Leucine and ACE inhibitors as therapies for sarcopenia: a two by two factorial randomised placebo controlled trial

Statistical Analysis Plan

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

(including Study abbreviations)

AE	Adverse Event
AR	Adverse Reaction
BIA	BioImpedance Assay
CNORIS	Clinical Negligence and Other Risks Scheme
CRF	Case Report Form
DXA	Dual Energy X-ray Absorptiometry
EQ5D	EuroQol 5D
EWGSOP	European Working Group on Sarcopenia in Older People
FFQ	Food Frequency Questionnaire
GCP	Good Clinical Practice
IMP	Investigational Medicinal Product
ISF	Investigator Site File
MHRA	Medicines and Healthcare Products Regulatory Agency
NEADL	Nottingham Extended Activities of Daily Living
NRES	National Research Ethics Service
REC	Research Ethics Committee
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SPPB	Short Physical Performance Battery
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UAR	Unexpected Adverse Reaction
6MWD	Six Minute Walking Distance

STUDY SUMMARY

Hypothesis and aims: We hypothesise that supplementation with the essential amino acid leucine and/or the angiotensin converting enzyme inhibitor perindopril will improve muscle mass and function in older people with sarcopenia. Leucine supplementation has been shown to counter the resistance to muscle protein synthesis in older people, reduce inflammatory markers, improve insulin resistance, increase satellite cells (indicating muscle regeneration) and improve mitochondrial function in muscle. Perindopril (a common cardiovascular medication) has been shown to reduce inflammatory cytokines and myokines, improve insulin resistance and mitochondrial function in skeletal muscle.

We have previously found that perindopril improves exercise capacity (equivalent to that seen after 6 months of exercise training) in older people. While both interventions can positively affect many factors implicated in the development of sarcopenia their effect has not been tried in this group. We will study the effect of leucine and perindopril on muscle performance and muscle mass in sarcopenia. We will also investigate biomarkers which predict response to interventions and examine mechanisms of faction of these interventions on sarcopenia.

Design: Multicentre double blind randomised controlled 2x2 factorial trial

Population: >/=70 years with sarcopenia as per the EWGSOP definition (loss of both muscle mass and function). Excluding those on or with contraindications to perindopril.

Interventions: 4 groups receiving one of perindopril+leucine, perindopril+placebo, leucine+placebo or placebo+placebo for 12 months. Leucine dose will be 2.5g, 3 times/day and perindopril will be 4mg/day.

Outcomes: Primary outcome is the difference in Short Physical Performance Battery between leucine vs. placebo and perindopril vs placebo. Secondary outcomes are change in muscle mass, grip strength, quadriceps strength, 6 minute walking distance, quality of life (EuroQol)

Assessments: Performed at baseline, 6 and 12 months. Safety assessments will be done 3 monthly.

Sample size: 440 participants (n=110 per group) will have 90% power at 0.05 alpha to identify a change of 0.5 (SD 2.7) in primary outcome allowing for a 20% drop out rate.

Statistical analysis: Change in outcome measures between interventions will be analysed using repeated measures using mixed modelling.

Potential benefit: Sarcopenia is a major contributor to loss of mobility, falls, institutionalisation, morbidity and mortality in older people. Both leucine and perindopril are inexpensive. If found to be acceptable for older people and effective in improving sarcopaenia, these agents will be value for money, costing less than £300 per patient per year.

Biomarker substudy: Blood will be analysed for biomarkers that predict improvement in muscle mass and function. Changes in microRNA, myokines, cytokines and hormones in blood, plus selected gene polymorphisms will be measured to examine predictors of response to therapies and changes in biological pathways in sarcopenia in response to interventions.

Derby substudy: A further substudy will be performed on participants at the Derby site; muscle mass will be estimated at baseline, 6 and 12 months using D3 creatine (a stable isotope tracer), and muscle turnover will be estimated using 3-methylhistidine as a stable tracer.

Scope of this SAP

The main trial analysis will be performed by Tayside CTU and is specified in this SAP. This SAP will not specify other analyses using data collected as part of the trial; these additional analyses will be managed as follows:

- Cross-sectional analysis of baseline data will be performed by investigators in NIHR Newcastle BRC and will be detailed in a separate SAP
- Biomarker substudy analyses will be performed by investigators at Imperial College London, and will be detailed in a separate SAP

- The D3 creatine dilution substudy will be analysed by collaborators in Derby, and will be detailed in a separate SAP

1 INTRODUCTION

1.1 BACKGROUND

Sarcopenia (the age-related loss of muscle mass and function) is a major contributor to loss of mobility, falls, loss of independence, morbidity and mortality in older people. The mechanisms behind the development of sarcopenia are not fully understood, but accumulating evidence suggests that it is multifactorial in aetiology.

Non-exercise interventions to prevent or counter the effects of sarcopenia are thus urgently required. A range of potential interventions have been proposed, including protein supplementation, myostatin inhibitors, testosterone and selective androgen receptor modifiers, growth hormone and novel interventions including activin ligands. We propose to study the efficacy of two promising interventions (leucine and angiotensin converting enzyme inhibition) that can potentially improve muscle mass and function in people with sarcopenia as defined by the European Working Group on Sarcopenia (EWGSOP).

1.2 RATIONALE FOR STUDY

We aim to investigate two interventions with potential to improve both muscle mass and function independently of exercise. Both have potential to improve factors contributing to sarcopenia, using different mechanisms.

Leucine
Muscle protein synthesis (MPS) in older people in response to protein ingestion is attenuated compared to younger people – i.e. there is anabolic resistance to protein supplementation. Increasing the amount of protein ingested is one way of overcoming this issue, but older, frail people typically already have suboptimal protein intakes and increasing protein intake may be challenging in practice.
Leucine, a branched-chain amino acid, is known to have important regulatory actions, mediated at least in part via the mTOR pathway. Leucine affects both aspects of protein turnover, by reducing proteolysis and enhancing protein synthesis in vitro. Studies in healthy older people confirm that addition of leucine to a protein meal enhances muscle protein synthesis; previous studies suggest that approximately 2.5g of leucine per meal is sufficient to generate the effect. In addition, leucine stimulates insulin release by pancreatic beta cells; insulin signalling not only improves glucose uptake by skeletal muscle, but is also an important anabolic signal for skeletal muscle.

Angiotensin Converting Enzyme inhibitor drugs (ACEi)
Renin-angiotensin-aldosterone system (RAAS) activity has been implicated in skeletal muscle dysfunction via multiple biological pathways. Angiotensin II impairs endothelial function and hence muscle blood supply; is associated with increased levels of inflammation, suppression of IGF-1 and has important effects on mitochondrial function. Aldosterone too has deleterious effects, including lowering serum potassium, impairment of endothelial function, and promotion of fibrosis.
Conversely, ACEi drugs have been shown to have multiple potentially beneficial effects on skeletal muscle function. ACEi improve endothelial function, angiogenesis and reduce inflammation, which translates to improved blood flow and exercise capacity in patients with peripheral vascular disease. They improve mitochondrial function, enhance IGF-1 levels, and suppress pro-inflammatory cytokine levels including IL-6; thought to be a key inflammatory mediator of sarcopenia. ACEi have also been shown to promote skeletal muscle glucose uptake, and promote a shift from type 1 to type 2 muscle fibres (the type preferentially affected by sarcopenia). Finally, there is evidence that ACEi may enhance neuromuscular junction activity, and selected ACEi may also have beneficial central CNS actions improving mood and motivation, which are likely to have important consequent benefits on physical activity levels.

2 STUDY OBJECTIVES FOR MAIN TRIAL ANALYSIS

2.1 OBJECTIVES

2.1.1 Primary Objective

To determine the efficacy of leucine and perindopril in improving physical function in older people with sarcopenia diagnosed using the EWGSOP definition.

2.1.2 Secondary Objectives

To evaluate the effect of leucine and perindopril on muscle mass, quality of life, activities of daily living and bone mineral density in older people.

2.2 OUTCOMES

2.2.1 Primary Outcomes

The primary outcome will be the between group difference in Short Physical Performance Battery (SPPB) score over 12 months of follow up.

2.2.2 Secondary Outcomes

Between group differences in:

- Appendicular muscle mass/height squared (measured by dual energy X-ray absorptiometry),
- Grip strength
- Quadriceps strength (handheld dynamometry)
- 6 minute walk test
- Gait speed (4m walk)
- Chair stands (Sit to stand test x 5)
- Activities of daily living (Nottingham extended ADL questionnaire) and quality of life (EuroQol 5D questionnaire).
-
- Hip Bone mineral density

In addition, data will be collected on adverse events, medication adherence by tablet counting and leucine tub container weight, falls. Hospitalisation, death and admission to institutional care will be obtained from SAE data; consent will be obtained for open-label follow up of participants for these outcomes collected from routine clinical data following the end of the 1 year study period but these data will not form part of the main trial analysis specified here. Data will be collected for diet at baseline and 12 months using the Scottish Collaborative Group Food Frequency Questionnaire (FFQ); this will allow the interaction between treatment and protein intake (total, plant vs animal protein) to be ascertained.

3 STUDY DESIGN

3.1 STUDY DESCRIPTION

LACE is a multicentre, double blind, placebo-controlled, 2x2 factorial randomised controlled trial. Participants will be randomised to receive either perindopril 4mg once daily or matching placebo, and to receive leucine 2.5g three times per day or matching placebo to mask treatment allocation to participants, investigators and usual healthcare providers. Intervention and follow up will be for 1 year. All trial outcomes will be measured and analysed masked to treatment allocation group. Randomisation will be performed via a central, web-based allocation system, using a minimisation algorithm to ensure balanced groups for key baseline factors (age, sex, SPPB, grip strength, comorbid disease, recruitment centre).

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

The LACE trial aims to recruit a total of 440 participants, from a minimum of 15 centres across the UK. Recruitment will take place over an initial 18 month period, with extension if required due to low recruitment rates. We anticipate a dropout rate of 20% at 1 year, and thus aim to recruit a final evaluable sample of 352 patients. Dropout rates will be reviewed after 9 months and 12 months of recruitment, and the total recruitment target will be adjusted based on projected dropout rates at these time points to ensure a final evaluable sample size of 352. The substudy performed at Derby aims to recruit a minimum of 30 participants

4.2 INCLUSION CRITERIA

Age 70 and over

Sarcopenia criteria according to EWGSOP definition, based on:

- low height-adjusted total skeletal muscle mass on BioImpedance Assay (BIA) using the BIA 101 device and the Sergi equation. Cutoffs will vary with body mass index and sex as follows:

BMI	Males	Females
<18.5 kg/m^2	<=6.02 kg/m^2	<=5.25 kg/m^2
18.5 – 24.9	<=7.14	<=5.70
25.0 – 29.9	<=8.00	<=6.19
>=30 kg/m^2	<=8.77	<=6.72

- and either low gait speed (<0.8 m/s on 4m walk) or low handgrip strength (<20Kg for women, <30Kg for men)

4.3 EXCLUSION CRITERIA

a) Contraindications or existing indications to therapies or placebo

-Known clinical diagnosis of chronic heart failure (by European Society of Cardiology criteria)
-Confirmed LV systolic dysfunction on any imaging modality
-Known aortic stenosis (peak gradient >30mmHg)
-Systolic BP<90 mmHg (supine)
-Dizziness on standing associated with a postural drop of >20/10mmHg (asymptomatic orthostatic hypotension will not be a contraindication)
-Serum Creatinine >170 umol/L or eGFR<30ml/min by MDRD4 calculation
-K>5.0 mmol/L; Na<130 mmol/L
-Using ACEi, Angiotensin receptor blocker, aldosterone blocker or leucine already
-Previous adverse reaction to ACEi or leucine
-Current use of oral NSAIDs (aspirin is permitted, as are topical NSAIDs)
-Current use of potassium supplements, aliskiren, spironolactone or other potassium-sparing diuretics

-Hereditary or idiopathic angioedema
-Lactose intolerance

b) Contraindications to consent or undertaking study outcomes
-Implantable cardioverter defibrillator or pacemaker with atrial sensing lead (pacemakers with ventricular sensing lead only are allowed)
-Peripheral oedema present above knee level
-Unable to mobilise without human assistance (walking aids allowed)
-Unable to give written informed consent
-Currently enrolled in another intervention research study, or less than 30 days since completing another intervention research study. Concomitant enrolment in observational studies is permitted.

c) Overlap with other myopathic conditions or important confounders
-Currently enrolled in a time-limited exercise-based rehabilitation programme
-Any progressive neurological or malignant condition with life expectancy <6 months
-Severe COPD (GOLD stage IV)
-Known myositis or other established myopathy
-Self-reported weight loss of >10% in last 6 months (to exclude significant cachexia)
-Known uncontrolled thyrotoxicosis
-7.5mg/day or greater prednisolone use (or equivalent)

4.4 RANDOMISATION

4.4.1 Randomisation

Randomisation will be via a centrally controlled web-based GCP compliant randomisation system, TRuST, run by Tayside Clinical Trials Unit (TCTU). Randomisation will be stratified by site. To ensure balanced assignment across critical variables, a minimisation algorithm will be employed, using baseline age, sex, SPPB score, grip strength and Charlson comorbidity score.

4.4.2 Treatment Allocation

The starting dose of perindopril will be 2mg daily which will be up-titrated to 4mg after 2 weeks if tolerated and renal function / potassium levels remain within defined limits (see Section 5.5.4 and 6.4). Participants in the placebo group will also undergo a ‘mock’ up-titration. If the 4 mg daily dose is not tolerated, participants will be down-titrated to the original 2mg daily. If 2mg is also not tolerated, medication will be withdrawn.

Medication will be dispensed by the Clinical Trials Pharmacist on receipt of a prescription with the participant number. Perindopril/placebo will be delivered to the participants on the day dispensed in sealed bottles with child proof caps. If the participant is unable to open child proof caps, a spare ordinary cap will be issued. Participants will be instructed to take one capsule every morning. This instruction is also printed on the bottle label.

Adherence will be checked and documented using tablet counts at each visit. If non-adherent, they will be encouraged to continue taking medication. If they persist as non-adherent, they will stay in the study, in order to do an “intention to treat” analysis.

Leucine and placebo will be dispensed as 400g tamper-evident tubs of identical powder (sufficient for 1 month) with a scoop to measure 4.5mls of powder (3 levelled scoops, 1.5ml each). Three level scoops will be taken with each meal. Instructions will be printed on the tub. Adherence will be checked by weighing the tubs at each study visit, with non-adherence treated as for perindopril.

4.4.3 Withdrawal procedures

Participants will not receive further doses of perindopril medication or placebo if:

- They exhibit serum potassium levels of >5.5mmol/L,
- Serum sodium < 130mmol/L,
- Serum creatinine rises from baseline by >60 umol/L or serum creatinine is >180 umol/L
- If systolic BP falls to <90mmHg (lying) when on 2mg perindopril/placebo.

They will however remain in the study and undergo their remaining follow-up visits to preserve intention to treat analysis in these cases.

Participants will not receive further doses of leucine if:

- They undergo an adverse reaction (e.g. allergic reaction) attributable to leucine or leucine placebo

Withdrawal from one medication/placebo does not affect administration of the other medication/placebo, which will continue until the end of the trial.

The reasons for withdrawal will be noted in the participant's CRF and casenotes. If withdrawal is due to an AE it will be logged as such on the Adverse Event Log.
Participants are free to withdraw from the study at any time. Data collected up to the point of withdrawal will be kept on file unless the participant requests that it is removed. Tissue will also be kept in storage unless the patient participant requests that it is removed.

Although a participant is not obliged to give reason(s) for withdrawing prematurely, if the participant appears lost to follow up, the CI will make a **reasonable** effort to ascertain the reason(s), while fully respecting the individual's rights, and will demonstrate that everything possible was done in an attempt to find any participant lost to follow-up. Those lost to follow-up or withdrawn will be identified and a descriptive analysis of them provided, including the reasons for their loss and its relationship to treatment and outcome.

As for active study medication, this will be under the supervision of the local Clinical Trials Pharmacist at each trial site as per the drug accountability and pharmacovigilance guidelines from TASC.

4.5 PARTICIPANT ADHERENCE

Adherence to perindopril or placebo will be checked by tablet counting, and of leucine or placebo by weighing container tubs, at each safety visit. In addition, serum ACE activity will be measured as a further test of adherence to perindopril therapy; this has been shown to have good sensitivity and specificity to adherence to ACE inhibitors.

5 DATA COLLECTION & MANAGEMENT

5.1 DATA COLLECTION

It is the CI responsibility to ensure the accuracy of all data entered and recorded in the CRFs and the database. The Delegation of Responsibilities Log will identify all trial personnel responsible for data collection, entry, handling and managing the database.

The data will be collected by the RN, the Site PI and/or or other local delegated individual onto a paper CRF with subsequent transcription to the eCRF. Where there is electronic storage of non-identifiable data it will be on a password protected device and/or database.

The study questionnaires will be completed at each study visit by the patient with the assistance of the research nurse, directly onto a paper format with subsequent transcription to the eCRF.

NHS laboratory derived blood tests and DXA results will be held on local NHS clinical systems databases in an identifiable format and for an indefinite time frame which can be assessed by primary and secondary care practitioners for future health care of patients. All research data and data established from the NHS tests will be stored in an unidentifiable format on password protected disaster recovery formatted OpenClinica database on a University of Dundee server. Quality control of data will be maintained by the Data Monitoring Committee. Patients will be informed of data storage and consent will be sought.

All research blood samples (link-anonymised) will be processed at each site (as per Working Practice Guidelines in the Study Operations Manual) and transported to the relevant laboratory for analysis at the end of the study for batch analysis. Depending upon volume and composition of each blood sample additional/surplus blood serum/plasma samples will be stored (with consent) and transported at the end of the study to the clinical laboratory at University of Dundee under the custodianship of the CI for future research which will be scientifically and ethically reviewed.

The medical notes can act as source data for past medical history, subsequent medical conditions, hospital admissions, diagnostic reports and blood and urine results.

5.2 MISSING DATA

Missing data will be handled in the analyses via two sensitivity analyses; a) by multiple imputation, and b) by assigning the worst possible value to missing data

5.3 TRANSFER OF DATA

Data transfer will occur via the secure, web-based electronic CRF hosted by Tayside Clinical Trials Unit. Data that are not part of the CRF (e.g. batched biomarker results analysed at the end of the trial) will be analysed via their unique trial number, and results without personal identifiers will be transferred to TCTU via encrypted spreadsheet for amalgamation with the main trial database.

5.4 DATA MANAGEMENT SYSTEM

A data management system will be provided by TCTU using OpenClinica. The study system will be based on the protocol and case report form (CRF) for the study and individual requirements of the investigators. Development and validation of the study database and QC and extraction of data will be done according to TCTU procedures and TASC SOPs. Extracts for analysis will be based on the dummy data tables provided by the study team.

6 STATISTICS AND DATA ANALYSIS

6.1 SAMPLE SIZE CALCULATION

We have taken a deliberately conservative approach and used the minimum clinically important difference (MCID) for SPPB to ensure we have proof of efficacy. In order to detect the MCID in SPPB of 0.5 points (anticipated SPPB of 8 in placebo group and 8.5 in intervention group, SD = 2.7 points) with a power of 90% at alpha 0.05, and assuming a correlation between time points of 0.7 as seen from our previous work, we would require a total of 352 participants for each of the 4 groups (88 per group). Assuming 20% dropout at 12 months, we would therefore need to recruit 440 patients. This sample size would also have 90% power to detect a 5% difference in muscle mass at 12 months, assuming a baseline value of 19kg (SD 2.8)

6.2 PROPOSED ANALYSES

Analyses will be according to Intention-to-Treat and comply with the ICH E9 'Statistical Principles for Clinical Trials'. A two-sided p value of <0.05 will be taken as significant for all analyses. No adjustment for multiple comparisons will be made. The primary analysis will be a repeated measures mixed model between-group comparison of SPPB utilising all available data points during follow-up. Initially, a test for treatment interaction will be carried out and if not significant the main analysis will proceed using the full power of the factorial design. Between-group differences for all primary and secondary outcomes will be adjusted for baseline values, age, sex and minimisation variables.

Secondary outcomes will be analysed with similar methodology using repeated measures mixed model between-group comparisons. Unadjusted and adjusted analyses will be presented as above. Sensitivity analyses will be performed to further test the effect of missing data by assigning worst possible result status to missing datapoints. A supplementary per-protocol analysis will be performed to examine adverse events in those taking at least 80% of study medication.

Mixed models have the added advantage of dealing with data that is missing at random (MAR) where all data present at each time point is used in the analyses.

Prespecified subgroup analyses:

- Age >80 vs <80
- Male vs Female
- Above and below median protein intake

For the quality assurance, the analysis of the primary outcome will be repeated by an independent statistician prior to the dissemination of results.

Analyses will be carried out using SAS 9.4 software.

A summary of objectives, outcomes, proposed statistical analysis and variable location in the dataset is presented in the Table 1 and 2.

Table 1: Summary of Objectives, outcomes, proposed statistical analysis.

Objectives/ Research question	Outcome measure	Statistical analysis / variables
Primary		
To determine the efficacy of leucine and perindopril in improving physical function in older people with sarcopenia diagnosed using the EWGSOP definition.	The primary outcome will be the between group difference in Short Physical Performance Battery (SPPB) score over 12 months follow up.	Mixed effects models for repeated measures
Secondary		
To evaluate the effect of leucine and perindopril on muscle mass in older people.	Between group differences in: - Appendicular muscle mass/height squared (measured by dual energy X-ray absorptiometry), - Grip strength - Quadriceps strength (handheld dynamometry) - 6 minute walk test - Gait speed (4m walk) - Chair stands (Sit to stand test x 5) - Activities of daily living (Nottingham extended ADL questionnaire) and quality of life (EuroQol 5D questionnaire). - Hip Bone mineral density	Mixed effects models for repeated measures
Sensitivity analysis		
To determine the efficacy of leucine and perindopril in improving physical function in older people with sarcopenia diagnosed using the EWGSOP definition.	The primary outcome will be reanalysed as the between group difference in Short Physical Performance Battery (SPPB) score at 12 months.	Mixed effects models

Table 2: Summary of Outcome measure and Variable source.

Objectives	Outcome	Variable location (dataset name)	Variable name	Calculation	Comments
Primary	P1 -Physical Performance Battery (SPPB) score at 12 months	DATA.SPPB	SPPBSC_C	None	Measured at V1, V7 & V9
Secondary					
S1	Appendicular muscle mass/height squared (measured by dual energy X-ray absorptiometry)	DATA.DXA	LEANMASS (in g). If each arm and leg are separate, add leanmass for R arm, L arm, R leg and L leg	(LEANMASS/1000)/ ((HEIGHT/100)^2)	measured at V2 and V9
		DATA.BIOIMP	HEIGHT (in cm)		
S2	Grip strength	DATA.HANDGRIP	GRIP	None	measured at V1, V7 & V9
S3	Quadriceps strength (handheld dynamometry)	DATA.QUADSTR	QUADSTR1, QUADSTR2, QUADSTR3	Use maximum of these three measures	measured at V2, V7 & V9
S4	6 minute walk test	DATA.SIXMW	SIXMWDIS	None	measured at V2, V7 & V9
S5	Gait speed (4m walk)	DATA.SPPB	GAITSPD (time in seconds)	4 / GAITSPD	measured at V1, V7 & V9
S6	Chair stands (Sit to stand test x 5)	DATA.SPPB	CHAIRTIM (time in seconds)	None	measured at V1, V7 & V9
S7.1	Activities of daily living (Nottingham extended ADL questionnaire)	DATA.NEADL	All variables exc for SIGNDT and SIGNED	Add variables according to NEADL scoring instructions	measured at V2, V7 & V9
S7.2	EuroQoL 5D	DATA.EQ5D	ANXIETY, MOBILITY, PAINDISC, SELFCARE, USUALACT	Derive score that uses UK normative weights for health status (Petra has the docs from BiCARB)	measured at V2, V7 & V9
S7.3	EuroQoL thermometer	DATA.EQ5D	HEALTHST	None	measured at V2, V7 & V9
S8	HOMA IR (Homeostatic Index of insulin resistance; measured from glucose and insulin levels)	DATA.BLOODS	Glucose: Insulin values will be uploaded from an external dataset	Insulin (milliU/L x Glucose (mmol/L) / 22.5	
S9	Hip Bone mineral density	DATA.DXA	TSCORE	None (most values will be negative; this is normal)	measured at V2 & V9
Explanatory variables	age, gender, minimisation variables	DATA.DEMO DATA.SPPB DATA.HANDGRIP DATA.CHARLSON	AGE SEX SPPBSC_C GRIP CCTLSC_C	None	

7 DOCUMENT HISTORY

This is document was developed following the study protocol V8
The final version of Statistical Analysis Plan for all analyses of LACE trial is dated 02 January 2020.

8 REPORTING CONVENTIONS

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as “<0.001”. The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

9 TECHNICAL DETAILS

All analysis will be performed using SAS 9.4. All data, analysis programs and output will be kept on the TCTU Server and backed up according to the internal IT SOPs.

Analysis programs will be required to run without errors or warnings. The analysis programs for outcomes will be reviewed by a second statistician, and any irregularities within the programs will be investigated and fixed and date of finalised analysis programs will be signed and recorded.

10 STATISTICAL REPORT

A statistical report will be created with all analyses presented according to the analyses outlined in the SAP. This will form the basis of any future paper or report and will be assessed for consistency.