisation); or (3) a protocol deviation that results in a significant risk to the person's safety. Discontinuation of participation may also occur in the event of (1) death, (2) discharge from the aged care facility or (3) withdrawal of consent by the participant.

Withdrawal of consent may be made at any time and for any reason, with details documented. Participants may withdraw consent to (1) no longer participate in the entire study, (2) not participate in a particular part of the study or aspects of assessments, (3) not participate in further visits or assessments or (4) have any further study related contact. In this case, contact would only be made for safety reasons. In the case of (1), (2) and (4) study

treatment will be discontinued, and no further assessments will be conducted.

Loss to follow-up

For participants whose status is unclear because they fail to undergo study visits without stating an intention to discontinue, the investigator will show ‘due diligence’ by documenting steps taken to determine their absence, for example, view medical records or inquiry through facility staff. A participant should not be considered lost to follow-up until their scheduled end of study visit has occurred. Participants who are discontinued from the study for any reason will not be replaced.

Study completion and post-study treatment

Each participant is planned to be followed up for 6 months; 3 months of intervention + 3 months of observation post-intervention. The study will be considered complete when the last participant completes their final visit, and any repeat assessments associated with this visit have been documented and followed up appropriately by the investigator.

Outcomes

To determine the efficacy of two daily doses of a multi-nutrient supplement on the immune system and muscle and respiratory function of older adults in residential aged care, the following outcomes will be assessed:

Primary outcome

T-cell subsets CD8 + and CD28null measured at baseline and months 1 and 3.

Secondary outcomes

1. Incidence and severity of COVID-19 and/or respiratory viral infections and their associated complications (ie, hospitalisations, complications and mortality) assessed by review of medical records at 3 and 6 months.
2. Handgrip strength measured at baseline and 3 months.
3. Multiple immunosenescence markers measured at baseline, and 1 and 3 months (table 2 and online supplemental appendix 2)
4. Respiratory function (FEV₁, FVC and FEV₁/FVC ratio) recorded at baseline and 3 months.
5. Gait velocity, measured at baseline and 3 months.
6. Appendicular lean mass measured by bioelectrical impedance, measured at baseline and 3 months.
7. SPPB measured at baseline and 3 months.
8. Serum haemoglobin, measured at baseline and 3 months.
9. Serum albumin measured at baseline and 3 months.
10. Serum vitamin D (25OHD) measured at baseline and 3 months.
11. Dietary intake recorded at baseline and 3 months.
12. QoL (EQ-5D-5L), recorded at baseline and 3 months.

Statistics

Sample size calculation

Available data on normal levels of T-cell subset CD28null indicated the standard deviation (SD) for men and women aged 60–80 year-old is 16% and 21% for those aged 80–100 years.⁶¹ A 10% difference in T-cell subset CD28null is considered clinically relevant.¹⁰ Assuming an SD of 20%, to detect a 10% difference with 80% power at the 5% significance level, 128 participants will be required. Allowing for 20% attrition, 160 participants (80 per group) are required.

Statistical analysis

Descriptive statistics will be used to summarise information collected for each outcome at each time point. The primary outcome of the study is the comparison of T-cell subsets CD8 + and CD28null at months 1 and 3 from baseline, which will be assessed using a generalised linear mixed model adjusted for baseline levels. If the model is of poor fit by visual inspection of residuals, the outcome will be transformed using natural logarithm. If the model fit is still insufficient, non-parametric tests will be used for analysis.

The analysis will be on an intention-to-treat basis with additional per-protocol analysis for the primary outcome. If there is a large proportion of participants violating the protocol, per-protocol analysis for all outcomes will be performed to determine the efficacy of the supplement. The extent of missing data will be evaluated. Missing data will be handled within the generalised linear mixed model. Where non-parametric analysis is required, complete case analysis will be performed (if missing data is minimal), or simple imputation will be performed. Secondary outcomes will be analysed in a similar manner, using mixed-effects linear regression for continuous outcomes and mixed-effects negative binomial regression for counts. Statistical significance will be assumed at $p < 0.05$. No adjustments for multiple comparisons will be made; however, secondary outcomes will be interpreted considering multiple comparisons.

Data collection and storage

Data management, security and handling

The investigators are responsible for ensuring the accuracy, completeness, legibility and timeliness of data reported. Designated research staff are provided with an individual log-in to enter data required by the protocol into the electronic Case Report Form (eCRF) within a secure electronic password-protected database (REDCap) hosted on a secure server by the University of Melbourne. All data will have an external originating source (either written or electronic). Paper-based source data will be stored in a locked office at AIMSS. Automatic validation syntax set within REDCap will check for data discrepancies and generating appropriate error messages will allow for data to be confirmed or corrected. Participants will be de-identified and given a unique participant number.

Data sharing

No study data or information will be released to any unauthorised third party without prior written approval by the University of Melbourne. Recipients will treat the data according to the Australian Privacy Principles or similar privacy legislation. The recipients will not use or disclose the information untowardly or outside the parameters of the agreement between them and the institution (University of Melbourne). No individual will be identified in reports or publications; only group-level data will be presented. Participants will only be identified by a unique participant number. The investigator will maintain a confidential participant identification list that allows the unambiguous identification of each participant. All relevant and applicable laws and guidelines will be applied to any data that is leaving the Institution. After the study is complete, a study summary will be provided to participating facilities and individual participants.

Site monitoring

During the study, the delegated monitor (eg, study coordinator independent of the study) will regularly check the completeness of study records and the accuracy of entries in the eCRFs, to ensure adherence to the protocol and to Good Clinical Practice. Designated investigator site staff will be required to respond to queries and confirm or correct the data as required during the ongoing monitoring of the study.

Record retention

All study documents will be retained for a minimum of 15 years after study completion and will be disposed of in a standard secure manner at the end of the archival period. Only authorised study staff will have access to the data.

ETHICS AND DISSEMINATION

This study was approved by Melbourne Health Human Research Ethics Committee (Ref No HREC/73985/MH-2021, ERM Ref No RMH73985, Melbourne Health Site Ref No 2021.115). Written informed consent will be obtained from participants. Results will be published in peer-reviewed journals and made available to aged care stakeholders, including providers, residents and government bodies.

Proposed timeline

The expected start date is 28 April 2022, when the first participant will be recruited. Expected timeline for completion of the study is mid-September 2023.

DISCUSSION

We aim to determine whether 3 months of multi-nutrient supplementation improves the immune system and muscle and respiratory function in older adults living in aged care who are at high risk of adverse outcomes due to seasonal influenza and other viral infections such as COVID-19. Older adults, particularly aged care residents,

are prioritised for influenza and COVID-19 vaccinations. However, low immune responses to standard vaccines because of immunosenescence may compromise vaccine effectiveness^{6 62}; therefore, vaccination efficacy may be improved by priming the immune system of older adults.

The proposed outcome from this work is that multi-nutrient supplementation will improve cellular immunity. Viral infections have several features that make them useful for studying T-cell immune responses as the productive infection is localised to lung tissue, and no persisting virus can be detected.⁶³ These features make influenza/COVID-19 infections a good model for studying both the effector and the memory phases of T-cell response. Multiple studies have tested proliferative responses to *in vitro* challenges with influenza antigens via examined total T-cell proliferation, and it is known that proliferative responses to influenza vaccine are generally higher within CD4 than CD8 T-cell subsets.^{64–66} These data indicate that specific nutritional supplements can enhance T-cell proliferative responses to viral challenges. Noteworthy, measuring immune competency in older adults should involve several criteria because immune changes during ageing and from malnutrition are similar; nutrient supplementation may improve immune status and clinical outcomes in older adults^{67 68} and those at risk of sarcopenia.⁶⁹ These criteria must include full blood counts, T and B cell counts and their function and subsets (particularly T-cell subsets CD8+, CD28null, Treg and dendritic cells), and concentrations of serum interleukins (particularly IL-7).^{19 10 70}

As the population ages and the risk of potential pandemics remain, it is reasonable to prepare vulnerable older adults for viral assaults by implementing efficacious evidence-based interventions using specialised nutritional supplements.^{39 71–74} Nutritional interventions using vitamin D, protein, zinc and selenium enhanced anti-viral resistance against COVID-19 in older adults.^{61 75} Among COVID-19 inpatients (n=134), 19% of patients in intensive care units had serum 25(OH)D levels above 50 nmol/L compared with 39% of those in conventional medical wards.⁷⁶ In a randomised placebo-controlled trial involving 38 older adults, vitamin D supplementation (100 000 IU/15 days) promoted a higher transforming growth factor beta (TGF β) plasma level (20.8 ng/mL) in response to influenza vaccination and directed the lymphocyte polarisation toward a tolerogenic immune response.⁷⁷ Furthermore, vitamin D has been associated with improved pulmonary function and reduced incidence of airway infections. A RCT of 86 older adults showed that consumption of 50 000 IU vitamin D supplementation in a daily diet could increase QoL and pulmonary function.⁷⁸ Moreover, higher serum vitamin D levels (>50 nmol/L) among adults are associated with decreased odds of obstructive lung disease in the general population.⁷⁹ In addition, vitamin D supplementation (2000 IU/day) reduced the risk for pneumonia, acute exacerbations of respiratory diseases and lung function decline in older adults.⁸⁰ Therefore, vitamin D supplementation

may have a beneficial role against viral infections in aged care residents.

Strengths of this study include the comprehensive immune, respiratory and functional assessments performed in aged care residents after receiving a multi-nutrient supplement that is commercially available. This study is randomised, and the assessor will be blinded to treatment allocation. Furthermore, the type of intervention used enables rapid implementation into practice and could help prime the immune system in older adults in aged care to combat viral infection and future strains of COVID-19. Limitations of this study are that any biological effect observed cannot be attributed to one single component of the multi-nutrient supplement, and the potential for group differences in energy intake can only be monitored by regularly assessing dietary intake and weight changes during the study period.

The main purpose of this study is to prepare aged care residents against viral infections, improve their immune, muscle and respiratory function and QoL. Therefore, this study may have significant health impacts that are broader than the preparation for or prevention of COVID-19. Outcomes from this study may provide evidence-based clinical care pathways to support scalable and pragmatic aged care-based nutrition support programmes that reduce the severity of seasonal influenza or other viral infections.

In summary, the Pomerium Study will determine the efficacy of a multi-nutrient supplement on immune, muscle and respiratory function and QoL of older adults in aged care. Further outcomes include a reduction in the incidence of COVID-19 and seasonal viral infections and their associated complications in supplemented participants. The study results may support the provision of multi-nutrient supplements to older adults in aged care prior to and during viral outbreaks as a strategy to reduce the onset and severity of viral infections.

Author affiliations

¹Medicine-Western Health, The University of Melbourne, Melbourne, Victoria, Australia

²Australian Institute for Musculoskeletal Science (AIMSS), St Albans, Victoria, Australia

³School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia

⁴Johns Hopkins Center on Aging and Immune Remodeling, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

⁵Nepean Clinical School, The University of Sydney, Sydney, New South Wales, Australia

Contributors Gustavo Duque conceived the trial and is the chief investigator in the MRFF grant. Ahmed Al Saedi, Ben Kirk, Sandra Iuliano, Jesse Zanker, Sara Vogrin, Lata Jayaram, Shane Thomas, Christine Golding, Diana Navarro-Perez, Petra Marusic, Sean Leng, Ralph Nanan and Gustavo Duque participated in the design of the trial and read and approved the final manuscript.

Funding This study was funded by a grant from the Australian Medical Research Future Fund 2020; Rare Cancers Rare Diseases and Unmet Need (Grant number: 2005987). Sponsor: University of Melbourne (Melbourne, Australia) to AAS, SI and GD.

Disclaimer The sponsor and funding agency will not play any role in study design; collection, management, analysis and interpretation of data; writing of the report; or the decision to submit the report for publication.

Competing interests GD and SI-B serve as members of the Advisory Board of Abbott Australia. GD serves on the Scientific Advisory Board at TSI Pharmaceuticals. BK is currently supported by a research fellowship from TSI Pharmaceuticals. All the other authors declare that they have no competing interests.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Gustavo Duque <http://orcid.org/0000-0001-8126-0637>

REFERENCES

- Leng SX, Margolick JB. Aging, sex, inflammation, frailty, and CMV and HIV infections. *Cell Immunol* 2020;348:104024.
- Cunha LL, Perazzo SF, Azzi J, *et al*. Remodeling of the immune response with aging: Immunosenescence and its potential impact on COVID-19 immune response. *Front Immunol* 2020;11:1748.
- Fulop T, Larbi A, Dupuis G, *et al*. Immunosenescence and Inflamm-Aging as two sides of the same coin: friends or foes? *Front Immunol* 2017;8:1960. doi:10.3389/fimmu.2017.01960
- Li H, Manwani B, Leng SX, Frailty LSX. Frailty, inflammation, and immunity. *Aging Dis* 2011;2:466–73.
- Chen Y, Liu S, Leng SX. Chronic low-grade inflammatory phenotype (clip) and senescent immune dysregulation. *Clin Ther* 2019;41:400–9.
- Woods JL, Iuliano-Burns S, Walker KZ. Immunological and nutritional factors in elderly people in low-level care and their association with mortality. *Immun Ageing* 2013;10:32.
- Sugg MM, Spaulding TJ, Lane SJ, *et al*. Mapping community-level determinants of COVID-19 transmission in nursing homes: a multi-scale approach. *Sci Total Environ* 2021;752:141946.
- Iuliano S, Poon S, Wang X, *et al*. Dairy food supplementation may reduce malnutrition risk in institutionalised elderly. *Br J Nutr* 2017;117:142–7.
- Nalin D. Immunosenescence and severe acute respiratory syndrome coronavirus 2 vaccine development. *J Infect Dis* 2020;222:2114.
- Fagnoni FF, Vescovini R, Mazzola M, *et al*. Expansion of cytotoxic CD8+ CD28- T cells in healthy ageing people, including centenarians. *Immunology* 1996;88:501–7.
- Adams RL, Broughton KS. Insulinotropic effects of whey: mechanisms of action, recent clinical trials, and clinical applications. *Ann Nutr Metab* 2016;69:56–63.
- Vyas N, Kurian SJ, Bagchi D, *et al*. Vitamin D in prevention and treatment of COVID-19: current perspective and future prospects. *J Am Coll Nutr* 2021;40:632–45.
- Liu BA, Gordon M, Labranche JM, *et al*. Seasonal prevalence of vitamin D deficiency in institutionalized older adults. *J Am Geriatr Soc* 1997;45:598–603.
- Arnljots R, Thorn J, Elm M, *et al*. Vitamin D deficiency was common among nursing home residents and associated with dementia: a cross sectional study of 545 Swedish nursing home residents. *BMC Geriatr* 2017;17:229.

- 15 Stow R, Ives N, Smith C, *et al.* A cluster randomised feasibility trial evaluating nutritional interventions in the treatment of malnutrition in care home adult residents. *Trials* 2015;16:433.
- 16 Deutz NEP, Bauer JM, Barazzoni R, *et al.* Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN expert group. *Clin Nutr* 2014;33:929–36.
- 17 Zochling J, Chen JS, Seibel M, *et al.* Calcium metabolism in the frail elderly. *Clin Rheumatol* 2005;24:576–82.
- 18 Sim M, Zhu K, Lewis JR, *et al.* Association between vitamin D status and long-term falls-related hospitalization risk in older women. *J Am Geriatr Soc* 2021;69:3114–23.
- 19 Hoong CWS, Huilin K, Cho S, *et al.* Are adequate vitamin D levels helpful in fighting COVID-19? A look at the evidence. *Horm Metab Res* 2020;52:775–83.
- 20 Radujkovic A, Hippchen T, Tiwari-Heckler S, *et al.* Vitamin D deficiency and outcome of COVID-19 patients. *Nutrients* 2020;12:2757. doi:10.3390/nu12092757
- 21 Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;393:2636–46.
- 22 Cruz-Jentoft AJ, Bahat G, Bauer J, *et al.* Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;48:16–31.
- 23 Zhu N, Zhang D, Wang W, *et al.* A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
- 24 Carrara G, Pecorelli N, De Cobelli F, *et al.* Preoperative sarcopenia determinants in pancreatic cancer patients. *Clin Nutr* 2017;36:1649–53.
- 25 Vang P, Vasdev A, Zhan W-Z, *et al.* Diaphragm muscle sarcopenia into very old age in mice. *Physiol Rep* 2020;8:e14305.
- 26 Nishimura JM, Ansari AZ, D'Souza DM, *et al.* Computed Tomography-Assessed skeletal muscle mass as a predictor of outcomes in lung cancer surgery. *Ann Thorac Surg* 2019;108:1555–64.
- 27 Kim G, Kang SH, Kim MY, *et al.* Prognostic value of sarcopenia in patients with liver cirrhosis: a systematic review and meta-analysis. *PLoS One* 2017;12:e0186990.
- 28 Rovere-Querini P, Tresoldi C, Conte C, *et al.* Biobanking for COVID-19 research. *Panminerva Med* 2020. doi:10.23736/S0031-0808.20.04168-3. [Epub ahead of print: 19 Oct 2020].
- 29 Ufuk F, Demirci M, Sagtas E, *et al.* The prognostic value of pneumonia severity score and pectoralis muscle area on chest CT in adult COVID-19 patients. *Eur J Radiol* 2020;131:109271.
- 30 Kim J-W, Yoon JS, Kim EJ, *et al.* Prognostic implication of baseline sarcopenia for length of hospital stay and survival in patients with coronavirus disease 2019. *J Gerontol A Biol Sci Med Sci* 2021;76:e110–6.
- 31 Feng Z, Zhao H, Kang W, *et al.* Association of paraspinous muscle measurements on chest computed tomography with clinical outcomes in patients with severe coronavirus disease 2019. *J Gerontol A Biol Sci Med Sci* 2021;76:e78–84.
- 32 Kara Özgür, Kara M, Akın ME, *et al.* Grip strength as a predictor of disease severity in hospitalized COVID-19 patients. *Heart Lung* 2021;50:743–7.
- 33 Cheval B, Sieber S, Maltagliati S, *et al.* Muscle strength is associated with COVID-19 hospitalization in adults 50 years of age or older. *J Cachexia Sarcopenia Muscle* 2021;12:1136–43.
- 34 Welch C, Greig C, Masud T, *et al.* COVID-19 and acute sarcopenia. *Aging Dis* 2020;11:1345–51.
- 35 Piotrowicz K, Gałowski J, Michel J-P, *et al.* Post-COVID-19 acute sarcopenia: pathophysiology and management. *Aging Clin Exp Res* 2021;33:2887–98.
- 36 Murphy CH, Saddler NI, Devries MC, *et al.* Leucine supplementation enhances integrative myofibrillar protein synthesis in free-living older men consuming lower- and higher-protein diets: a parallel-group crossover study. *Am J Clin Nutr* 2016;104:1594–606.
- 37 Moore DR, Churchward-Venne TA, Witard O, *et al.* Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol A Biol Sci Med Sci* 2015;70:57–62.
- 38 Wilkinson DJ, Hossain T, Hill DS, *et al.* Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. *J Physiol* 2013;591:2911–23.
- 39 Oktaviana J, Zanker J, Vogrin S, *et al.* The effect of β-hydroxy-β-methylbutyrate (HMB) on sarcopenia and functional frailty in older persons: a systematic review. *J Nutr Health Aging* 2019;23:145–50.
- 40 Kirk B, Prokopenko K, Duque G. Nutrients to mitigate osteosarcopenia: the role of protein, vitamin D and calcium. *Curr Opin Clin Nutr Metab Care* 2021;24:25–32.
- 41 Kirk B, Feehan J, Lombardi G, *et al.* Muscle, bone, and fat crosstalk: the biological role of myokines, osteokines, and adipokines. *Curr Osteoporos Rep* 2020;18:388–400.
- 42 Chanet A, Salles J, Guillet C, *et al.* Vitamin D supplementation restores the blunted muscle protein synthesis response in deficient old rats through an impact on ectopic fat deposition. *J Nutr Biochem* 2017;46:30–38.
- 43 Verlaan S, Maier AB, Bauer JM, *et al.* Sufficient levels of 25-hydroxyvitamin D and protein intake required to increase muscle mass in sarcopenic older adults – the provide study. *Clinical Nutrition* 2018;37:551–7.
- 44 Kositsawat J, Duque G, Kirk B. Nutrients with anabolic/anticatabolic, antioxidant, and anti-inflammatory properties: targeting the biological mechanisms of aging to support musculoskeletal health. *Exp Gerontol* 2021;154:111521.
- 45 Langkamp-Henken B, Wood SM, Herlinger-Garcia KA, *et al.* Nutritional formula improved immune profiles of seniors living in nursing homes. *J Am Geriatr Soc* 2006;54:1861–70.
- 46 Akatsu H, Nagafuchi S, Kurihara R, *et al.* Enhanced vaccination effect against influenza by prebiotics in elderly patients receiving enteral nutrition. *Geriatr Gerontol Int* 2016;16:205–13.
- 47 Aspinall R, Lang PO. Interventions to restore appropriate immune function in the elderly. *Immun Ageing* 2018;15:5.
- 48 Schulz KF, Altman DG, Moher D. *Consort 2010 statement: updated guidelines for reporting parallel group randomised trials.* . 2010: 115, 1063–70.
- 49 Al Saedi A, Gunawardene P, Bermeo S, *et al.* Lamin A expression in circulating osteoprogenitors as a potential biomarker for frailty: the Nepean osteoporosis and frailty (nof) study. *Exp Gerontol* 2018;102:69–75.
- 50 Gunawardene P, Bermeo S, Vidal C, *et al.* Association between circulating osteogenic progenitor cells and disability and frailty in older persons: the Nepean osteoporosis and frailty study. *J Gerontol A Biol Sci Med Sci* 2016;71:1124–30.
- 51 Bhasin S, Travison TG, Manini TM, *et al.* Sarcopenia definition: the position statements of the sarcopenia definition and outcomes Consortium. *J Am Geriatr Soc* 2020;68:1410–8.
- 52 Glendenning P, Inderjeeth CA. Vitamin D: methods of 25 hydroxyvitamin D analysis, targeting at risk populations and selecting thresholds of treatment. *Clin Biochem* 2012;45:901–6.
- 53 Phu S, Vogrin S, Al Saedi A, *et al.* Balance training using virtual reality improves balance and physical performance in older adults at high risk of falls. *Clin Interv Aging* 2019;14:1567–77.
- 54 Guralnik JM, Simonsick EM, Ferrucci L, *et al.* A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;49:M85–94.
- 55 Phu S, Kirk B, Bani Hassan E, *et al.* The diagnostic value of the short physical performance battery for sarcopenia. *BMC Geriatr* 2020;20:242.
- 56 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
- 57 Vellas B, Guigoz Y, Garry PJ, *et al.* The mini nutritional assessment (MNA) and its use in grading the nutritional state of elderly patients. *Nutrition* 1999;15:116–22.
- 58 Abimanyi-Ochom J, Watts JJ, Borgström F, *et al.* Changes in quality of life associated with fragility fractures: Australian arm of the International cost and utility related to osteoporotic fractures study (AusLCUROS). *Osteoporos Int* 2015;26:1781–90.
- 59 Tinetti ME, Ginter SF. The nursing home life-space diameter. A measure of extent and frequency of mobility among nursing home residents. *J Am Geriatr Soc* 1990;38:1311–5.
- 60 Sherwin AJ, Nowson CA, McPhee J. Nutrient intake at meals in residential care facilities for the aged: validated visual estimation of plate waste. *Austr J Nutr Diet* 1998;55:188–93.
- 61 Beck AM, Christensen AG, Hansen BS, *et al.* Multidisciplinary nutritional support for undernutrition in nursing home and home-care: a cluster randomized controlled trial. *Nutrition* 2016;32:199–205.
- 62 Schaffner W, van Buynder P, McNeil S, *et al.* Seasonal influenza immunisation: strategies for older adults. *Int J Clin Pract* 2018;72:e13249.
- 63 Doherty PC, Topham DJ, Tripp RA, *et al.* Effector CD4+ and CD8+ T-cell mechanisms in the control of respiratory virus infections. *Immunol Rev* 1997;159:105–17.
- 64 Herrera MT, Gonzalez Y, Juárez E, *et al.* Humoral and cellular responses to a non-adjuvanted monovalent H1N1 pandemic influenza vaccine in hospital employees. *BMC Infect Dis* 2013;13:544.
- 65 Chapman TJ, Castrucci MR, Padrick RC, *et al.* Antigen-Specific and non-specific CD4+ T cell recruitment and proliferation during influenza infection. *Virology* 2005;340:296–306.
- 66 Schmidt T, Dirks J, Enders M, *et al.* Cd4+ T-cell immunity after pandemic influenza vaccination cross-reacts with seasonal antigens

- and functionally differs from active influenza infection. *Eur J Immunol* 2012;42:1755–66.
- 67 Meydani SN, Leka LS, Fine BC, *et al.* Vitamin E and respiratory tract infections in elderly nursing home residents: a randomized controlled trial. *JAMA* 2004;292:828–36.
 - 68 Girodon F, Lombard M, Galan P, *et al.* Effect of micronutrient supplementation on infection in institutionalized elderly subjects: a controlled trial. *Ann Nutr Metab* 1997;41:98–107.
 - 69 Kirk B, Mooney K, Vogrin S, *et al.* Leucine-enriched whey protein supplementation, resistance-based exercise, and cardiometabolic health in older adults: a randomized controlled trial. *J Cachexia Sarcopenia Muscle* 2021;12:2022–33.
 - 70 Nguyen V, Mendelsohn A, Larrick JW. Interleukin-7 and Immunosenescence. *J Immunol Res* 2017;2017:4807853.
 - 71 Constantin D, Menon MK, Houchen-Wolloff L, *et al.* Skeletal muscle molecular responses to resistance training and dietary supplementation in COPD. *Thorax* 2013;68:625–33.
 - 72 EFAD guidelines. *Nutritional guidelines and menu checklist for residential and nursing homes*, 2014.
 - 73 Dietitians of Canada. *Best practices for nutrition, food service and dining in long term care homes*, 2019.
 - 74 Caccialanza R, Laviano A, Lobascio F, *et al.* Early nutritional supplementation in non-critically ill patients hospitalized for the 2019 novel coronavirus disease (COVID-19): rationale and feasibility of a shared pragmatic protocol. *Nutrition* 2020;74:110835.
 - 75 Alexander J, Tinkov A, Strand TA, *et al.* Early nutritional interventions with zinc, selenium and vitamin D for raising anti-viral resistance against progressive COVID-19. *Nutrients* 2020;12. doi:10.3390/nu12082358. [Epub ahead of print: 07 Aug 2020].
 - 76 Panagiotou G, Tee SA, Ihsan Y, *et al.* Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity. *Clin Endocrinol* 2020;93:508–11.
 - 77 Goncalves-Mendes N, Talvas J, Dualé C, *et al.* Impact of vitamin D supplementation on influenza vaccine response and immune functions in deficient elderly persons: a randomized placebo-controlled trial. *Front Immunol* 2019;10:65.
 - 78 Alavi Foumani A, Mehrdad M, Jafarinezhad A, *et al.* Impact of vitamin D on spirometry findings and quality of life in patients with chronic obstructive pulmonary disease: a randomized, double-blinded, placebo-controlled clinical trial. *Int J Chron Obstruct Pulmon Dis* 2019;14:1495–501.
 - 79 Rafiq R, Prins HJ, Boersma WG, *et al.* Effects of daily vitamin D supplementation on respiratory muscle strength and physical performance in vitamin D-deficient COPD patients: a pilot trial. *Int J Chron Obstruct Pulmon Dis* 2017;12:2583–92.
 - 80 Gold DR, Litonjua AA, Carey VJ, *et al.* Lung vital: rationale, design, and baseline characteristics of an ancillary study evaluating the effects of vitamin D and/or marine omega-3 fatty acid supplements on acute exacerbations of chronic respiratory disease, asthma control, pneumonia and lung function in adults. *Contemp Clin Trials* 2016;47:185–95.