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Study protocol - A protein-enriched, milk-based supplement to counteract sarcopenia in acutely ill geriatric patients offered resistance exercise training during and after hospitalization – a randomized, double-blind, multicenter trial

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Manuscript

Title: 'Study protocol - A protein-enriched, milk-based supplement to counteract sarcopenia in acutely ill geriatric patients offered resistance exercise training during and after hospitalization – a randomized, double-blind, multicenter trial'

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Public trials registry: The study has been approved by the Danish Regional Ethical Committee (reference no. H-16018240), and the Danish Data Protection Agency (reference no. HGH-2016-050), and it is registered in ClinicalTrials.gov (identifier: NCT02717819).

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ABSTRACT

Introduction: Age-related loss of muscle mass and strength, sarcopenia, is a great burden to many older adults, and the process is accelerated with bedrest, protein intakes below requirements, and the catabolic effect of certain illnesses. Thus, acutely ill older adults admitted to hospital are a particular vulnerable population. Protein supplementation has been shown in some studies to preserve muscle mass and/or strength, and combining this with resistance exercise training (RT), may have additional benefits. Therefore, the purpose of this study is to investigate the effect of protein supplementation in addition to offering RT among older adults while admitted to the geriatric ward and after discharge, which have not previously been investigated.

Methods and analysis: In a block-randomised, double-blind, multicentre intervention study 165 older adults above 70 years, fulfilling the eligibility criteria, will be included consecutively from three Medical Departments (blocks of n=20, stratified by recruitment site). After inclusion, participants will be randomly allocated (1:1) to receive either protein-enriched, milk-based supplements (27.5 g protein/d) or iso-energetic placebo products (<1.5 g protein/d), as a supplement to their habitual diet. Both groups will be offered a standardized RT program. The study period starts during their hospital stay and continue12 weeks after discharge. The primary endpoint is lower extremity muscle strength and function (30-s chair-stand-test). Secondary endpoints include muscle mass, measures of physical function, and measures related to cost-effectiveness.

Ethics and dissemination: Approval is given by the Research Ethic Committee of the Capital Region of Denmark (reference no. H-16018240) and the Danish Data Protection Agency (reference no. HGH-2016-050). There are no expected risks associated with participation, and we expect each participant to benefit from the RT. The results of the study will be published in peer-reviewed international journals and presented at national and international congresses and symposiums.

Trial Registration: ClinicalTrials.gov: NCT02717819 (March 9, 2016).

Strengths and limitations of this study:

- To our knowledge this is the first study to investigate the effect of protein supplementation in addition to RT among acutely ill geriatric patients, while admitted and after discharge, and it adds new information to the evidence-based health care.
- The study is randomized and double-blinded which minimizes the risk of selection, performance, and detection bias, and the multi-center trial design increases the generalizability of the results.
- The lack of supervised RT after discharge might lower compliance to the RT, although it is more realistic that self-training at home can be implemented in a real world setting.
- Acutely ill older adults are a difficult population to maintain in a long duration intervention study, which increases the risk of drop outs and/or low compliance.
- Registration of compliance in dietary studies is always associated with a risk of bias, but by asking the participants to register their daily intake, save empty bottles, and by calling them on a weekly basis to check on compliance, this is minimized.

INTRODUCTION

Sarcopenia is the loss of muscle mass with ageing and is an unavoidable process with a multifactorial aetiology [1,2]. The decrease in lean body mass (LBM), and thus muscle strength and power, are important predictors of impaired balance, falls, and mortality [3]. Also, sarcopenia is associated with a 3- to 4-fold increased risk of disability, which in turn is associated with substantial socio-economic and health care spending [4]. Sarcopenia is estimated to affect about 5-10 % of people > 65 years, with the number being as high as 50 % in individuals > 80 years [1]. Globally the percentage of older adults increases rapidly. Thus, studies on how to counteract sarcopenia are highly relevant.

Acute illness might result in stress metabolism which further increases the loss of protein and the anabolic resistance in older adults, leading to increased loss of lean body mass (LBM) [5]. With advancing age it becomes more likely that acute illness necessitating a period of bed rest could initiate a serious decline in LBM, muscle strength, and functional capacity, which can be hard for the older adult to fully recover from. Even a short hospital stay increases the risk of losing functional capacity and losing ability to cope with activities of daily living [6]. For older medical patients it is shown that only one in three have reached back to their original physical function one year after discharge [7]. Any additional catabolic crisis, e.g. episodes of illnesses and readmissions to hospital, will result in an accelerated episodic loss of LBM and functional abilities. The consequences of the accelerated loss of LBM in bed-ridden older adults during acute illness may be further complicated by the fact that up to two-third of the patients can already be characterised as moderately sarcopenic prior to admission [5]. Also, many older adults consume relatively small amounts of protein, important for maintenance and buildup of LBM, and loss of appetite as a consequence of acute illness may further decrease the protein consumption. Furthermore, a substantial number of geriatric patients are severely limited in their ability to take care of their own nutrition, due to e.g. their cognitive or general status [8,9]. This is very critical, as research has shown that the protein requirement increases with age. Also, research indicate, that a higher amount

of protein per meal is needed to maximally stimulate muscle hypertrophy [10]. Hence, interdisciplinary interventions to counteract sarcopenia become even more relevant in the acutely ill older patients.

The beneficial effect of resistance exercise training (RT) on counteracting sarcopenia is quite well established [11,12], and the effect of protein supplementation alone has also been documented [13]. Less well studied is the potential benefit of a higher protein intake or supplementation when older adults are offered RT at the same time. A recent systematic review by Malafarina et al. (2013) and a meta-analysis by Cermak et al. (2012) have both concluded that protein supplementation increases muscle mass, and in some studies also muscle strength, during prolonged RT in older adults [13,14]. Furthermore, some reviews stresses that the evidence is sparse in the frailest older adults, who often have a low dietary protein intake, and based on their findings the hypothesis is that this sub population will benefit even more from a combined intervention [14-16]. This said, to our knowledge, no studies have yet investigated the effect of a protein supplementation among hospitalized, acutely ill old adults offered RT, which is a population where many have a high risk of malnutrition and experience accelerated loss of muscle mass and strength, loss of function, and (further) development of sarcopenia.

METHODS AND ANALYSIS

Study design

The study design is a block randomised, double-blind, placebo-controlled, multicentre intervention study. A total of 165 participants will be included consecutively from the Medical Departments of three Hospitals in the Capital Region of Denmark **(**Gentofte and Herlev University Hospital and Rigshospitalet-Glostrup, n=55 from each place). Recruitment takes place a maximum of 72 hours after admission. After inclusion, participants will be randomly allocated (1:1) to receive either protein-enriched milk-based supplements (whey protein) or an iso-energetic placebo product, as a supplement to their habitual diet. Both groups follow the same RT program and are daily supplemented with vitamin D. The intervention starts at the hospital while admitted and continues

12 weeks after discharge. Recruitment and data collection started in April 2016, and will end in June 2018.

Study population

Inclusion criteria for participation are; men and women aged ≥ 70 years, able to speak and understand Danish, expected length of stay > 3 days (evaluated by medical staff at department), ability to stand independently for at least 30 seconds, and admission to the medical departments of Gentofte Hospital, Herlev Hospital or Rigshospitalet-Glostrup. Exclusion criteria are: active cancer, renal insufficiency (eGFR < 30 mL/min/1.73m^2), cognitive impairment (not able to comprehend the purpose of the study/give informed consent), terminal disease, exclusively receiving enteral or parenteral nutrition, milk/lactose allergy or intolerance, planning to lose weight/go on a special diet, planned transfer to other hospitals/departments and pacemaker/other implanted electrical stimulants (due to Bio-Impedance Analysis (BIA) measurements). Participants will be withdrawn from the study if they die during admission (does not apply to subsequent admissions) or are discharged/transferred from the medical department before the intervention has started.

Randomization and blinding

After collection of baseline measurements and characteristics, participants are randomized to either the intervention or the control group using sealed, opaque envelopes containing a paper with either an ‘A’ or a ‘B’. Each Hospital site has its own pile of envelopes in order to allow for block-randomization. Within each site, 10 A’s and 10 B’s (20 in total) are put in the pile over three rounds, to ensure a more even allocation of participants in the two groups at any time. Participants, hospital staff, and study investigators will all be blinded towards the randomization. If a situation arises where unblinding may be considered for the benefit of the participant, this will be decided on an individual basis taking the specific situation into account. Enrollment and randomization is performed by study investigators.

Intervention

Protein-enriched, milk-based supplements and Placebo

Depending on their allocation, participants will receive either a protein-enriched milk-based supplement beverage (Arla Foods®: 781 kJ, 10 g whey protein, 10 g fat, and 13 g carbohydrate per 100 ml) (intervention group) or an iso-energetic placebo beverage (Arla Foods®: 797 kJ, 0,58 g protein, 10.2 g fat, and 24,14 g carbohydrate per 100 ml) (control group). Both products have a flavour of raspberry. From January 2017 and on, the protein-enriched milk-based supplement will have vitamin D added in amounts of 1.125 µg per 100 ml. During the whole study period (while hospitalized and 12 weeks post discharge) the participants will be instructed to drink a total of 250 ml per day, divided into two servings of 125 ml. Thus, the intervention group will get a total of 27.5 g extra whey protein per day. The beverages come in white bottles with either a 'group A' or 'group B' label on. While hospitalized, the timing of the intake is as follows; one serving at breakfast (or at lunch, if not consumed at breakfast for any reasons, e.g. fasting necessary, or if the RT is performed right after breakfast) and one serving directly after the RT. In the 12 weeks after discharge the participants will be instructed to drink one serving at breakfast and one serving with the next cold main meal, irrespective of the meal is eaten at lunch or at dinner time. If the participants forget to drink the beverages at the specific times, they will be told to drink it when they become aware of it. The participants will not be instructed to make other dietary changes during the study period. If participants are prescribed/recommended by hospital staff to take oral nutritional supplements, this is not an exclusion criterion, but participants will be instructed to take any additional supplements on a given day only after intake of the 'study beverages'.

Vitamin D supplements

Vitamin D supplementation has been shown to have an independent effect on muscle [17]. To reduce the potential confounder of a large difference in intake of vitamin D between groups, all participants will get vitamin D supplements handed out after enrolment, and be instructed to take a supplement of 20 µg/day (two tablets of 10 µg), as recommended by the Danish National Board of Health [18]. Exceptions to this are those participants whose serum-vitamin D levels have been measured to ≥ 100 nmol/L at the time of study inclusion to avoid reaching toxic levels. The

participants have to register their intake of vitamin D in a diary along with their intake of the intervention products. Also, at the last visit in study week 12, the number of tablets left in the container will be counted to verify the registrations. If participants already take vitamin D supplements in combination tablets with other vitamins and/or minerals corresponding to 20 µg/day or more, they will be instructed to keep taking their own tablets and register this. The exact amount of vitamin D in these tablets will be recorded. An average intake of vitamin D per day during the intervention period will be used to compare if the intake of vitamin D is different between the two groups.

Resistance exercise training (RT)

The RT program is developed by experienced physiotherapists and is consistent with the official statements from the American College of Sports Medicine on recommendations for RT in older adults [19]. It focuses on strength training primarily of the big muscle groups of the lower limbs, and can be performed without any training equipment. One training session consists of three exercises; 'lifting-and-lowering the pelvic' from a crook-lying position, 'sit-to-stand from a chair', and 'lifting-and-lowering the heels' in a standing position – i.e. performing heel-raises. All exercises are performed in three sets, aiming at 10 repetitions, pursuing an intensity of 8-12 repetition maximum. The repetition velocity will be performed at the participants own preferred speed. There will be a time interval of 1-3 minutes between sets and exercises, depending on the individual need for rest. Each of the three exercises can be performed in five different modes (A-B-C-D-E), graduated in terms of increasing resistance, by applying the participants' own body weight and different starting positions. Thus, the program can be individualized corresponding to the participants abilities, and adjustments will be made to ensure progression.

While admitted to hospital, supervised RT is offered daily by physiotherapists in addition to the standard of care. After discharge, the participants are encouraged to perform the same RT program as self-training four times per week. They will be instructed to have at least 24 hours between training sessions. During the hospital stay it is expected that the participants have a very limited

amount of physical activity besides the RT program offered, and that the intensity by which they can perform the RT is rather low. This is why the frequency of the RT differs between the hospital and discharge setting. To instruct the participants in regard to the RT, and to ensure progression (or regression if necessary), they receive follow-up home visits by a physiotherapist in study week 1, 3, 6, 9, and after discharge from any readmissions. The adjustments are made after standardized procedures.

Participants who are discharged with a plan of rehabilitation including ambulatory training at a center or supervised training at home, to be provided by their municipally, will be asked to perform the full RT study program until their rehabilitation program starts up (a wait of 2-6 weeks are normal). Each training session performed as part of a rehabilitation program will replace *one* self-training session of the RT study program. The same applies, if participants are discharged from the hospital directly to a 24-h rehabilitation center and they are performing RT in their regimen. This is to allow for proper restitution. The offer of supervised training applies only to the first hospital stay, but if readmitted to hospital participants will be encouraged to do the RT themselves to the extent possible.

Compliance

While hospitalized, the participants will get the product handed out along with the vitamin D supplements. Investigators and physiotherapists register overall study compliance, that is daily ingestion of the intervention or placebo supplements (time for handout and amount ingested), vitamin D (dose, yes/no), and performance of the RT (number of sets and repetitions for each exercise). Empty bottles are saved so that study investigators can verify the amount of intervention product consumed.

After discharge, the amount of intervention or placebo supplement consumed and the RT performed for each participant will be assessed by daily records in a 'beverage and exercise diary', specifically designed for the study and handed out to be filled in by the participants. The participants, e.g. with help from their relatives, are asked to daily register the amount of beverage consumed; 0 %, 25 %,

50 %, 75 %, or 100 % of each of the two servings by ticking of the corresponding circular illustration, along with ticking of the intake of vitamin D. Participants also have to register execution of the RT, and specify for each of the three exercises the number of sets and repetitions performed. If they are exercise training at a rehabilitation center this can be registered in the relevant boxes. In case of deviations, four pre-specified explanations are given that they can tick off, both in regard to the intake of supplements and the execution of the RT. To verify the participants' records they are asked to save and store empty bottles, which will be picked up by investigators on days with home-visits. At the same time study investigators will help the participants' to retrospectively fill out any missing registrations. Participants who are discharged to a 24-hour rehabilitation centre will get the intervention products handed out by the staff, who will also save empty bottles. On the first visit after discharge the participants will receive thorough instructions on how to register compliance in the 'beverage and exercise diary', and upcoming visits will be planned. Both groups will receive daily standard messages on their cell phone (if they have one and agrees to this) and weekly phone calls, kindly reminding them to consume the supplement and vitamin D, perform the RT, and register compliance. Furthermore, as part of the phone call, they will be asked about compliance and any deviations or e.g. upstart of training at a rehabilitation center will be registered and validated/compared later on with their own diaries, and they will be reminded of upcoming home visits.

Outcome parameters

The baseline characteristics will be collected at inclusion to the study. To standardize the endpoint measures, especially that of LBM, these will be assessed 1.5-2 hours after a light breakfast. Thus, if inclusion happens in the afternoon, then baseline measurements will be assessed the following day, prior to any study interventions. The measurements will be assessed in a predefined order to reduce fatigue and follow standardized procedures, and they will be repeated within 72 hours after discharge and 12 weeks (± 2 days) after discharge. If possible, before each endpoint examination the participants will be asked to consume a breakfast, similar to that consumed at the hospital before

the baseline measurements. The assessments after discharge will be performed in the participants own home. Follow-up assessments, including only admission to hospital and mortality, will be assessed six months after the intervention period. In general, if participants are readmitted to hospital, if possible, assessments will be performed there and otherwise at a replacement visit after discharge. All data collection is performed by study investigators. Table 1 gives an overview of the study period and the different time points for meetings and tests.

Table 1. Flow-chart of the study period, including meetings and tests

Flow-Chart of study period	**Baseline**	**In-hospital intervention**	**Post-hospital intervention[d]**					**Follow-up**
Study week no.	-	-	1	3	6	9	12	38
Meetings incl. tests	1+2	-	3	4	5	6	7	-
In- and exclusion criteria	X							
Informed consent	X							
Baseline characteristics	X							
Baseline endpoint assessment[a]	X							
Randomization	X							
LOS (in-hospital intervention period)		X						
Dietary registration		X (4 days in total)						
Daily compliance registrations		X	X					
Endpoint assessment[a]			X[b]				X[c]	
Exercise adjustments			X	X	X	X		
24-h dietary interview				X	X	X	X	
Exercise interview				X	X	X	X	
Evaluation-questionnaire							X	
Delivery of intervention products		X (ongoing basis)	X (deliveries after appointment)					
Collection of empty intervention bottles			X	X	X	X	X	
Readmissions, LOS, and mortality							X	X

a: *assessed 1.5-2 hours after a light breakfast (preferably the same meal every time).* ***b:*** *assessed within 72 hours after discharge.* ***c:*** *assessed 12 weeks (±2 days) after discharge.* ***d:*** *assessments and meeting are taking place where the participant's live.*

Primary endpoint

30-second chair-stand-test (30-s CST) gives a measure of the muscle strength in the lower extremities. It exists in both a standardized and a modified version. The standardized 30-s CST measures the number of times the participant can rise-and-sit from a standard chair (height of 43-45 cm) in 30 seconds with the arms folded across the chest, starting from a sitting position. Only full stands will count – i.e. full extension of the knees and hips. Those who cannot stand from the chair

without using the arm rest will get a score of 0 [20]. In the modified 30-s CST the participant is allowed to use the arm rests [21]. If participants are only able to perform the modified version at baseline, for the following assessments they will be asked to do the same. If they are able to do the standardized version they will be asked to do that as well after a 15 minutes rest.

Secondary endpoints

Muscle mass is assessed by Bio-impedance Analysis (BIA) using the portable InBody-230 body composition analyzer (dual frequency (20 kHz, 100 kHz), tetra polar 8-Point Tactile Electrode System (InBody, Copenhagen, Denmark)). Direct segmental measurement technology is used, meaning that no calculations, and thus empirical factors and imputations, are needed. Measures of total, appendicular, and trunk LBM is registered (kg and percent). Various factors can affect BIA measurements such as previous exercise, body position, skin temperature, dietary intake, and hydration state [22]. Thus, in order to standardize the measurements these will be performed in the morning 1.5-2 hours after a light breakfast and bladder emptying (preferably also bowel emptying), and before any exercise. Participants will be asked to wear light clothes and no shoes. They will be instructed to stand upright with the feet on the build-in electrodes embedded in the scale platform, grasp the handles of the analyzer while spreading the arms as much as they can, and look straight ahead.

Hand grip strength (HGS) is measured in kg using the second handle position with a DHD-1 Digital Hand Dynamometer (Saehan Medical, 2012, Roskilde Denmark). The second handle position is recommended as a standard position, as it is suitable for most hand sizes. An investigator will instruct the participants to be seated with their feet on the ground, shoulders adducted and neutrally rotated, elbow flexed at a 90° angle and supported on the armrests of the chair or a table, and forearm and wrist in neutral position, as recommended by Roberts et al. (2011) [23]. They will be asked to perform three maximum force trials with their dominant hand, and the highest value will be registered. They will be instructed to squeeze the handle as hard as they can for 5 seconds, and the test will be repeated within 15 seconds.

4-meter gait speed (4-m GS) is used to assess the usual gait speed (m/s) over a short distance. Participants will be placed behind a starting line and instructed to start walking at their usual pace after the investigators command. To reduce the effect of acceleration and deceleration, each participant will be instructed to walk towards a visual goal for 5 meters. The time will be started after the participant has walked 0.5 meter and stopped after 4.5 meters, counted from the first foot-step that crosses the 4-m start line and end line, respectively. The fastest of two attempts is recorded. If it is not possible to establish a 5 m test track, a shorter track with a minimum length of 3.5 m in total will be used instead, and this will be registered as bias [24,25]. The participants are allowed to use a gait aid, which will be registered as well.

Functional ability is measured using the modified Barthel Index (Barthel-100) [26,27]. The Barthel-100 contains 10 measures of every-day and mobility activities, and the ability to master these activities reflects the level of functioning. Each measure has five levels of functioning, and for all 10 measures a maximum of 100 points can be achieved, corresponding to fully independent. The Barthel-100 will be scored by the investigators, and rated based on the amount of assistance required to complete each activity or by observing, and clarifying questions will be asked when necessary.

Mobility is assessed by De Morton Mobility Index (DEMMI), which provides a 15-item unidimensional measure of mobility across the spectrum from bed bound to independent Mobility, specifically developed for geriatric patients [28]. It has 5 categories in which the participants are tested; bed (3 test scores), chair (3 test scores), static balance (4 test scores), walking (2 test scores), and dynamic balance (3 test scores). A total test score from 0-19 can be achieved, and this raw score is converted to an interval DEMMI score from 0-100, where 100 is represents independent mobility.

Cognitive function is measured using the Mini Mental State Examination (MMSE), which consists of small simple tasks to elucidate eight different cognitive functions; orientation, episodic memory, concentration, function of language, practical exercise, reading skills, writing skills, and visual-

spatial construction. The performances are scored to give a raw score ranging from 0-30, where 30 represent the best/optimal function [29].

Social support is evaluated using registrations of home care (yes/no, if yes, then divided into practical help, personal care, and both) and residence (own home, nursing home/assisted living facility, 24-hour rehabilitation facility).

Use of gait aid is registered as yes (incl. specific gait aid), no, or cannot walk.

Length of hospital stay (LOS) corresponds to the in-hospital intervention period (days from recruitment until discharge) which is registered from the electronic patient register.

Readmission to hospital and mortality. Readmission to hospital is registered both with regard to frequency and the total LOS, from the electronic patient register. These data are summed up after the intervention period and after the follow-up period, respectively.

Health related Quality of life (QOL) is assessed by using the generic questionnaire, Euroqol EQ-5D-3L [30]. The questionnaire is self-reported, and reflects the participant's current situation. Scores for the EQ-5D-3L are generated from the ability of the individual to function in five dimensions; mobility, pain/discomfort, self-care, anxiety/depression, and usual activities. Each dimension has three possible answers; no problem, some problems, and major problems. Also, the participants rate their current health state on a visual-analogue-scale ranging from 0-100 (reflecting a health state from 'worst' to 'best').

Body weight is measured to the nearest 0.1 kg using the BIA equipment InBody-230, and follows the same standardized procedures as described under the endpoint 'muscle mass'.

Product-evaluation-questionnaire. Both the intervention and placebo product is evaluated using a self-report questionnaire. The evaluation questionnaire concerns overall liking, side effects related to consumption, taste fatigue, texture, dosage, and manageability.

Control for confounders - other registrations and precautions

Actions are taken to actively reduce or register known or possible confounders. Thus, at baseline, confounders such as nutritional risk (NRS 2002) [31], sarcopenia [3,32], depression [33], and

mobility [34,35] are evaluated, among other. Furthermore, besides register vitamin D intakes, throughout the study the following two measures are collected on an ongoing basis.

Protein and energy intake. During hospitalization the participants' protein (g/kg) and energy (kJ/kg) intake will be registered for four days, or shorter if the participants' are discharged. The hospitals' food and drink registration schemes will be used. Participants will be asked to fill in the food registration schemes themselves with help from the nurses and study investigators. The participant's body weight at inclusion will be used to calculate the intake per kg body weight. During the 12-week post-hospital intervention the participants protein and energy intake will be estimated based on the average of four 24-hour dietary-recall interviews performed at study week 3, 6, 9, and 12 at home visits, or by phone if the participant' are no longer compliant in the study with regard to the intervention products and the RT. To minimize the risk of recall bias a checklist of specific foods and beverages will be used to verify the reported intake. Furthermore, when interviewing face to face, picture series of portion sizes of different foods will be used to estimate the amounts ingested [36]. The foods and drinks will be entered in the software program Madlog Vita® to calculate the intake of protein (g) and energy (kJ). Four days of registration/dietary recalls are considered adequate to assess this information with a high correlation [37]. An average of the participant's body weight after discharge and in week 12 will be used to calculate the intake per kg body weight. The cut-off for suspecting underreporting will be evaluated retrospectively on an individual basis taking any illness, readmissions, loss of body weight, activity level etc. into account.

Daily activity level. In a semi-structured interview the participants are asked about exercise-related activities besides the RT program. This happens four times after discharge in study week 3, 6, 9, and 12 at home visits, or by phone if the participant' is no longer compliant in the study with regard to the intervention products and the RT. Depending on the answers given, the participants will be divided into increasing activity levels from 1-5, after predefined criteria, inspired by Saltin & Grimby (1968) [38].

Statistics

Power calculation

The primary endpoint is muscle strength measured by the 30-s CST. The clinical relevant difference for this test is found to be 2.0-2.6, when assessed in older populations with hip and knee osteoarthritis [39]. Jones et al. (1999) has used the standardized 30-s CST on community-dwelling older people and found a SD of 3.0 and 3.6 for people in the age range of 70-79 and 80-89, respectively [20]. This gives a pooled SD of 3.31, which is used in this power calculation, and it corresponds well with measures of SD found in the modified test version [40].

In order to be able to detect a difference of 2.0, with a power of 80 % and a two-sided alpha-error of 0.05, the required sample size is 80 participants in each group, given an anticipated combined rate of drop-outs and non-compliance of 45 %. This rate is chosen since studies with resistance training in older people both while hospitalized [41] and in a community-dwelling setting [42], have experienced drop-outs of 30 %. Moreover, an additional 15 % is added to account for participants with a low compliance to the intervention, to be able to maintain the statistical power of the study in the intention-to-treat analysis as well as in the per protocol analysis. For practical reasons, if possible within the time schedule, 55 participants will be included at each of the three sites, resulting in a total inclusion of 165 participants.

Feasibility of recruitment and sample size

The three hospitals where recruitment is going to take place had between 525-687 geriatric patients in year 2014, with a median LOS ranging from 8-11 (5-16) days. The median age for women was in the range of 84-87 years and 83-84 years for men [43]. To meet the timetable the expected recruitment rate is a minimum of two participants per week which based on these data is considered realistic.

Statistical tests

The primary analysis will be performed by the intention-to-treat principle. In addition, a predefined per-protocol analysis will be performed including participants with a high compliance only (consumption of the intervention product ≥ 75 %). Furthermore, endpoints will be compared

adjusting for randomization bias (defined as $p < 0.05$ between groups). Analysis will be done both with and without imputation techniques for missing values, but drop-outs will be encouraged to participate in follow-up examinations, including interviews concerning dietary intake and activity level. Sensitivity analysis will be performed without outliers, defined as a value of 3 SD above or below the mean. To investigate whether the intervention will have different impacts in different groups of patients, e.g. those who are at nutritional risk or sarcopenic, subgroup analysis will be performed looking at treatment effect in the subgroups and interactions between treatment effect and subgroups. Furthermore, observational analysis will be performed, investigating the importance of total protein- and energy intake and total activity level on outcome measures. The two groups will be compared looking at the hospitalization intervention period and the 12 week post discharge intervention period both separately and as a whole.

Results will be presented as median (range) or mean (SD or 95 % CI) and number (absolute frequencies) for continuous and categorical variables, respectively. Inspection for normality will be done by visual inspection (QQ-plot), and parametric or nonparametric statistical tests will be used in accordance with the distribution of the variables. Statistical comparisons will be made between the two groups by using the Mann-Whitney U-test or Students t-test for continuous variables, and the Chi-square test (X^2) or Fisher's Exact Test (in case of expected cell count < 5) for the comparison of categorical variables. ANCOVA will be used for continuous outcomes and binary logistic regression for binary outcomes if/when adjusting for confounders and testing for subgroup interaction. The Spearman-Rank Correlation Test or General Linear Model will be used to test for correlations between independent variables. All tests are two-tailed and an alpha-level of $P < 0.05$ will be used to determine statistical significance in all analyses.

With regard to the primary endpoint, 30-s CST, the changes in performance from baseline (both with and without pooling standardized and modified test results) will be measured and compared between the two groups. Furthermore, performance will be scored into one of three categories; 1. ability to rise from the chair with arms folded across the chest, 2. ability to rise from the chair using

the arm rest, and 3. not able to rise independently from the chair. Also, compared to baseline, performance will be scored into either 'better', 'worse' or 'unchanged'.

ETICHS AND DISSEMINATION

The study will be conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. Thus, precautions will be taken to protect the privacy and confidentiality of research subjects. Approval is given by the Danish Data Protection Agency (HGH-2016-050) and the Research Ethic Committee of the Capital Region of Denmark (H-16018240), and the study is registered in the clinical.trial.gov database (NCT02717819). Any amendments to the protocol will be made public at clinical.trial.gov. All participants receive written and oral information from study investigators about all relevant aspects of the study before making decision about participation, and they are informed that they can withdraw from the study at any time**.** The participants receive no payment and will have no expenses associated with participation in the study. There are no expected risks associated with participation, and we expect each participant to benefit from the RT. The results of the study will be published in international peer-reviewed journals and presented at national and international congresses and symposiums.

DISCUSSION

This study investigates the effect of protein supplementation in addition to offering RT among older adults while admitted to the geriatric ward and after discharge. The acutely ill 'geriatric patient' is a heterogeneous patient group with various (non-surgical) diseases and often existing comorbidities. The goals are to counteract sarcopenia, maintain or improve physical function, and reduce health care costs in this specific population. Thus, with this study we wish to add knowledge about effective secondary prevention and interdisciplinary rehabilitation strategies to the large population of acutely ill older adults admitted to hospital. The eligibility criteria are very broad, however, the weakest patients (no stand function) are excluded, as these will not be able to participate in a RT program and perform the endpoint measurements. The participants in the current study are included within three days of admission. It is possible that the weakest geriatric patients with no stand

function, currently excluded, will gain their stand function later during their hospitalization (>3 days). Thus, the results from the current study may also be relevant to this group of patients, although not examined. A common confounder is that people agreeing to participate in an intervention trial are more motivated to lifestyle changes, which is an important factor for the compliance and possible success of this intervention.

Use of placebo beverages allows blinding of participants and researchers. Thus, performance and detection bias are minimized. Another strength is the randomization procedure, which will limit selection bias and hopefully balance different confounders which could potentially influence the results. The multi-center trial design furthermore increases the generalizability of the results. The activity and dietary interviews are conducted in order to be able to correct statistically for differences in protein intake and activity levels between groups. In addition, it will also enable us to investigate the importance of overall protein and energy intake on the results.

A majority of older adults in Denmark take vitamin D supplements as recommended by the Danish Health Authority [18]. Studies have shown that vitamin D has an independent positive effect on muscle strength [44]. In order to investigate the effect of the protein supplementation alone, vitamin D supplements will be given to all participants with serum-vitamin D levels ≤ 100 nmol/L at inclusion, to insure similar vitamin D intakes. Another reason for ensuring that all participants are supplemented with vitamin D is that the protein-enriched beverage approximately half-way through the intervention period will have vitamin D added to the product. However, the fortification level is quite low, adding an extra amount of only 3.5 µg vitamin D per day from the beverages, which e.g. corresponds to 13 g of salmon [45]. Also, compared to the daily vitamin D supplementation of minimum 20 µg (some older adults' takes even higher amounts, as prescribed by their doctor) it is considered insignificant.

In regard to ensure compliance to the RT program, it is a weakness of the study that the RT at home after discharge is not supervised. On the other hand, an aim of the current study is to test the effect of an interdisciplinary rehabilitation regime that is cost-effective and could easily be implemented.

Supervised RT four times per week would have required a lot of resources, which most likely would not be possible to implement in the real world. If a positive effect is found of an intervention only consisting of extra protein and self-training after discharge, potential implementation in clinical practice will be more feasible. The current study can also give valuable insights into which sub groups of the geriatric patients that would be able to benefit from a rehabilitation regime based on self-training and protein intervention. The high rate of readmissions to hospital among older adults [46] indicates that there is room for improvement in regard to secondary prevention strategies.

The specific endpoints included in the current study were chosen in order to be suitable, feasible and valid for this specific population of older adults. Thus, a low amount of missing data is expected due to low feasibility. The 30-s CST, DEMMI, and Barthel-100 are part of the normal routine tests for geriatric patients admitted to the medical departments (they are included in The Danish National Geriatric Data Base), and all tests and questionnaires are developed and/or validated in older adults [24,27-29]. Furthermore, the Danish Board of Health recommends the use of 30-s CST, 4-m GS, MMSE, and EQ-5D-3L as tests in older geriatric patients [25]. Also, LBM measured by BIA, has been proposed as a feasible measurement tool in this population [3,47], and a portable BIA is a practical tool suitable for home visits.

Specifically for the primary endpoint, the 30-s CST has been shown to be a reliable and valid indicator of lower body strength in generally active, community-dwelling older adults, when validated against maximum weight-adjusted leg-press performance [20]. The Standardized 30-s CST version has been shown to have low feasibility (54 %) in acutely admitted old medical patients, and to have lower inter-rater reliability than in medically stable patients. However, the Modified 30-s CST has been shown to be both feasible and having a high inter-rater reliability [24]. Thus, we believe that all participants will be able to perform either the standardized or the modified version, supported by the inclusion criteria, that only patients who can stand independently are recruited, eliminating those in poorest conditions. This is also in accordance with experience from

our former intervention studies performed in geriatric patients [48,49], and also applies to the other secondary endpoints.

For the secondary endpoint, LBM measured by a portable BIA, Moon *et al.* (2013) have shown that single frequency BIA in elderly men and women (72 men and women, > 65 years) correlate well with Dual Energy X-ray Absorptiometry (DXA) measurements, as well as the 4-compartment model, at single time points as well as for tracking changes in LBM. They concluded that DXA and BIA can be used interchangeably as valid methods to measure LBM when looking at a population basis of more than 15-22 people [47]. Furthermore, Karelis *et al.* (2013) has validated the portable, dual-frequency InBody-230 BIA against DXA in a healthy mixed population (145 men and women, 44.6±20 years) and found a significant high correlation when looking at fat mass, percent body fat, and total LBM [50]. Thus, it is expected that using the InBody-230 BIA equipment, besides being practical in regard to home visits, will be a reasonable valid method to assess total muscle mass in a population of 165 older adults.

DECLARATIONS

Authors' contributions

AMB prepared the grant application. AMB and JG conceived the overall study draft, and JG created the detailed study protocol. AA, AV, BC, TWK, and CB participated in its design and coordination. JG and research assistants collect the data under the supervision of AA, AV, AMB, BC and CB. JG drafted the manuscript. All authors reviewed the article critically and contributed significantly to the final content. All authors have read and approved the final manuscript.

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Danish Dairy Foundation will not be involved in the conduction of the study or interpretation of results. A Scientist from Copenhagen University have been involved with the study design, and will be involved in all steps from analysis and interpretation to publication of the results.

Competing interests

None of the authors have financial or personal conflicting interests. The sponsor (Danish Dairy Research Foundation) and the producer of the intervention and placebo products (Arla Foods) will not have any influence on the analysis and interpretation of the results.

Acknowledgements

We want to thank the sponsors. Also, we want to thank everybody who contributed to this article as well as those who participated in the planning of study practicalities. A special thanks to Maria Aagensen who participated in developing the standardized resistance training program.

List of abbreviations

BIA, Bio-Impedance Analysis; DEMMI, De Morton Mobility Index; DXA, Dual-energy X-ray Absorptiometry; LBM, Lean Body Mass; MPS, Muscle Protein Synthesis; NRS 2002, Nutritional Risk Screening 2002; RT, Resistance exercise Training; 30-s CST, 30-second chair-stand-test.

Consent for publication

The model in Figure 1. *'Standardized Resistance training program'* (supplemental material) has given written consent to publish the pictures.

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Tables and Figure legends

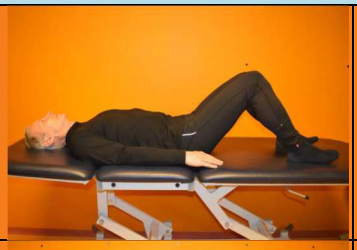
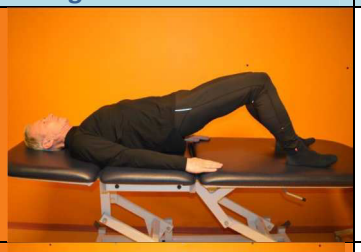
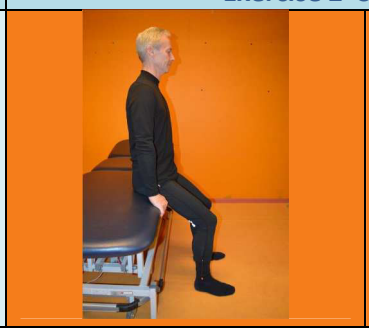
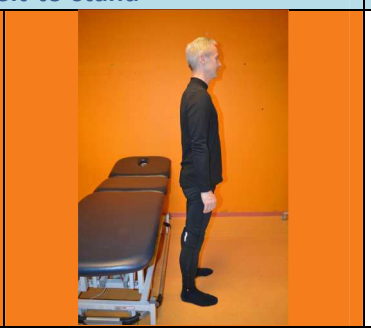
Table 2. Flow-chart of the study period, including meetings and tests

Supplemental material

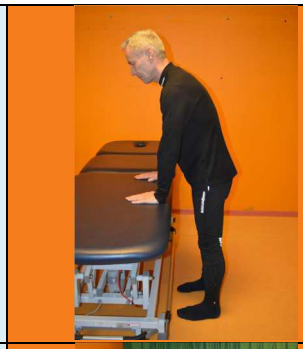
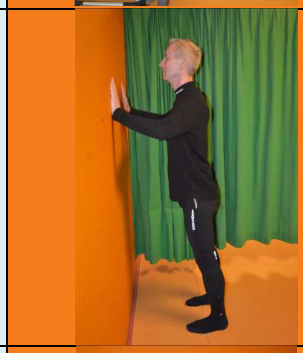
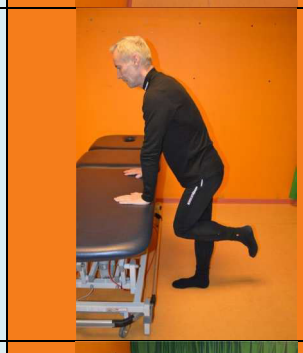
Figure 1. Standardized resistance training program

Supplemental material

Figure 1. Standardized Resistance training program

Level of resistance	Exercise 1 'Bridge'		Description of starting position
A			On the back with knees bent and feet flat on the floor/bed/table. Feet hip-width apart and hands by your side.
B			On the back with knees bent and feet flat on the floor/bed/table. Feet hip-width apart and arms crossed.
C			On the back with knees bent and feet flat on the floor/bed/table. Feet in semi tandem stand position and hip-width apart. Hands by your side. Repeated with the opposite leg in front. 3 x 10 on both legs.
D			On the back with knees bent and feet flat on the floor/bed/table. Feet in semi tandem stand position and hip-width apart. Arms crossed. Repeated with the opposite leg in front. 3 x 10 on both legs.
E			On the back with knees bent and feet flat on the floor/ bed/table. One knee bent and other leg on the ground with hands by your side. Repeated with the opposite leg in stretched. 3 x 10 on both legs.
Level of resistance	**Exercise 2 'Sit-to-stand'**		**Description of starting position**
A			Sitting on an elevated bed/table/chair. Feet hip-width apart. Stand up using the arms to push off.

B			Sitting on a chair with armrest. Feet hip-width apart. Stand up using the arms and arm rests to push off.
C			Sitting on a chair. Feet hip-width apart. Stand up from chair with arms crossed.
D			Sitting on a chair with armrest. Feet in semi tandem stand position and hip-width apart. Stand up using the arms and arm rests to push off. Repeated with the opposite leg in front. 3 x 10 on both legs.
E			Sitting on a chair. Feet in semi tandem stand position and hip-width apart. Stand up from chair with arms crossed. Repeated with the opposite leg in front. 3 x 10 on both legs.
Level of resistance	**Exercise 3 'Calf-rasises'**		**Description of starting position**
A			Sitting on a chair. Lifting the heels off the floor as high as possible. If it is really easy, extra weight can be added by leaning forward and pushing downwards with the hands on the knees.

B			Standing, using an elevated bed or table for balance/support. Heels are lifted off the floor as high as possible.
C			Standing, using a wall for balance. Heels are lifted off the floor as high as possible.
D			Standing on one leg, using a table for balance/support. The heel is lifted off the floor as high as possible. Repeated on both legs.
E			Standing on one leg, using a wall for balance. The heel is lifted off the floor as high as possible. Repeated on both legs.

Progression/regression: The level of resistance A-E is modified applying only the participants' own body weight and different starting positions. One session consists of 3 sets of 10 repetitions. An intensity of 8-12 repetition maximum (RM) is pursued. If participants can do more than 12 repetitions of an exercise in each of two consecutive sets they are told to progress to the next level of resistance for that particular exercise. They progress to the next level of resistance even though they cannot do 3 x 10 repetitions of that exercise in the very beginning. If their performance exceeds that of the highest level of resistance (E), they will be instructed to increase the number of repetitions to 3 x 15 of 'exercise E'. If they can do less than 8 repetitions in the last set of the exercise, they will be instructed in an exercise mode with a lower level of resistance.
The model has given written consent to publish this material.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1+2
	2b	All items from the World Health Organization Trial Registration Data Set	Relevant items throughout the manuscript
Protocol version	3	Date and version identifier	_____
Funding	4	Sources and types of financial, material, and other support	21+22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1+21
	5b	Name and contact information for the trial sponsor	21+suppl. Material (letter)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	21+22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4+5
	6b	Explanation for choice of comparators	4+5
Objectives	7	Specific objectives or hypotheses	4+5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9-10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-15
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5+10-11+table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	16
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-15
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10+16-17

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18 (follow the rules of the Danish Data Protection Agency – security and storage)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16-18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	16-18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Already approved

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6+18
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	22
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	18
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21+22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	22
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not relevant

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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A protein-enriched, milk-based supplement to counteract sarcopenia in acutely ill geriatric patients offered resistance exercise training during and after hospitalization: study protocol for a randomized, double-blind, multicenter trial

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Manuscript

Title: 'A protein-enriched, milk-based supplement to counteract sarcopenia in acutely ill geriatric patients offered resistance exercise training during and after hospitalization: study protocol for a randomized, double-blind, multicenter trial'

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Public trials registry: The study has been approved by the Danish Regional Ethical Committee (reference no. H-16018240), and the Danish Data Protection Agency (reference no. HGH-2016-050), and it is registered in ClinicalTrials.gov (identifier: NCT02717819).

Word count: 7246

ABSTRACT

Introduction: Age-related loss of muscle mass and strength, sarcopenia, burdens many older adults. The process is accelerated with bedrest, protein intakes below requirements, and the catabolic effect of certain illnesses. Thus, acutely ill, hospitalized older adults are particularly vulnerable. Protein supplementation can preserve muscle mass and/or strength, and combining this with resistance exercise training (RT), may have additional benefits. Therefore, this study investigates the effect of protein supplementation as an addition to offering RT among older adults while admitted to the geriatric ward and after discharge. This has not previously been investigated.

Methods and analysis: In a block-randomised, double-blind, multicentre intervention study, 165 older adults above 70 years, fulfilling the eligibility criteria, will be included consecutively from three Medical Departments (blocks of n=20, stratified by recruitment site). After inclusion, participants will be randomly allocated (1:1) to receive either ready-to-drink, protein-enriched, milk-based supplements (a total of 27.5 g whey protein/day) or iso-energetic placebo products (<1.5 g protein/day), twice daily as a supplement to their habitual diet. Both groups will be offered a standardized RT program for lower extremity muscle strength (daily while hospitalized and 4x/week after discharge). The study period starts during their hospital stay and continues12 weeks after discharge. The primary endpoint is lower extremity muscle strength and function (30-s chair-stand-test). Secondary endpoints include muscle mass, measures of physical function, and measures related to cost-effectiveness.

Ethics and dissemination: Approval is given by the Research Ethic Committee of the Capital Region of Denmark (reference no. H-16018240) and the Danish Data Protection Agency (reference no. HGH-2016-050). There are no expected risks associated with participation, and each participant is expected to benefit from the RT. Results will be published in peer-reviewed international journals and presented at national and international congresses and symposiums.

Trial Registration: ClinicalTrials.gov: NCT02717819 (March 9, 2016).

Strengths and limitations of this study:

- To our knowledge this is the first study to investigate the effect of protein supplementation in addition to RT among acutely ill geriatric patients, while admitted and after discharge, and it adds new information to the evidence-based health care.
- The study is randomized and double-blinded which minimizes the risk of selection, performance, and detection bias, and the multi-center trial design increases the generalizability of the results.
- The lack of supervised RT after discharge might lower compliance to the RT, although it is more realistic that self-training at home can be implemented in a real world setting.
- Acutely ill older adults are a difficult population to maintain in a long duration intervention study, which increases the risk of drop outs and/or low compliance.
- Registration of compliance in dietary studies is always associated with a risk of bias, but by asking the participants to register their daily intake, save empty bottles, and by calling them on a weekly basis to check on compliance, this is minimized.

INTRODUCTION

Sarcopenia is the loss of muscle mass and strength with ageing. It is an unavoidable process with a multifactorial aetiology [1,2] associated to impaired balance and increased risk of falls and mortality [3]. Also, sarcopenia is associated with a 3- to 4-fold increased risk of disability, which in turn is related to substantial socio-economic and health care spending [4]. Acute illness might result in stress metabolism which further increases the loss of protein and the anabolic resistance in older adults, leading to increased loss of lean body mass (LBM) [5], and this is further accelerated by bed-rest during hospitalization. Also, many older adults consume relatively small amounts of protein, important for maintenance and buildup of LBM, and loss of appetite as a consequence of acute illness may further decrease the protein consumption [6,7]. This is very critical, as research has shown that the protein requirement increases with age. [8]. Even a short hospital stay increases the risk of losing functional capacity and the ability to cope with activities of daily living [9]. For older medical patients it has been shown that only one in three regained their habitual physical function one year after discharge [10]. Hence, interdisciplinary interventions to counteract sarcopenia become even more relevant in the acutely ill older patients.

The beneficial effect of resistance exercise training (RT) on counteracting sarcopenia is quite well established [11,12], and the effect of protein supplementation alone has also been documented [13]. Less well studied is the potential benefit of a higher protein intake or supplementation as an addition to offering RT among older adults. A recent systematic review by Malafarina et al. (2013) and a meta-analysis by Cermak et al. (2012) have concluded that in older adults, protein supplementation increases muscle mass, and in some studies also muscle strength, during prolonged RT [13,14]. However, the evidence is sparse in the frailest older adults, who often have a low dietary protein intake, and based on findings in systematic reviews, they might benefit even more from a combined intervention [14-16]. To our knowledge, no studies have yet investigated the effect of protein supplementation in addition to offering RT among hospitalized, acutely ill old adults – a population at great risk of a rapid functional deterioration. Thus, the present study aims at

investigated this, and in addition the intervention will continue after discharge from the hospital. The novelty of this study is two-fold. Firstly the intervention involves hospitalized older adults, and secondly the intervention continues after discharge. To the best of the authors' knowledge, previous studies were only performed in one setting.

METHODS AND ANALYSIS

Study design

The study design is a block-randomised, double-blind, placebo-controlled, multicentre intervention study. A total of 165 participants will be included consecutively from the Medical Departments of three Hospitals in the Capital Region of Denmark **(**Gentofte and Herlev University Hospital and Rigshospitalet-Glostrup, n=55 from each place). Recruitment takes place a maximum of 72 hours after admission. After inclusion, participants will be randomly allocated (1:1) to receive either protein-enriched milk-based supplements (whey protein) or an iso-energetic placebo product, as a supplement to their habitual diet. Both groups follow the same RT program and are daily supplemented with vitamin D. The intervention starts at the hospital while admitted and continues 12 weeks after discharge. Recruitment and data collection started in April 2016, and will end in June 2018.

Study population

Inclusion criteria for participation are; men and women aged ≥ 70 years, able to speak and understand Danish, expected length of stay > 3 days (evaluated by medical staff at department), ability to stand independently for at least 30 seconds, and admission to the medical departments of Gentofte Hospital, Herlev Hospital or Rigshospitalet-Glostrup. Exclusion criteria are: active cancer, renal insufficiency (eGFR < 30 mL/min/1.73m^2), cognitive impairment (not able to comprehend the purpose of the study/give informed consent), terminal disease, exclusively receiving enteral or parenteral nutrition, milk/lactose allergy or intolerance, planning to lose weight/go on a special diet, planned transfer to other hospitals/departments and pacemaker/other implanted electrical stimulants (due to Bio-Impedance Analysis (BIA) measurements). Participants will be withdrawn from the

study if they die during admission (does not apply to subsequent admissions) or are discharged/transferred from the medical department before the intervention has started.

Randomization and blinding

After collection of baseline measurements and characteristics, participants are randomized to either the intervention or the control group using sealed, opaque envelopes containing a paper with either an 'A' or a 'B'. Each Hospital site has its own pile of envelopes in order to allow for block-randomization. Within each site, 10 A's and 10 B's (20 in total) are put in the pile over three rounds, to ensure a more even allocation of participants in the two groups at any time. Participants, hospital staff, and study investigators will all be blinded towards the randomization. If a situation arises where unblinding may be considered for the benefit of the participant, this will be decided on an individual basis taking the specific situation into account. Enrollment and randomization is performed by study investigators.

Intervention

Protein-enriched, milk-based supplements and Placebo

Depending on their allocation, participants will receive either a protein-enriched, milk-based supplement beverage (Arla Foods®: 781 kJ, 10.5 g whey protein concentrate and 0.5 g casein, 10 g fat, and 13 g carbohydrate per 100 ml) (intervention group) or an iso-energetic placebo beverage (Arla Foods®: 797 kJ, 0.6 g protein, 10 g fat, and 24 g carbohydrate per 100 ml) (control group). The amino acid profile of the intervention product is shown in the supplemental material, table 1. Both products have a flavour of raspberry and come in ready-to-drink preparations. From January 2017 and on, the protein-enriched milk-based supplement will have vitamin D added in amounts of 1.125 µg per 100 ml. During the whole study period (while hospitalized and 12 weeks post discharge) the participants will be instructed to drink a total of 250 ml per day, divided into two servings of 125 ml. Thus, the intervention group will get a total of 27.5 g extra protein per day, equal to 26.25 g whey protein containing a total of ~ 2.5 g leucine. This amount of protein supplementation is chosen, based on previous studies finding positive effects from similar or

smaller dosages [17-19]. Furthermore, protein supplementation is satiating, and if given in higher amounts might compromise habitual food intake to a great extend – especially among older adults with low appetite. The total dosage is divided into two servings (breakfast and next cold main meal), as research indicate that 25-30 grams of high quality protein is needed per main meal to maximally stimulate post prandial protein synthesis [8]. The beverages come in white bottles with either a 'group A' or 'group B' label on. While hospitalized, the timing of the intake is as follows; one serving at breakfast (or at lunch, if not consumed at breakfast for any reasons, e.g. fasting necessary, or if the RT is performed right after breakfast) and one serving directly after the RT. In the 12 weeks after discharge the participants will be instructed to drink one serving at breakfast and one serving with the next cold main meal, irrespective of the meal is eaten at lunch or at dinner time. If the participants forget to drink the beverages at the specific times, they will be told to drink it when they become aware of it. The participants will not be instructed to make other dietary changes during the study period. If participants are prescribed/recommended by hospital staff to take oral nutritional supplements, this is not an exclusion criterion, but participants will be instructed to take any additional supplements on a given day only after intake of the 'study beverages'. If for some reason (e.g. uncontrolled diabetes or severe reduction of habitual food intake), the participant is advised by medical doctors'/nutritional therapists' to stop taking the supplement, this advice will always be followed.

Vitamin D supplements

Vitamin D supplementation has been shown to have an independent effect on muscle [20]. To reduce the potential confounder of a large difference in intake of vitamin D between groups, all participants will get vitamin D supplements handed out after enrolment, and be instructed to take a supplement of 20 µg/day (two tablets of 10 µg), as recommended by the Danish National Board of Health [21]. Exceptions to this are those participants whose serum-vitamin D levels have been measured to ≥ 100 nmol/L at the time of study inclusion to avoid reaching toxic levels. The participants have to register their intake of vitamin D in a diary along with their intake of the

intervention products. Also, at the last visit in study week 12, the number of tablets left in the container will be counted to verify the registrations. If participants already take vitamin D supplements in combination tablets with other vitamins and/or minerals corresponding to 20 µg/day or more, they will be instructed to keep taking their own tablets and register this. The exact amount of vitamin D in these tablets will be recorded. An average intake of vitamin D per day during the intervention period will be used to compare if the intake of vitamin D is different between the two groups.

Resistance exercise training (RT)

The RT program is developed by experienced physiotherapists and is consistent with the official statements from the American College of Sports Medicine on recommendations for RT in older adults [22]. It focuses on strength training primarily of the big muscle groups of the lower limbs, and can be performed without any training equipment. One training session consists of three exercises; 'lifting-and-lowering the pelvic' from a crook-lying position, 'sit-to-stand from a chair', and 'lifting-and-lowering the heels' in a standing position – i.e. performing heel-raises. All exercises are performed in three sets, aiming at 10 repetitions, pursuing an intensity of 8-12 repetition maximum. The repetition velocity will be performed at the participants own preferred speed. There will be a time interval of 1-3 minutes between sets and exercises, depending on the individual need for rest. Each of the three exercises can be performed in five different modes (A-B-C-D-E), graduated in terms of increasing resistance, by applying the participants' own body weight and different starting positions. Thus, the program can be individualized corresponding to the participants abilities, and adjustments will be made to ensure progression. The illustrated RT-program can be seen in the supplemental material, figure 1. Participants can be asked to leave out a specific exercise, if there are safety concerns (e.g. severe dizziness or worsening of a condition) or if they experience pain related to performance a certain exercise.

While admitted to hospital, supervised RT is offered daily by physiotherapists in addition to the standard of care. After discharge, the participants are encouraged to perform the same RT program

as self-training four times per week. They will be instructed to have at least 24 hours between training sessions. During the hospital stay it is expected that the participants have a very limited amount of physical activity besides the RT program offered, and that the intensity by which they can perform the RT is rather low. This is why the frequency of the RT differs between the hospital and discharge setting. To instruct the participants in regard to the RT, and to ensure progression (or regression if necessary), they receive follow-up home visits by a physiotherapist in study week 1, 3, 6, 9, and after discharge from any readmissions. The adjustments are made after standardized procedures.

Participants who are discharged with a plan of rehabilitation including ambulatory training at a center or supervised training at home, to be provided by their municipally, will be asked to perform the full RT study program until their rehabilitation program starts up (a wait of 2-6 weeks are normal). Each training session performed as part of a rehabilitation program will replace *one* self-training session of the RT study program. The same applies, if participants are discharged from the hospital directly to a 24-h rehabilitation center and they are performing RT in their regimen. This is to allow for proper restitution. The offer of supervised training applies only to the first hospital stay, but if readmitted to hospital participants will be encouraged to do the RT themselves to the extent possible.

Compliance

While hospitalized, the participants will get the product handed out along with the vitamin D supplements. Investigators and physiotherapists register overall study compliance, that is daily ingestion of the intervention or placebo supplements (time for handout and amount ingested), vitamin D (dose, yes/no), and performance of the RT (number of sets and repetitions for each exercise). Empty bottles are saved so that study investigators can verify the amount of intervention product consumed.

After discharge, the amount of intervention or placebo supplement consumed and the RT performed for each participant will be assessed by daily records in a 'beverage and exercise diary', specifically

designed for the study and handed out to be filled in by the participants. The participants, e.g. with help from their relatives, are asked to daily register the amount of beverage consumed; 0 %, 25 %, 50 %, 75 %, or 100 % of each of the two servings by ticking of the corresponding circular illustration, along with ticking of the intake of vitamin D. Participants also have to register execution of the RT, and specify for each of the three exercises the number of sets and repetitions performed. If they are exercise training at a rehabilitation center this can be registered in the relevant boxes. In case of deviations, four pre-specified explanations are given that they can tick off, both in regard to the intake of supplements and the execution of the RT. To verify the participants' records they are asked to save and store empty bottles, which will be picked up by investigators on days with home-visits. At the same time study investigators will help the participants' to retrospectively fill out any missing registrations. Participants who are discharged to a 24-hour rehabilitation centre will get the intervention products handed out by the staff, who will also save empty bottles. On the first visit after discharge the participants will receive thorough instructions on how to register compliance in the 'beverage and exercise diary', and upcoming visits will be planned. Both groups will receive daily standard messages on their cell phone (if they have one and agrees to this) and weekly phone calls, kindly reminding them to consume the supplement and vitamin D, perform the RT, and register compliance. Furthermore, as part of the phone call, they will be asked about compliance and any deviations or e.g. upstart of training at a rehabilitation center will be registered and validated/compared later on with their own diaries, and they will be reminded of upcoming home visits.

Outcome parameters

The baseline characteristics will be collected at inclusion to the study. To standardize the endpoint measures, especially that of LBM, these will be assessed 1.5-2 hours after a light breakfast. Thus, if inclusion happens in the afternoon, then baseline measurements will be assessed the following day, prior to any study interventions. The measurements will be assessed in a predefined order to reduce fatigue and follow standardized procedures, and they will be repeated within 72 hours after

discharge and 12 weeks (± 2 days) after discharge. If possible, before each endpoint examination the participants will be asked to consume a breakfast, similar to that consumed at the hospital before the baseline measurements. The assessments after discharge will be performed in the participants own home. Follow-up assessments, including only admission to hospital and mortality, will be assessed six months after the intervention period. In general, if participants are readmitted to hospital, if possible, assessments will be performed there and otherwise at a replacement visit after discharge. All data collection is performed by study investigators. Table 1 gives an overview of the study period and the different time points for meetings and tests.

Table 1. Flow-chart of the study period, including meetings and tests

Flow-Chart of study period	Baseline	In-hospital intervention	Post-hospital intervention[d]					Follow-up
Study week no.	-	-	1	3	6	9	12	38
Meetings incl. tests	1+2	-	3	4	5	6	7	-
In- and exclusion criteria	X							
Informed consent	X							
Baseline characteristics	X							
Baseline endpoint assessment[a]	X							
Randomization	X							
LOS (in-hospital intervention period)		X						
Dietary registration		X (4 days in total)						
Daily compliance registrations		X	X					
Endpoint assessment[a]			X[b]				X[c]	
Exercise adjustments			X	X	X	X		
Weekly phone call			X					
24-h dietary interview				X	X	X	X	
Exercise interview				X	X	X	X	
Evaluation-questionnaire							X	
Delivery of intervention products		X (ongoing basis)	X (deliveries after appointment)					
Collection of empty intervention bottles			X	X	X	X	X	
Readmissions, LOS, and mortality							X	X

a: *assessed 1.5-2 hours after a light breakfast (preferably the same meal every time).* ***b:*** *assessed within 72 hours after discharge.* ***c:*** *assessed 12 weeks (±2 days) after discharge.* ***d:*** *assessments and meeting are taking place where the participant's live.*

Primary endpoint

Lower extremity muscle strength is measured by the 30-second chair-stand-test (30-s CST). The test exists in both a standardized and a modified version. The standardized 30-s CST measures the

number of times the participant can rise-and-sit from a standard chair (height of 43-45 cm) in 30 seconds with the arms folded across the chest, starting from a sitting position. Only full stands will count – i.e. full extension of the knees and hips. Those who cannot stand from the chair without using the arm rest will get a score of 0 [23]. In the modified 30-s CST the participant is allowed to use the arm rests [24]. If participants are only able to perform the modified version at baseline, for the following assessments they will be asked to do the same. If they are able to do the standardized version they will be asked to do that as well after a 15 minutes rest. A change of 2.0-2.6 stands is considered to be clinically relevant based on data from a population of older adults with hip and knee osteoarthritis [25].

Secondary endpoints

Total, appendicular, and trunk LBM (kg and percent) is assessed by Bio-impedance Analysis (BIA) using the portable InBody-230 body composition analyzer (dual frequency (20 kHz, 100 kHz), tetra polar 8-Point Tactile Electrode System (InBody, Copenhagen, Denmark)). Direct segmental measurement technology is used, meaning that no calculations, and thus empirical factors and imputations, are needed. Various factors can affect BIA measurements such as previous exercise, body position, skin temperature, dietary intake, and hydration state [26]. Thus, in order to standardize the measurements these will be performed in the morning 1.5-2 hours after a light breakfast and bladder emptying (preferably also bowel emptying), and before any exercise. Participants will be asked to wear light clothes and no shoes. They will be instructed to stand upright with the feet on the build-in electrodes embedded in the scale platform, grasp the handles of the analyzer while spreading the arms as much as they can, and look straight ahead. The reliability of the InBody-230 body composition analyzer will be measured and used to establish the threshold of change needed beyond measurement error.

Hand grip strength (HGS) is a proxy measure of upper extremity strength, and is measured in kg using the second handle position with a DHD-1 Digital Hand Dynamometer (Saehan Medical, 2012, Roskilde Denmark). The second handle position is recommended as a standard position, as it

is suitable for most hand sizes. An investigator will instruct the participants to be seated with their feet on the ground, shoulders adducted and neutrally rotated, elbow flexed at a 90° angle and supported on the armrests of the chair or a table, and forearm and wrist in neutral position, as recommended by Roberts et al. (2011) [27]. They will be asked to perform three maximum force trials with their dominant hand, and the highest value will be registered. They will be instructed to squeeze the handle as hard as they can for 5 seconds, and the test will be repeated within 15 seconds.

4-meter gait speed (4-m GS) is used to assess the usual gait speed (m/s) over a short distance. Participants will be placed behind a starting line and instructed to start walking at their usual pace after the investigators command. To reduce the effect of acceleration and deceleration, each participant will be instructed to walk towards a visual goal for 5 meters. The time will be started after the participant has walked 0.5 meter and stopped after 4.5 meters, counted from the first foot-step that crosses the 4-m start line and end line, respectively. The fastest of two attempts is recorded. If it is not possible to establish a 5 m test track, a shorter track with a minimum length of 3.5 m in total will be used instead, and this will be registered as bias [28,29]. The participants are allowed to use a gait aid, which will be registered as well. In sedentary older adults, a clinical relevant difference is found to be 0.03-0.05 m/s, while 0.08 m/s is found to be a substantial relevant difference [30].

Functional ability is measured using the modified Barthel Index (Barthel-100) [31,32]. The Barthel-100 contains 10 measures of every-day and mobility activities, and the ability to master these activities reflects the level of functioning. Each measure has five levels of functioning, and for all 10 measures a maximum of 100 points can be achieved, corresponding to fully independent. The Barthel-100 will be scored by the investigators, and rated based on the amount of assistance required to complete each activity or by observing, and clarifying questions will be asked when necessary.

Mobility is assessed by De Morton Mobility Index (DEMMI), which provides a 15-item unidimensional measure of mobility across the spectrum from bed bound to independent mobility, specifically developed for geriatric patients [33]. It has 5 categories in which the participants are tested; bed (3 test scores), chair (3 test scores), static balance (4 test scores), walking (2 test scores), and dynamic balance (3 test scores). A total test score from 0-19 can be achieved, and this raw score is converted to an interval DEMMI score from 0-100, where 100 is represents independent mobility. In older acute medical patients, the clinical relevant difference is found to be 10 points on the converted scale [33].

Cognitive function is measured using the Mini Mental State Examination (MMSE), which consists of small simple tasks to elucidate eight different cognitive functions; orientation, episodic memory, concentration, function of language, practical exercise, reading skills, writing skills, and visual-spatial construction. The performances are scored to give a raw score ranging from 0-30, where 30 represent the best/optimal function [34].

Social support is evaluated using registrations of home care (yes/no, if yes, then divided into practical help, personal care, and both) and residence (own home, nursing home/assisted living facility, 24-hour rehabilitation facility).

Use of gait aid is registered as yes (incl. specific gait aid), no, or cannot walk.

Length of hospital stay (LOS) corresponds to the in-hospital intervention period (days from recruitment until discharge) which is registered from the electronic patient register.

Readmission to hospital and mortality. Readmission to hospital is registered both with regard to frequency and the total LOS, from the electronic patient register. These data are summed up after the intervention period and after the follow-up period, respectively.

Health related Quality of life (QOL) is assessed by using the generic questionnaire, Euroqol EQ-5D-3L [35]. The questionnaire is self-reported, and reflects the participant's current situation. Scores for the EQ-5D-3L are generated from the ability of the individual to function in five dimensions; mobility, pain/discomfort, self-care, anxiety/depression, and usual activities. Each

dimension has three possible answers; no problem, some problems, and major problems. Also, the participants rate their current health state on a visual-analogue-scale ranging from 0-100 (reflecting a health state from 'worst' to 'best').

Body weight is measured to the nearest 0.1 kg using the BIA equipment InBody-230, and follows the same standardized procedures as described under the endpoint 'muscle mass'.

Product-evaluation-questionnaire. Both the intervention and placebo product is evaluated using a self-report questionnaire. The evaluation questionnaire concerns overall liking, side effects related to consumption, taste fatigue, texture, dosage, and manageability.

Control for confounders - other registrations and precautions

Actions are taken to actively reduce or register known or possible confounders. Thus, at baseline, confounders such as admission diagnosis, chronic diseases, nutritional risk (NRS 2002) [36], sarcopenia [3,37], depression [38], and mobility [39,40] are evaluated, among other. Nutritional risk is determined based on a combination of factors: unintended weight loss within the last three months, loss of appetite within the last week, body mass index, disease severity, and age. Patients screened to be at risk are expected to benefit from nutritional intervention. Sarcopenia is assessed according to the definition proposed by the European Working Group on Sarcopenia in Older People (EWGSOP). This is based on the assessments of LBM (measured by BIA), muscle strength (measured HGS), and physical performance (measured by 4-m gait speed). Furthermore, besides register vitamin D intakes, throughout the study the following two measures are collected on an ongoing basis.

Protein and energy intake. During hospitalization the participants' protein (g/kg) and energy (kJ/kg) intake will be registered for four days, or shorter if the participants' are discharged. The hospitals' food and drink registration schemes will be used. Participants will be asked to fill in the food registration schemes themselves with help from the nurses and study investigators. The participant's body weight at inclusion will be used to calculate the intake per kg body weight. During the 12-week post-hospital intervention the participants protein and energy intake will be estimated based

on the average of four 24-hour dietary-recall interviews performed at study week 3, 6, 9, and 12 at home visits, or by phone if the participant are no longer compliant in the study with regard to the intervention products and the RT. As the home visits will be planned in collaboration with the participants, and has to be fitted into other study tasks and visits, these practicalities decide what day of the week the recall interview is covering. To minimize the risk of recall bias a checklist of specific foods and beverages will be used to verify the reported intake. Furthermore, when interviewing face to face, picture series of portion sizes of different foods will be used to estimate the amounts ingested [41]. The foods and drinks will be entered in the software program Madlog Vita® to calculate the intake of protein (g) and energy (kJ). Four days of registration/dietary recalls are considered adequate to assess this information with a high correlation [42]. An average of the participant's body weight after discharge and in week 12 will be used to calculate the intake per kg body weight. The cut-off for suspecting underreporting will be evaluated retrospectively on an individual basis taking any illness, readmissions, loss of body weight, activity level etc. into account.

Daily activity level. In a semi-structured interview the participants are asked about exercise-related activities besides the RT program. This is reported four times after discharge in study week 3, 6, 9, and 12 at home visits, or by phone if the participant' is no longer compliant in the study with regard to the intervention products and the RT. Depending on the answers given, the participants will be divided into activity levels from 1-5 after predefined criteria, inspired by Saltin & Grimby (1968) [43]. The scale is ordinal, and activity level 1 represents the least active and level 5 the most active. It is the time used on different activities and the intensities of these (low, moderate, or high) that determine the activity level.

Statistics

Power calculation

The primary endpoint is muscle strength measured by the 30-s CST. The clinical relevant difference for this test is found to be 2.0-2.6, when assessed in older populations with hip and knee

osteoarthritis [25]. Jones et al. (1999) has used the standardized 30-s CST on community-dwelling older people and found a SD of 3.0 and 3.6 for people in the age range of 70-79 and 80-89, respectively [23]. This gives a pooled SD of 3.31, which is used in this power calculation, and it corresponds well with measures of SD found in the modified test version [44].

In order to be able to detect a difference of 2.0, with a power of 80 % and a two-sided alpha-error of 0.05, the required sample size is 80 participants in each group, given an anticipated combined rate of drop-outs and non-compliance of 45 %. This rate is chosen since studies with resistance training in older people both while hospitalized [45] and in a community-dwelling setting [46], have experienced drop-outs of 30 %. Moreover, an additional 15 % is added to account for participants with a low compliance to the intervention, to be able to maintain the statistical power of the study in the intention-to-treat analysis as well as in the per protocol analysis. For practical reasons, if possible within the time schedule, 55 participants will be included at each of the three sites, resulting in a total inclusion of 165 participants.

Feasibility of recruitment and sample size

The three hospitals where recruitment is going to take place had between 525-687 geriatric patients in year 2014, with a median LOS ranging from 8-11 (5-16) days. The median age for women was in the range of 84-87 years and 83-84 years for men [47]. To meet the timetable the expected recruitment rate is a minimum of two participants per week which based on these data is considered realistic.

Statistical tests

The primary analysis will be performed by the intention-to-treat principle. In addition, a predefined per-protocol analysis will be performed including participants with a high compliance only (consumption of the intervention product ≥ 75 %). Furthermore, endpoints will be compared adjusting for randomization bias (defined as $p < 0.05$ between groups), and confounding factors (total activity level and total protein- and energy intake). Analysis will be done both with and without imputation techniques for missing values, but drop-outs will be encouraged to participate in

follow-up examinations, including interviews concerning dietary intake and activity level. Sensitivity analysis will be performed without outliers, defined as a value of 3 SD above or below the mean. To investigate whether the intervention will have different impacts in different groups of patients, e.g. those who are at nutritional risk or sarcopenic, subgroup analysis will be performed looking at treatment effect in the subgroups and interactions between treatment effect and subgroups. Furthermore, observational analysis will be performed, investigating the importance of total protein- and energy intake and total activity level on outcome measures. The two groups will be compared looking at the hospitalization intervention period and the 12 week post discharge intervention period both separately and as a whole.

Results will be presented as median (range) or mean (SD or 95 % CI) and number (absolute frequencies) for continuous and categorical variables, respectively. Inspection for normality will be done by visual inspection (QQ-plot), and parametric or nonparametric statistical tests will be used in accordance with the distribution of the variables. Statistical comparisons will be made between the two groups by using the Mann-Whitney U-test or Students t-test for continuous variables, and the Chi-square test (X^2) or Fisher's Exact Test (in case of expected cell count < 5) for the comparison of categorical variables. ANCOVA will be used for continuous outcomes and binary logistic regression for binary outcomes if/when adjusting for confounders and testing for subgroup interaction. The Spearman-Rank Correlation Test or General Linear Model will be used to test for correlations between independent variables. All tests are two-tailed and an alpha-level of $P < 0.05$ will be used to determine statistical significance in all analyses.

With regard to the primary endpoint, 30-s CST, the changes in performance from baseline (both with and without pooling standardized and modified test results) will be measured and compared between the two groups. Furthermore, performance will be scored into one of three categories; 1. ability to rise from the chair with arms folded across the chest, 2. ability to rise from the chair using the arm rest, and 3. not able to rise independently from the chair. Also, compared to baseline, performance will be scored into either 'better', 'worse' or 'unchanged'.

ETICHS AND DISSEMINATION

The study will be conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. Thus, precautions will be taken to protect the privacy and confidentiality of research subjects. Approval is given by the Danish Data Protection Agency (HGH-2016-050) and the Research Ethic Committee of the Capital Region of Denmark (H-16018240), and the study is registered in the clinical.trial.gov database (NCT02717819). Any amendments to the protocol will be made public at clinical.trial.gov. All participants receive written and oral information from study investigators about all relevant aspects of the study before making decision about participation, and they are informed that they can withdraw from the study at any time**.** The participants receive no payment and will have no expenses associated with participation in the study. There are no expected risks associated with participation, and we expect each participant to benefit from the RT. The results of the study will be published in international peer-reviewed journals and presented at national and international congresses and symposiums.

DISCUSSION

This study investigates the effect of protein supplementation in addition to offering RT among older adults while admitted to the geriatric ward and after discharge. The acutely ill 'geriatric patient' is a heterogeneous patient group with various (non-surgical) diseases and often existing comorbidities. The goals are to counteract sarcopenia, maintain or improve physical function, and reduce health care costs in this specific population. Thus, with this study we wish to add knowledge about effective secondary prevention and interdisciplinary rehabilitation strategies to the large population of acutely ill older adults admitted to hospital. The eligibility criteria are very broad, however, the weakest patients (no stand function) are excluded, as these will not be able to participate in a RT program and perform the endpoint measurements. The participants in the current study are included within three days of admission. It is possible that the weakest geriatric patients with no stand function, currently excluded, will gain their stand function later during their hospitalization (>3 days). Thus, the results from the current study may also be relevant to this group of patients,

although not examined. A common confounder is that people agreeing to participate in an intervention trial are more motivated to lifestyle changes, which is an important factor for the compliance and possible success of this intervention.

Use of placebo beverages allows blinding of participants and researchers. Thus, performance and detection bias are minimized. Another strength is the randomization procedure, which will limit selection bias and hopefully balance different confounders which could potentially influence the results. The multi-center trial design furthermore increases the generalizability of the results. The activity and dietary interviews are conducted in order to be able to correct statistically for differences in protein intake and activity levels between groups. In addition, it will also enable us to investigate the importance of overall protein and energy intake on the results.

The majority of older adults in Denmark take vitamin D supplements as recommended by the Danish Health Authority [21]. Studies have shown that vitamin D has an independent positive effect on muscle strength [48]. In order to investigate the effect of the protein supplementation alone, vitamin D supplements will be given to all participants with serum-vitamin D levels ≤ 100 nmol/L at inclusion, to ensure similar vitamin D intakes. Another reason for ensuring that all participants are supplemented with vitamin D is that the protein-enriched beverage approximately half-way through the intervention period will have vitamin D added to the product. However, the fortification level is quite low, adding an extra amount of only 3.5 µg vitamin D per day from the beverages, which e.g. corresponds to 13 g of salmon [49]. Also, compared to the daily vitamin D supplementation of minimum 20 µg (some older adults' takes even higher amounts, as prescribed by their doctor) it is considered insignificant.

In regard to ensure compliance to the RT program, it is a weakness of the study that the RT at home after discharge is not supervised. On the other hand, an aim of the current study is to test the effect of an interdisciplinary rehabilitation regime that is cost-effective and could easily be implemented. Supervised RT four times per week would have required a lot of resources, which most likely would not be possible to implement in the real world. If a positive effect is found of an intervention

only consisting of extra protein and self-training after discharge, potential implementation in clinical practice will be more feasible. The current study can also give valuable insights into which sub groups of the geriatric patients that would be able to benefit from a rehabilitation regime based on self-training and protein intervention. The high rate of readmissions to hospital among older adults [50] indicates that there is room for improvement in regard to secondary prevention strategies.

The specific endpoints included in the current study were chosen in order to be suitable, feasible and valid for this specific population of older adults. Thus, a low amount of missing data is expected due to low feasibility. The 30-s CST, DEMMI, and Barthel-100 are part of the normal routine tests for geriatric patients admitted to the medical departments (they are included in The Danish National Geriatric Data Base), and all tests and questionnaires are developed and/or validated in older adults [28,32-34]. Furthermore, the Danish Board of Health recommends the use of 30-s CST, 4-m GS, MMSE, and EQ-5D-3L as tests in older geriatric patients [29]. Also, LBM measured by BIA, has been proposed as a feasible measurement tool in this population [3,51], and a portable BIA is a practical tool suitable for home visits.

Specifically for the primary endpoint, the 30-s CST has been shown to be a reliable and valid indicator of lower body strength in generally active, community-dwelling older adults, when validated against maximum weight-adjusted leg-press performance [23]. The Standardized 30-s CST version has been shown to have low feasibility (54 %) in acutely admitted old medical patients, and to have lower inter-rater reliability than in medically stable patients. However, the Modified 30-s CST has been shown to be both feasible and having a high inter-rater reliability [28]. Thus, we believe that all participants will be able to perform either the standardized or the modified version, supported by the inclusion criteria, that only patients who can stand independently are recruited, eliminating those in poorest conditions. This is also in accordance with experience from our former intervention studies performed in geriatric patients [52,53], and also applies to the other secondary endpoints.

For the secondary endpoint, LBM measured by a portable BIA, Moon *et al.* (2013) have shown that single frequency BIA in elderly men and women (72 men and women, > 65 years) correlate well with Dual Energy X-ray Absorptiometry (DXA) measurements, as well as the 4-compartment model, at single time points as well as for tracking changes in LBM. They concluded that DXA and BIA can be used interchangeably as valid methods to measure LBM when looking at a population basis of more than 15-22 people [51]. Furthermore, Karelis *et al.* (2013) has validated the portable, dual-frequency InBody-230 BIA against DXA in a healthy mixed population (145 men and women, 44.6±20 years) and found a significant high correlation when looking at fat mass, percent body fat, and total LBM [54]. Thus, it is expected that using the InBody-230 BIA equipment, besides being practical in regard to home visits, will be a reasonable valid method to assess total muscle mass in a population of 165 older adults.

DECLARATIONS

Authors' contributions

AMB prepared the grant application. AMB and JG conceived the overall study draft, and JG created the detailed study protocol. AA, AV, BC, TWK, and CB participated in its design and coordination. JG and research assistants collect the data under the supervision of AA, AV, AMB, BC and CB. JG drafted the manuscript. All authors reviewed the article critically and contributed significantly to the final content. All authors have read and approved the final manuscript.

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results. A Scientist from Copenhagen University have been involved with the study design, and will be involved in all steps from analysis and interpretation to publication of the results.

Competing interests

None of the authors have financial or personal conflicting interests. The sponsor (Danish Dairy Research Foundation) and the producer of the intervention and placebo products (Arla Foods) will not have any influence on the analysis and interpretation of the results.

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List of abbreviations

BIA, Bio-Impedance Analysis; DEMMI, De Morton Mobility Index; DXA, Dual-energy X-ray Absorptiometry; LBM, Lean Body Mass; MPS, Muscle Protein Synthesis; NRS 2002, Nutritional Risk Screening 2002; RT, Resistance exercise Training; 30-s CST, 30-second chair-stand-test.

Consent for publication

The model in Figure 1. *'Standardized Resistance training program'* (supplemental material) has given written consent to publish the pictures.

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Tables and Figure legends

Table 2. Flow-chart of the study period, including meetings and tests

Supplemental material

Figure 1. Standardized resistance training program

Table 1. Amino acid profile of the intervention product

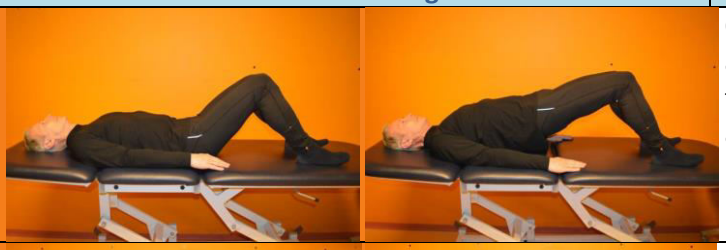
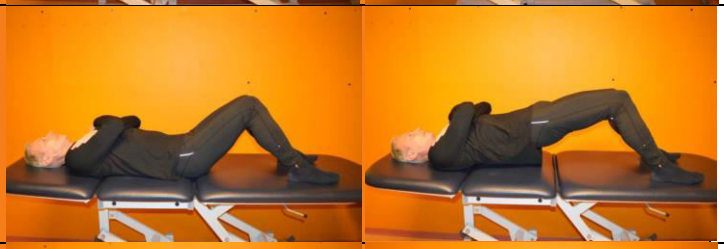
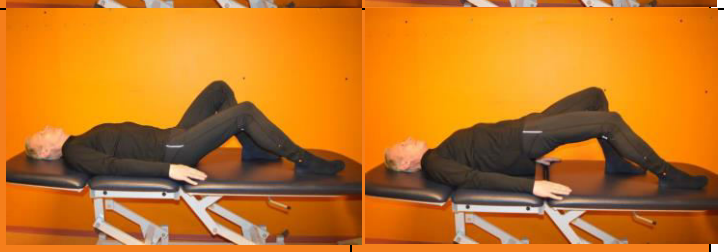
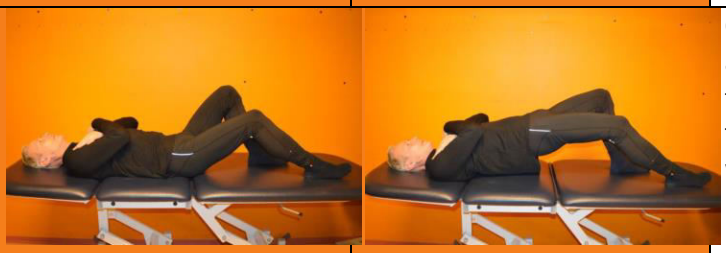
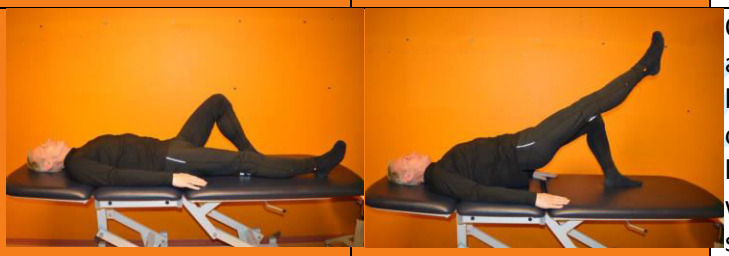
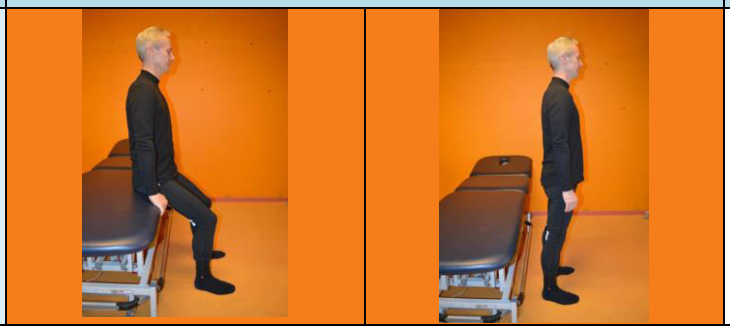
Supplemental material

Table 1. Amino acid profile of the intervention product

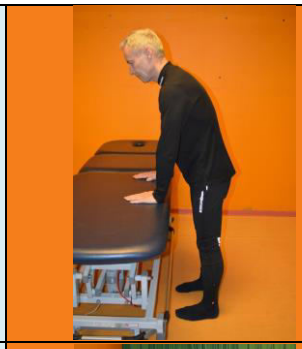
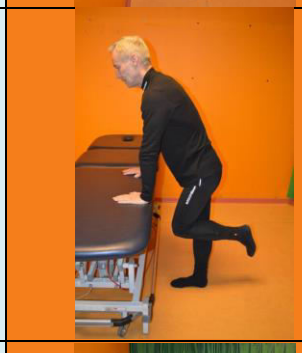
Amino acid	Grams per 100 gram
Serine	0.473
Glutamic acid	1.58
Proline	0.560
Glycine	0.166
Alanine	0.499
Valine	0.527
Isoleucine	0.546
Leucine	0.998
Tyrosine	0.258
Phenylalanine	0.291
Lysine	0.852
Histidine	0.170
Arginine	0.242
Aspartic acid	0.947
Threonine	0.620
Tryptophan	0.137
Cystein +Cystine	0.181
Methionine	0.210
Particulated whey protein concentrate, name: Nutrilac YO-8078. Analysis performed by Eurofins Steins Laboratorium A/S, DK.	

Supplemental material

Figure 1. Standardized Resistance training program

Level of resistance	Exercise 1 'Bridge'	Description of starting position
A		On the back with knees bent and feet flat on the floor/bed/table. Feet hip-width apart and hands by your side.
B		On the back with knees bent and feet flat on the floor/bed/table. Feet hip-width apart and arms crossed.
C		On the back with knees bent and feet flat on the floor/bed/table. Feet in semi tandem stand position and hip-width apart. Hands by your side. Repeated with the opposite leg in front. 3 x 10 on both legs.
D		On the back with knees bent and feet flat on the floor/bed/table. Feet in semi tandem stand position and hip-width apart. Arms crossed. Repeated with the opposite leg in front. 3 x 10 on both legs.
E		On the back with knees bent and feet flat on the floor/ bed/table. One knee bent and other leg on the ground with hands by your side. Repeated with the opposite leg in stretched. 3 x 10 on both legs.
Level of resistance	**Exercise 2 'Sit-to-stand'**	**Description of starting position**
A		Sitting on an elevated bed/table/chair. Feet hip-width apart. Stand up using the arms to push off.

B			Sitting on a chair with armrest. Feet hip-width apart. Stand up using the arms and arm rests to push off.
C			Sitting on a chair. Feet hip-width apart. Stand up from chair with arms crossed.
D			Sitting on a chair with armrest. Feet in semi tandem stand position and hip-width apart. Stand up using the arms and arm rests to push off. Repeated with the opposite leg in front. 3 x 10 on both legs.
E			Sitting on a chair. Feet in semi tandem stand position and hip-width apart. Stand up from chair with arms crossed. Repeated with the opposite leg in front. 3 x 10 on both legs.
Level of resistance	**Exercise 3 'Calf-rasises'**		**Description of starting position**
A			Sitting on a chair. Lifting the heels off the floor as high as possible. If it is really easy, extra weight can be added by leaning forward and pushing downwards with the hands on the knees.

B			Standing, using an elevated bed or table for balance/support. Heels are lifted off the floor as high as possible.
C			Standing, using a wall for balance. Heels are lifted off the floor as high as possible.
D			Standing on one leg, using a table for balance/support. The heel is lifted off the floor as high as possible. Repeated on both legs.
E			Standing on one leg, using a wall for balance. The heel is lifted off the floor as high as possible. Repeated on both legs.

Progression/regression: The level of resistance A-E is modified applying only the participants' own body weight and different starting positions. One session consists of 3 sets of 10 repetitions. An intensity of 8-12 repetition maximum (RM) is pursued. If participants can do more than 12 repetitions of an exercise in each of two consecutive sets they are told to progress to the next level of resistance for that particular exercise. They progress to the next level of resistance even though they cannot do 3 x 10 repetitions of that exercise in the very beginning. If their performance exceeds that of the highest level of resistance (E), they will be instructed to increase the number of repetitions to 3 x 15 of 'exercise E'. If they can do less than 8 repetitions in the last set of the exercise, they will be instructed in an exercise mode with a lower level of resistance.
The model has given written consent to publish this material.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1+2
	2b	All items from the World Health Organization Trial Registration Data Set	Relevant items throughout the manuscript
Protocol version	3	Date and version identifier	_____
Funding	4	Sources and types of financial, material, and other support	21+22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1+21
	5b	Name and contact information for the trial sponsor	21+suppl. Material (letter)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	21+22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4+5
	6b	Explanation for choice of comparators	4+5
Objectives	7	Specific objectives or hypotheses	4+5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9-10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-15
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5+10-11+table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	16
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-15
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10+16-17

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18 (follow the rules of the Danish Data Protection Agency – security and storage)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16-18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	16-18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Already approved

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6+18
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	22
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	18
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21+22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	22
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not relevant

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.