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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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22 ABSTRACT

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

31 **Methods and analysis:** In a block-randomised, double-blind, multicentre intervention study 165
32 older adults above 70 years, fulfilling the eligibility criteria, will be included consecutively from
33 three Medical Departments (blocks of n=20, stratified by recruitment site). After inclusion,
34 participants will be randomly allocated (1:1) to receive either protein-enriched, milk-based
35 supplements (27.5 g protein/d) or iso-energetic placebo products (<1.5 g protein/d), as a supplement
36 to their habitual diet. Both groups will be offered a standardized RT program. The study period
37 starts during their hospital stay and continues 12 weeks after discharge. The primary endpoint is
38 lower extremity muscle strength and function (30-s chair-stand-test). Secondary endpoints include
39 muscle mass, measures of physical function, and measures related to cost-effectiveness.

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

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

46 **Strengths and limitations of this study:**

- 1
2 47 • To our knowledge this is the first study to investigate the effect of protein supplementation
3
4 48 in addition to RT among acutely ill geriatric patients, while admitted and after discharge,
5
6 49 and it adds new information to the evidence-based health care.
7
8 50 • The study is randomized and double-blinded which minimizes the risk of selection,
9
10 51 performance, and detection bias, and the multi-center trial design increases the
11
12 52 generalizability of the results.
13
14 53 • The lack of supervised RT after discharge might lower compliance to the RT, although it is
15
16 54 more realistic that self-training at home can be implemented in a real world setting.
17
18 55 • Acutely ill older adults are a difficult population to maintain in a long duration intervention
19
20 56 study, which increases the risk of drop outs and/or low compliance.
21
22 57 • Registration of compliance in dietary studies is always associated with a risk of bias, but by
23
24 58 asking the participants to register their daily intake, save empty bottles, and by calling them
25
26 59 on a weekly basis to check on compliance, this is minimized.
27
28
29
30
31 60

61 INTRODUCTION

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

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

1 87 of protein per meal is needed to maximally stimulate muscle hypertrophy [10]. Hence,
2
3 88 interdisciplinary interventions to counteract sarcopenia become even more relevant in the acutely ill
4
5 89 older patients.
6
7
8 90 The beneficial effect of resistance exercise training (RT) on counteracting sarcopenia is quite well
9
10 91 established [11,12], and the effect of protein supplementation alone has also been documented [13].
11
12 92 Less well studied is the potential benefit of a higher protein intake or supplementation when older
13
14 93 adults are offered RT at the same time. A recent systematic review by Malafarina et al. (2013) and
15
16 94 a meta-analysis by Cermak et al. (2012) have both concluded that protein supplementation increases
17
18 95 muscle mass, and in some studies also muscle strength, during prolonged RT in older adults
19
20 96 [13,14]. Furthermore, some reviews stresses that the evidence is sparse in the frailest older adults,
21
22 97 who often have a low dietary protein intake, and based on their findings the hypothesis is that this
23
24 98 sub population will benefit even more from a combined intervention [14-16]. This said, to our
25
26 99 knowledge, no studies have yet investigated the effect of a protein supplementation among
27
28 100 hospitalized, acutely ill old adults offered RT, which is a population where many have a high risk of
29
30 101 malnutrition and experience accelerated loss of muscle mass and strength, loss of function, and
31
32 102 (further) development of sarcopenia.
33
34
35

36 37 103 **METHODS AND ANALYSIS**

38 39 104 **Study design**

40
41 105 The study design is a block randomised, double-blind, placebo-controlled, multicentre intervention
42
43 106 study. A total of 165 participants will be included consecutively from the Medical Departments of
44
45 107 three Hospitals in the Capital Region of Denmark (Gentofte and Herlev University Hospital and
46
47 108 Rigshospitalet-Glostrup, n=55 from each place). Recruitment takes place a maximum of 72 hours
48
49 109 after admission. After inclusion, participants will be randomly allocated (1:1) to receive either
50
51 110 protein-enriched milk-based supplements (whey protein) or an iso-energetic placebo product, as a
52
53 111 supplement to their habitual diet. Both groups follow the same RT program and are daily
54
55 112 supplemented with vitamin D. The intervention starts at the hospital while admitted and continues
56
57
58
59
60

1 113 12 weeks after discharge. Recruitment and data collection started in April 2016, and will end in
2
3
4 114 June 2018.

5 115 **Study population**

6 116 Inclusion criteria for participation are; men and women aged ≥ 70 years, able to speak and
7
8 117 understand Danish, expected length of stay > 3 days (evaluated by medical staff at department),
9
10 118 ability to stand independently for at least 30 seconds, and admission to the medical departments of
11
12 119 Gentofte Hospital, Herlev Hospital or Rigshospitalet-Glostrup. Exclusion criteria are: active cancer,
13
14 120 renal insufficiency (eGFR < 30 mL/min/1.73m²), cognitive impairment (not able to comprehend the
15
16 121 purpose of the study/give informed consent), terminal disease, exclusively receiving enteral or
17
18 122 parenteral nutrition, milk/lactose allergy or intolerance, planning to lose weight/go on a special diet,
19
20 123 planned transfer to other hospitals/departments and pacemaker/other implanted electrical stimulants
21
22 124 (due to Bio-Impedance Analysis (BIA) measurements). Participants will be withdrawn from the
23
24 125 study if they die during admission (does not apply to subsequent admissions) or are
25
26 126 discharged/transferred from the medical department before the intervention has started.
27
28
29
30

31 127 **Randomization and blinding**

32 128 After collection of baseline measurements and characteristics, participants are randomized to either
33
34 129 the intervention or the control group using sealed, opaque envelopes containing a paper with either
35
36 130 an 'A' or a 'B'. Each Hospital site has its own pile of envelopes in order to allow for block-
37
38 131 randomization. Within each site, 10 A's and 10 B's (20 in total) are put in the pile over three
39
40 132 rounds, to ensure a more even allocation of participants in the two groups at any time. Participants,
41
42 133 hospital staff, and study investigators will all be blinded towards the randomization. If a situation
43
44 134 arises where unblinding may be considered for the benefit of the participant, this will be decided on
45
46 135 an individual basis taking the specific situation into account. Enrollment and randomization is
47
48 136 performed by study investigators.
49
50
51
52

53 137 **Intervention**

54 138 *Protein-enriched, milk-based supplements and Placebo*

1 139 Depending on their allocation, participants will receive either a protein-enriched milk-based
2
3 140 supplement beverage (Arla Foods®: 781 kJ, 10 g whey protein, 10 g fat, and 13 g carbohydrate per
4
5 141 100 ml) (intervention group) or an iso-energetic placebo beverage (Arla Foods®: 797 kJ, 0,58 g
6
7 142 protein, 10.2 g fat, and 24,14 g carbohydrate per 100 ml) (control group). Both products have a
8
9 143 flavour of raspberry. From January 2017 and on, the protein-enriched milk-based supplement will
10
11 144 have vitamin D added in amounts of 1.125 µg per 100 ml. During the whole study period (while
12
13 145 hospitalized and 12 weeks post discharge) the participants will be instructed to drink a total of 250
14
15 146 ml per day, divided into two servings of 125 ml. Thus, the intervention group will get a total of 27.5
16
17 147 g extra whey protein per day. The beverages come in white bottles with either a 'group A' or 'group
18
19 148 B' label on. While hospitalized, the timing of the intake is as follows; one serving at breakfast (or at
20
21 149 lunch, if not consumed at breakfast for any reasons, e.g. fasting necessary, or if the RT is performed
22
23 150 right after breakfast) and one serving directly after the RT. In the 12 weeks after discharge the
24
25 151 participants will be instructed to drink one serving at breakfast and one serving with the next cold
26
27 152 main meal, irrespective of the meal is eaten at lunch or at dinner time. If the participants forget to
28
29 153 drink the beverages at the specific times, they will be told to drink it when they become aware of it.
30
31 154 The participants will not be instructed to make other dietary changes during the study period. If
32
33 155 participants are prescribed/recommended by hospital staff to take oral nutritional supplements, this
34
35 156 is not an exclusion criterion, but participants will be instructed to take any additional supplements
36
37 157 on a given day only after intake of the 'study beverages'.

158 *Vitamin D supplements*

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

1
2 165 participants have to register their intake of vitamin D in a diary along with their intake of the
3
4 166 intervention products. Also, at the last visit in study week 12, the number of tablets left in the
5
6 167 container will be counted to verify the registrations. If participants already take vitamin D
7
8 168 supplements in combination tablets with other vitamins and/or minerals corresponding to 20 µg/day
9
10 169 or more, they will be instructed to keep taking their own tablets and register this. The exact amount
11
12 170 of vitamin D in these tablets will be recorded. An average intake of vitamin D per day during the
13
14 171 intervention period will be used to compare if the intake of vitamin D is different between the two
15
16 172 groups.

173 *Resistance exercise training (RT)*

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

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

1
2 191 amount of physical activity besides the RT program offered, and that the intensity by which they
3
4 192 can perform the RT is rather low. This is why the frequency of the RT differs between the hospital
5
6 193 and discharge setting. To instruct the participants in regard to the RT, and to ensure progression (or
7
8 194 regression if necessary), they receive follow-up home visits by a physiotherapist in study week 1, 3,
9
10 195 6, 9, and after discharge from any readmissions. The adjustments are made after standardized
11
12 196 procedures.

13
14 197 Participants who are discharged with a plan of rehabilitation including ambulatory training at a
15
16 198 center or supervised training at home, to be provided by their municipality, will be asked to perform
17
18 199 the full RT study program until their rehabilitation program starts up (a wait of 2-6 weeks are
19
20 200 normal). Each training session performed as part of a rehabilitation program will replace *one* self-
21
22 201 training session of the RT study program. The same applies, if participants are discharged from the
23
24 202 hospital directly to a 24-h rehabilitation center and they are performing RT in their regimen. This is
25
26 203 to allow for proper restitution. The offer of supervised training applies only to the first hospital stay,
27
28 204 but if readmitted to hospital participants will be encouraged to do the RT themselves to the extent
29
30 205 possible.

31 32 206 **Compliance**

33
34 207 While hospitalized, the participants will get the product handed out along with the vitamin D
35
36 208 supplements. Investigators and physiotherapists register overall study compliance, that is daily
37
38 209 ingestion of the intervention or placebo supplements (time for handout and amount ingested),
39
40 210 vitamin D (dose, yes/no), and performance of the RT (number of sets and repetitions for each
41
42 211 exercise). Empty bottles are saved so that study investigators can verify the amount of intervention
43
44 212 product consumed.

45
46 213 After discharge, the amount of intervention or placebo supplement consumed and the RT performed
47
48 214 for each participant will be assessed by daily records in a 'beverage and exercise diary', specifically
49
50 215 designed for the study and handed out to be filled in by the participants. The participants, e.g. with
51
52 216 help from their relatives, are asked to daily register the amount of beverage consumed; 0 %, 25 %, 50 %, 75 %, 100 %.

1 217 50 %, 75 %, or 100 % of each of the two servings by ticking of the corresponding circular
2
3 218 illustration, along with ticking of the intake of vitamin D. Participants also have to register
4
5 219 execution of the RT, and specify for each of the three exercises the number of sets and repetitions
6
7
8 220 performed. If they are exercise training at a rehabilitation center this can be registered in the
9
10 221 relevant boxes. In case of deviations, four pre-specified explanations are given that they can tick
11
12 222 off, both in regard to the intake of supplements and the execution of the RT. To verify the
13
14 223 participants' records they are asked to save and store empty bottles, which will be picked up by
15
16 224 investigators on days with home-visits. At the same time study investigators will help the
17
18 225 participants' to retrospectively fill out any missing registrations. Participants who are discharged to
19
20 226 a 24-hour rehabilitation centre will get the intervention products handed out by the staff, who will
21
22 227 also save empty bottles. On the first visit after discharge the participants will receive thorough
23
24 228 instructions on how to register compliance in the 'beverage and exercise diary', and upcoming visits
25
26 229 will be planned. Both groups will receive daily standard messages on their cell phone (if they have
27
28 230 one and agrees to this) and weekly phone calls, kindly reminding them to consume the supplement
29
30 231 and vitamin D, perform the RT, and register compliance. Furthermore, as part of the phone call,
31
32 232 they will be asked about compliance and any deviations or e.g. upstart of training at a rehabilitation
33
34 233 center will be registered and validated/compared later on with their own diaries, and they will be
35
36 234 reminded of upcoming home visits.

37 235 **Outcome parameters**

38 236 The baseline characteristics will be collected at inclusion to the study. To standardize the endpoint
39
40 237 measures, especially that of LBM, these will be assessed 1.5-2 hours after a light breakfast. Thus, if
41
42 238 inclusion happens in the afternoon, then baseline measurements will be assessed the following day,
43
44 239 prior to any study interventions. The measurements will be assessed in a predefined order to reduce
45
46 240 fatigue and follow standardized procedures, and they will be repeated within 72 hours after
47
48 241 discharge and 12 weeks (± 2 days) after discharge. If possible, before each endpoint examination
49
50 242 the participants will be asked to consume a breakfast, similar to that consumed at the hospital before
51
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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
			1	3	6	9	12	
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.

249

250 Primary endpoint

251 30-second chair-stand-test (30-s CST) gives a measure of the muscle strength in the lower
 252 extremities. It exists in both a standardized and a modified version. The standardized 30-s CST
 253 measures the number of times the participant can rise-and-sit from a standard chair (height of 43-45
 254 cm) in 30 seconds with the arms folded across the chest, starting from a sitting position. Only full
 255 stands will count – i.e. full extension of the knees and hips. Those who cannot stand from the chair

1
2 256 without using the arm rest will get a score of 0 [20]. In the modified 30-s CST the participant is
3
4 257 allowed to use the arm rests [21]. If participants are only able to perform the modified version at
5
6 258 baseline, for the following assessments they will be asked to do the same. If they are able to do the
7
8 259 standardized version they will be asked to do that as well after a 15 minutes rest.

10 260 **Secondary endpoints**

11
12 261 *Muscle mass* is assessed by Bio-impedance Analysis (BIA) using the portable InBody-230 body
13
14 262 composition analyzer (dual frequency (20 kHz, 100 kHz), tetra polar 8-Point Tactile Electrode
15
16 263 System (InBody, Copenhagen, Denmark)). Direct segmental measurement technology is used,
17
18 264 meaning that no calculations, and thus empirical factors and imputations, are needed. Measures of
19
20 265 total, appendicular, and trunk LBM is registered (kg and percent). Various factors can affect BIA
21
22 266 measurements such as previous exercise, body position, skin temperature, dietary intake, and
23
24 267 hydration state [22]. Thus, in order to standardize the measurements these will be performed in the
25
26 268 morning 1.5-2 hours after a light breakfast and bladder emptying (preferably also bowel emptying),
27
28 269 and before any exercise. Participants will be asked to wear light clothes and no shoes. They will be
29
30 270 instructed to stand upright with the feet on the build-in electrodes embedded in the scale platform,
31
32 271 grasp the handles of the analyzer while spreading the arms as much as they can, and look straight
33
34 272 ahead.

35
36 273 *Hand grip strength* (HGS) is measured in kg using the second handle position with a DHD-1
37
38 274 Digital Hand Dynamometer (Saehan Medical, 2012, Roskilde Denmark). The second handle
39
40 275 position is recommended as a standard position, as it is suitable for most hand sizes. An investigator
41
42 276 will instruct the participants to be seated with their feet on the ground, shoulders adducted and
43
44 277 neutrally rotated, elbow flexed at a 90° angle and supported on the armrests of the chair or a table,
45
46 278 and forearm and wrist in neutral position, as recommended by Roberts et al. (2011) [23]. They will
47
48 279 be asked to perform three maximum force trials with their dominant hand, and the highest value
49
50 280 will be registered. They will be instructed to squeeze the handle as hard as they can for 5 seconds,
51
52 281 and the test will be repeated within 15 seconds.

1
2 282 *4-meter gait speed* (4-m GS) is used to assess the usual gait speed (m/s) over a short distance.
3
4 283 Participants will be placed behind a starting line and instructed to start walking at their usual pace
5
6 284 after the investigators command. To reduce the effect of acceleration and deceleration, each
7
8 285 participant will be instructed to walk towards a visual goal for 5 meters. The time will be started
9
10 286 after the participant has walked 0.5 meter and stopped after 4.5 meters, counted from the first foot-
11
12 287 step that crosses the 4-m start line and end line, respectively. The fastest of two attempts is
13
14 288 recorded. If it is not possible to establish a 5 m test track, a shorter track with a minimum length of
15
16 289 3.5 m in total will be used instead, and this will be registered as bias [24,25]. The participants are
17
18 290 allowed to use a gait aid, which will be registered as well.
19
20
21 291 *Functional ability* is measured using the modified Barthel Index (Barthel-100) [26,27]. The Barthel-
22
23 292 100 contains 10 measures of every-day and mobility activities, and the ability to master these
24
25 293 activities reflects the level of functioning. Each measure has five levels of functioning, and for all
26
27 294 10 measures a maximum of 100 points can be achieved, corresponding to fully independent. The
28
29 295 Barthel-100 will be scored by the investigators, and rated based on the amount of assistance
30
31 296 required to complete each activity or by observing, and clarifying questions will be asked when
32
33 297 necessary.
34
35
36 298 *Mobility* is assessed by De Morton Mobility Index (DEMMI), which provides a 15-item
37
38 299 unidimensional measure of mobility across the spectrum from bed bound to independent
39
40 300 Mobility, specifically developed for geriatric patients [28]. It has 5 categories in which the
41
42 301 participants are tested; bed (3 test scores), chair (3 test scores), static balance (4 test scores),
43
44 302 walking (2 test scores), and dynamic balance (3 test scores). A total test score from 0-19 can be
45
46 303 achieved, and this raw score is converted to an interval DEMMI score from 0-100, where 100 is
47
48 304 represents independent mobility.
49
50
51 305 *Cognitive function* is measured using the Mini Mental State Examination (MMSE), which consists
52
53 306 of small simple tasks to elucidate eight different cognitive functions; orientation, episodic memory,
54
55 307 concentration, function of language, practical exercise, reading skills, writing skills, and visual-

1
2 308 spatial construction. The performances are scored to give a raw score ranging from 0-30, where 30
3
4 309 represent the best/optimal function [29].

5
6 310 *Social support* is evaluated using registrations of home care (yes/no, if yes, then divided into
7
8 311 practical help, personal care, and both) and residence (own home, nursing home/assisted living
9
10 312 facility, 24-hour rehabilitation facility).

11
12 313 *Use of gait aid* is registered as yes (incl. specific gait aid), no, or cannot walk.

13
14 314 *Length of hospital stay (LOS)* corresponds to the in-hospital intervention period (days from
15
16 315 recruitment until discharge) which is registered from the electronic patient register.

17
18 316 *Readmission to hospital and mortality.* Readmission to hospital is registered both with regard to
19
20 317 frequency and the total LOS, from the electronic patient register. These data are summed up after
21
22 318 the intervention period and after the follow-up period, respectively.

23
24 319 *Health related Quality of life (QOL)* is assessed by using the generic questionnaire, Euroqol EQ-
25
26 320 5D-3L [30]. The questionnaire is self-reported, and reflects the participant's current situation.

27
28 321 Scores for the EQ-5D-3L are generated from the ability of the individual to function in five
29
30 322 dimensions; mobility, pain/discomfort, self-care, anxiety/depression, and usual activities. Each
31
32 323 dimension has three possible answers; no problem, some problems, and major problems. Also, the
33
34 324 participants rate their current health state on a visual-analogue-scale ranging from 0-100 (reflecting
35
36 325 a health state from 'worst' to 'best').

37
38 326 *Body weight* is measured to the nearest 0.1 kg using the BIA equipment InBody-230, and follows
39
40 327 the same standardized procedures as described under the endpoint 'muscle mass'.

41
42 328 *Product-evaluation-questionnaire.* Both the intervention and placebo product is evaluated using a
43
44 329 self-report questionnaire. The evaluation questionnaire concerns overall liking, side effects related
45
46 330 to consumption, taste fatigue, texture, dosage, and manageability.

47 331 **Control for confounders - other registrations and precautions**

48
49 332 Actions are taken to actively reduce or register known or possible confounders. Thus, at baseline,
50
51 333 confounders such as nutritional risk (NRS 2002) [31], sarcopenia [3,32], depression [33], and

1 334 mobility [34,35] are evaluated, among other. Furthermore, besides register vitamin D intakes,
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3
4 335 throughout the study the following two measures are collected on an ongoing basis.
5
6 336 *Protein and energy intake.* During hospitalization the participants' protein (g/kg) and energy (kJ/kg)
7
8 337 intake will be registered for four days, or shorter if the participants' are discharged. The hospitals'
9
10 338 food and drink registration schemes will be used. Participants will be asked to fill in the food
11
12 339 registration schemes themselves with help from the nurses and study investigators. The participant's
13
14 340 body weight at inclusion will be used to calculate the intake per kg body weight. During the 12-
15
16 341 week post-hospital intervention the participants protein and energy intake will be estimated based
17
18 342 on the average of four 24-hour dietary-recall interviews performed at study week 3, 6, 9, and 12 at
19
20 343 home visits, or by phone if the participant' are no longer compliant in the study with regard to the
21
22 344 intervention products and the RT. To minimize the risk of recall bias a checklist of specific foods
23
24 345 and beverages will be used to verify the reported intake. Furthermore, when interviewing face to
25
26 346 face, picture series of portion sizes of different foods will be used to estimate the amounts ingested
27
28 347 [36]. The foods and drinks will be entered in the software program Madlog Vita® to calculate the
29
30 348 intake of protein (g) and energy (kJ). Four days of registration/dietary recalls are considered
31
32 349 adequate to assess this information with a high correlation [37]. An average of the participant's
33
34 350 body weight after discharge and in week 12 will be used to calculate the intake per kg body weight.
35
36 351 The cut-off for suspecting underreporting will be evaluated retrospectively on an individual basis
37
38 352 taking any illness, readmissions, loss of body weight, activity level etc. into account.
39
40 353 *Daily activity level.* In a semi-structured interview the participants are asked about exercise-related
41
42 354 activities besides the RT program. This happens four times after discharge in study week 3, 6, 9,
43
44 355 and 12 at home visits, or by phone if the participant' is no longer compliant in the study with regard
45
46 356 to the intervention products and the RT. Depending on the answers given, the participants will be
47
48 357 divided into increasing activity levels from 1-5, after predefined criteria, inspired by Saltin &
49
50 358 Grimby (1968) [38].
51
52
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56 359 **Statistics**

1 360 *Power calculation*

2 361 The primary endpoint is muscle strength measured by the 30-s CST. The clinical relevant difference
3
4 362 for this test is found to be 2.0-2.6, when assessed in older populations with hip and knee
5
6 363 osteoarthritis [39]. Jones et al. (1999) has used the standardized 30-s CST on community-dwelling
7
8 364 older people and found a SD of 3.0 and 3.6 for people in the age range of 70-79 and 80-89,
9
10 365 respectively [20]. This gives a pooled SD of 3.31, which is used in this power calculation, and it
11
12 366 corresponds well with measures of SD found in the modified test version [40].
13
14

15
16 367 In order to be able to detect a difference of 2.0, with a power of 80 % and a two-sided alpha-error of
17
18 368 0.05, the required sample size is 80 participants in each group, given an anticipated combined rate
19
20 369 of drop-outs and non-compliance of 45 %. This rate is chosen since studies with resistance training
21
22 370 in older people both while hospitalized [41] and in a community-dwelling setting [42], have
23
24 371 experienced drop-outs of 30 %. Moreover, an additional 15 % is added to account for participants
25
26 372 with a low compliance to the intervention, to be able to maintain the statistical power of the study in
27
28 373 the intention-to-treat analysis as well as in the per protocol analysis. For practical reasons, if
29
30 374 possible within the time schedule, 55 participants will be included at each of the three sites,
31
32 375 resulting in a total inclusion of 165 participants.
33
34

35
36 376 *Feasibility of recruitment and sample size*

37
38 377 The three hospitals where recruitment is going to take place had between 525-687 geriatric patients
39
40 378 in year 2014, with a median LOS ranging from 8-11 (5-16) days. The median age for women was in
41
42 379 the range of 84-87 years and 83-84 years for men [43]. To meet the timetable the expected
43
44 380 recruitment rate is a minimum of two participants per week which based on these data is considered
45
46 381 realistic.
47

48
49 382 *Statistical tests*

50
51 383 The primary analysis will be performed by the intention-to-treat principle. In addition, a predefined
52
53 384 per-protocol analysis will be performed including participants with a high compliance only
54
55 385 (consumption of the intervention product ≥ 75 %). Furthermore, endpoints will be compared
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1
2 386 adjusting for randomization bias (defined as $p < 0.05$ between groups). Analysis will be done both
3
4 387 with and without imputation techniques for missing values, but drop-outs will be encouraged to
5
6 388 participate in follow-up examinations, including interviews concerning dietary intake and activity
7
8 389 level. Sensitivity analysis will be performed without outliers, defined as a value of 3 SD above or
9
10 390 below the mean. To investigate whether the intervention will have different impacts in different
11
12 391 groups of patients, e.g. those who are at nutritional risk or sarcopenic, subgroup analysis will be
13
14 392 performed looking at treatment effect in the subgroups and interactions between treatment effect
15
16 393 and subgroups. Furthermore, observational analysis will be performed, investigating the importance
17
18 394 of total protein- and energy intake and total activity level on outcome measures. The two groups
19
20 395 will be compared looking at the hospitalization intervention period and the 12 week post discharge
21
22 396 intervention period both separately and as a whole.

23
24 397 Results will be presented as median (range) or mean (SD or 95 % CI) and number (absolute
25
26 398 frequencies) for continuous and categorical variables, respectively. Inspection for normality will be
27
28 399 done by visual inspection (QQ-plot), and parametric or nonparametric statistical tests will be used
29
30 400 in accordance with the distribution of the variables. Statistical comparisons will be made between
31
32 401 the two groups by using the Mann-Whitney U-test or Students t-test for continuous variables, and
33
34 402 the Chi-square test (X^2) or Fisher's Exact Test (in case of expected cell count < 5) for the
35
36 403 comparison of categorical variables. ANCOVA will be used for continuous outcomes and binary
37
38 404 logistic regression for binary outcomes if/when adjusting for confounders and testing for subgroup
39
40 405 interaction. The Spearman-Rank Correlation Test or General Linear Model will be used to test for
41
42 406 correlations between independent variables. All tests are two-tailed and an alpha-level of $P < 0.05$
43
44 407 will be used to determine statistical significance in all analyses.

45
46 408 With regard to the primary endpoint, 30-s CST, the changes in performance from baseline (both
47
48 409 with and without pooling standardized and modified test results) will be measured and compared
49
50 410 between the two groups. Furthermore, performance will be scored into one of three categories; 1.
51
52 411 ability to rise from the chair with arms folded across the chest, 2. ability to rise from the chair using
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1
2 412 the arm rest, and 3. not able to rise independently from the chair. Also, compared to baseline,
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4 413 performance will be scored into either 'better', 'worse' or 'unchanged'.
5

6 414 **ETICHS AND DISSEMINATION**

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8
9 415 The study will be conducted in accordance with the principles of the World Medical Association
10
11 416 Declaration of Helsinki. Thus, precautions will be taken to protect the privacy and confidentiality of
12
13 417 research subjects. Approval is given by the Danish Data Protection Agency (HGH-2016-050) and
14
15 418 the Research Ethic Committee of the Capital Region of Denmark (H-16018240), and the study is
16
17 419 registered in the clinical.trial.gov database (NCT02717819). Any amendments to the protocol will
18
19 420 be made public at clinical.trial.gov. All participants receive written and oral information from study
20
21 421 investigators about all relevant aspects of the study before making decision about participation, and
22
23 422 they are informed that they can withdraw from the study at any time. The participants receive no
24
25 423 payment and will have no expenses associated with participation in the study. There are no expected
26
27 424 risks associated with participation, and we expect each participant to benefit from the RT. The
28
29 425 results of the study will be published in international peer-reviewed journals and presented at
30
31 426 national and international congresses and symposiums.
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34 35 427 **DISCUSSION**

36
37 428 This study investigates the effect of protein supplementation in addition to offering RT among older
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39 429 adults while admitted to the geriatric ward and after discharge. The acutely ill 'geriatric patient' is a
40
41 430 heterogeneous patient group with various (non-surgical) diseases and often existing comorbidities.
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43 431 The goals are to counteract sarcopenia, maintain or improve physical function, and reduce health
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45 432 care costs in this specific population. Thus, with this study we wish to add knowledge about
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47 433 effective secondary prevention and interdisciplinary rehabilitation strategies to the large population
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49 434 of acutely ill older adults admitted to hospital. The eligibility criteria are very broad, however, the
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51 435 weakest patients (no stand function) are excluded, as these will not be able to participate in a RT
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53 436 program and perform the endpoint measurements. The participants in the current study are included
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55 437 within three days of admission. It is possible that the weakest geriatric patients with no stand
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1
2 438 function, currently excluded, will gain their stand function later during their hospitalization (>3
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4 439 days). Thus, the results from the current study may also be relevant to this group of patients,
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6 440 although not examined. A common confounder is that people agreeing to participate in an
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8 441 intervention trial are more motivated to lifestyle changes, which is an important factor for the
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10 442 compliance and possible success of this intervention.

11
12 443 Use of placebo beverages allows blinding of participants and researchers. Thus, performance and
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14 444 detection bias are minimized. Another strength is the randomization procedure, which will limit
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16 445 selection bias and hopefully balance different confounders which could potentially influence the
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18 446 results. The multi-center trial design furthermore increases the generalizability of the results. The
19
20 447 activity and dietary interviews are conducted in order to be able to correct statistically for
21
22 448 differences in protein intake and activity levels between groups. In addition, it will also enable us to
23
24 449 investigate the importance of overall protein and energy intake on the results.

25
26
27 450 A majority of older adults in Denmark take vitamin D supplements as recommended by the Danish
28
29 451 Health Authority [18]. Studies have shown that vitamin D has an independent positive effect on
30
31 452 muscle strength [44]. In order to investigate the effect of the protein supplementation alone, vitamin
32
33 453 D supplements will be given to all participants with serum-vitamin D levels ≤ 100 nmol/L at
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35 454 inclusion, to insure similar vitamin D intakes. Another reason for ensuring that all participants are
36
37 455 supplemented with vitamin D is that the protein-enriched beverage approximately half-way through
38
39 456 the intervention period will have vitamin D added to the product. However, the fortification level is
40
41 457 quite low, adding an extra amount of only 3.5 μg vitamin D per day from the beverages, which e.g.
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43 458 corresponds to 13 g of salmon [45]. Also, compared to the daily vitamin D supplementation of
44
45 459 minimum 20 μg (some older adults' takes even higher amounts, as prescribed by their doctor) it is
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47 460 considered insignificant.

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49
50
51 461 In regard to ensure compliance to the RT program, it is a weakness of the study that the RT at home
52
53 462 after discharge is not supervised. On the other hand, an aim of the current study is to test the effect
54
55 463 of an interdisciplinary rehabilitation regime that is cost-effective and could easily be implemented.

1
2 464 Supervised RT four times per week would have required a lot of resources, which most likely
3
4 465 would not be possible to implement in the real world. If a positive effect is found of an intervention
5
6 466 only consisting of extra protein and self-training after discharge, potential implementation in
7
8 467 clinical practice will be more feasible. The current study can also give valuable insights into which
9
10 468 sub groups of the geriatric patients that would be able to benefit from a rehabilitation regime based
11
12 469 on self-training and protein intervention. The high rate of readmissions to hospital among older
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14 470 adults [46] indicates that there is room for improvement in regard to secondary prevention
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16 471 strategies.

17
18 472 The specific endpoints included in the current study were chosen in order to be suitable, feasible
19
20 473 and valid for this specific population of older adults. Thus, a low amount of missing data is
21
22 474 expected due to low feasibility. The 30-s CST, DEMMI, and Barthel-100 are part of the normal
23
24 475 routine tests for geriatric patients admitted to the medical departments (they are included in The
25
26 476 Danish National Geriatric Data Base), and all tests and questionnaires are developed and/or
27
28 477 validated in older adults [24,27-29]. Furthermore, the Danish Board of Health recommends the use
29
30 478 of 30-s CST, 4-m GS, MMSE, and EQ-5D-3L as tests in older geriatric patients [25]. Also, LBM
31
32 479 measured by BIA, has been proposed as a feasible measurement tool in this population [3,47], and a
33
34 480 portable BIA is a practical tool suitable for home visits.

35
36 481 Specifically for the primary endpoint, the 30-s CST has been shown to be a reliable and valid
37
38 482 indicator of lower body strength in generally active, community-dwelling older adults, when
39
40 483 validated against maximum weight-adjusted leg-press performance [20]. The Standardized 30-s
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42 484 CST version has been shown to have low feasibility (54 %) in acutely admitted old medical
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44 485 patients, and to have lower inter-rater reliability than in medically stable patients. However, the
45
46 486 Modified 30-s CST has been shown to be both feasible and having a high inter-rater reliability [24].
47
48 487 Thus, we believe that all participants will be able to perform either the standardized or the modified
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50 488 version, supported by the inclusion criteria, that only patients who can stand independently are
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52 489 recruited, eliminating those in poorest conditions. This is also in accordance with experience from
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1
2 490 our former intervention studies performed in geriatric patients [48,49], and also applies to the other
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4 491 secondary endpoints.
5
6 492 For the secondary endpoint, LBM measured by a portable BIA, Moon *et al.* (2013) have shown that
7
8 493 single frequency BIA in elderly men and women (72 men and women, > 65 years) correlate well
9
10 494 with Dual Energy X-ray Absorptiometry (DXA) measurements, as well as the 4-compartment
11
12 495 model, at single time points as well as for tracking changes in LBM. They concluded that DXA and
13
14 496 BIA can be used interchangeably as valid methods to measure LBM when looking at a population
15
16 497 basis of more than 15-22 people [47]. Furthermore, Karelis *et al.* (2013) has validated the portable,
17
18 498 dual-frequency InBody-230 BIA against DXA in a healthy mixed population (145 men and women,
19
20 499 44.6±20 years) and found a significant high correlation when looking at fat mass, percent body fat,
21
22 500 and total LBM [50]. Thus, it is expected that using the InBody-230 BIA equipment, besides being
23
24 501 practical in regard to home visits, will be a reasonable valid method to assess total muscle mass in a
25
26 502 population of 165 older adults.
27
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29

30 503 **DECLARATIONS**

31 504 **Authors' contributions**

32 505 AMB prepared the grant application. AMB and JG conceived the overall study draft, and JG
33
34 506 created the detailed study protocol. AA, AV, BC, TWK, and CB participated in its design and
35
36 507 coordination. JG and research assistants collect the data under the supervision of AA, AV, AMB,
37
38 508 BC and CB. JG drafted the manuscript. All authors reviewed the article critically and contributed
39
40 509 significantly to the final content. All authors have read and approved the final manuscript.
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43
44

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47
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49
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51
52 514 faculty of Nutrition, Exercise and Sports. Representatives from Arla Food have been involved in the
53
54 515 study design, but will not be involved in collection, analysis and interpretation of the data. The
55
56
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1 516 Danish Dairy Foundation will not be involved in the conduction of the study or interpretation of
2
3
4 517 results. A Scientist from Copenhagen University have been involved with the study design, and will
5
6 518 be involved in all steps from analysis and interpretation to publication of the results.
7

8 519 **Competing interests**

9
10 520 None of the authors have financial or personal conflicting interests. The sponsor (Danish Dairy
11
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13
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21
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23
24
25

26 527 **List of abbreviations**

27
28 528 BIA, Bio-Impedance Analysis; DEMMI, De Morton Mobility Index; DXA, Dual-energy X-ray
29
30 529 Absorptiometry; LBM, Lean Body Mass; MPS, Muscle Protein Synthesis; NRS 2002, Nutritional
31
32 530 Risk Screening 2002; RT, Resistance exercise Training; 30-s CST, 30-second chair-stand-test.
33

34 531 **Consent for publication**

35
36 532 The model in Figure 1. '*Standardized Resistance training program*' (supplemental material) has
37
38 533 given written consent to publish the pictures.
39
40

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2 658 **Tables and Figure legends**

3 659 Table 2. Flow-chart of the study period, including meetings and tests
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6 660 **Supplemental material**












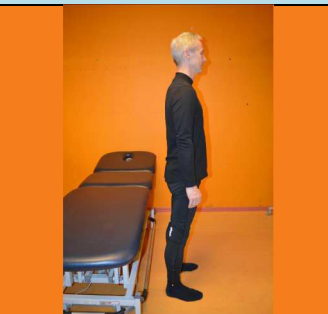
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8 661 Figure 1. Standardized resistance training program
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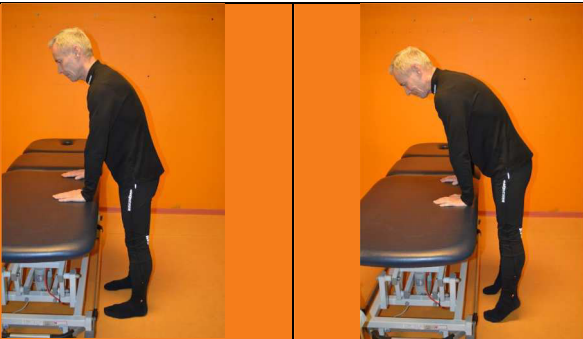







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Supplemental material

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.

<p>B</p>			<p>Sitting on a chair with armrest. Feet hip-width apart. Stand up using the arms and arm rests to push off.</p>
<p>C</p>			<p>Sitting on a chair. Feet hip-width apart. Stand up from chair with arms crossed.</p>
<p>D</p>			<p>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.</p>
<p>E</p>			<p>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.</p>
<p>Level of resistance</p>	<p>Exercise 3 'Calf-rasises'</p>		<p>Description of starting position</p>
<p>A</p>			<p>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.</p>

1 2 3 4 5 6 7 8 9 10 11 12 B			Standing, using an elevated bed or table for balance/support. Heels are lifted off the floor as high as possible.
13 14 15 16 17 18 19 20 21 22 C			Standing, using a wall for balance. Heels are lifted off the floor as high as possible.
23 24 25 26 27 28 29 30 31 32 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.
34 35 36 37 38 39 40 41 42 43 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.
<p>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.</p> <p>The model has given written consent to publish this material.</p>			



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)	_____

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1				
2				
3	Introduction			
4				
5	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
6				
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8		6b	Explanation for choice of comparators	4+5
9				
10	Objectives	7	Specific objectives or hypotheses	4+5
11				
12	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
13				
14				
15	Methods: Participants, interventions, and outcomes			
16				
17	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
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20	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
21				
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23	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-9
24				
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26		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
27				
28				
29		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9-10
30				
31				
32		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-9
33				
34	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
35				
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39	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
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1				
2	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
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	16
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8	Methods: Assignment of interventions (for controlled trials)			
9	Allocation:			
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11	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
12				
13	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
14				
15	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
16				
17	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
18				
19		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
20				
21				
22	Methods: Data collection, management, and analysis			
23				
24	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
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26		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
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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

1				
2				
3	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes,	18
4	amendments		analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,	
5			regulators)	
6				
7	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	6+18
8			how (see Item 32)	
9				
10		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	22
11			studies, if applicable	
12				
13	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	18
14			in order to protect confidentiality before, during, and after the trial	
15				
16	Declaration of	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21+22
17	interests			
18				
19	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	_____
20			limit such access for investigators	
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22	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial	_____
23	trial care		participation	
24				
25	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals,	18
26			the public, and other relevant groups (eg, via publication, reporting in results databases, or other data	
27			sharing arrangements), including any publication restrictions	
28				
29		31b	Authorship eligibility guidelines and any intended use of professional writers	_____
30				
31		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
32				
33	Appendices			
34				
35	Informed consent	32	Model consent form and other related documentation given to participants and authorised surrogates	22
36	materials			
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38	Biological	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular	Not relevant
39	specimens		analysis in the current trial and for future use in ancillary studies, if applicable	
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for an 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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-019210.R1
Article Type:	Protocol
Date Submitted by the Author:	12-Oct-2017
Complete List of Authors:	Gade, Josephine; Herlev and Gentofte University Hospital, Dietetics and Clinical Nutrition Research Unit Beck, AM; Herlev Hospital, Bitz, Christian; Bispebjerg and Frederiksberg Hospital, Kitchen Unit Christensen, Britt; Arla Foods amla Klausen, Tobias; Herlev and Gentofte University Hospital, Department of Haematology Vinther, Anders; Herlev and Gentofte University Hospital, Department of Rehabilitation Astrup, Arne; University of Copenhagen, Department of Nutrition, Exercise and Sports,
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Nutrition and metabolism, Evidence based practice
Keywords:	GERIATRIC MEDICINE, NUTRITION & DIETETICS, REHABILITATION MEDICINE

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Manuscripts

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’

Authors: Josephine Gade^{1,6}, Anne Marie Beck¹, Christian Bitz², Britt Christensen³, Tobias Wrenfeldt Klausen⁴, Anders Vinther⁵, Arne Astrup^{1,6}

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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

23 ABSTRACT

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

31 **Methods and analysis:** In a block-randomised, double-blind, multicentre intervention study, 165
32 older adults above 70 years, fulfilling the eligibility criteria, will be included consecutively from
33 three Medical Departments (blocks of n=20, stratified by recruitment site). After inclusion,
34 participants will be randomly allocated (1:1) to receive either ready-to-drink, protein-enriched,
35 milk-based supplements (a total of 27.5 g whey protein/day) or iso-energetic placebo products (<1.5
36 g protein/day), twice daily as a supplement to their habitual diet. Both groups will be offered a
37 standardized RT program for lower extremity muscle strength (daily while hospitalized and
38 4x/week after discharge). The study period starts during their hospital stay and continues 12 weeks
39 after discharge. The primary endpoint is lower extremity muscle strength and function (30-s chair-
40 stand-test). Secondary endpoints include muscle mass, measures of physical function, and measures
41 related to cost-effectiveness.

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

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

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1
2 49 **Strengths and limitations of this study:**
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- 4 50 • To our knowledge this is the first study to investigate the effect of protein supplementation
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6 51 in addition to RT among acutely ill geriatric patients, while admitted and after discharge,
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8 52 and it adds new information to the evidence-based health care.
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10 53 • The study is randomized and double-blinded which minimizes the risk of selection,
11
12 54 performance, and detection bias, and the multi-center trial design increases the
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14 55 generalizability of the results.
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16 56 • The lack of supervised RT after discharge might lower compliance to the RT, although it is
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18 57 more realistic that self-training at home can be implemented in a real world setting.
19
20 58 • Acutely ill older adults are a difficult population to maintain in a long duration intervention
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22 59 study, which increases the risk of drop outs and/or low compliance.
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26 60 • Registration of compliance in dietary studies is always associated with a risk of bias, but by
27
28 61 asking the participants to register their daily intake, save empty bottles, and by calling them
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30 62 on a weekly basis to check on compliance, this is minimized.
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63 INTRODUCTION

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

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

1 89 investigated this, and in addition the intervention will continue after discharge from the hospital.

2 90 The novelty of this study is two-fold. Firstly the intervention involves hospitalized older adults, and
3
4 91 secondly the intervention continues after discharge. To the best of the authors' knowledge, previous
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6 92 studies were only performed in one setting.
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10 93 **METHODS AND ANALYSIS**

11 94 **Study design**

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14 95 The study design is a block-randomised, double-blind, placebo-controlled, multicentre intervention
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16 96 study. A total of 165 participants will be included consecutively from the Medical Departments of
17
18 97 three Hospitals in the Capital Region of Denmark (Gentofte and Herlev University Hospital and
19
20 98 Rigshospitalet-Glostrup, n=55 from each place). Recruitment takes place a maximum of 72 hours
21
22 99 after admission. After inclusion, participants will be randomly allocated (1:1) to receive either
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24 100 protein-enriched milk-based supplements (whey protein) or an iso-energetic placebo product, as a
25
26 101 supplement to their habitual diet. Both groups follow the same RT program and are daily
27
28 102 supplemented with vitamin D. The intervention starts at the hospital while admitted and continues
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30 103 12 weeks after discharge. Recruitment and data collection started in April 2016, and will end in
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32 104 June 2018.
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36 105 **Study population**

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38 106 Inclusion criteria for participation are; men and women aged ≥ 70 years, able to speak and
39
40 107 understand Danish, expected length of stay > 3 days (evaluated by medical staff at department),
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42 108 ability to stand independently for at least 30 seconds, and admission to the medical departments of
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44 109 Gentofte Hospital, Herlev Hospital or Rigshospitalet-Glostrup. Exclusion criteria are: active cancer,
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46 110 renal insufficiency (eGFR < 30 mL/min/1.73m²), cognitive impairment (not able to comprehend the
47
48 111 purpose of the study/give informed consent), terminal disease, exclusively receiving enteral or
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50 112 parenteral nutrition, milk/lactose allergy or intolerance, planning to lose weight/go on a special diet,
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52 113 planned transfer to other hospitals/departments and pacemaker/other implanted electrical stimulants
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54 114 (due to Bio-Impedance Analysis (BIA) measurements). Participants will be withdrawn from the
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1 115 study if they die during admission (does not apply to subsequent admissions) or are
2
3 116 discharged/transferred from the medical department before the intervention has started.

6 117 **Randomization and blinding**

8 118 After collection of baseline measurements and characteristics, participants are randomized to either
9
10 119 the intervention or the control group using sealed, opaque envelopes containing a paper with either
11
12 120 an 'A' or a 'B'. Each Hospital site has its own pile of envelopes in order to allow for block-
13
14 121 randomization. Within each site, 10 A's and 10 B's (20 in total) are put in the pile over three
15
16 122 rounds, to ensure a more even allocation of participants in the two groups at any time. Participants,
17
18 123 hospital staff, and study investigators will all be blinded towards the randomization. If a situation
19
20 124 arises where unblinding may be considered for the benefit of the participant, this will be decided on
21
22 125 an individual basis taking the specific situation into account. Enrollment and randomization is
23
24 126 performed by study investigators.

27 127 **Intervention**

29 128 *Protein-enriched, milk-based supplements and Placebo*

31 129 Depending on their allocation, participants will receive either a protein-enriched, milk-based
32
33 130 supplement beverage (Arla Foods®: 781 kJ, 10.5 g whey protein concentrate and 0.5 g casein, 10 g
34
35 131 fat, and 13 g carbohydrate per 100 ml) (intervention group) or an iso-energetic placebo beverage
36
37 132 (Arla Foods®: 797 kJ, 0.6 g protein, 10 g fat, and 24 g carbohydrate per 100 ml) (control group).
38
39 133 The amino acid profile of the intervention product is shown in the supplemental material, table 1.
40
41 134 Both products have a flavour of raspberry and come in ready-to-drink preparations. From January
42
43 135 2017 and on, the protein-enriched milk-based supplement will have vitamin D added in amounts of
44
45 136 1.125 µg per 100 ml. During the whole study period (while hospitalized and 12 weeks post
46
47 137 discharge) the participants will be instructed to drink a total of 250 ml per day, divided into two
48
49 138 servings of 125 ml. Thus, the intervention group will get a total of 27.5 g extra protein per day,
50
51 139 equal to 26.25 g whey protein containing a total of ~ 2.5 g leucine. This amount of protein
52
53 140 supplementation is chosen, based on previous studies finding positive effects from similar or
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1
2 141 smaller dosages [17-19]. Furthermore, protein supplementation is satiating, and if given in higher
3
4 142 amounts might compromise habitual food intake to a great extent – especially among older adults
5
6 143 with low appetite. The total dosage is divided into two servings (breakfast and next cold main
7
8 144 meal), as research indicate that 25-30 grams of high quality protein is needed per main meal to
9
10 145 maximally stimulate post prandial protein synthesis [8]. The beverages come in white bottles with
11
12 146 either a ‘group A’ or ‘group B’ label on. While hospitalized, the timing of the intake is as follows;
13
14 147 one serving at breakfast (or at lunch, if not consumed at breakfast for any reasons, e.g. fasting
15
16 148 necessary, or if the RT is performed right after breakfast) and one serving directly after the RT. In
17
18 149 the 12 weeks after discharge the participants will be instructed to drink one serving at breakfast and
19
20 150 one serving with the next cold main meal, irrespective of the meal is eaten at lunch or at dinner
21
22 151 time. If the participants forget to drink the beverages at the specific times, they will be told to drink
23
24 152 it when they become aware of it. The participants will not be instructed to make other dietary
25
26 153 changes during the study period. If participants are prescribed/recommended by hospital staff to
27
28 154 take oral nutritional supplements, this is not an exclusion criterion, but participants will be
29
30 155 instructed to take any additional supplements on a given day only after intake of the ‘study
31
32 156 beverages’. If for some reason (e.g. uncontrolled diabetes or severe reduction of habitual food
33
34 157 intake), the participant is advised by medical doctors’/nutritional therapists’ to stop taking the
35
36 158 supplement, this advice will always be followed.

37 159 *Vitamin D supplements*

38
39 160 Vitamin D supplementation has been shown to have an independent effect on muscle [20]. To
40
41 161 reduce the potential confounder of a large difference in intake of vitamin D between groups, all
42
43 162 participants will get vitamin D supplements handed out after enrolment, and be instructed to take a
44
45 163 supplement of 20 µg/day (two tablets of 10 µg), as recommended by the Danish National Board of
46
47 164 Health [21]. Exceptions to this are those participants whose serum-vitamin D levels have been
48
49 165 measured to ≥ 100 nmol/L at the time of study inclusion to avoid reaching toxic levels. The
50
51 166 participants have to register their intake of vitamin D in a diary along with their intake of the
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1 167 intervention products. Also, at the last visit in study week 12, the number of tablets left in the
2
3 168 container will be counted to verify the registrations. If participants already take vitamin D
4
5 169 supplements in combination tablets with other vitamins and/or minerals corresponding to 20 µg/day
6
7
8 170 or more, they will be instructed to keep taking their own tablets and register this. The exact amount
9
10 171 of vitamin D in these tablets will be recorded. An average intake of vitamin D per day during the
11
12 172 intervention period will be used to compare if the intake of vitamin D is different between the two
13
14 173 groups.

174 *Resistance exercise training (RT)*

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

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

1 193 as self-training four times per week. They will be instructed to have at least 24 hours between
2
3
4 194 training sessions. During the hospital stay it is expected that the participants have a very limited
5
6 195 amount of physical activity besides the RT program offered, and that the intensity by which they
7
8 196 can perform the RT is rather low. This is why the frequency of the RT differs between the hospital
9
10 197 and discharge setting. To instruct the participants in regard to the RT, and to ensure progression (or
11
12 198 regression if necessary), they receive follow-up home visits by a physiotherapist in study week 1, 3,
13
14 199 6, 9, and after discharge from any readmissions. The adjustments are made after standardized
15
16 200 procedures.

17
18 201 Participants who are discharged with a plan of rehabilitation including ambulatory training at a
19
20 202 center or supervised training at home, to be provided by their municipality, will be asked to perform
21
22 203 the full RT study program until their rehabilitation program starts up (a wait of 2-6 weeks are
23
24 204 normal). Each training session performed as part of a rehabilitation program will replace *one* self-
25
26 205 training session of the RT study program. The same applies, if participants are discharged from the
27
28 206 hospital directly to a 24-h rehabilitation center and they are performing RT in their regimen. This is
29
30 207 to allow for proper restitution. The offer of supervised training applies only to the first hospital stay,
31
32 208 but if readmitted to hospital participants will be encouraged to do the RT themselves to the extent
33
34 209 possible.

38 210 **Compliance**

39
40 211 While hospitalized, the participants will get the product handed out along with the vitamin D
41
42 212 supplements. Investigators and physiotherapists register overall study compliance, that is daily
43
44 213 ingestion of the intervention or placebo supplements (time for handout and amount ingested),
45
46 214 vitamin D (dose, yes/no), and performance of the RT (number of sets and repetitions for each
47
48 215 exercise). Empty bottles are saved so that study investigators can verify the amount of intervention
49
50 216 product consumed.

51
52
53 217 After discharge, the amount of intervention or placebo supplement consumed and the RT performed
54
55 218 for each participant will be assessed by daily records in a 'beverage and exercise diary', specifically

1
2 219 designed for the study and handed out to be filled in by the participants. The participants, e.g. with
3
4 220 help from their relatives, are asked to daily register the amount of beverage consumed; 0 %, 25 %, 25
5
6 221 50 %, 75 %, or 100 % of each of the two servings by ticking of the corresponding circular
7
8 222 illustration, along with ticking of the intake of vitamin D. Participants also have to register
9
10 223 execution of the RT, and specify for each of the three exercises the number of sets and repetitions
11
12 224 performed. If they are exercise training at a rehabilitation center this can be registered in the
13
14 225 relevant boxes. In case of deviations, four pre-specified explanations are given that they can tick
15
16 226 off, both in regard to the intake of supplements and the execution of the RT. To verify the
17
18 227 participants' records they are asked to save and store empty bottles, which will be picked up by
19
20 228 investigators on days with home-visits. At the same time study investigators will help the
21
22 229 participants' to retrospectively fill out any missing registrations. Participants who are discharged to
23
24 230 a 24-hour rehabilitation centre will get the intervention products handed out by the staff, who will
25
26 231 also save empty bottles. On the first visit after discharge the participants will receive thorough
27
28 232 instructions on how to register compliance in the 'beverage and exercise diary', and upcoming visits
29
30 233 will be planned. Both groups will receive daily standard messages on their cell phone (if they have
31
32 234 one and agrees to this) and weekly phone calls, kindly reminding them to consume the supplement
33
34 235 and vitamin D, perform the RT, and register compliance. Furthermore, as part of the phone call,
35
36 236 they will be asked about compliance and any deviations or e.g. upstart of training at a rehabilitation
37
38 237 center will be registered and validated/compared later on with their own diaries, and they will be
39
40 238 reminded of upcoming home visits.

41 239 **Outcome parameters**

42 240 The baseline characteristics will be collected at inclusion to the study. To standardize the endpoint
43
44 241 measures, especially that of LBM, these will be assessed 1.5-2 hours after a light breakfast. Thus, if
45
46 242 inclusion happens in the afternoon, then baseline measurements will be assessed the following day,
47
48 243 prior to any study interventions. The measurements will be assessed in a predefined order to reduce
49
50 244 fatigue and follow standardized procedures, and they will be repeated within 72 hours after
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245 discharge and 12 weeks (± 2 days) after discharge. If possible, before each endpoint examination
 246 the participants will be asked to consume a breakfast, similar to that consumed at the hospital before
 247 the baseline measurements. The assessments after discharge will be performed in the participants
 248 own home. Follow-up assessments, including only admission to hospital and mortality, will be
 249 assessed six months after the intervention period. In general, if participants are readmitted to
 250 hospital, if possible, assessments will be performed there and otherwise at a replacement visit after
 251 discharge. All data collection is performed by study investigators. Table 1 gives an overview of the
 252 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.

253

254 Primary endpoint

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

1
2 257 number of times the participant can rise-and-sit from a standard chair (height of 43-45 cm) in 30
3
4 258 seconds with the arms folded across the chest, starting from a sitting position. Only full stands will
5
6 259 count – i.e. full extension of the knees and hips. Those who cannot stand from the chair without
7
8 260 using the arm rest will get a score of 0 [23]. In the modified 30-s CST the participant is allowed to
9
10 261 use the arm rests [24]. If participants are only able to perform the modified version at baseline, for
11
12 262 the following assessments they will be asked to do the same. If they are able to do the standardized
13
14 263 version they will be asked to do that as well after a 15 minutes rest. A change of 2.0-2.6 stands is
15
16 264 considered to be clinically relevant based on data from a population of older adults with hip and
17
18 265 knee osteoarthritis [25].

21 266 **Secondary endpoints**

23 267 *Total, appendicular, and trunk LBM (kg and percent)* is assessed by Bio-impedance Analysis (BIA)
24
25 268 using the portable InBody-230 body composition analyzer (dual frequency (20 kHz, 100 kHz), tetra
26
27 269 polar 8-Point Tactile Electrode System (InBody, Copenhagen, Denmark)). Direct segmental
28
29 270 measurement technology is used, meaning that no calculations, and thus empirical factors and
30
31 271 imputations, are needed. Various factors can affect BIA measurements such as previous exercise,
32
33 272 body position, skin temperature, dietary intake, and hydration state [26]. Thus, in order to
34
35 273 standardize the measurements these will be performed in the morning 1.5-2 hours after a light
36
37 274 breakfast and bladder emptying (preferably also bowel emptying), and before any exercise.
38
39 275 Participants will be asked to wear light clothes and no shoes. They will be instructed to stand
40
41 276 upright with the feet on the build-in electrodes embedded in the scale platform, grasp the handles of
42
43 277 the analyzer while spreading the arms as much as they can, and look straight ahead. The reliability
44
45 278 of the InBody-230 body composition analyzer will be measured and used to establish the threshold
46
47 279 of change needed beyond measurement error.

51 280 *Hand grip strength* (HGS) is a proxy measure of upper extremity strength, and is measured in kg
52
53 281 using the second handle position with a DHD-1 Digital Hand Dynamometer (Saehan Medical,
54
55 282 2012, Roskilde Denmark). The second handle position is recommended as a standard position, as it

1
2 283 is suitable for most hand sizes. An investigator will instruct the participants to be seated with their
3
4 284 feet on the ground, shoulders adducted and neutrally rotated, elbow flexed at a 90° angle and
5
6 285 supported on the armrests of the chair or a table, and forearm and wrist in neutral position, as
7
8 286 recommended by Roberts et al. (2011) [27]. They will be asked to perform three maximum force
9
10 287 trials with their dominant hand, and the highest value will be registered. They will be instructed to
11
12 288 squeeze the handle as hard as they can for 5 seconds, and the test will be repeated within 15
13
14 289 seconds.

15
16 290 *4-meter gait speed* (4-m GS) is used to assess the usual gait speed (m/s) over a short distance.
17
18 291 Participants will be placed behind a starting line and instructed to start walking at their usual pace
19
20 292 after the investigators command. To reduce the effect of acceleration and deceleration, each
21
22 293 participant will be instructed to walk towards a visual goal for 5 meters. The time will be started
23
24 294 after the participant has walked 0.5 meter and stopped after 4.5 meters, counted from the first foot-
25
26 295 step that crosses the 4-m start line and end line, respectively. The fastest of two attempts is
27
28 296 recorded. If it is not possible to establish a 5 m test track, a shorter track with a minimum length of
29
30 297 3.5 m in total will be used instead, and this will be registered as bias [28,29]. The participants are
31
32 298 allowed to use a gait aid, which will be registered as well. In sedentary older adults, a clinical
33
34 299 relevant difference is found to be 0.03-0.05 m/s, while 0.08 m/s is found to be a substantial relevant
35
36 300 difference [30].

37
38 301 *Functional ability* is measured using the modified Barthel Index (Barthel-100) [31,32]. The Barthel-
39
40 302 100 contains 10 measures of every-day and mobility activities, and the ability to master these
41
42 303 activities reflects the level of functioning. Each measure has five levels of functioning, and for all
43
44 304 10 measures a maximum of 100 points can be achieved, corresponding to fully independent. The
45
46 305 Barthel-100 will be scored by the investigators, and rated based on the amount of assistance
47
48 306 required to complete each activity or by observing, and clarifying questions will be asked when
49
50 307 necessary.
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1
2 308 *Mobility* is assessed by De Morton Mobility Index (DEMMI), which provides a 15-item
3
4 309 unidimensional measure of mobility across the spectrum from bed bound to independent
5
6 310 mobility, specifically developed for geriatric patients [33]. It has 5 categories in which the
7
8 311 participants are tested; bed (3 test scores), chair (3 test scores), static balance (4 test scores),
9
10 312 walking (2 test scores), and dynamic balance (3 test scores). A total test score from 0-19 can be
11
12 313 achieved, and this raw score is converted to an interval DEMMI score from 0-100, where 100 is
13
14 314 represents independent mobility. In older acute medical patients, the clinical relevant difference is
15
16 315 found to be 10 points on the converted scale [33].

17
18 316 *Cognitive function* is measured using the Mini Mental State Examination (MMSE), which consists
19
20 317 of small simple tasks to elucidate eight different cognitive functions; orientation, episodic memory,
21
22 318 concentration, function of language, practical exercise, reading skills, writing skills, and visual-
23
24 319 spatial construction. The performances are scored to give a raw score ranging from 0-30, where 30
25
26 320 represent the best/optimal function [34].

27
28 321 *Social support* is evaluated using registrations of home care (yes/no, if yes, then divided into
29
30 322 practical help, personal care, and both) and residence (own home, nursing home/assisted living
31
32 323 facility, 24-hour rehabilitation facility).

33
34 324 *Use of gait aid* is registered as yes (incl. specific gait aid), no, or cannot walk.

35
36 325 *Length of hospital stay* (LOS) corresponds to the in-hospital intervention period (days from
37
38 326 recruitment until discharge) which is registered from the electronic patient register.

39
40 327 *Readmission to hospital and mortality*. Readmission to hospital is registered both with regard to
41
42 328 frequency and the total LOS, from the electronic patient register. These data are summed up after
43
44 329 the intervention period and after the follow-up period, respectively.

45
46 330 *Health related Quality of life* (QOL) is assessed by using the generic questionnaire, Euroqol EQ-
47
48 331 5D-3L [35]. The questionnaire is self-reported, and reflects the participant's current situation.
49
50 332 Scores for the EQ-5D-3L are generated from the ability of the individual to function in five
51
52 333 dimensions; mobility, pain/discomfort, self-care, anxiety/depression, and usual activities. Each
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1
2 334 dimension has three possible answers; no problem, some problems, and major problems. Also, the
3
4 335 participants rate their current health state on a visual-analogue-scale ranging from 0-100 (reflecting
5
6 336 a health state from 'worst' to 'best').

7
8 337 *Body weight* is measured to the nearest 0.1 kg using the BIA equipment InBody-230, and follows
9
10 338 the same standardized procedures as described under the endpoint 'muscle mass'.

11
12 339 *Product-evaluation-questionnaire*. Both the intervention and placebo product is evaluated using a
13
14 340 self-report questionnaire. The evaluation questionnaire concerns overall liking, side effects related
15
16 341 to consumption, taste fatigue, texture, dosage, and manageability.

17 342 **Control for confounders - other registrations and precautions**

18
19 343 Actions are taken to actively reduce or register known or possible confounders. Thus, at baseline,
20
21 344 confounders such as admission diagnosis, chronic diseases, nutritional risk (NRS 2002) [36],
22
23 345 sarcopenia [3,37], depression [38], and mobility [39,40] are evaluated, among other. Nutritional risk
24
25 346 is determined based on a combination of factors: unintended weight loss within the last three
26
27 347 months, loss of appetite within the last week, body mass index, disease severity, and age. Patients
28
29 348 screened to be at risk are expected to benefit from nutritional intervention. Sarcopenia is assessed
30
31 349 according to the definition proposed by the European Working Group on Sarcopenia in Older
32
33 350 People (EWGSOP). This is based on the assessments of LBM (measured by BIA), muscle strength
34
35 351 (measured HGS), and physical performance (measured by 4-m gait speed). Furthermore, besides
36
37 352 register vitamin D intakes, throughout the study the following two measures are collected on an
38
39 353 ongoing basis.

40
41 354 *Protein and energy intake*. During hospitalization the participants' protein (g/kg) and energy (kJ/kg)
42
43 355 intake will be registered for four days, or shorter if the participants' are discharged. The hospitals'
44
45 356 food and drink registration schemes will be used. Participants will be asked to fill in the food
46
47 357 registration schemes themselves with help from the nurses and study investigators. The participant's
48
49 358 body weight at inclusion will be used to calculate the intake per kg body weight. During the 12-
50
51 359 week post-hospital intervention the participants protein and energy intake will be estimated based
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1
2 360 on the average of four 24-hour dietary-recall interviews performed at study week 3, 6, 9, and 12 at
3
4 361 home visits, or by phone if the participant are no longer compliant in the study with regard to the
5
6 362 intervention products and the RT. As the home visits will be planned in collaboration with the
7
8 363 participants, and has to be fitted into other study tasks and visits, these practicalities decide what
9
10 364 day of the week the recall interview is covering. To minimize the risk of recall bias a checklist of
11
12 365 specific foods and beverages will be used to verify the reported intake. Furthermore, when
13
14 366 interviewing face to face, picture series of portion sizes of different foods will be used to estimate
15
16 367 the amounts ingested [41]. The foods and drinks will be entered in the software program Madlog
17
18 368 Vita® to calculate the intake of protein (g) and energy (kJ). Four days of registration/dietary recalls
19
20 369 are considered adequate to assess this information with a high correlation [42]. An average of the
21
22 370 participant's body weight after discharge and in week 12 will be used to calculate the intake per kg
23
24 371 body weight. The cut-off for suspecting underreporting will be evaluated retrospectively on an
25
26 372 individual basis taking any illness, readmissions, loss of body weight, activity level etc. into
27
28 373 account.

31
32 374 *Daily activity level.* In a semi-structured interview the participants are asked about exercise-related
33
34 375 activities besides the RT program. This is reported four times after discharge in study week 3, 6, 9,
35
36 376 and 12 at home visits, or by phone if the participant' is no longer compliant in the study with regard
37
38 377 to the intervention products and the RT. Depending on the answers given, the participants will be
39
40 378 divided into activity levels from 1-5 after predefined criteria, inspired by Saltin & Grimby (1968)
41
42 379 [43]. The scale is ordinal, and activity level 1 represents the least active and level 5 the most active.
43
44 380 It is the time used on different activities and the intensities of these (low, moderate, or high) that
45
46 381 determine the activity level.

49 382 **Statistics**

51 383 *Power calculation*

53 384 The primary endpoint is muscle strength measured by the 30-s CST. The clinical relevant difference
54
55 385 for this test is found to be 2.0-2.6, when assessed in older populations with hip and knee
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1
2 386 osteoarthritis [25]. Jones et al. (1999) has used the standardized 30-s CST on community-dwelling
3
4 387 older people and found a SD of 3.0 and 3.6 for people in the age range of 70-79 and 80-89,
5
6 388 respectively [23]. This gives a pooled SD of 3.31, which is used in this power calculation, and it
7
8 389 corresponds well with measures of SD found in the modified test version [44].
9
10 390 In order to be able to detect a difference of 2.0, with a power of 80 % and a two-sided alpha-error of
11
12 391 0.05, the required sample size is 80 participants in each group, given an anticipated combined rate
13
14 392 of drop-outs and non-compliance of 45 %. This rate is chosen since studies with resistance training
15
16 393 in older people both while hospitalized [45] and in a community-dwelling setting [46], have
17
18 394 experienced drop-outs of 30 %. Moreover, an additional 15 % is added to account for participants
19
20 395 with a low compliance to the intervention, to be able to maintain the statistical power of the study in
21
22 396 the intention-to-treat analysis as well as in the per protocol analysis. For practical reasons, if
23
24 397 possible within the time schedule, 55 participants will be included at each of the three sites,
25
26 398 resulting in a total inclusion of 165 participants.
27
28

29 399 *Feasibility of recruitment and sample size*

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31
32 400 The three hospitals where recruitment is going to take place had between 525-687 geriatric patients
33
34 401 in year 2014, with a median LOS ranging from 8-11 (5-16) days. The median age for women was in
35
36 402 the range of 84-87 years and 83-84 years for men [47]. To meet the timetable the expected
37
38 403 recruitment rate is a minimum of two participants per week which based on these data is considered
39
40 404 realistic.
41

42 405 *Statistical tests*

43
44
45 406 The primary analysis will be performed by the intention-to-treat principle. In addition, a predefined
46
47 407 per-protocol analysis will be performed including participants with a high compliance only
48
49 408 (consumption of the intervention product ≥ 75 %). Furthermore, endpoints will be compared
50
51 409 adjusting for randomization bias (defined as $p < 0.05$ between groups), and confounding factors
52
53 410 (total activity level and total protein- and energy intake). Analysis will be done both with and
54
55 411 without imputation techniques for missing values, but drop-outs will be encouraged to participate in
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1
2 412 follow-up examinations, including interviews concerning dietary intake and activity level.
3
4 413 Sensitivity analysis will be performed without outliers, defined as a value of 3 SD above or below
5
6 414 the mean. To investigate whether the intervention will have different impacts in different groups of
7
8 415 patients, e.g. those who are at nutritional risk or sarcopenic, subgroup analysis will be performed
9
10 416 looking at treatment effect in the subgroups and interactions between treatment effect and
11
12 417 subgroups. Furthermore, observational analysis will be performed, investigating the importance of
13
14 418 total protein- and energy intake and total activity level on outcome measures. The two groups will
15
16 419 be compared looking at the hospitalization intervention period and the 12 week post discharge
17
18 420 intervention period both separately and as a whole.

19 421 Results will be presented as median (range) or mean (SD or 95 % CI) and number (absolute
20
21 422 frequencies) for continuous and categorical variables, respectively. Inspection for normality will be
22
23 423 done by visual inspection (QQ-plot), and parametric or nonparametric statistical tests will be used
24
25 424 in accordance with the distribution of the variables. Statistical comparisons will be made between
26
27 425 the two groups by using the Mann-Whitney U-test or Students t-test for continuous variables, and
28
29 426 the Chi-square test (X^2) or Fisher's Exact Test (in case of expected cell count < 5) for the
30
31 427 comparison of categorical variables. ANCOVA will be used for continuous outcomes and binary
32
33 428 logistic regression for binary outcomes if/when adjusting for confounders and testing for subgroup
34
35 429 interaction. The Spearman-Rank Correlation Test or General Linear Model will be used to test for
36
37 430 correlations between independent variables. All tests are two-tailed and an alpha-level of $P < 0.05$
38
39 431 will be used to determine statistical significance in all analyses.

40
41 432 With regard to the primary endpoint, 30-s CST, the changes in performance from baseline (both
42
43 433 with and without pooling standardized and modified test results) will be measured and compared
44
45 434 between the two groups. Furthermore, performance will be scored into one of three categories; 1.
46
47 435 ability to rise from the chair with arms folded across the chest, 2. ability to rise from the chair using
48
49 436 the arm rest, and 3. not able to rise independently from the chair. Also, compared to baseline,
50
51 437 performance will be scored into either 'better', 'worse' or 'unchanged'.

438 ETICHS AND DISSEMINATION

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

451 DISCUSSION

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

1
2 464 although not examined. A common confounder is that people agreeing to participate in an
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4 465 intervention trial are more motivated to lifestyle changes, which is an important factor for the
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6 466 compliance and possible success of this intervention.
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8 467 Use of placebo beverages allows blinding of participants and researchers. Thus, performance and
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10 468 detection bias are minimized. Another strength is the randomization procedure, which will limit
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12 469 selection bias and hopefully balance different confounders which could potentially influence the
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14 470 results. The multi-center trial design furthermore increases the generalizability of the results. The
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16 471 activity and dietary interviews are conducted in order to be able to correct statistically for
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18 472 differences in protein intake and activity levels between groups. In addition, it will also enable us to
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20 473 investigate the importance of overall protein and energy intake on the results.
21
22

23 474 The majority of older adults in Denmark take vitamin D supplements as recommended by the
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25 475 Danish Health Authority [21]. Studies have shown that vitamin D has an independent positive effect
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27 476 on muscle strength [48]. In order to investigate the effect of the protein supplementation alone,
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29 477 vitamin D supplements will be given to all participants with serum-vitamin D levels ≤ 100 nmol/L
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31 478 at inclusion, to ensure similar vitamin D intakes. Another reason for ensuring that all participants
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33 479 are supplemented with vitamin D is that the protein-enriched beverage approximately half-way
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35 480 through the intervention period will have vitamin D added to the product. However, the fortification
36
37 481 level is quite low, adding an extra amount of only 3.5 μg vitamin D per day from the beverages,
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39 482 which e.g. corresponds to 13 g of salmon [49]. Also, compared to the daily vitamin D
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41 483 supplementation of minimum 20 μg (some older adults' takes even higher amounts, as prescribed
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43 484 by their doctor) it is considered insignificant.
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46
47 485 In regard to ensure compliance to the RT program, it is a weakness of the study that the RT at home
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49 486 after discharge is not supervised. On the other hand, an aim of the current study is to test the effect
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51 487 of an interdisciplinary rehabilitation regime that is cost-effective and could easily be implemented.
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53 488 Supervised RT four times per week would have required a lot of resources, which most likely
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55 489 would not be possible to implement in the real world. If a positive effect is found of an intervention
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1
2 490 only consisting of extra protein and self-training after discharge, potential implementation in
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4 491 clinical practice will be more feasible. The current study can also give valuable insights into which
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6 492 sub groups of the geriatric patients that would be able to benefit from a rehabilitation regime based
7
8 493 on self-training and protein intervention. The high rate of readmissions to hospital among older
9
10 494 adults [50] indicates that there is room for improvement in regard to secondary prevention
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12 495 strategies.

13
14 496 The specific endpoints included in the current study were chosen in order to be suitable, feasible
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16 497 and valid for this specific population of older adults. Thus, a low amount of missing data is
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18 498 expected due to low feasibility. The 30-s CST, DEMMI, and Barthel-100 are part of the normal
19
20 499 routine tests for geriatric patients admitted to the medical departments (they are included in The
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22 500 Danish National Geriatric Data Base), and all tests and questionnaires are developed and/or
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24 501 validated in older adults [28,32-34]. Furthermore, the Danish Board of Health recommends the use
25
26 502 of 30-s CST, 4-m GS, MMSE, and EQ-5D-3L as tests in older geriatric patients [29]. Also, LBM
27
28 503 measured by BIA, has been proposed as a feasible measurement tool in this population [3,51], and a
29
30 504 portable BIA is a practical tool suitable for home visits.

31
32 505 Specifically for the primary endpoint, the 30-s CST has been shown to be a reliable and valid
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34 506 indicator of lower body strength in generally active, community-dwelling older adults, when
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36 507 validated against maximum weight-adjusted leg-press performance [23]. The Standardized 30-s
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38 508 CST version has been shown to have low feasibility (54 %) in acutely admitted old medical
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40 509 patients, and to have lower inter-rater reliability than in medically stable patients. However, the
41
42 510 Modified 30-s CST has been shown to be both feasible and having a high inter-rater reliability [28].
43
44 511 Thus, we believe that all participants will be able to perform either the standardized or the modified
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46 512 version, supported by the inclusion criteria, that only patients who can stand independently are
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48 513 recruited, eliminating those in poorest conditions. This is also in accordance with experience from
49
50 514 our former intervention studies performed in geriatric patients [52,53], and also applies to the other
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52 515 secondary endpoints.
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1
2 516 For the secondary endpoint, LBM measured by a portable BIA, Moon *et al.* (2013) have shown that
3
4 517 single frequency BIA in elderly men and women (72 men and women, > 65 years) correlate well
5
6 518 with Dual Energy X-ray Absorptiometry (DXA) measurements, as well as the 4-compartment
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8 519 model, at single time points as well as for tracking changes in LBM. They concluded that DXA and
9
10 520 BIA can be used interchangeably as valid methods to measure LBM when looking at a population
11
12 521 basis of more than 15-22 people [51]. Furthermore, Karelis *et al.* (2013) has validated the portable,
13
14 522 dual-frequency InBody-230 BIA against DXA in a healthy mixed population (145 men and women,
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16 523 44.6±20 years) and found a significant high correlation when looking at fat mass, percent body fat,
17
18 524 and total LBM [54]. Thus, it is expected that using the InBody-230 BIA equipment, besides being
19
20 525 practical in regard to home visits, will be a reasonable valid method to assess total muscle mass in a
21
22 526 population of 165 older adults.

25 527 **DECLARATIONS**

28 528 **Authors' contributions**

29
30 529 AMB prepared the grant application. AMB and JG conceived the overall study draft, and JG
31
32 530 created the detailed study protocol. AA, AV, BC, TWK, and CB participated in its design and
33
34 531 coordination. JG and research assistants collect the data under the supervision of AA, AV, AMB,
35
36 532 BC and CB. JG drafted the manuscript. All authors reviewed the article critically and contributed
37
38 533 significantly to the final content. All authors have read and approved the final manuscript.

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42
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44
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46
47 537 Research Foundation, Arla Foods Amba and Arla Foods Ingredients, and Copenhagen University,
48
49 538 faculty of Nutrition, Exercise and Sports. Representatives from Arla Food have been involved in the
50
51 539 study design, but will not be involved in collection, analysis and interpretation of the data. The
52
53 540 Danish Dairy Foundation will not be involved in the conduction of the study or interpretation of

1 541 results. A Scientist from Copenhagen University have been involved with the study design, and will
2
3
4 542 be involved in all steps from analysis and interpretation to publication of the results.

5 543 **Competing interests**

6
7
8 544 None of the authors have financial or personal conflicting interests. The sponsor (Danish Dairy
9
10 545 Research Foundation) and the producer of the intervention and placebo products (Arla Foods) will
11
12 546 not have any influence on the analysis and interpretation of the results.

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14
15
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17
18 549 well as those who participated in the planning of study practicalities. A special thanks to Maria
19
20 550 Aagensen who participated in developing the standardized resistance training program.

21 551 **List of abbreviations**

22
23
24 552 BIA, Bio-Impedance Analysis; DEMMI, De Morton Mobility Index; DXA, Dual-energy X-ray
25
26 553 Absorptiometry; LBM, Lean Body Mass; MPS, Muscle Protein Synthesis; NRS 2002, Nutritional
27
28 554 Risk Screening 2002; RT, Resistance exercise Training; 30-s CST, 30-second chair-stand-test.

29 555 **Consent for publication**

30
31
32 556 The model in Figure 1. '*Standardized Resistance training program*' (supplemental material) has
33
34 557 given written consent to publish the pictures.

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702 **Tables and Figure legends**

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

704 **Supplemental material**

705 Figure 1. Standardized resistance training program

706 Table 1. Amino acid profile of the intervention product

707

For peer review only

Supplemental material











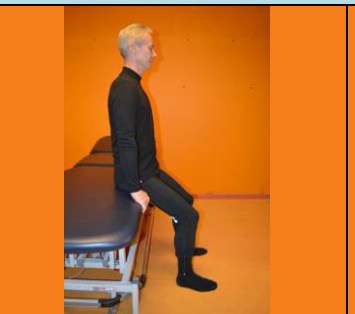
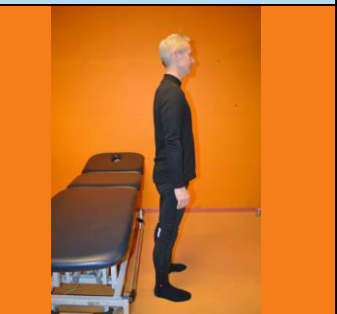
Table 1. Amino acid profile of the intervention product











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

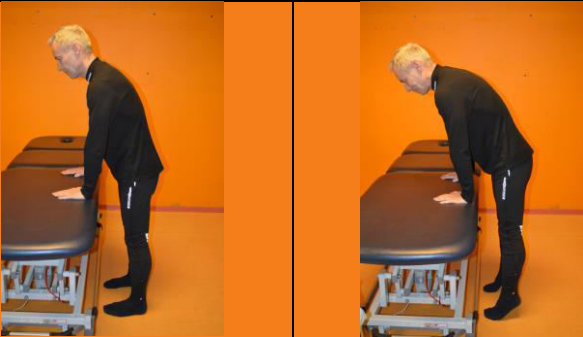







Particulated whey protein concentrate, name: Nutrilac YO-8078.
Analysis performed by Eurofins Steins Laboratorium A/S, DK.

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.

<p>B</p>			<p>Sitting on a chair with armrest. Feet hip-width apart. Stand up using the arms and arm rests to push off.</p>
<p>C</p>			<p>Sitting on a chair. Feet hip-width apart. Stand up from chair with arms crossed.</p>
<p>D</p>			<p>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.</p>
<p>E</p>			<p>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.</p>
<p>Level of resistance</p>	<p>Exercise 3 'Calf-rasises'</p>		<p>Description of starting position</p>
<p>A</p>			<p>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.</p>

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.
<p>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.</p> <p>The model has given written consent to publish this material.</p>			



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)	_____

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3	Introduction			
4				
5	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
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8		6b	Explanation for choice of comparators	4+5
9				
10	Objectives	7	Specific objectives or hypotheses	4+5
11				
12	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
13				
14				
15	Methods: Participants, interventions, and outcomes			
16				
17	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
18				
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20	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
21				
22				
23	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6-9
24				
25				
26		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
27				
28				
29		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9-10
30				
31				
32		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6-9
33				
34	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
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39	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
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2	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
3				
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5	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	16
6				
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8	Methods: Assignment of interventions (for controlled trials)			
9	Allocation:			
10				
11	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
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17	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
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21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
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24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
25				
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27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
28				
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31	Methods: Data collection, management, and analysis			
32				
33	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
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38		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
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3	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)
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9	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
10				
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12		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16-18
13				
14		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
15				
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18	Methods: Monitoring			
19				
20	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	_____
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25		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	_____
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28	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	_____
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31	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____
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35	Ethics and dissemination			
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37	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Already approved
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3	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes,	18
4	amendments		analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,	
5			regulators)	
6				
7	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	6+18
8			how (see Item 32)	
9				
10		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	22
11			studies, if applicable	
12				
13	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	18
14			in order to protect confidentiality before, during, and after the trial	
15				
16	Declaration of	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21+22
17	interests			
18				
19	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	_____
20			limit such access for investigators	
21				
22	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial	_____
23	trial care		participation	
24				
25	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals,	18
26			the public, and other relevant groups (eg, via publication, reporting in results databases, or other data	
27			sharing arrangements), including any publication restrictions	
28				
29		31b	Authorship eligibility guidelines and any intended use of professional writers	_____
30				
31		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____
32				
33	Appendices			
34				
35	Informed consent	32	Model consent form and other related documentation given to participants and authorised surrogates	22
36	materials			
37				
38	Biological	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular	Not relevant
39	specimens		analysis in the current trial and for future use in ancillary studies, if applicable	
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for an 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](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

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