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Effectiveness and cost-effectiveness of personalized dietary advice aiming at increasing protein intake on physical functioning in community-dwelling older adults with lower habitual protein intake: rationale and design of the PROMISS trial

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3 1 **Effectiveness and cost-effectiveness of personalized dietary advice aiming at increasing**
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5 2 **protein intake on physical functioning in community-dwelling older adults with lower**
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7 3 **habitual protein intake: rationale and design of the PROMISS trial**
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9 4

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For peer review only

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2
3 **30 Abstract**
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5 **31 Background:** Short-term metabolic and observational studies suggest that protein intake
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32 above the recommended dietary allowance of 0.83 g/kg body weight (BW)/d may support
33 preservation of lean body mass and physical function in old age, but evidence from RCTs is
34 inconclusive.

35 **35 Methods:** The PROMISS trial examines effects of personalized dietary advice aiming at
36 increasing protein intake with or without advice regarding timing of protein intake to close
37 proximity of any usual physical activity, on change in physical functioning after 6 months
38 among community-dwelling older adults (≥ 65 y) with a habitual protein intake of < 1.0 g/kg
39 adjusted (a)BW/d. Participants (n=264) will be recruited in Finland and the Netherlands, and
40 will be randomized into three groups; two intervention groups and one control group.
41 Intervention group 1 (n=88) receives personalized dietary advice and protein enriched food
42 products in order to increase their protein intake to at least 1.2 g/kg aBW/d. Intervention
43 group 2 (n=88) receives the same advice as intervention group 1, and advice to consume 7.5-
44 10 g protein through protein (en)rich(ed) foods within half an hour after performing any usual
45 physical activity. The control group (n=88) receives no intervention. All participants will be
46 invited to attend lectures not related to health. The primary outcome is 6-months change in
47 physical functioning measured by change in walk time using a 400-m walk test. Secondary
48 outcomes are: 6-months change in the Short Physical Performance Battery score, muscle
49 strength, body composition, self-reported mobility limitations, quality of life, and incidence
50 of frailty, sarcopenia and malnutrition. We also investigate cost-effectiveness by change in
51 health care costs.

52 **52 Discussion:** The PROMISS trial will provide evidence whether increasing protein intake, and
53 additionally optimizing timing of protein intake, has a positive effect on the course of
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3 54 physical functioning among community-dwelling older adults with a habitual protein intake
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5 55 of < 1.0 g/kg aBW/d.
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10 57 **Strengths and limitations of this study**

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12 58 • The effectiveness of advising to increase protein intake on 6-month change in
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14 59 physical functioning will be investigated;
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17 60 • the combined benefit of increasing protein intake and timing of protein intake will
18
19 61 be investigated;
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22 62 • Only community-dwelling older adults with a habitual protein intake of < 1.0 g/kg
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24 63 aBW/d will be included;
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26 64 • The intervention is based on personalized dietary advice;
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28
29 65 • The biological value of the total protein intake will not be known.
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33 67 **Trial registration:** ClinicalTrials.gov. Number of identification: NCT03712306. Registered
34
35 68 19 October 2018,
36
37
38 69 <https://clinicaltrials.gov/ct2/show/NCT03712306?cond=protein&cntry=NL&city=Amsterda>
39
40 70 [m&draw=2&rank=1](https://clinicaltrials.gov/ct2/show/NCT03712306?cond=protein&cntry=NL&city=Amsterdam&draw=2&rank=1)
41
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45 72 **Keywords:** older adults, protein intake, physical functioning, RCT, malnutrition, protein
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47 73 recommendation, 400 meter walk.
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74 **Background**

75 There is an ongoing debate on whether or not older adults should be recommended a protein
76 intake above the current recommended daily allowance (RDA) established by the European
77 Food Safety Authority (EFSA) of 0.83 g/kg body weight (BW)/d for adults (1). International
78 panels of geriatricians, nutritional experts and scientists have proposed at least 1.0-1.2 g
79 protein/kg BW/d for healthy older adults in order to maintain and regain muscle mass,
80 strength and function (2, 3).

81 The proposed increase of the RDA for older adults is merely based on results from
82 short term metabolic and epidemiological studies. Several metabolic studies showed that
83 older adults (≥ 65 y) have a lower muscle protein synthesis (MPS) following protein intake
84 compared to younger adults (4-6), and that higher protein intake enhances MPS in older
85 adults when compared to lower protein intake (1.2 g/kg BW/d vs. 0.8 g/kg BW/d (7), or ≥ 30
86 g/d vs. 15 g/d (8)). In addition, the anabolic threshold (i.e. optimal dose of dietary protein in a
87 meal that stimulates MPS) is 70% higher in older compared to younger adults (5).
88 Epidemiological studies have shown that higher dietary protein intake in older adults, defined
89 as > 0.9 g/kg BW/d (9) or > 1.0 g/kg BW/d (10-12) is associated with lower risk of weight
90 loss (11), better disability trajectories (12), less loss of lean mass (9), or lower risk of
91 developing functional impairments (10).

92 Despite the evidence from metabolic and epidemiological studies, causal evidence to
93 support beneficial effects of protein intake at or above 1.0 g/kg BW/d based on randomized
94 controlled trials (RCTs) is not conclusive. One systematic review showed no beneficial effect
95 of increasing protein intake on lean body mass, muscle cross-sectional area, muscle strength,
96 or physical performance (13). Of the 36 studies included in the systematic review, 26 studies
97 presented mean habitual protein intake of the study participants which ranged between 0.78
98 and 1.5 g/kg BW/d, with only one study below the protein RDA of 0.8 g/kg BW/d (13). The

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3 99 relatively high mean habitual protein intake may explain the absence of a beneficial effect of
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5 100 additional protein. Another explanation may be that the amount of protein provided might not
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7 101 have been sufficient to augment MPS. I.e. a protein intake of 25 – 30 g is required to
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10 102 stimulate MPS and maintain muscle mass (14, 15), though the amounts provided varied
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12 103 between 10 g/d (3d/wk) and total intake of 125 g/day or were not reported. Of the trials
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14 104 published after the systematic review, Park et al. showed that intake of 1.5 g/kg BW/d for 12
15
16 105 weeks resulted in higher muscle mass and improved gait speed compared to intake of 0.8
17
18 106 g/kg BW/d in undernourished pre-frail and frail older adults (16). Ten Haaf et al. showed a
19
20 107 positive effect of increasing protein intake for 12 weeks on lean body mass in active older
21
22 108 adults with a habitual protein intake of < 1.0 g/kg BW/d (17). Beelen et al. found no effects
23
24 109 of protein supplementation on physical performance among older adults after hospital
25
26 110 discharge (18), however, baseline protein intake was already 1.0 g/kg BW/d in the control
27
28 111 group and 1.5 g/kg BW/d in the intervention group. Finally, Bhasin et al. showed no
29
30 112 beneficial effects other than a decrease in fat mass after a controlled diet with 1.3 g/kg BW/d
31
32 113 of protein for 6 months compared to a control diet consisting of 0.8 g protein /kg BW/d (19)
33
34 114 among functionally limited community-dwelling men aged ≥ 65 years. However, mean BMI
35
36 115 of the participants was quite high (30.3 kg/m²), which may have resulted in an overestimation
37
38 116 of baseline protein requirements. Based on inconsistent findings, more RCTs in older adults
39
40 117 with lower habitual protein intake are needed to determine the potential effect of increasing
41
42 118 protein intake on physical functioning outcomes.

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45 119 Previous studies among older adults showed that protein supplementation in
46
47 120 combination with resistance exercise has more beneficial effects on body composition,
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49 121 muscle strength and physical function compared to resistance exercise alone (20-26). The
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51 122 underlying hypothesis is that protein supplementation augments the adaptive response of
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53 123 skeletal muscle to resistance exercise. In addition, there is evidence that timing of protein
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3 124 intake in close proximity of physical activity stimulated MPS to greater extent than when
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5 125 timed at other hours during the day (27). To our knowledge, there are no RCTs investigating
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7 126 the effect of timing protein intake in in close proximity of physical activities on physical
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10 127 functioning.

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12 128 The Prevention Of Malnutrition In Senior Subjects in the EU (PROMISS) trial is
13
14 129 designed to fill in some of the current knowledge gaps on the optimal amount of dietary
15
16 130 protein in older community dwelling adults and timing of protein intake in relation to
17
18 131 physical activity. Its primary objective is to examine the effectiveness of personalized dietary
19
20 132 advice aiming at increasing protein intake to at least 1.2 g/kg adjusted (a)BW/d on change in
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22 133 physical functioning after 6 months measured by change in walk time using a 400-m walk
23
24 134 test among community-dwelling older adults with a habitual protein intake of < 1.0 g/kg
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26 135 aBW/d. Additionally, it examines the combined effect of personalized dietary advice aiming
27
28 136 at increasing protein intake to at least 1.2 g/kg aBW/d and advice aiming at optimizing the
29
30 137 timing of protein intake in close proximity of any usual physical activity. The secondary
31
32 138 objectives are to examine the effectiveness of personalized dietary advice aiming at
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34 139 increasing protein intake to at least 1.2 g/kg adjusted on 6-month changes in physical
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36 140 functioning measured by the Short Physical Performance Battery (SPPB) score, muscle
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38 141 strength, body composition, self-reported mobility limitations, quality of life, incidence of
39
40 142 frailty, incidence of sarcopenia, and incidence of malnutrition and change in health care costs.

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42 143 In three ancillary studies the following additional objectives are addressed; 1) the
43
44 144 effect of using persuasive technology on adherence to personalized dietary advice aiming at
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46 145 increasing protein to at least 1.2 g/kg aBW/d, 2) the effect of personalized dietary advice
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48 146 aiming at increasing protein intake to at least 1.2 g/kg aBW/d on the oral and gut microbiota
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50 147 composition, and 3) the effect of personalized dietary advice aiming at increasing protein
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3 148 intake to at least 1.2 g/kg aBW/d on central neural responses to food-cues in brain areas of
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5 149 interest.

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10 151 **Methods/Design**

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12 152 *Study design*

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14 153 The PROMISS trial is a multicentre randomized controlled trial (ClinicalTrials.gov identifier:
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16 NCT03712306) designed to examine the effectiveness of personalized dietary advice aiming
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18 154 at increasing protein intake and advice on optimizing the timing of protein intake in close
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20 155 proximity of any usual physical activity on change in physical functioning after 6 months.
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22 156 Participants will be randomised into three groups: one control group (no intervention);
23
24 157 intervention group 1) receiving personalized dietary advice aiming at increasing protein
25
26 158 intake to at least 1.2 g/kg aBW/d; and intervention group 2) receiving personalized dietary
27
28 159 advice aiming at increasing protein intake to at least 1.2 g/kg aBW/d, including personalized
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30 160 advice to optimize the consumption of protein in close proximity of any usual physical
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32 161 activity.
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37 163 Researchers, nutritionists, and statisticians are responsible to the design of the trial. Older
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39 164 adults were not involved in the design or conduct of the research.

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44 166 *Eligibility criteria*

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47 167 The eligibility criteria are proposed to include a study group of older adults (65+) with a
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49 168 habitual protein intake < 1.0 g/kg aBW/d. Inclusion and exclusion criteria are listed in Table
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51 169 1, and some are described in more detail below.

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54 170 Older adults with a BMI of < 18.5 kg/m² will be excluded, because these participants
55
56 171 are likely to be undernourished (28) and should preferably receive general nutritional care
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58 172 that is not provided in this trial. Those with a BMI of > 32.0 kg/m² will be also excluded,
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3 173 because they should be advised to lose weight, which is not the aim of the present study and
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5 174 may interfere with the study objective. Because participants of intervention group 2 will be
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8 175 advised to consume protein rich foods in close proximity of any usual physical activity, older
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10 176 adults who are bedridden, wheelchair bound or do not go outside will be excluded from the
11
12 177 trial. Older adults with a diagnosis of severe kidney disease (i.e. treatment of a nephrologist
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14 178 and/or protein-restricted diet, self-reported) will also be excluded as they should be advised to
15
16 179 limit their protein intake (2, 29-31). Older adults with a low cognitive status (Mini-Mental
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18 180 State Examination (MMSE) score ≤ 20 (32)) will be excluded, as participants should be able
19
20 181 to understand and follow dietary advice if randomized to one of the intervention groups.
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26 183 Calculation of protein intake using adjusted body weight

28 184 To calculate habitual protein intake in g/kg aBW/d for all (potential) participants and
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30 185 recommended protein intake (participants in the two intervention groups), we apply adjusted
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33 186 BW depending on participants' age and BMI. We use adjusted body weight because
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35 187 underweight persons require extra protein to build muscle tissue, while in overweight
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37 188 persons, much 'extra weight' is adipose tissue. Protein intake in g/kg aBW/d is based on self-
38
39 189 reported BW during screening and afterwards based on measured BW during the baseline
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42 190 assessment, which is further used throughout the study. For those with a BMI > 25.0 to 32.0
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44 191 kg/m^2 (age ≤ 70 y) or > 27.0 to 32.0 kg/m^2 (age > 70 y) we apply aBW corresponding to a
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46 192 BMI of respectively 25.0 or 27.0 kg/m^2 . For those with a BMI > 18.5 to < 22.0 kg/m^2 (age $>$
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48 193 70 y) we apply aBW corresponding to a BMI of respectively 18.5 or 22.0 kg/m^2 (33). For the
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50 194 recommended protein intake, we apply adjusted BW which is based on baseline measured
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53
54 195 BW.

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58 197 *Recruitment and screening*

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3 198 Two hundred and sixty-four community-dwelling adults aged 65 years and older will be
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5 199 recruited at two study sites (metropolitan area of Finland including Helsinki, Espoo, Vantaa,
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7 200 Kauniainen, and Amsterdam, the Netherlands). The recruitment strategy includes mass
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9 201 mailing using addresses obtained from a random sample of the Finnish Population Registry
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11 202 (in Finland only), newspaper advertisements, media coverage, lectures, oral presentations to
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13 203 the target group, informing professionals working with older adults and flyers which will be
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15 204 distributed at locations where many community-dwelling older adults visit.
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19 205 Older adults who are interested in participating will be asked to contact the local
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21 206 PROMISS research team (by phone or by e-mail). Thereafter, screening by phone takes
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23 207 place, only when verbal informed consent is given, in which the majority of the eligibility
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25 208 criteria will be assessed along with an explanation of the study. Only those with a lower
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27 209 habitual protein intake (< 1.0 g/kg aBW/d) will be invited for the first clinic visit. Assessment
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29 210 of habitual protein intake will be estimated in two steps: 1) initial screening by phone; 2) a
30
31 211 full dietary assessment based on a combination of three food diaries and three 24-h dietary
32
33 212 recalls to confirm lower habitual protein intake. Step 1, the initial screening is performed by
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35 213 phone using the Protein Screener 55+ (Pro55+, available for use in English, Finnish and
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37 214 Dutch: see www.proteinscreener.nl/#/). This screening tool was specifically developed and
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39 215 validated for this purpose (34). The screening results in a probability score (0-100%) of
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41 216 having a protein intake below 1.0 g/kg aBW/d. At a probability of $> 30\%$, sensitivity and
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43 217 specificity are optimally balanced (34). In the PROMISS trial we select persons with a
44
45 218 probability score varying between $> 15\%$ (when initial response rates to recruitment
46
47 219 strategies are low) and $> 30\%$ (when initial response rates are high), for the second step of
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49 220 assessing habitual protein intake. Those who fulfill the eligibility criteria receive further
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51 221 information on the study and a food diary with a booklet with pictures of portion sizes by post
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53 222 to support the 24-h dietary recalls. After a minimum of one week of consideration, the
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3 223 research staff contacts the older adults, and among those who are still willing to participate
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5 224 the full dietary assessment will take place (step 2). These potential participants will be asked
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8 225 to keep track of their dietary intake by filling out the provided food diary for three
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10 226 consecutive days (three weekdays; or two weekdays and one weekend day). The booklet with
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12 227 pictures of portion sizes that they received earlier will help them accurately filling out the
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14 228 diary. Each day after, they will be called by a nutritionist to go through their food diary of the
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17 229 day before (24-h dietary recall). Potential participants are asked whether these days are
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19 230 representative for their habitual diet. In case one of the three days is not representative, mean
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21 231 protein intake is based on two instead of three days. In case of more than one non-
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23
24 232 representative day, the person will be excluded. The food intake data based on the 24-h
25
26 233 dietary recall will be entered into the program 'Fineli' for the Finnish data (35) and into the
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28 234 program 'Eetmeter' of the Dutch Nutrition Center using an extended version of the Dutch
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30 235 Food Composition Table of 2016 for the Dutch data (36) to calculate intake of macronutrients
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33 236 and micronutrients (vitamin D and vitamin B12). Participants with an actual protein intake \geq
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35 237 1.0 g/kg aBW/d (based on self-reported BW) will be excluded.

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38 238 Potential participants with a mean habitual protein intake < 1.0 g/kg aBW/d (based on
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40 239 the three 24-h dietary recalls), will be invited for the clinic visit, where final eligibility
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42 240 criteria will be assessed; MMSE > 20 , ability to walk 400 m within 15 minutes (the use of a
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44 241 cane is allowed, but without the use of a walker and no rest longer than 60 seconds), and BMI
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46 242 of ≥ 18.5 kg/m² and ≤ 32.0 kg/m² based on measured BW and body height. When all
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48 243 eligibility criteria are met, participants are included in the PROMISS trial.

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52 53 245 *Randomization, allocation and masking*

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56 246 Randomization by means of a stratified block randomization procedure will be performed by
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58 247 an independent statistician. Participants will be allocated in a 1:1:1 ratio to the three groups.
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3 248 The size of the randomization blocks is three. Participants will be stratified according to their
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5 249 baseline habitual protein intake (< 0.9 or 0.9-1.0 g/kg aBW/d) and sex to ensure
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8 250 homogeneous distribution of baseline habitual protein intake and sex in the three groups
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10 251 across the two recruitment sites, because there may be a different intervention effect by
11
12 252 baseline habitual protein or sex. In case couples are eligible we will allocate them to the same
13
14 253 intervention group to limit interference between intervention groups. We will randomly select
15
16
17 254 on which partner the randomization for the intervention group is based. Any resulting
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19 255 unbalance in the number of subjects per treatment arm will be corrected in the randomization
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21 256 of the next block. Due to the nature of the study, researchers, nutritionists and participants are
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23
24 257 not blinded to the study group.
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26 258

28 259 *Study timeline*

30 260 The first clinic visit starts with written informed consent, and when participants are eligible,
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32 261 the baseline assessment will be performed. The baseline assessment consists of
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34 262 questionnaires (frailty status, risk of sarcopenia, self-reported mobility limitations, quality of
35
36 263 life (QoL) and health care costs) and measurements (physical function, muscle strength and
37
38 264 body composition). See below 'primary and secondary outcomes' and 'other measures' for
39
40 265 details on these assessments. An accelerometer will be attached to measure physical activity
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43 266 for 7 subsequent days.

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46 267 After the baseline assessment, participants will be randomised to one of the three
47
48 268 study groups done by the nutritionists and they will inform the participants in which group
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50 269 they are allocated to. Participants randomized to one of the two intervention groups will be
51
52 270 invited for a consultation meeting at the clinic to receive their personalized dietary advice,
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54 271 and personalized advice on optimizing the timing of protein intake in close proximity of any
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56 272 usual physical activity (intervention group 2 only). This will take place within 2 weeks after
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3 273 the baseline assessment since the personalized advice needs to be composed by the
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5 274 nutritionist. The baseline assessment is considered the start of the study period for
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7
8 275 participants of the control group, while the consultation meeting is considered the start of the
9
10 276 study period for participants of the intervention groups.

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12 277 One week prior to the 3-month follow-up visit, dietary intake will be assessed again
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14 278 by means of a combination of three food diaries and three 24-h dietary recalls. The 3-month
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16
17 279 follow-up visit will take place at the clinic and includes measurement of BW, assessment of
18
19 280 self-reported mobility limitations, risk of sarcopenia, QoL, health care costs and the
20
21 281 accelerometer will be attached to measure physical activity for 7 subsequent days.

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23
24 282 One week prior to the 6-month follow-up visit (final measurement) dietary intake will
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26 283 again be assessed by means of a combination of three food diaries and three 24-h dietary
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28 284 recalls, which allows us to determine compliance to the dietary advice. The 6-month follow-
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30 285 up visit at the clinic includes all measurements performed during the baseline visit and the
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32 286 accelerometer is attached again to measure physical activity for the next 7 days. Finally,
33
34 287 among participants of the intervention groups only, several questions regarding the
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36 288 appreciation and adherence of the intervention and participants' intention to follow the
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38 289 dietary advice in the future will be asked in order to perform a process-evaluation. Figure 1
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40 290 shows the study timeline and Table 2 provides an overview of all measurements.
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46 292 *Intervention*

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49 293 Participants in the intervention group 1 will receive a personalized dietary advice by
50
51 294 nutritionists dedicated to this study aiming at increasing their protein intake to at least 1.2
52
53 295 g/kg aBW/d (without increasing total daily energy intake), based on their habitual dietary
54
55 296 characteristics, protein intake and measured BW assessed at baseline. The advice includes the
56
57 297 use of regular protein rich food products and protein enriched food products provided by the
58
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1
2
3 298 research team, and will be based on personal dietary preferences. Protein enriched food
4
5 299 products that can be incorporated within the regular diet include bars, cereals, puddings,
6
7
8 300 coconut water and whey powder, which will be freely provided and shipped to participants'
9
10 301 home. Those products can be incorporated in the dietary advice as they can make it easier to
11
12 302 increase protein intake due to their high protein content. Participants receive guidelines how
13
14 303 to incorporate the protein enriched food products within their diet. The dietary advice will
15
16
17 304 also incorporate the advice to consume at least one daily meal consisting of ≥ 35 g protein, as
18
19 305 studies have shown that this amount increases MPS in older adults (37-39).

20
21 306 Participants in intervention group 2 will receive the same dietary advice as
22
23 307 intervention group 1, and the personalized advice to consume at least 7.5-10 g protein
24
25 308 through protein (en)rich(ed) food products within half an hour after performing any usual
26
27
28 309 physical activity as this may enhance resistance exercise induced MPS (27). One RCT among
29
30 310 older adults has shown that protein supplementation in combination with resistance exercise
31
32 311 had beneficial effects on e.g. muscle mass and function, but no differences in effect were
33
34 312 found between protein consumption pre versus post resistance exercise (40). We therefore
35
36 313 recommend protein intake after physical activity as this is a uniform and more feasible advice
37
38 314 compared to 'in close proximity of', and might also result in less stomach discomfort as
39
40 315 compared to protein consumption prior to physical activity. Usual physical activity is defined
41
42 316 as either physical exercise (e.g. biking, swimming, tennis) or the most intensive activities of
43
44 317 daily living when the participant does not engage in physical exercise (e.g. gardening,
45
46 318 housekeeping, doing groceries) for a minimum of 30 minutes. The advice is linked to most
47
48 319 extensive or longest physical activity. Participants are instructed not to become more or less
49
50 320 physically active but merely to shift their physical activity or protein intake moment.

51 321 During the intervention period, nutritionists will plan follow-up phone calls in
52
53
54 322 consultation with the participants during week 2, week 4, week 8, week 16 and week 20 to
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2
3 323 ask if they have understood the advice and are able to adhere to the advice. In addition, any
4
5 324 issues related to the use of protein enriched food product can be discussed (intervention
6
7 325 groups only). If necessary, changes in the dietary advice will be made, for example when
8
9 326 weight change > 2 kg has occurred (based on self-assessment). Participants allocated to the
10
11 327 control group do not receive any intervention, but are contacted on similar time points as the
12
13 328 intervention groups to ask how they are doing.
14
15

16
17 329 All participants are invited to a minimum of one organized lecture on non-health related
18
19 330 themes and other social events during the trial in order to stimulate their commitment to the
20
21 331 trial. Separate lectures/events (with the same topic) are organized for the intervention groups
22
23 332 and the control group to prevent interference between intervention arms.
24
25

26 333

27 28 334 Compliance

29
30 335 We will collect dietary intake prior to the 3-month follow-up visit (by means of the
31
32 336 combination of three food diaries and three 24-h dietary recalls) to assess compliance to the
33
34 337 dietary advice. This information allows the nutritionists to provide additional advice – if
35
36 338 needed – for participants in the intervention groups, which will be provided during the 3-
37
38 339 month follow-up visit. Dietary intake will again be assessed at follow-up and compliance to
39
40 340 the dietary advice will be determined.
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44 341

45 46 342 Intervention fidelity

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48
49 343 To ensure good adherence to the intervention protocols at both study sites, all personnel
50
51 344 working for the trial have undergone extensive training. The nutritionists will follow written
52
53 345 standardized operational procedures to develop and provide the personalized dietary advice
54
55 346 (with or without additional advice to consume protein within half an hour after any usual
56
57 347 physical activity). Four times during the conduct of the trial, the nutritionists from one site
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1
2
3 348 will visit the other site to attend assessments, and potentially notice and correct differences in
4
5 349 order to ensure identical execution of the trial at both sites. In addition, monthly Skype-
6
7 350 meetings will be held between all staff involved in the execution of the trial at both sites to
8
9 351 solve any potential day-to-day issues in a standardized way. Furthermore, identical
10
11 352 participant brochures and other printed materials have been developed and translated to
12
13 353 Dutch and Finnish language.
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18
19 355 *Outcomes*

20
21 356 Primary outcome

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23
24 357 The primary outcome of the PROMISS trial is 6-months change in physical functioning
25
26 358 measured by change in walk time using a 400-m walk test (Long Distance Corridor Walk)
27
28 359 (41, 42). This test is predictive for higher risk of mortality, incident cardiovascular disease
29
30 360 and mobility limitation and disability (43). One advantage of this continuous outcome is that
31
32 361 it enables discrimination between categories of risk among participants (44), and it is less
33
34 362 prone to a ceiling effect as compared to other functional outcome measures (e.g. SPPB) (45).
35
36 363 The course for the 400-m test is 20-m long and marked by a traffic cone and tape line at the
37
38 364 beginning and end. For all participants, the test will begin with a mandatory 40-m walk
39
40 365 (warm-up) at their usual pace. Thereafter the 400-m test starts with the feet behind and just
41
42 366 touching the starting line and ends after 10 complete rounds when one foot is behind the end
43
44 367 line. For the 400-m test, older adults will be instructed to walk as fast as possible at a pace
45
46 368 they can maintain for 400 m. Standardized encouragement will be given each lap, including
47
48 369 the number of laps remaining. At the 6-month follow-up visit, older adults are allowed to use
49
50 370 a cane, can take rest as needed (but no rest longer than 60 seconds) and there will be a time
51
52 371 limit of 17 minutes. Time will be recorded to the nearest second.
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3 373 Secondary outcomes
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5 374 The secondary outcomes are changes in physical functioning, muscle strength, body
6
7 375 composition, self-reported mobility limitations, QoL, incidence of frailty, incidence of
8
9 376 sarcopenia, and incidence of malnutrition. We will also investigate change in health care
10
11 377 costs.
12
13

14 378 Physical functioning will be assessed by means of the Short Physical Performance
15
16 379 Battery (SPPB) (46). The SPPB assesses lower extremity function which consists of three
17
18 380 timed tests: repeated (5x) chair stands test, 4-meter walk test and three standing balance tests
19
20 381 (ability to stand with the feet together in the side-by-side, semi-tandem, and tandem
21
22 382 positions). The total score ranges from 0-12. A higher score indicates better physical
23
24 383 functioning.
25
26

27 384 Muscle strength will be determined by hand-grip strength (kg). Hand-grip strength is
28
29 385 an indicator of overall muscle strength (47) and a higher hand grip strength is associated with
30
31 386 decreased risk of physical disabilities (48) and all-cause mortality in old age (48, 49).
32
33

34 387 Maximum grip strength will be measured three times at each hand during baseline and 6-
35
36 388 month follow-up visit. We will use a digital dynamometer (Saehan Digital Hand held
37
38 389 dynamometer) adjusted for hand size. Participants will be measured in an upright sitting
39
40 390 position with the forearms supported by the armrest of a chair according to a standardized
41
42 391 protocol (50). The mean of the maximum score of left and right hand will be used for
43
44 392 analyses. Muscle strength will also be determined by leg extension strength (N). A higher leg
45
46 393 extension strength is associated with decreased risk of mobility disability (51, 52) and higher
47
48 394 risk of early mortality (53-55). Leg extension strength will be assessed using a chair designed
49
50 395 to measure leg extension strength (56). Maximum leg extension strength will be measured
51
52 396 three times for each leg during baseline and 6-month follow-up visit. The mean of the
53
54 397 maximum score of left and right leg will be used for analyses.
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3 398 Body composition will be estimated by means of bioelectrical impedance using the
4
5 399 BodyStat 1500MDD device, using the Kyle equation to determine fat percentage (%), fat
6
7 400 mass and fat-free mass (60) and the Sergi equation to determine appendicular skeletal muscle
8
9 401 mass (ASMM, kg) (61). Additionally, at the Dutch study site, body composition (fat free
10
11 402 mass (kg), fat mass and fat percentage (%)) will be measured by air displacement
12
13
14 403 plethysmography (62).

15
16
17 404 Self-reported mobility limitations will be assessed by means of a questionnaire;
18
19 405 “Because of your health, how much difficulty do you have walking 400 meter?” and
20
21 406 “Because of your health, how much difficulty do you have climbing 10 steps?” Participants
22
23 407 will respond using a five level Likert scale: ‘no difficulty’, ‘a little difficulty’, ‘some
24
25 408 difficulty’, ‘a lot of difficulty’ and ‘unable to do the activity’.

26
27
28 409 QoL will be measured using the EuroQol 5D – 5L questionnaire (63).

29
30
31 410 Incident frailty will be assessed using the Fried criteria (57). Participants will be
32
33 411 considered ‘frail’ when three or more components are present. Those with no components
34
35 412 will be considered ‘robust’, whereas those with one or two components will be considered
36
37 413 ‘prefrail’. The criteria include 1) self-reported unintentional weight loss (> 4 kg in past 6
38
39 414 months), 2) self-reported exhaustion (based on two questions from the Center for
40
41 415 Epidemiologic Studies Depression (CES-D) scale on exhaustion in the past week at baseline
42
43 416 and follow-up: “I felt that everything I did was an effort” and “I could not get going”. Scores
44
45 417 ranges from 1 ‘rarely or none of the time’ to 4 ‘always or most of the time’. A score of 3 or 4
46
47 418 on either question indicates exhaustion (58), 3) weakness (grip strength in the lowest 20% of
48
49 419 the whole study population based on the mean of the maximum scores, adjusted for gender
50
51 420 and BMI), 4) slow walking speed (walk time on the 4m walk test in the slowest 20% of the
52
53 421 whole study population, adjusted for gender and height), and 5) low physical activity (total
54
55 422 counts per week based on the accelerometer data in the lowest 20% of physical activity for
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1
2
3 423 each gender). Incident frailty is considered deterioration of frailty status; i.e. from robust at
4
5 424 baseline to pre-frail or frail at follow-up or from pre-frail at baseline to frail at follow. Frail
6
7
8 425 participants at baseline will be excluded from these analyses.

9
10 426 Incidence of sarcopenia will be assessed with the SARC-F questionnaire (59); how
11
12 427 much effort do you experience when 1) lifting and carrying a bag of 4.5 kilo, 2) walking
13
14 428 across a room, 3) transferring from a chair or bed, 4) climbing a flight of 10 stairs, and 5)
15
16
17 429 how many times have you fallen in the past year. Answering option include no effort (0
18
19 430 points), a bit of effort (1 point) and a lot of effort (2 points), where a score equal to or greater
20
21 431 than 4 is predictive of sarcopenia and poor outcomes. Participants with sarcopenia at baseline
22
23
24 432 will be excluded from these analyses.

25
26 433 Incidence of malnutrition will be defined as BMI < 22.0 kg/m² or unintentional
27
28 434 weight loss > 5% in the last 6 months. Malnourished participants at baseline will be excluded
29
30
31 435 from these analyses.

32
33 436 A modified version of the Resource Utilization in Dementia Questionnaire (RUD)
34
35 437 (64) will be used to collect data on health care and social utilization costs over the period
36
37 438 three month prior to baseline, three months prior to the 3-month follow-up visit and three
38
39
40 439 months prior to 6-month follow-up visit. Costs include costs of primary and secondary care,
41
42 440 complementary care, informal care and home care.

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45 441

46 47 442 Other measures

48
49 443 BW will be measured without shoes in underwear to the nearest 0.1 kg using a digital
50
51 444 calibrated scale (Finland; SECA 877, the Netherlands; Marsden M-520). Body height will be
52
53 445 measured to the nearest millimeter using a SECA stadiometer for mobile height
54
55
56 446 measurements (Finland; SECA 217, the Netherlands; SECA 214). Corrections will be made
57
58 447 to adjust the measured body weight for clothing, shoes or a cast (minus 1 kg for each
59
60

1
2
3 448 element), and to adjust the measured body height for shoes (minus 1 cm). Physical activity
4
5 449 will be objectively assessed by means of an accelerometer (Axivity, AX3) during 7
6
7
8 450 subsequent days after each clinic visit (baseline, 3-month follow-up visit and 6-month follow-
9
10 451 up visit). The accelerometer will be attached by a nutritionist to the frontal part of the right
11
12 452 thigh in the mid-point between iliac crest and patella bone when sitting down, with a surgical
13
14 453 plaster. Participants can perform any physical activity as the accelerometer is water resistant.
15
16
17 454 Appetite will be measured with SNAQ-Appetite questionnaire (65). Dietary intake will be
18
19 455 assessed by means of a combination of three food diaries and three 24-h dietary recalls prior
20
21 456 the 3-month follow-up visit and the 6-month follow-up visit.
22
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26 458 *Sample size and statistical analyses*27
28 459 Sample size

29
30 460 The study is powered to detect a substantial meaningful change of 28 sec (SD=61 sec) (66)
31
32 461 between the respective intervention groups and the control group on the primary outcome
33
34 462 walk time on the 400-m walk test, assuming a 2-sided test at $\alpha=0.05$ with a power of 0.8. For
35
36 463 this, 75 participants per group are needed, which is 225 in total. Assuming a drop-out of 15%
37
38 464 (which was reported in a comparable study of Bhasin et al. 2018 (19), the total number of
39
40 465 study participants to be included in the study will be n=264. Thus, a total of n=132 at each
41
42 466 study site (n=44 per group per study site).
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49 468 Statistical analyses plan

50
51 469 Statistical reporting will be according to the CONSORT standards (67). The collected data at
52
53 470 the two study sites will be pooled together at the Amsterdam site, with a variable indicating
54
55 471 study site. All statistical analyses on primary and secondary outcome measures will be
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1
2
3 472 performed by an independent statistician blinded for group allocation. Baseline
4
5 473 characteristics will be described (percentages, means \pm standard deviations) by study group.
6

7
8 474 The primary analyses will be based on the intention-to-treat principle, i.e. data from
9
10 475 participants allocated to the intervention groups will be analyzed as part of those groups,
11
12 476 irrespectively of their level of adherence to the advice. Multiple Imputation using
13
14 477 multivariate Imputation by Chained Equations will be used to impute missing cost and effect
15
16 478 data. The continuous primary outcome (change in 400-m walk time) will be analyzed using
17
18 479 mixed model regression analyses with study site and participating partner as random variable.
19
20 480 We will adjust for baseline 400-m walk time as well as baseline protein intake (g/kg aBW/d)
21
22 481 and sex (the stratification factors for randomization). We will compare intervention effects of
23
24 482 the respective intervention groups versus the control group. Effects will also be expressed in
25
26 483 Cohen's d and the corresponding 95% confidence intervals will be calculated, which allows
27
28 484 comparison between intervention effect estimates between different outcome measures. The
29
30 485 secondary outcomes and other measures will be analyzed using mixed model regression
31
32 486 analogously to the primary outcome. For binary secondary outcomes, generalized estimating
33
34 487 equation models will be used. With regard to time-to-event analyses (incident sarcopenia and
35
36 488 mobility limitations) Cox proportional hazard models will be used. Time-to-event is defined
37
38 489 as the time of the start of the study period to the date of the first occurrence of the event (3-
39
40 490 month follow-up visit or 6-month follow-up visit). Participants who do not meet these criteria
41
42 491 will be censored at the latest time we had information available. We will perform subgroup
43
44 492 analyses stratified by baseline protein intake (< 0.9 or $0.9-1.0$ g/kg aBW/d), sex and baseline
45
46 493 400-m time (based on median) for the primary and secondary outcomes.
47
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50
51 494 Per-protocol analyses will also be conducted as a sensitivity analysis. Effect estimates
52
53 495 for change in primary and secondary outcome measures will be calculated for participants
54
55 496 from the intervention groups who reached the protein target of at least 1.2 g/kg aBW/d after
56
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3 497 both 3 and after 6 months (mean protein intake based on the three 24-h dietary recalls) vs.
4
5 498 participants from the control group. Data will be analyzed using SPSS (IBM SPSS Statistics.
6
7 499 Armonk, NY). A two-sided *P*-value of 0.05 is considered statistical significant.
8
9

10 500 The cost-effectiveness analysis will be performed from a healthcare perspective. Total
11
12 501 mean costs during the study will be related to physical functioning (the 400-m walk test) and
13
14 502 change in Quality-Adjusted Life-Years based on the EuroQol 5D questionnaire. Mixed model
15
16 503 regression analyses will be used to estimate differences in the primary outcome of the
17
18 504 respective interventions groups versus the control group. Linear regression analyses will be
19
20 505 used to estimate differences in QoL (expressed as Quality-Adjusted Life-Years) and
21
22 506 healthcare costs. Incremental cost-effectiveness ratios will be calculated by dividing the
23
24 507 difference in costs by the difference in effects. Statistical uncertainty will be estimated using
25
26 508 bias-corrected accelerated bootstrapping (5000 replications) and will be presented using cost-
27
28 509 effectiveness planes and cost-effectiveness acceptability curves.
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33 510

34 35 511 *Participants' safety*

36
37 512 In case any (medical) questions arise during the screening or intervention period, participants
38
39 513 can consult an independent medical doctor. All adverse events and serious adverse events
40
41 514 will be tracked by the nutritionists during the follow-up phone calls, 3-month follow-up visit
42
43 515 and 6-month follow-up visit to assess their potential relationship to the intervention at both
44
45 516 sites and will be documented in the final report. Adverse events will be reported within 7 days
46
47 517 (death or life threatening situations) or within 15 days (in case of other adverse events) of
48
49 518 first knowledge to The Medical Ethical Committee of the Amsterdam UMC, location Vumc
50
51 519 (required for the Dutch site only).
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56 520

57 58 521 *Data quality assurance and data management*

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3 522 Research data will be collected at each site and each visit (baseline, 3-month follow-up visit
4
5 523 and 6-month follow-up visit) using a standardized protocol with the same order of
6
7 524 assessments, and entered twice in separate electronic datasets. When discrepancies between
8
9 525 the datasets are found, the original questionnaire will be consulted. Questionnaire items and
10
11 526 measurements will include the corresponding variable names to minimize errors in data
12
13 527 entering. Finally the two final electronic dataset (one from each site) containing all data will
14
15 528 be pooled. A data catalogue and codebook will be developed.

16
17
18
19 529 Original questionnaires will be stored in a secure manner at each site in an area with
20
21 530 limited access. All records that contain names (i.e. informed consent forms), will be stored
22
23 531 separately from study records identified by code number. All databases will be secured with
24
25 532 password-protected access systems.
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31 534 *Ancillary studies*

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33 535 Within the PROMISS trial, three ancillary studies will be conducted: 1) persuasive
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35 536 technology study, 2) microbiota study, and 3) fMRI study.
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40 538 1. Persuasive technology study

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42 539 The primary aim of this study is to examine the effect of persuasive technology on adherence
43
44 540 to the personalized dietary advice aiming at increasing protein intake to at least 1.2 g/kg
45
46 541 aBW/d in a sub-sample of Dutch participants from intervention group 1 (n=24) and
47
48 542 intervention group 2 (n=24), i.e. the first 24 participants of intervention group 1 and the first
49
50 543 24 participants of intervention group 2 that consent to it (writing informed consent will be
51
52 544 signed).
53
54

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56 545 Participants will be provided with a food storage box that registers which provided
57
58 546 protein enriched food products are taken out. The food box is used to store the protein
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3 547 enriched food products provided by the research team. Participants will also receive a tablet
4
5 548 that allows participants to register any consumed protein enriched food products and supports
6
7 549 them in finding alternative food products that contain a comparable amount of protein. For
8
9 550 this, the system uses the personalized dietary advice as provided by the nutritionist and data
10
11 551 from the storage box. The tablet application aims to stimulate adherence to the dietary advice
12
13 552 by providing tailored and personalized messages. In addition, personality characteristics and
14
15 553 communication style preferences that are determined via a questionnaire completed at
16
17 554 baseline are used to tailor the style and tone of these messages (68).

18
19
20
21 555 In addition to the personalized messages, half of the participants from intervention
22
23 556 group 1 and 2 who participate in the persuasive technology sub-study will also receive a
24
25 557 gamified version of the tablet application (n=12 + n=12). In this version, participants can earn
26
27 558 game-points by registering their consumed protein (en)rich(ed) food products and by playing
28
29 559 mini-games about the protein content of foods (i.e. guess the protein content, more-or-less
30
31 560 protein). The distribution of receiving the gamified version vs. standard version is quasi-
32
33 561 randomized, where we will balance the group size.

34
35
36
37 562 At the consultation meeting, participants receive their food storage box and tablet.
38
39 563 Both are fully configured, i.e. they are loaded with their personal dietary advice. After the 6-
40
41 564 month follow-up visit, participants will be asked to return the equipment and fill out
42
43 565 questions on the feasibility and user experience of the provided persuasive technology.

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46
47 566 The secondary objectives are 1) to investigate to what extent participants perceive
48
49 567 messages of which the style and tone are adapted to their personal characteristics as
50
51 568 personalized and adequate, and 2) to determine the effect of gamification on the effectiveness
52
53 569 and feasibility of the persuasive technology.

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57
58 571 2. Microbiota study

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2
3 572 In the microbiota study, the effect of personalized dietary advice aiming at increasing protein
4
5 573 intake in community-dwelling older adults with lower habitual protein intake on both the oral
6
7 574 and gut microbiota is investigated. The study will be conducted at both study sites.
8
9

10 575 The human microbiota consists of the 4×10^{13} micro-organisms that inhabit the body
11
12 576 (69). The emergence of next generation DNA sequencing techniques at the start of the 21st
13
14 577 century has allowed more detailed study of the microbiota and since then, the microbiota
15
16 578 composition has been associated with both health and disease (70), as well as aging itself (71,
17
18 579 72). Moreover, several interventional studies proved that dietary changes also affect the gut
19
20 580 microbiota, with the first microbial shifts being evident within 48 hours (73). The altered
21
22 581 microbiota in turn, can differentially affect the human host metabolism through the
23
24 582 production of metabolically active metabolites. Less is known about the oral microbiota. It
25
26 583 was found to be associated with oral health and function and even nutritional status (74, 75),
27
28 584 but its possible role in undernutrition in older adults has not been investigated.
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33 585 A fresh frozen fecal sample and tongue swab is collected at baseline and 6-month
34
35 586 follow-up visit once written informed consent is provided. Participants from either the control
36
37 587 group or intervention group 1 can be included in this study. Participants from the intervention
38
39 588 group 2 are excluded to limit the number of groups and parameters in this exploratory study.
40
41 589 Additional exclusion criteria are: use of systemic antibiotics in the three months prior to the
42
43 590 first sampling visit, diagnosis with inflammatory bowel disease and prolonged
44
45 591 institutionalization (> 4 weeks) in the three months prior to the first sampling visit. There is
46
47 592 no restriction other than consent rate to the number of PROMISS participants that will be
48
49 593 included in this side study.
50
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54 594 Once all samples from all participants are collected, fecal samples are shipped to the
55
56 595 Wallenberg Laboratory of Cardiovascular and Metabolic research (at the University of
57
58 596 Gothenburg, in Sweden) for 16S rRNA sequencing using sequencing methods previously
59
60

1
2
3 597 described (76). The tongue swabs will be send to the Netherlands Organisation for Applied
4
5 598 Scientific Research for 16S rRNA sequencing as is previously described (77).
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10 600 3. fMRI study
11

12 601 In the fMRI study, we will investigate the effect of personalized dietary advice aiming at
13
14 602 increasing protein intake in community-dwelling older adults with lower habitual protein
15
16 603 intake on central brain circuits involved in the regulation of appetite. Several studies
17
18 604 demonstrated that increasing protein intake affects appetite (78) and the gut microbiota (79).
19
20 605 However, none have studied the effects on both simultaneously, or the interaction. A
21
22 606 functional MRI (fMRI) scan will be used to measure the brain responses to visual or actual
23
24 607 food cues. Brain activity in response to food cues will also be related to (shifts) in the gut
25
26 608 microbiota. Therefore, only participants from the microbiota side study can be included in
27
28 609 this study, with additional exclusion criteria: being claustrophobic, being diagnosed with a
29
30 610 mental disorder (e.g. depression or addiction), being uncorrectable visually or hearing
31
32 611 impaired, or having a contra-indication for MRI-scans (e.g. having a pacemaker). Up to 50
33
34 612 participants will be included in this side study. This side study will only be conducted at the
35
36 613 Dutch study site.
37
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41

42 614 Once written informed consent is provided, participants who are included in this side
43
44 615 study will be asked to visit the Amsterdam University Medical Centre, location VUmc, for an
45
46 616 fMRI scan twice during the study period: at baseline and at 6-month follow-up visit. Prior to
47
48 617 the fMRI-scan, additional salivary and blood samples will be collected for determination of
49
50 618 additional nutritional and microbial biomarkers. The protocol for the fMRI experiments have
51
52 619 been previously described (80, 81).
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57
58 621 **Discussion**
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60

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2
3 622 There is an ongoing discussion whether the EFSA RDA of 0.8 g protein /kg BW/d is
4
5 623 sufficient for older adults and whether it should be increased to at least 1.0-1.2 g protein/kg
6
7 624 BW/d to support muscle health and functioning. National guidelines of some European
8
9 625 countries already increased their RDA, i.e. the RDA of the German-speaking countries (D-A-
10
11 626 CH) is increased to 1.0 g/kg BW/d (82), and the Nordic Nutrition Recommendation has
12
13 627 increased their RDA to 1.2 g/kg BW/d (83). The PROMISS trial is the first RCT which will
14
15 628 investigate the effect of personalized dietary advice aiming at increasing protein intake and
16
17 629 the combined effect of personalized dietary advice aiming at increasing protein and optimally
18
19 630 timing protein intake in close proximity of any usual physical activity, on change in physical
20
21 631 functioning after 6 months among community-dwelling older adults (≥ 65 y) with a habitual
22
23 632 protein intake of < 1.0 g/kg adjusted (a)BW/d. The PROMISS trial will therefore provide
24
25 633 additional insight to the question whether the current EFSA RDA for protein for older adults
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27 634 should be increased to 1.2 g/kg aBW/d, and whether optimal timing of protein intake will
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29 635 additionally benefit physical functioning.
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38 637 A strong and unique aspect of the PROMISS trial is that we will include participants with a
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40 638 habitual protein intake < 1.0 g/kg aBW/d, excluding those with a BMI < 18.5 and > 32.0
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42 639 kg/m². This will allow us to examine the effects of increasing protein intake from < 1.0 to at
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44 640 least 1.2 g/kg aBW/d. An innovative component of our study is that we will investigate the
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46 641 combined benefit of increasing protein intake and timing of protein intake with any usual
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48 642 physical activity on physical functioning and other health related outcomes. Another strength
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50 643 is that in our study the intervention is based on personalized dietary advice which is likely
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52 644 more feasible in the long term to maintain in everyday life, compared to providing custom-
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54 645 prepared meals (19) or protein supplements (84, 85), as done in most other studies. Finally,
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56 646 we will be able to investigate the effect of persuasive technology on adherence to the dietary
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3 647 advice strategy, and the effect of the dietary advice on the microbiota composition and on
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5 648 central responses to food-cues in brain areas involved in appetite regulation.
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10 650 In summary, this randomized controlled trial will demonstrate the effectiveness of
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12 651 personalized dietary advice aiming at increasing protein intake to at least 1.2 g protein/kg
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14 652 BW/d on physical functioning in older adults with a lower habitual protein intake, with or
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17 653 without the advice to consume protein in close proximity of any usual physical activity.
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3 **656 List of abbreviations**
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5 657 aBW = adjusted body weight
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8 658 BMI = body mass index
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10 659 BW = body weight
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12 660 fMRI-scan: Functional Magnetic Resonance Imaging scan
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14 661 MMSE = Mini Mental State Examination
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16 662 MPS = muscle protein synthesis
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18 663 PROMISS = PRevention Of Malnutrition In Senior Subjects
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20 664 QoL = quality of life
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22 665 RCT = randomized controlled trial
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24 666 RDA = recommended daily allowance
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26 667 RUD = Resource Utilization in Dementia Questionnaire
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28 668 SARC-F = Simple Questionnaire to Rapidly Diagnose Sarcopenia
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30 669 SD = standard deviation
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32 670 SPPB = Short Physical Performance Battery
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3 **671 Declarations**
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8 **673 Ethics approval and consent to participate**
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10 674 The study has been approved by the Ethics Committee of the Helsinki University Central
11 Hospital, Finland and The Medical Ethical Committee of the Amsterdam UMC, location
12 675 VUmc, Amsterdam, the Netherlands. Oral informed consent will be obtained from each
13
14 676 participants before the screening procedure and written informed (please see appendix I)
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16 677 consent will be obtained from each participant before any measurement take place.
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24 **680 Consent for publication**
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26 681 Not applicable. Personal data were not identifiable during the analysis.
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31 **683 Availability of data and materials**
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33 684 Datasets from this research will be stored at the repository of the Vrije Universiteit
34
35 685 Amsterdam, the Netherlands and potentially available for other researchers after submitting a
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37 686 research proposal.
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42 **688 Competing interests**
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44 689 The authors declare that they have no competing interests.
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48
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50

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52
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54
55 694 did not participate in the study design, collection, management, analysis and interpretation of
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2
3 695 data; or writing of the manuscript. They did not participate in the decision to submit the
4
5 696 report for publication, nor had ultimate authority over any of these activities.
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10 698 **Protein enriched food products**

11
12 699 Protein enriched food products are provided by Kellogg and Fonterra. Costs for these
13
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17 701

18
19 702 **Author Contributions**

20
21 703 *IR, HAHW, IAB, MRO* and *MV* obtained funding for the PROMISS project. *IR* and *HAHW*
22
23 704 coordinate the trial center at the Vrije Universiteit Amsterdam, the Netherlands. *SKJ* and
24
25 705 *MHS* coordinate the trial center at the Helsinki University, Finland. All authors contributed to
26
27 706 conception of and designing the trial. *IR* drafted the manuscript. *JB* provided cost-
28
29 707 effectiveness expertise in clinical trial design. *LDK* provided statistical expertise and will
30
31 708 conduct the primary statistical analysis. *KSF* drafted the sections for the microbiota and fMRI
32
33 709 ancillary studies. *MCAK* and *LML* drafted the section for the persuasive technology study.
34
35 710 *HAHW, SKJ, MHS, RN, IAB, MRO, KHP, RV* and *MV* critically reviewed the manuscript. All
36
37 711 authors approved the final version.
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43
44 713 **Acknowledgements**

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47
48 715 thank the study participants.
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52
53 717 **Trial status**

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55 718 The Trial was registered in ClinicalTrials.gov. Title of registration: The (Cost)Effectiveness
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57 719 of Increasing Protein Intake on Physical Functioning in Older Adults. Number of
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720 identification: NCT03712306. October 2018. Recruitment commenced in November 2018
721 and ended in November 2019.

For peer review only

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990 **Tables**

991

992 **Table 1.** Eligibility criteria for participation of the PROMISS trial

Inclusion criteria

Community-dwelling

Age \geq 65 yearsHabitual protein intake $<$ 1.0 g protein/kg aBW/dBMI \geq 18.5 kg/m² and \leq 32.0 kg/m² (based on measured weight and height)

Self-reported ability to eat independently

Ability to speak, write and read the local language (Finnish or Dutch)

Ability to walk 400 meters within 15 minutes, without the use of a walker and no rest

longer than 60 seconds

Exclusion criteria

Inability or unwillingness to provide informed consent

Current participation in supervised behavioural or lifestyle intervention that intervenes with the PROMISS trial

Not able to visit the research site in the following next 6 months

Bedridden, wheelchair bound or always being inside

Self-reported Parkinson's disease

Diagnosis of severe kidney disease (self-reported)

Diagnosis of type I diabetes mellitus (self-reported)

Diagnosis of type II diabetes mellitus and requiring use of insulin started within 6 months (self-reported)

Current treatment of cancer (with the exception of basal cell carcinoma)

Vegan diet

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3 Severe allergies to certain food product (peanut, gluten)
4

5 Diagnosis of an eating disorder (self-reported)
6

7 Purposefully lost/gained > 3 kg in the past three months
8

9
10 Heart problems in the past three months, defined as heart attack, angioplasty, heart surgery,
11
12 stroke, severe shortness of breath during physical activity (self-reported)
13

14 Alcohol abuse during past 6 months, defined as the AUDIT-C score ≥ 3 (86)
15

16 Low cognitive status, defined as the MMSE score ≤ 20 (32)
17

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19 993 Abbreviations: aBW; adjusted body weight, BMI; Body Mass Index, MMSE; Mini-Mental
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21 994 State Examination.
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996 **Table 2.** Measurements and time of measurements in the PROMISS trial

		Timeline				
		Screening	Screening	Baseline	3-month	6-month
		(phone)	visit	visit	FU visit	FU visit
			(prior			
			baseline)			
Topic	Specific variables					
Oral informed consent (phone)		✓				
Screening questionnaire (phone)	Sex, age, self-reported weight, height, eligibility criteria	✓				
Protein intake	Pro55+ screening (34) (phone)	✓				
Protein intake	Combination of three food diaries and three 24-h dietary recalls		✓		✓	✓
Written informed consent			✓			
Cognitive function	MMSE (32)		✓			

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1					
2					
3	Physical functioning	400-m walk test (41, 42)	✓		✓
4					
5					
6	Antropometrics	Measured body height	✓		
7					
8	Antropometrics	Measured body weight	✓	✓	✓
9					
10					
11	Demographic	Education, household		✓	
12					
13					
14	General characteristics	Perceived health, smoking status		✓	✓
15					
16	Body composition	Bio-electrical impedance		✓	✓
17					
18					
19	Body composition	Air displacement plethysmography (Dutch site		✓	✓
20		only)			
21					
22					
23					
24	Physical functioning	SPPB (46)		✓	✓
25					
26	Muscle strength	Handgrip strength		✓	✓
27					
28					
29	Muscle strength	Leg extension strength		✓	✓
30					
31					
32	Self-reported mobility limitations	Ability to walk 400m and climb one flight of		✓	✓
33		stairs			✓
34					
35					
36	Risk of sarcopenia	SARC-F questionnaire (59)		✓	✓
37					✓
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Malnutrition	GLIM phenotypic criteria (28)	✓		✓
Frailty	Frailty Fried Frailty Index (57)	✓		✓
Quality of Life	EuroQol 5D (63)	✓	✓	✓
Health care costs	RUD (64)	✓	✓	✓
Appetite	SNAQ-Appetite (65)	✓		✓
Physical activity	Accelerometers	✓	✓	✓
Process evaluation				✓
<i>Persuasive technology study</i>				
Communication style preferences	Personality traits form	✓		
Usage data of technology	Practical experiences, interaction data of technology (number of notifications, openings, registered food intake, games)		✓	✓
Attitude towards technology	Questionnaire			✓

Microbiota study

Oral health	Questionnaire	✓	✓
Oral microbiota	Tongue swab (16S rRNA sequencing)	✓	✓
Gut microbiota	Fresh frozen faecal sample (16S rRNA sequencing)	✓	✓

fMRI study

Oral microbiota	Fasted unstimulated salivary sample (16S rRNA sequencing)	✓	✓
Nutritional and microbial markers	Blood sample	✓	✓
Appetite	VAS-scores of appetite and central neural responses to food-cues measured by fMRI-scan	✓	✓

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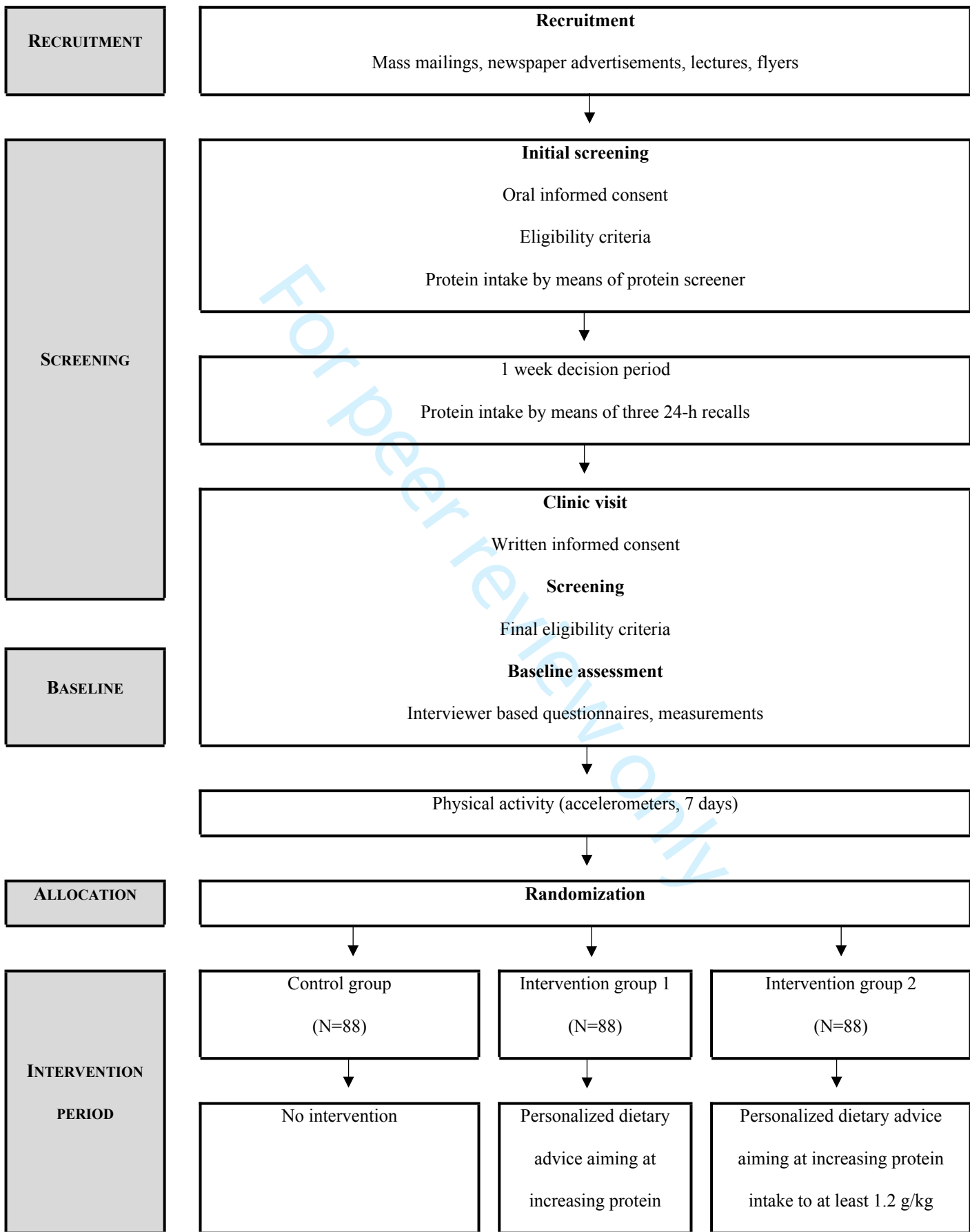
999 **Figure legends**

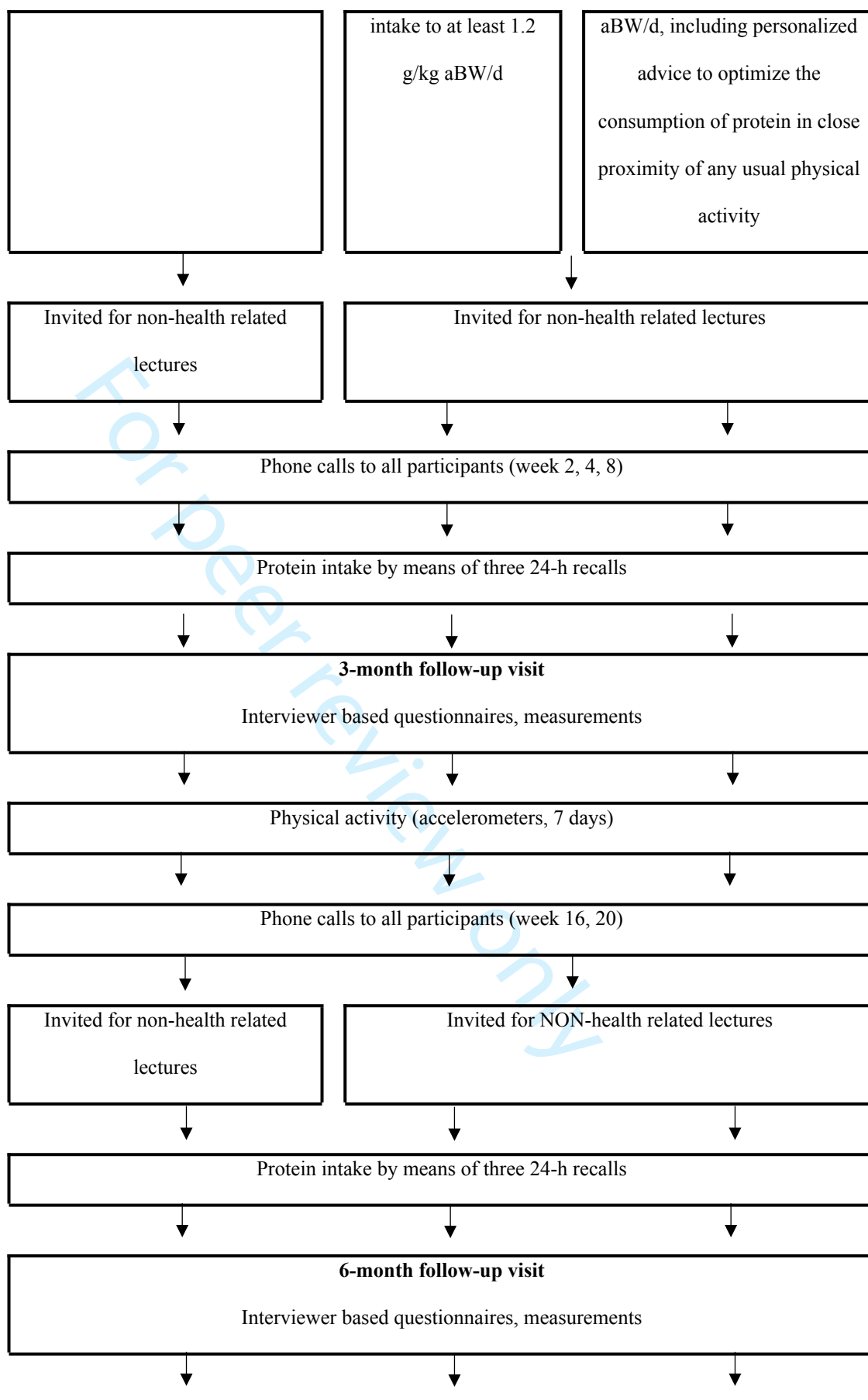
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1001 **Figure 1.** Study timeline of the PROMISS trial

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Physical activity (accelerometers, 7 days)

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Toestemmingsformulier proefpersoon

Effectiviteit van het verhogen van eiwitname op fysiek functioneren

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.
- Ik geef toestemming voor het informeren van mijn huisarts dat ik meedoe aan dit onderzoek.
- Ik geef toestemming voor het informeren van mijn huisarts over eventuele afwijkingen die tijdens het onderzoek gevonden worden.
- Ik geef toestemming voor het opvragen van informatie bij mijn huisarts over mijn nierfunctie, mochten er twijfels over de effecten op mijn gezondheid bestaan.
- Ik weet dat sommige mensen mijn gegevens kunnen inzien. Die mensen staan vermeld in deze informatiebrief.
- Ik geef toestemming voor het verzamelen en gebruiken van mijn gegevens op de manier en voor de doelen die in de informatiebrief staan.
- Ik geef toestemming om mijn gegevens op de onderzoekslocatie nog 15 jaar na dit onderzoek te bewaren.
- Ik wil meedoen aan dit onderzoek.

Naam proefpersoon:

Handtekening:

Datum : __ / __ / __

Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker (of diens vertegenwoordiger):

Handtekening:

Datum: __ / __ / __

Appendix

Appendix II

PROMISS project coordination, Vrije Universiteit Amsterdam, Department of Health Sciences, the Netherlands

Prof. Marjolein Visser, PhD – Principle investigator of the PROMISS project

Prof. Ingeborg A Brouwer, PhD – Project manager of the PROMISS project

Margreet Olthof, PhD – Financial manager of the PROMISS project

Rachel Vijlbrief – Assistant project manager of the PROMISS project

Trial sites

Vrije Universiteit Amsterdam, the Netherlands

Hanneke Wijnhoven, PhD - local principal investigator

Nanouk Bakker Schut – research intern

Judith Bosmans, PhD – researcher

Mariska Bout – dietician and research assistant

Ingeborg Brouwer, PhD – researcher

Nona Kerremans – research intern

Lothar Kuijper, PhD – researcher

Margreet Olthof, PhD – researcher

Ilse Reinders, PhD – local co-principal investigator

Marjon Veeke – dietician and research assistant

Rachel Vijlbrief – researcher

Marjolein Visser, PhD – researcher

Merel Vrijmoeth – dietician and research assistant

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8 University of Helsinki
9

10 Merja Suominen, PhD – local principal investigator
11

12 Kirsi Ali-Kovero – research assistant
13

14 Johannes Anttila – research intern
15

16 Aliisa Hyvönen – dietician and research assistant
17

18 Henriikka Jussila – research intern
19

20 Satu Jyväkorpi, PhD – local co-principal investigator
21

22 Riikka Niskanen – dietician and research assistant
23

24 Anna-Maria Piipponen – research intern and research assistant
25

26 Kaisu Pitkälä, PhD – researcher
27

28 Heli Salmenius-Suominen – researcher
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35 *Ancillary studies*
36

37 Persuasive technology study
38

39 Michel Klein, PhD – principal investigator of the persuasive technology study, *Vrije*
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41 *Universiteit Amsterdam, the Netherlands*
42

43 Laura van der Lubbe – researcher, *Vrije Universiteit Amsterdam, the Netherlands*
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49 Microbiota study and fMRI study
50

51 Fredrik Bäckhed, MD, PhD – researcher, *University of Gothenburg, Gothenburg, Sweden and*
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53 *University of Copenhagen, Copenhagen, Denmark*
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55 Kristien Fluitman, MD – researcher, *Amsterdam UMC, location VUmc, Amsterdam, the*
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57 *Netherlands*
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5 *Netherlands*
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8 Bart Keijser, PhD – researcher, *TNO earth, Zeist, the Netherlands and Academic Center for*
9
10 *Dentistry Amsterdam, the Netherlands*
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12
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15 *Amsterdam UMC, location AMC and location VUmc, Amsterdam, the Netherlands*
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page number, section
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	4; end of abstract, 8; study design, 31; trial status
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	32; trial status
Funding	4	Sources and types of financial, material, and other support	30; funding
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 31; author contributions
	5b	Name and contact information for the trial sponsor	-
	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	30, 31; funding
	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)	Appendix II

Introduction

1				
2	Background and	6a	Description of research question and justification for	5, 6, 7, 8
3	rationale		undertaking the trial, including summary of relevant	
4			studies (published and unpublished) examining	
5			benefits and harms for each intervention	
6				
7		6b	Explanation for choice of comparators	7
8				
9	Objectives	7	Specific objectives or hypotheses	7; second
10				paragraph
11				
12	Trial design	8	Description of trial design including type of trial (eg,	8; study design
13			parallel group, crossover, factorial, single group),	
14			allocation ratio, and framework (eg, superiority,	
15			equivalence, noninferiority, exploratory)	
16				
17				
18				
19	Methods: Participants, interventions, and outcomes			
20	Study setting	9	Description of study settings (eg, community clinic,	8; eligibility
21			academic hospital) and list of countries where data	criteria, 9-11;
22			will be collected. Reference to where list of study	recruitment and
23			sites can be obtained	screening
24				
25				
26	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If	8; eligibility
27			applicable, eligibility criteria for study centres and	criteria
28			individuals who will perform the interventions (eg,	
29			surgeons, psychotherapists)	
30				
31				
32	Interventions	11a	Interventions for each group with sufficient detail to	13-15;
33			allow replication, including how and when they will	intervention
34			be administered	
35				
36		11b	Criteria for discontinuing or modifying allocated	-
37			interventions for a given trial participant (eg, drug	
38			dose change in response to harms, participant	
39			request, or improving/worsening disease)	
40				
41				
42		11c	Strategies to improve adherence to intervention	14; compliance
43			protocols, and any procedures for monitoring	
44			adherence (eg, drug tablet return, laboratory tests)	
45				
46		11d	Relevant concomitant care and interventions that	-
47			are permitted or prohibited during the trial	
48				
49	Outcomes	12	Primary, secondary, and other outcomes, including	16; primary
50			the specific measurement variable (eg, systolic	outcome, 17-19;
51			blood pressure), analysis metric (eg, change from	secondary
52			baseline, final value, time to event), method of	outcomes, 19, 20;
53			aggregation (eg, median, proportion), and time point	other measures
54			for each outcome. Explanation of the clinical	
55			relevance of chosen efficacy and harm outcomes is	
56			strongly recommended	
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1				
2	Participant	13	Time schedule of enrolment, interventions (including	12, 13; study
3	timeline		any run-ins and washouts), assessments, and visits	timeline, Figure 1
4			for participants. A schematic diagram is highly	
5			recommended (see Figure)	
6				
7	Sample size	14	Estimated number of participants needed to achieve	20; sample size
8			study objectives and how it was determined,	
9			including clinical and statistical assumptions	
10			supporting any sample size calculations	
11				
12				
13	Recruitment	15	Strategies for achieving adequate participant	9-11; recruitment
14			enrolment to reach target sample size	and screening
15				

Methods: Assignment of interventions (for controlled trials)

Allocation:

16				
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18				
19				
20	Sequence	16a	Method of generating the allocation sequence (eg,	11, 12
21	generation		computer-generated random numbers), and list of	Randomization,
22			any factors for stratification. To reduce predictability	allocation and
23			of a random sequence, details of any planned	masking
24			restriction (eg, blocking) should be provided in a	
25			separate document that is unavailable to those who	
26			enrol participants or assign interventions	
27				
28				
29				
30	Allocation	16b	Mechanism of implementing the allocation sequence -	
31	concealment		(eg, central telephone; sequentially numbered,	
32	mechanism		opaque, sealed envelopes), describing any steps to	
33			conceal the sequence until interventions are	
34			assigned	
35				
36				
37	Implementation	16c	Who will generate the allocation sequence, who will	12; second
38			enrol participants, and who will assign participants to	paragraph of
39			interventions	study time line
40				
41	Blinding	17a	Who will be blinded after assignment to interventions	12;
42	(masking)		(eg, trial participants, care providers, outcome	Randomization,
43			assessors, data analysts), and how	allocation and
44				masking, 20;
45				statistical
46				analyses plan
47				
48				
49		17b	If blinded, circumstances under which unblinding is	-
50			permissible, and procedure for revealing a	
51			participant's allocated intervention during the trial	
52				
53				

Methods: Data collection, management, and analysis

1				
2	Data collection	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	12, 13; study timeline, 15; intervention fidelity, 16; primary outcome, 17, 18, 19; secondary outcomes, 19, 20; other measures
3	methods			
4				
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13				
14		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	-
15				
16				
17				
18				
19	Data	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	22; Data quality assurance and data management
20	management			
21				
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23				
24				
25				
26				
27	Statistical	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	20, 21, 22; Statistical analyses plan
28	methods			
29				
30				
31				
32				
33		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	20, 21, 22; Statistical analyses plan
34				
35				
36				
37				
38		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)	20, 21, 22; Statistical analyses plan
39				
40				
41				
42				
43				
44	Methods: Monitoring			
45	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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55		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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2	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	22; Participants' safety
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7	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-
8				
9				
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12	Ethics and dissemination			
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14	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	30; Ethics approval and consent to participate
15				
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18				
19	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-
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23				
24				
25				
26	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9, 10, 11 recruitment and screening, 12, 13; study timeline, 30; Ethics approval and consent to participate
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37		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	23, 24; Persuasive technology study, 24, 25, 26; Microbiota study, 26; fMRI study.
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45	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	22, 23; Data quality assurance and data management, 30; Consent for publication
46				
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53	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	30; Competing interests
54				
55				
56				
57	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	30; Availability of data and materials
58				
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2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care,	N/A
3	post-trial care		and for compensation to those who suffer harm from	
4			trial participation	
5				
6	Dissemination	31a	Plans for investigators and sponsor to communicate	-
7	policy		trial results to participants, healthcare professionals,	
8			the public, and other relevant groups (eg, via	
9			publication, reporting in results databases, or other	
10			data sharing arrangements), including any	
11			publication restrictions	
12				
13				
14		31b	Authorship eligibility guidelines and any intended	-
15			use of professional writers	
16				
17				
18		31c	Plans, if any, for granting public access to the full	-
19			protocol, participant-level dataset, and statistical	
20			code	
21				
22	Appendices			
23				
24	Informed consent	32	Model consent form and other related	Appendix I
25	materials		documentation given to participants and authorised	
26			surrogates	
27				
28				
29	Biological	33	Plans for collection, laboratory evaluation, and	N/A
30	specimens		storage of biological specimens for genetic or	
31			molecular analysis in the current trial and for future	
32			use in ancillary studies, if applicable	
33				

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

Effectiveness and cost-effectiveness of personalized dietary advice aiming at increasing protein intake on physical functioning in community-dwelling older adults with lower habitual protein intake: rationale and design of the PROMISS randomized controlled trial

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3 1 **Effectiveness and cost-effectiveness of personalized dietary advice aiming at increasing**
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5 2 **protein intake on physical functioning in community-dwelling older adults with lower**
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7 3 **habitual protein intake: rationale and design of the PROMISS randomized controlled**
8
9 4 **trial**

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For peer review only

1
2
3 31 **Abstract**
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5 32 **Introduction:** Short-term metabolic and observational studies suggest that protein intake
6
7
8 33 above the recommended dietary allowance of 0.83 g/kg body weight (BW)/d may support
9
10 34 preservation of lean body mass and physical function in old age, but evidence from
11
12 35 randomized controlled trials is inconclusive.

13
14 36 **Methods and analysis:** The PRevention Of Malnutrition In Senior Subjects in the EU
15
16
17 37 (PROMISS) trial examines the effect of personalized dietary advice aiming at increasing
18
19 38 protein intake with or without advice regarding timing of protein intake to close proximity of
20
21 39 usual physical activity, on change in physical functioning after 6 months among community-
22
23 40 dwelling older adults (≥ 65 y) with a habitual protein intake of < 1.0 g/kg adjusted (a)BW/d.
24
25 41 Participants (n=264) will be recruited in Finland and the Netherlands, and will be randomized
26
27 42 into three groups; two intervention groups and one control group. Intervention group 1
28
29 43 (n=88) receives personalized dietary advice and protein enriched food products in order to
30
31 44 increase their protein intake to at least 1.2 g/kg aBW/d. Intervention group 2 (n=88) receives
32
33 45 the same advice as described for intervention group 1, and in addition advice to consume 7.5-
34
35 46 10 g protein through protein (en)rich(ed) foods within half an hour after performing usual
36
37 47 physical activity. The control group (n=88) receives no intervention. All participants will be
38
39 48 invited to attend lectures not related to health. The primary outcome is 6-months change in
40
41 49 physical functioning measured by change in walk time using a 400-m walk test. Secondary
42
43 50 outcomes are: 6-months change in the Short Physical Performance Battery score, muscle
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45 51 strength, body composition, self-reported mobility limitations, quality of life, incidence of
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47 52 frailty, incidence of sarcopenia risk and incidence of malnutrition. We also investigate cost-
48
49 53 effectiveness by change in health care costs.
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54 54 **Discussion:** The PROMISS trial will provide evidence whether increasing protein intake, and
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56 55 additionally optimizing the timing of protein intake, has a positive effect on the course of
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3 56 physical functioning after 6 months among community-dwelling older adults with a habitual
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5 57 protein intake of < 1.0 g/kg aBW/d.
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8 58 **Ethics and disseminations:** The study has been approved by the Ethics Committee of the
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10 59 Helsinki University Central Hospital, Finland (ID of the approval; HUS/1530/2018) and The
11
12 60 Medical Ethical Committee of the Amsterdam UMC, location VUmc, Amsterdam, the
13
14 61 Netherlands (ID of the approval; 2018.399). All participants provided written informed
15
16 62 consent prior to being enrolled onto the study. Results will be submitted for publication in
17
18 63 peer-reviewed journals and will be made available to stakeholders (i.e. older adults, health
19
20 64 care professionals and industry).
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26 66 **Trial status**

27
28 67 The Trial was registered in ClinicalTrials.gov. Title of registration: The (Cost)Effectiveness
29
30 68 of Increasing Protein Intake on Physical Functioning in Older Adults. Number of
31
32 69 identification: NCT03712306. Registered 19 October 2018. Recruitment commenced in
33
34 70 October 2018 and ended in November 2019.
35
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37
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39

40 72 **Keywords:** older adults, protein intake, physical functioning, RCT, malnutrition, protein
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42 73 recommendation, 400 meter walk.
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3 74 **Strength and limitations of this study**
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- 5 75 • This large randomized controlled trial addresses a key question whether dietary advice
6 to increase protein intake to ≥ 1.2 g/kg adjusted body weight (aBW)/d is beneficial for
7 physical functioning in community-dwelling older adults.
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10 77
11
12 78 • This trial will also examine the additional effect of the timing of protein intake in close
13 proximity of usual physical activity on change in physical functioning.
14 79
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16 80 • Participants included had a habitual protein intake of < 1.0 g/kg aBW/d.
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18 81 • The lack of blinding of the study participants and nutritionists who also collect data on
19 all outcome measures is a limitation of the study design.
20 82
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22 83 • Another limitation of this study is that the biological value of the total protein intake (i.e.
23 type of amino acids) is unknown.
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85 Introduction

86 There is an ongoing debate on whether or not older adults should be recommended a protein
87 intake above the current recommended daily allowance (RDA) established by the European
88 Food Safety Authority (EFSA) of 0.83 g/kg body weight (BW)/d for adults (1). International
89 panels of geriatricians, nutritional experts and scientists have proposed at least 1.0-1.2 g
90 protein/kg BW/d for healthy older adults in order to maintain and regain muscle mass,
91 strength and function (2, 3).

92 The proposed increase of the RDA for older adults is merely based on results from
93 short term metabolic and epidemiological studies. Several metabolic studies showed that
94 older adults (≥ 65 y) have a lower muscle protein synthesis (MPS) following protein intake
95 compared to younger adults (4-6), and that higher protein intake enhances MPS in older
96 adults when compared to lower protein intake (1.2 g/kg BW/d vs. 0.8 g/kg BW/d (7), or ≥ 30
97 g/d vs. 15 g/d (8)). In addition, the anabolic threshold (i.e. optimal dose of dietary protein in a
98 meal that stimulates MPS) is 70% higher in older compared to younger adults (5).
99 Epidemiological studies have shown that higher dietary protein intake in older adults, defined
100 as > 0.9 g/kg BW/d (9) or > 1.0 g/kg BW/d (10-12) is associated with lower risk of weight
101 loss (11), better disability trajectories (12), less loss of lean mass (9), or lower risk of
102 developing functional impairments (10).

103 Despite the evidence from metabolic and epidemiological studies, causal evidence to
104 support beneficial effects of protein intake at or above 1.0 g/kg BW/d based on randomized
105 controlled trials (RCTs) is not conclusive. One systematic review showed no beneficial effect
106 of increasing protein intake on lean body mass, muscle cross-sectional area, muscle strength,
107 or physical performance (13). Of the 36 studies included in the systematic review, 26 studies
108 presented mean habitual protein intake of the study participants which ranged between 0.78
109 and 1.5 g/kg BW/d, with only one study below the protein RDA of 0.8 g/kg BW/d (13). The

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3 110 relatively high mean habitual protein intake may explain the absence of a beneficial effect of
4
5 111 additional protein. Another explanation may be that the amount of protein provided might not
6
7 112 have been sufficient to augment MPS. I.e. a protein intake of 25 – 30 g is required to
8
9 113 stimulate MPS and maintain muscle mass (14, 15), though the amounts provided varied
10
11 114 between 10 g/d (3d/wk) and total intake of 125 g/day or were not reported. Of the trials
12
13 115 published after the systematic review, Park et al. showed that intake of 1.5 g/kg BW/d for 12
14
15 116 weeks resulted in higher muscle mass and improved gait speed compared to intake of 0.8
16
17 117 g/kg BW/d in undernourished pre-frail and frail older adults (16). Ten Haaf et al. showed a
18
19 118 positive effect of increasing protein intake for 12 weeks on lean body mass in active older
20
21 119 adults with a habitual protein intake of < 1.0 g/kg BW/d (17). Beelen et al. found no effects
22
23 120 of protein supplementation on physical performance among older adults after hospital
24
25 121 discharge (18), however, baseline protein intake was already 1.0 g/kg BW/d in the control
26
27 122 group and 1.5 g/kg BW/d in the intervention group. Finally, Bhasin et al. showed no
28
29 123 beneficial effects other than a decrease in fat mass after a controlled diet with 1.3 g/kg BW/d
30
31 124 of protein for 6 months compared to a control diet consisting of 0.8 g protein /kg BW/d (19)
32
33 125 among functionally limited community-dwelling men aged ≥ 65 years. However, mean BMI
34
35 126 of the participants was quite high (30.3 kg/m²), which may have resulted in an overestimation
36
37 127 of baseline protein requirements. Based on inconsistent findings, more RCTs in older adults
38
39 128 with lower habitual protein intake are needed to determine the potential effect of increasing
40
41 129 protein intake on physical functioning outcomes.

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49 130 Previous studies among older adults showed that protein supplementation in
50
51 131 combination with resistance exercise has more beneficial effects on body composition,
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53 132 muscle strength and physical function compared to resistance exercise alone (20-26). The
54
55 133 underlying hypothesis is that protein supplementation augments the adaptive response of
56
57 134 skeletal muscle to resistance exercise. In addition, there is evidence that timing of protein
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3 135 intake in close proximity of physical activity stimulated MPS to greater extent than when
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5 136 timed at other hours during the day (27). To our knowledge, there are no RCTs investigating
6
7 137 the effect of timing protein intake in close proximity of physical activities on physical
8
9
10 138 functioning.

11
12 139 The PROMISS trial is designed to fill in some of the current knowledge gaps on the
13
14 140 optimal amount of dietary protein in older community-dwelling adults and timing of protein
15
16 141 intake in relation to physical activity. Its primary objective is to examine the effectiveness of
17
18 142 personalized dietary advice aiming at increasing protein intake to at least 1.2 g/kg adjusted
19
20 143 (a)BW/d on change in physical functioning after 6 months measured by change in walk time
21
22 144 using a 400-m walk test among community-dwelling older adults with a habitual protein
23
24 145 intake of < 1.0 g/kg aBW/d. Additionally, it examines the combined effect of personalized
25
26 146 dietary advice aiming at increasing protein intake to at least 1.2 g/kg aBW/d and advice
27
28 147 aiming at optimizing the timing of protein intake in close proximity of usual physical activity.
29
30
31 148 The secondary objectives are to examine the effectiveness of personalized dietary advice
32
33 149 aiming at increasing protein intake to at least 1.2 g/kg adjusted on 6-month changes in
34
35 150 physical functioning measured by the Short Physical Performance Battery (SPPB) score,
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37 151 muscle strength, body composition, self-reported mobility limitations, quality of life,
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39 152 incidence of frailty, incidence of sarcopenia risk, and incidence of malnutrition and change in
40
41 153 health care costs.

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46 154 In three ancillary studies the following additional objectives are addressed; 1) the
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48 155 effect of using persuasive technology on adherence to personalized dietary advice aiming at
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50 156 increasing protein to at least 1.2 g/kg aBW/d, 2) the effect of personalized dietary advice
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52 157 aiming at increasing protein intake to at least 1.2 g/kg aBW/d on the oral and gut microbiota
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54 158 composition, and 3) the effect of personalized dietary advice aiming at increasing protein
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3 159 intake to at least 1.2 g/kg aBW/d on central neural responses to food-cues in brain areas of
4
5 160 interest.
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9
10 162 **Methods and analysis**

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12 163 *Study design*

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14 164 The PROMISS trial is a multicentre randomized controlled trial (ClinicalTrials.gov identifier:
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17 165 NCT03712306) designed to examine the effectiveness of personalized dietary advice aiming
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19 166 at increasing protein intake and advice on optimizing the timing of protein intake in close
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21 167 proximity of usual physical activity on change in physical functioning after 6 months.

22
23 168 Participants will be randomised into three groups: one control group (no intervention);
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25
26 169 intervention group 1) receiving personalized dietary advice aiming at increasing protein
27
28 170 intake to at least 1.2 g/kg aBW/d; and intervention group 2) receiving personalized dietary
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30 171 advice aiming at increasing protein intake to at least 1.2 g/kg aBW/d, including personalized
31
32 172 advice to optimize the timing of protein intake in close proximity of usual physical activity.
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37 174 *Eligibility criteria*

38
39 175 The eligibility criteria are proposed to include a study group of community-dwelling older
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41 176 adults (65+) with a habitual protein intake < 1.0 g/kg aBW/d. Inclusion and exclusion criteria
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43 177 are listed in Table 1, and some are described in more detail below.
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179 **Table 1.** Eligibility criteria for participation of the PROMISS trial

Inclusion criteria

Community-dwelling

Age \geq 65 yearsHabitual protein intake $<$ 1.0 g protein/kg aBW/dBMI \geq 18.5 kg/m² and \leq 32.0 kg/m² (based on measured weight and height)

Ability to walk 400 meters within 15 minutes, without the use of a walker and no rest longer than 60 seconds

Exclusion criteria

Inability or unwillingness to provide informed consent

Not able to eat independently (self-reported)

Not able to speak, write and read the local language (Finnish or Dutch)

Current participation in supervised behavioural or lifestyle intervention that intervenes with the PROMISS trial

Not able to visit the research site in the following next 6 months

Bedridden, wheelchair bound or always being inside

Diagnosis of severe kidney disease (self-reported)

Parkinson's disease (self-reported)

Diagnosis of type I diabetes mellitus (self-reported)

Diagnosis of type II diabetes mellitus and requiring use of insulin started within 6 months (self-reported)

Current treatment of cancer (with the exception of basal cell carcinoma)

Vegan diet

Severe allergies to certain food product (peanut, gluten)

Diagnosis of an eating disorder (self-reported)

Purposefully lost/gained > 3 kg in the past three months

Heart problems in the past three months, defined as heart attack, angioplasty, heart surgery, stroke, severe shortness of breath during physical activity (self-reported)

Alcohol abuse during past 6 months, defined as the AUDIT-C score ≥ 3 (28)

Low cognitive status, defined as the MMSE score ≤ 20 (29)

180 Abbreviations: aBW; adjusted body weight, BMI; Body Mass Index, MMSE; Mini-Mental

181 State Examination.

182

183 Older adults with a BMI of < 18.5 kg/m² will be excluded, because these participants are
184 likely to be undernourished (30) and should preferably receive general nutritional care that is
185 not provided in this trial. Those with a BMI of > 32.0 kg/m² will be also excluded, because a
186 high BMI (> 30.0 kg/m²) is associated with poorer physical function (31) and disability (32)
187 in old age and intentional weight loss by lifestyle interventions lead to a reduced mortality
188 risk (33). In light of this evidence, older adults with a BMI > 32.0 kg/m² should preferably be
189 advised to lose weight, which is not the aim of the present study and may interfere with the
190 study objective. Because participants of intervention group 2 will be advised to consume
191 protein rich foods in close proximity of usual physical activity, older adults who are
192 bedridden, wheelchair bound or do not go outside will be excluded from the trial. Older
193 adults with a diagnosis of severe kidney disease (i.e. treatment of a nephrologist and/or
194 protein-restricted diet, self-reported) will also be excluded as they should be advised to limit
195 their protein intake (2, 34-36). Older adults with a low cognitive status (Mini-Mental State
196 Examination (MMSE) score ≤ 20 (29)) will be excluded, as participants should be able to
197 understand and follow dietary advice if randomized to one of the intervention groups.

198

199 Calculation of protein intake using adjusted body weight

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3 200 To calculate habitual protein intake in g/kg aBW/d for all (potential) participants and
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5 201 recommended protein intake (participants in the two intervention groups), we apply adjusted
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7 202 BW depending on participants' age and BMI. We use adjusted body weight because
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10 203 underweight persons require extra protein to build muscle tissue, while in overweight
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12 204 persons, much 'extra weight' is adipose tissue. Protein intake in g/kg aBW/d is based on self-
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14 205 reported BW during screening and afterwards based on measured BW during the baseline
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16 206 assessment, which is further used throughout the study. For those with a BMI > 25.0 to 32.0
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18 207 kg/m² (age ≤ 70 y) or > 27.0 to 32.0 kg/m² (age > 70 y) we apply aBW corresponding to a
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20 208 BMI of respectively 25.0 or 27.0 kg/m². For those with a BMI > 18.5 to < 22.0 kg/m² (age >
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22 209 70 y) we apply aBW corresponding to a BMI of respectively 18.5 or 22.0 kg/m² (37). For the
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24 210 recommended protein intake, we apply adjusted BW which is based on baseline measured
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26 211 BW.
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33 213 *Recruitment and screening*

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35 214 Two hundred and sixty-four community-dwelling adults aged 65 years and older will be
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37 215 recruited at two study sites (metropolitan area of Finland including Helsinki, Espoo, Vantaa,
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39 216 Kauniainen, and Amsterdam, the Netherlands). The recruitment strategy includes mass
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41 217 mailing using addresses obtained from a random sample of the Finnish Population Registry
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43 218 (in Finland only), newspaper advertisements, media coverage, lectures, oral presentations to
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45 219 the target group, informing professionals working with older adults and flyers which will be
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47 220 distributed at locations where many community-dwelling older adults visit.
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51 221 Older adults who are interested in participating will be asked to contact the local
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53 222 PROMISS research team (by phone or by e-mail). Thereafter, screening by phone takes
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55 223 place, only when verbal informed consent is given, in which the majority of the eligibility
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57 224 criteria will be assessed along with an explanation of the study. Only those with a lower
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3 225 habitual protein intake (< 1.0 g/kg aBW/d) will be invited for the first clinic visit. Assessment
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5 226 of habitual protein intake will be estimated in two steps: 1) initial screening by phone; 2) a
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7 227 full dietary assessment based on a combination of three food diaries and three 24-h dietary
8
9 228 recalls to confirm lower habitual protein intake. Step 1, the initial screening is performed by
10
11 229 phone using the Protein Screener 55+ (Pro55+, available for use in English, Finnish and
12
13 230 Dutch: see www.proteinscreener.nl/#/). This screening tool was specifically developed and
14
15 231 validated for this purpose (38). The screening results in a probability score (0-100%) of
16
17 232 having a protein intake below 1.0 g/kg aBW/d. At a probability of $> 30\%$, sensitivity and
18
19 233 specificity are optimally balanced (38). In the PROMISS trial we select persons with a
20
21 234 probability score varying between $> 15\%$ (when initial response rates to recruitment
22
23 235 strategies are low) and $> 30\%$ (when initial response rates are high), for the second step of
24
25 236 assessing habitual protein intake. Those who fulfill the eligibility criteria receive further
26
27 237 information on the study and a food diary with a booklet with pictures of portion sizes by post
28
29 238 to support the 24-h dietary recalls. After a minimum of one week of consideration, the
30
31 239 research staff contacts the older adults, and among those who are still willing to participate
32
33 240 the full dietary assessment will take place (step 2). These potential participants will be asked
34
35 241 to keep track of their dietary intake by filling out the provided food diary for three
36
37 242 consecutive days (three weekdays; or two weekdays and one weekend day). The booklet with
38
39 243 pictures of portion sizes that they received earlier will help them accurately filling out the
40
41 244 diary. Each day after, they will be called by a nutritionist to go through their food diary of the
42
43 245 day before (24-h dietary recall). Potential participants are asked whether these days are
44
45 246 representative for their habitual diet. In case one of the three days is not representative, mean
46
47 247 protein intake is based on two instead of three days. In case of more than one non-
48
49 248 representative day, the person will be excluded. The food intake data based on the 24-h
50
51 249 dietary recall will be entered into the program 'Fineli' for the Finnish data (39) and into the
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3 250 program 'Eetmeter' of the Dutch Nutrition Center using an extended version of the Dutch
4
5 251 Food Composition Table of 2016 for the Dutch data (40) to calculate intake of macronutrients
6
7 252 and micronutrients (vitamin D and vitamin B12). Participants with an actual protein intake \geq
8
9 253 1.0 g/kg aBW/d (based on self-reported BW) will be excluded.

10
11
12 254 Potential participants with a mean habitual protein intake < 1.0 g/kg aBW/d (based on
13
14 255 the three 24-h dietary recalls), will be invited for the clinic visit, where final eligibility
15
16 256 criteria will be assessed; MMSE > 20 , ability to walk 400 m within 15 minutes (the use of a
17
18 257 cane is allowed, but without the use of a walker and no rest longer than 60 seconds), and BMI
19
20 258 of ≥ 18.5 kg/m² and ≤ 32.0 kg/m² based on measured BW and body height. When all
21
22 259 eligibility criteria are met, participants are included in the PROMISS trial.
23
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27 260

28 261 *Randomization, allocation and masking*

29
30 262 Randomization by means of a stratified block randomization procedure will be performed by
31
32 263 an independent statistician. Participants will be allocated in a 1:1:1 ratio to the three groups.
33
34 264 The size of the randomization blocks is three. Participants will be stratified according to their
35
36 265 baseline habitual protein intake (< 0.9 or $0.9-1.0$ g/kg aBW/d) and sex to ensure
37
38 266 homogeneous distribution of baseline habitual protein intake and sex in the three groups
39
40 267 across the two recruitment sites, because there may be a different intervention effect by
41
42 268 baseline habitual protein or sex. In case couples are eligible we will allocate them to the same
43
44 269 intervention group to limit interference between intervention groups. We will randomly select
45
46 270 on which partner the randomization for the intervention group is based. Any resulting
47
48 271 unbalance in the number of subjects per treatment arm will be corrected in the randomization
49
50 272 of the next block. Due to the nature of the study, researchers, nutritionists and participants are
51
52 273 not blinded to the study group.
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3 275 *Study timeline*
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5 276 The first clinic visit starts with written informed consent, and when participants are eligible,
6
7 277 the baseline assessment will be performed. The baseline assessment consists of
8
9
10 278 questionnaires (frailty status, risk of sarcopenia, self-reported mobility limitations, quality of
11
12 279 life (QoL) and health care costs) and measurements (physical function, muscle strength and
13
14 280 body composition). See below ‘primary and secondary outcomes’ and ‘other measures’ for
15
16 281 details on these assessments. An accelerometer will be attached to measure physical activity
17
18 282 for 7 subsequent days.
19
20

21 283 After the baseline assessment, participants will be randomised to one of the three
22
23 284 study groups done by the nutritionists and they will inform the participants in which group
24
25 285 they are allocated to. Participants randomized to one of the two intervention groups will be
26
27 286 invited for a consultation meeting at the clinic to receive their personalized dietary advice,
28
29 287 and personalized advice on optimizing the timing of protein intake in close proximity of usual
30
31 288 physical activity (intervention group 2 only). This will take place within 2 weeks after the
32
33 289 baseline assessment since the personalized advice needs to be composed by the nutritionist.
34
35 290 The baseline assessment is considered the start of the study period for participants of the
36
37 291 control group, while the consultation meeting is considered the start of the study period for
38
39 292 participants of the intervention groups.
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43

44 293 One week prior to the 3-month follow-up visit, dietary intake will be assessed again
45
46 294 by means of a combination of three food diaries and three 24-h dietary recalls. The 3-month
47
48 295 follow-up visit will take place at the clinic and includes measurement of BW, assessment of
49
50 296 self-reported mobility limitations, risk of sarcopenia, QoL, health care costs and the
51
52 297 accelerometer will be attached to measure physical activity for 7 subsequent days.
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55 298 One week prior to the 6-month follow-up visit (final measurement) dietary intake will
56
57 299 again be assessed by means of a combination of three food diaries and three 24-h dietary
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3 300 recalls, which allows us to determine compliance to the dietary advice. The 6-month follow-
4
5 301 up visit at the clinic includes all measurements performed during the baseline visit and the
6
7 302 accelerometer is attached again to measure physical activity for the next 7 days. Finally,
8
9
10 303 among participants of the intervention groups only, several questions regarding the
11
12 304 appreciation and adherence of the intervention and participants' intention to follow the
13
14 305 dietary advice in the future will be asked in order to perform a process-evaluation. Figure 1
15
16
17 306 shows the study timeline and Table 2 provides an overview of all measurements.
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19
20 307

21 308 *Intervention*

22
23 309 Participants in the intervention group 1 will receive a personalized dietary advice face-to-face
24
25 310 by nutritionists dedicated to this study aiming at increasing their protein intake to at least 1.2
26
27 311 g/kg aBW/d (without increasing total daily energy intake), based on their habitual dietary
28
29 312 characteristics (based on three 24-h recalls), protein intake and measured BW assessed at
30
31 313 baseline. Participants will be asked if they usually prepare the main meal; whether they eat
32
33 314 the meal at a e.g. community home; whether they consume ready-to-eat meals; whether they
34
35 315 use meal services; and if they eat at family or friends' home. All their answers will be
36
37 316 incorporated in the personalized dietary advice. Participants will receive written dietary
38
39 317 advice accompanied by a verbal explanation from the nutritionist. Participants can contact the
40
41 318 nutritionist at any time by mail or phone in case any question arise. The advice includes the
42
43 319 use of regular protein rich food products and protein enriched food products provided by the
44
45 320 research team, and will be based on personal dietary preferences. Protein enriched food
46
47 321 products that can be incorporated within the regular diet include protein bars, cereals,
48
49 322 puddings, coconut water and whey powder, which will be freely provided and shipped to
50
51 323 participants' home. Those products can be incorporated in the dietary advice as they can
52
53 324 make it easier to increase protein intake due to their high protein content.
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325 **Table 2.** Measurements and time of measurements in the PROMISS trial

		Timeline				
		Screening	Screening	Baseline	3-	6-
		(phone)	visit	visit	month	month
			(prior		FU visit	FU visit
			baseline)			
Topic	Specific variables					
Oral informed consent (phone)		✓				
Screening questionnaire (phone)	Sex, age, self-reported weight, height, eligibility criteria	✓				
Protein intake	Pro55+ screening (38) (phone)	✓				
Protein intake	Combination of three food diaries and three 24-h dietary recalls		✓		✓	✓
Written informed consent			✓			
Cognitive function	MMSE (29)		✓			

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Physical functioning	400-m walk test (41, 42)	✓		✓
Antropometrics	Measured body height	✓		
Antropometrics	Measured body weight	✓	✓	✓
Demographic	Education, household		✓	
General characteristics	Perceived health, smoking status		✓	✓
Body composition	Bio-electrical impedance		✓	✓
Body composition	Air displacement plethysmography (Dutch site only)		✓	✓
Physical functioning	SPPB (43)		✓	✓
Muscle strength	Handgrip strength		✓	✓
Muscle strength	Leg extension strength		✓	✓
Self-reported mobility limitations	Ability to walk 400m and climb one flight of stairs		✓	✓
Risk of sarcopenia	SARC-F questionnaire (44)		✓	✓

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2					
3	Malnutrition	BMI < 22.0 kg/m ² or unintentional weight loss	✓		✓
4					
5		> 5% in the last 6 months			
6					
7					
8	Frailty	Frailty Fried Frailty Index (45)	✓		✓
9					
10	Quality of Life	EuroQol 5D (46)	✓	✓	✓
11					
12					
13	Health care costs	RUD (47)	✓	✓	✓
14					
15					
16	Appetite	SNAQ-Appetite (48)	✓		✓
17					
18	Physical activity	Accelerometers	✓	✓	✓
19					
20					
21	Process evaluation				✓
22					
23					
24					
25					
26	<i>Persuasive technology study</i>				
27					
28	Communication style preferences	Personality traits form	✓		
29					
30					
31	Usage data of technology	Practical experiences, interaction data of		✓	✓
32					
33		technology (number of notifications, openings,			
34					
35		registered food intake, games)			
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37					
38	Attitude towards technology	Questionnaire			✓
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Microbiota study

Oral health	Questionnaire	✓	✓
Oral microbiota	Tongue swab (16S rRNA sequencing)	✓	✓
Gut microbiota	Fresh frozen faecal sample (16S rRNA sequencing)	✓	✓

fMRI study

Oral microbiota	Fasted unstimulated salivary sample (16S rRNA sequencing)	✓	✓
Nutritional and microbial markers	Blood sample	✓	✓
Appetite	VAS-scores of appetite and central neural responses to food-cues measured by fMRI-scan	✓	✓

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3 327 Participants receive guidelines how to incorporate the protein enriched food products within
4
5 328 their diet. The dietary advice will also incorporate the advice to consume at least one daily
6
7 329 meal consisting of ≥ 35 g protein, as studies have shown that this amount increases MPS in
8
9 330 older adults (49-51).

10
11
12 331 Participants in intervention group 2 will receive the same dietary advice as
13
14 332 intervention group 1, and the personalized advice to consume at least 7.5-10 g protein
15
16 333 through protein (en)rich(ed) food products within half an hour after performing usual
17
18 334 physical activity as this may enhance resistance exercise induced MPS (27). One RCT among
19
20 335 older adults has shown that protein supplementation in combination with resistance exercise
21
22 336 had beneficial effects on e.g. muscle mass and function, but no differences in effect were
23
24 337 found between protein consumption pre versus post resistance exercise (52). We therefore
25
26 338 recommend protein intake after physical activity as this is a uniform and more feasible advice
27
28 339 compared to ‘in close proximity of’, and might also result in less stomach discomfort as
29
30 340 compared to protein consumption prior to physical activity. Usual physical activity is defined
31
32 341 as either physical exercise (e.g. biking, swimming, tennis) or the most intensive activities of
33
34 342 daily living when the participant does not engage in physical exercise (e.g. gardening,
35
36 343 housekeeping, doing groceries) for a minimum of 30 minutes. The advice is linked to most
37
38 344 extensive or longest physical activity. Participants are instructed not to become more or less
39
40 345 physically active but merely to shift their physical activity or protein intake moment.

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43 346 During the intervention period, nutritionists will plan follow-up phone calls in
44
45 347 consultation with the participants during week 2, week 4, week 8, week 16 and week 20 to
46
47 348 ask if they have understood the advice and are able to adhere to the advice. In addition, any
48
49 349 issues related to the use of protein enriched food product can be discussed (intervention
50
51 350 groups only). If necessary, changes in the dietary advice will be made, for example when
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53 351 weight change > 2 kg has occurred (based on self-assessment). Participants allocated to the
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3 352 control group do not receive any intervention, but are contacted on similar time points as the
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5 353 intervention groups to ask how they are doing.

7 354 All participants are invited to a minimum of one organized lecture on non-health related
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9
10 355 themes and other social events during the trial in order to stimulate their commitment to the
11
12 356 trial. Separate lectures/events (with the same topic) are organized for the intervention groups
13
14 357 and the control group to prevent interference between intervention arms. Participants can
15
16 358 freely attend those lectures and all travel costs will be reimbursed.

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21 360 Compliance

23
24 361 We will collect dietary intake prior to the 3-month follow-up visit (by means of the
25
26 362 combination of three food diaries and three 24-h dietary recalls) to assess compliance to the
27
28 363 dietary advice. This information allows the nutritionists to provide additional advice – if
29
30 364 needed – for participants in the intervention groups, which will be provided during the 3-
31
32 365 month follow-up visit. Dietary intake will again be assessed at follow-up and compliance to
33
34 366 the dietary advice will be determined.

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39 368 Intervention fidelity

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42 369 To ensure good adherence to the intervention protocols at both study sites, all personnel
43
44 370 working for the trial have undergone extensive training. The nutritionists will follow written
45
46 371 standardized operational procedures to develop and provide the personalized dietary advice
47
48 372 (with or without additional advice to consume protein within half an hour after usual physical
49
50 373 activity). Four times during the conduct of the trial, the nutritionists from one site will visit
51
52 374 the other site to attend assessments, and potentially notice and correct differences in order to
53
54 375 ensure identical execution of the trial at both sites. In addition, monthly Skype-meetings will
55
56 376 be held between all staff involved in the execution of the trial at both sites to solve any
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3 377 potential day-to-day issues in a standardized way. Furthermore, identical participant
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5 378 brochures and other printed materials have been developed and translated to Dutch and
6
7
8 379 Finnish language.
9

10 380

11
12 381 *Outcomes*

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14 382 Primary outcome

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16
17 383 The primary outcome of the PROMISS trial is 6-months change in physical functioning
18
19 384 measured by change in walk time using a 400-m walk test (Long Distance Corridor Walk)
20
21 385 (41, 42). This test is predictive for higher risk of mortality, incident cardiovascular disease
22
23 386 and mobility limitation and disability (53). One advantage of this continuous outcome is that
24
25 387 it enables discrimination between categories of risk among participants (54), and it is less
26
27 388 prone to a ceiling effect as compared to other functional outcome measures (e.g. SPPB) (55).
28
29 389 The course for the 400-m test is 20-m long and marked by a traffic cone and tape line at the
30
31 390 beginning and end. For all participants, the test will begin with a mandatory 40-m walk
32
33 391 (warm-up) at their usual pace. Thereafter the 400-m test starts with the feet behind and just
34
35 392 touching the starting line and ends after 10 complete rounds when one foot is behind the end
36
37 393 line. For the 400-m test, older adults will be instructed to walk as fast as possible at a pace
38
39 394 they can maintain for 400 m. Standardized encouragement will be given each lap, including
40
41 395 the number of laps remaining. At the 6-month follow-up visit, older adults are allowed to use
42
43 396 a cane, can take rest as needed (but no rest longer than 60 seconds) and there will be a time
44
45 397 limit of 17 minutes. Time will be recorded to the nearest second.
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51 398

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53 399 Secondary outcomes

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56 400 The secondary outcomes are changes in physical performance, muscle strength, body
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58 401 composition, self-reported mobility limitations, QoL, incidence of frailty, incidence of
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3 402 sarcopenia risk, and incidence of malnutrition. We will also investigate change in health care
4
5 403 costs.

6
7
8 404 Physical performance will be assessed by means of the Short Physical Performance
9
10 405 Battery (SPPB) (43). The SPPB assesses lower extremity function which consists of three
11
12 406 timed tests: repeated (5x) chair stands test, 4-meter walk test and three standing balance tests
13
14 407 (ability to stand with the feet together in the side-by-side, semi-tandem, and tandem
15
16 408 positions). The total score ranges from 0-12. A higher score indicates better physical
17
18 409 functioning.

19
20
21 410 Muscle strength will be determined by hand-grip strength (kg). Hand-grip strength is
22
23 411 an indicator of overall muscle strength (56) and a higher hand grip strength is associated with
24
25 412 decreased risk of physical disabilities (57) and all-cause mortality in old age (57, 58).

26
27 413 Maximum grip strength will be measured three times at each hand during baseline and 6-
28
29 414 month follow-up visit. We will use a digital dynamometer (Saehan Digital Hand held
30
31 415 dynamometer) adjusted for hand size. Participants will be measured in an upright sitting
32
33 416 position with the forearms supported by the armrest of a chair according to a standardized
34
35 417 protocol (59). The mean of the maximum score of left and right hand will be used for
36
37 418 analyses. Muscle strength will also be determined by leg extension strength (N). A higher leg
38
39 419 extension strength is associated with decreased risk of mobility disability (60, 61) and lower
40
41 420 risk of early mortality (62-64). Leg extension strength will be assessed using a chair designed
42
43 421 to measure leg extension strength (65). Maximum leg extension strength will be measured
44
45 422 three times for each leg during baseline and 6-month follow-up visit. The mean of the
46
47 423 maximum score of left and right leg will be used for analyses.

48
49 424 Body composition will be estimated by means of bioelectrical impedance using the
50
51 425 BodyStat 1500MDD device, using the Kyle equation to determine fat percentage (%), fat
52
53 426 mass and fat-free mass (66) and the Sergi equation to determine appendicular skeletal muscle
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3 427 mass (ASMM, kg) (67). Additionally, at the Dutch study site, body composition (fat free
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5 428 mass (kg), fat mass and fat percentage (%)) will be measured by air displacement
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8 429 plethysmography (68).

9
10 430 Self-reported mobility limitations will be assessed by means of a questionnaire;
11
12 431 “Because of your health, how much difficulty do you have walking 400 meter?” and
13
14 432 “Because of your health, how much difficulty do you have climbing 10 steps?” Participants
15
16 433 will respond using a five level Likert scale: ‘no difficulty’, ‘a little difficulty’, ‘some
17
18 434 difficulty’, ‘a lot of difficulty’ and ‘unable to do the activity’. Mobility limitation is defined
19
20 435 as two consecutive reports of having any difficulty walking 400 meter or climbing 10 steps
21
22 436 without resting due to a health or a physical problem.

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26 437 QoL will be measured using the EuroQol 5D – 5L questionnaire (46).

27
28 438 Incident frailty will be assessed using the Fried criteria (45). Participants will be
29
30 439 considered ‘frail’ when three or more components are present. Those with no components
31
32 440 will be considered ‘robust’, whereas those with one or two components will be considered
33
34 441 ‘prefrail’. The criteria include 1) self-reported unintentional weight loss (> 4 kg in past 6
35
36 442 months), 2) self-reported exhaustion (based on two questions from the Center for
37
38 443 Epidemiologic Studies Depression (CES-D) scale on exhaustion in the past week at baseline
39
40 444 and follow-up: “I felt that everything I did was an effort” and “I could not get going”. Scores
41
42 445 ranges from 1 ‘rarely or none of the time’ to 4 ‘always or most of the time’. A score of 3 or 4
43
44 446 on either question indicates exhaustion (69), 3) weakness (grip strength in the lowest 20% of
45
46 447 the whole study population based on the mean of the maximum scores, adjusted for gender
47
48 448 and BMI), 4) slow walking speed (walk time on the 4m walk test in the slowest 20% of the
49
50 449 whole study population, adjusted for gender and height), and 5) low physical activity (total
51
52 450 counts per week based on the accelerometer data in the lowest 20% of physical activity for
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54 451 each gender). Incident frailty is considered deterioration of frailty status; i.e. from robust at
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3 452 baseline to pre-frail or frail at follow-up or from pre-frail at baseline to frail at follow. Frail
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5 453 participants at baseline will be excluded from these analyses.

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7 454 Incidence of sarcopenia risk will be assessed with the SARC-F questionnaire (44);
8
9 455 how much effort do you experience when 1) lifting and carrying a bag of 4.5 kilo, 2) walking
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11 456 across a room, 3) transferring from a chair or bed, 4) climbing a flight of 10 stairs, and 5)
12
13 457 how many times have you fallen in the past year. Answering option include no effort (0
14
15 458 points), a bit of effort (1 point) and a lot of effort (2 points), where a score equal to or greater
16
17 459 than 4 is predictive of sarcopenia and poor outcomes. Participants with risk of sarcopenia at
18
19 460 baseline will be excluded from these analyses.

20
21 461 Incidence of malnutrition will be defined as BMI < 22.0 kg/m² or unintentional
22
23 462 weight loss > 5% in the last 6 months. Malnourished participants at baseline will be excluded
24
25 463 from these analyses.

26
27 464 A modified version of the Resource Utilization in Dementia Questionnaire (RUD)
28
29 465 (47) will be used to collect data on health care and social utilization costs over the period
30
31 466 three month prior to baseline, three months prior to the 3-month follow-up visit and three
32
33 467 months prior to 6-month follow-up visit. Costs include costs of primary and secondary care,
34
35 468 complementary care, informal care and home care.

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37 469

38
39 470 Other measures

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41 471 BW will be measured without shoes in underwear to the nearest 0.1 kg using a digital
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43 472 calibrated scale (Finland; SECA 877, the Netherlands; Marsden M-520). Body height will be
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45 473 measured to the nearest millimeter using a SECA stadiometer for mobile height
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47 474 measurements (Finland; SECA 217, the Netherlands; SECA 214). Corrections will be made
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49 475 to adjust the measured body weight for clothing, shoes or a cast (minus 1 kg for each
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51 476 element), and to adjust the measured body height for shoes (minus 1 cm). Change in BW and
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3 477 BMI at 3-month and 6-month will be calculated. As the personalized dietary advice is
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5 478 isocaloric, no significant difference in BW or BMI are expected. Physical activity will be
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7 479 objectively assessed by means of an accelerometer (Axivity, AX3) during 7 subsequent days
8
9 480 after each clinic visit (baseline, 3-month follow-up visit and 6-month follow-up visit). The
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11 481 accelerometer will be attached by a nutritionist to the frontal part of the right thigh in the
12
13 482 mid-point between iliac crest and patella bone when sitting down, with a surgical plaster.
14
15 483 Participants can perform any physical activity as the accelerometer is water resistant.
16
17 484 Appetite will be measured with SNAQ-Appetite questionnaire (48). Dietary intake will be
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19 485 assessed by means of a combination of three food diaries and three 24-h dietary recalls prior
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21 486 the 3-month follow-up visit and the 6-month follow-up visit.
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28 488 *Sample size and statistical analyses*

30 489 Sample size

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33 490 The study is powered to detect a substantial meaningful change of 28 sec (SD=61 sec) (70)
34
35 491 between the respective intervention groups and the control group on the primary outcome
36
37 492 walk time on the 400-m walk test, assuming a 2-sided test at $\alpha=0.05$ with a power of 0.8. For
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39 493 this, 75 participants per group are needed, which is 225 in total. Assuming a drop-out of 15%
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41 494 (which was reported in a comparable study of Bhasin et al. 2018 (19), the total number of
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43 495 study participants to be included in the study is $n=264$. Therefore, we aim to include a total of
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45 496 $n=132$ at each study site ($n=44$ per study group per study site).
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49 497

51 498 Statistical analyses plan

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54 499 Statistical reporting will be according to the CONSORT standards (71). The collected data at
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56 500 the two study sites will be pooled together at the Amsterdam site, with a variable indicating
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58 501 study site. All statistical analyses on primary and secondary outcome measures will be
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3 502 performed by an independent statistician blinded for group allocation. Baseline
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5 503 characteristics will be described (percentages, means \pm standard deviations) by study group.

6
7 504 The primary analyses will be based on the intention-to-treat principle, i.e. data from
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10 505 participants allocated to the intervention groups will be analyzed as part of those groups,
11
12 506 irrespectively of their level of adherence to the advice. Multiple Imputation using
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14 507 multivariate Imputation by Chained Equations will be used to impute missing cost and effect
15
16 508 data. The continuous primary outcome (change in 400-m walk time) will be analyzed using
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18 509 mixed model regression analyses with study site as random variable. We will adjust for
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20 510 baseline 400-m walk time as well as baseline protein intake (g/kg aBW/d) and sex (the
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22 511 stratification factors for randomization). We will compare intervention effects of the
23
24 512 respective intervention groups versus the control group. Effects will also be expressed in
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26 513 Cohen's d and the corresponding 95% confidence intervals will be calculated, which allows
27
28 514 comparison between intervention effect estimates between different outcome measures. The
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30 515 secondary outcomes and other measures will be analyzed using mixed model regression
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32 516 analogously to the primary outcome. For binary secondary outcomes, generalized estimating
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34 517 equation models will be used. With regard to time-to-event analyses (incident sarcopenia risk
35
36 518 and mobility limitations) Cox proportional hazard models will be used. Time-to-event is
37
38 519 defined as the time of the start of the study period to the date of the first occurrence of the
39
40 520 event (3-month follow-up visit or 6-month follow-up visit). Participants who do not meet
41
42 521 these criteria will be censored at the latest time we had information available. We will
43
44 522 perform subgroup analyses stratified by baseline protein intake (< 0.9 or 0.9-1.0 g/kg
45
46 523 aBW/d), sex and baseline 400-m time (based on median) for the primary and secondary
47
48 524 outcomes.

49 525 Per-protocol analyses will also be conducted as a sensitivity analysis. Effect estimates
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51 526 for change in primary and secondary outcome measures will be calculated for participants
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3 527 from the intervention groups who reached the protein target of at least 1.2 g/kg aBW/d after
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5 528 both 3 and after 6 months (mean protein intake based on the three 24-h dietary recalls) vs.
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7
8 529 participants from the control group. Data will be analyzed using SPSS (IBM SPSS Statistics.
9
10 530 Armonk, NY). A two-sided *P*-value of 0.05 is considered statistical significant.

11
12 531 The cost-effectiveness analysis will be performed from a healthcare perspective. Total
13
14 532 mean costs during the study will be related to physical functioning (the 400-m walk test) and
15
16 533 change in Quality-Adjusted Life-Years based on the EuroQol 5D questionnaire. Mixed model
17
18 534 regression analyses will be used to estimate differences in the primary outcome of the
19
20 535 respective interventions groups versus the control group. Linear regression analyses will be
21
22 536 used to estimate differences in QoL (expressed as Quality-Adjusted Life-Years) and
23
24 537 healthcare costs. Incremental cost-effectiveness ratios will be calculated by dividing the
25
26 538 difference in costs by the difference in effects. Statistical uncertainty will be estimated using
27
28 539 bias-corrected accelerated bootstrapping (5000 replications) and will be presented using cost-
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30 540 effectiveness planes and cost-effectiveness acceptability curves.

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37 542 *Participants' safety*

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39 543 In case any (medical) questions arise during the screening or intervention period, participants
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41 544 can consult an independent medical doctor. All adverse events and serious adverse events
42
43 545 will be tracked by the nutritionists during the follow-up phone calls, 3-month follow-up visit
44
45 546 and 6-month follow-up visit to assess their potential relationship to the intervention at both
46
47 547 sites and will be documented in the final report. Adverse events will be reported within 7 days
48
49 548 (death or life threatening situations) or within 15 days (in case of other adverse events) of
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51 549 first knowledge to The Medical Ethical Committee of the Amsterdam UMC, location Vumc
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53 550 (required for the Dutch site only).

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3 552 *Data quality assurance and data management*
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5 553 Research data will be collected at each site and each visit (baseline, 3-month follow-up visit
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7 554 and 6-month follow-up visit) using a standardized protocol with the same order of
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9
10 555 assessments, and entered twice in separate electronic datasets. When discrepancies between
11
12 556 the datasets are found, the original questionnaire will be consulted. Questionnaire items and
13
14 557 measurements will include the corresponding variable names to minimize errors in data
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16
17 558 entering. Finally the two final electronic dataset (one from each site) containing all data will
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19 559 be pooled. A data catalogue and codebook will be developed.
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21 560 Original questionnaires will be stored in a secure manner at each site in an area with
22
23 561 limited access. All records that contain names (i.e. informed consent forms), will be stored
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25 562 separately from study records identified by code number. All databases will be secured with
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27
28 563 password-protected access systems.
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33 565 *Patient and Public Involvement*
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35 566 The PROMISS randomized controlled trial is designed by the Faculty of Science (VU
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37 567 Amsterdam, the Netherlands) and the Department of General Practice and Primary Health
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39 568 Care (University of Helsinki, Finland) (please see Appendix I for the PROMIS trial group), a
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41
42 569 collaboration of the EU Horizon 2020 PROMISS Project. Two medical and one ethical
43
44 570 advisor are involved in the study. As part of the PROMISS project, we previously performed
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46
47 571 three pilot studies as a preparation of the long term PROMISS randomized trial, of which one
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49 572 is published (72). We included the feedback of the participants in designing the long term
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51 573 PROMISS randomized trial; participants enjoyed participating in the pilot studies and they
52
53 574 liked the frequent contact with the nutritionist. We also tested which protein enriched food
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56 575 products they preferred and included those products in the long term PROMISS randomized
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58 576 trial which they liked the most. Older adults are not involved in recruitment of participants or
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3 577 conduct of the study. Results of this study will be disseminated to participants through
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5 578 sending them a lay abstract with the results and conclusions of the study. At the end of the
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7
8 579 study, each participant will receive a fact-sheet with personal results of dietary intake data,
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10 580 hand grip strenght, body composition mesures and body weight. Participant burden of the
11
12 581 pilot intervention was assessed using informal feedback from older adults participating in one
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14
15 582 of three pilot studies.
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19 584 *Ancillary studies*

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21 585 Within the PROMISS trial, three ancillary studies will be conducted: 1) persuasive
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23 586 technology study, 2) microbiota study, and 3) fMRI study.
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27
28 588 1. Persuasive technology study

29
30 589 The primary aim of this study is to examine the effect of persuasive technology on adherence
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32
33 590 to the personalized dietary advice aiming at increasing protein intake to at least 1.2 g/kg
34
35 591 aBW/d in a sub-sample of Dutch participants from intervention group 1 (n=24) and
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37 592 intervention group 2 (n=24), i.e. the first 24 participants of intervention group 1 and the first
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39 593 24 participants of intervention group 2 that consent to it (writting informed consent will be
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41 594 signed).
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44 595 Participants will be provided with a food storage box that registers which provided
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46 596 protein enriched food products are taken out. The food box is used to store the protein
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48 597 enriched food products provided by the research team. Participants will also receive a tablet
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50 598 that allows participants to register any consumed protein enriched food products and supports
51
52 599 them in finding alternative food products that contain a comparable amount of protein. For
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54 600 this, the system uses the personalized dietary advice as provided by the nutritionist and data
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56 601 from the storage box. The tablet application aims to stimulate adherence to the dietary advice
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3 602 by providing tailored and personalized messages. In addition, personality characteristics and
4
5 603 communication style preferences that are determined via a questionnaire completed at
6
7 604 baseline are used to tailor the style and tone of these messages (73).

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10 605 In addition to the personalized messages, half of the participants from intervention
11
12 606 group 1 and 2 who participate in the persuasive technology sub-study will also receive a
13
14 607 gamified version of the tablet application ($n=12 + n=12$). In this version, participants can earn
15
16 608 game-points by registering their consumed protein (en)rich(ed) food products and by playing
17
18 609 mini-games about the protein content of foods (i.e. guess the protein content, more-or-less
19
20 610 protein). The distribution of receiving the gamified version vs. standard version is quasi-
21
22 611 randomized, where we will balance the group size.

23
24
25 612 At the consultation meeting, participants receive their food storage box and tablet.
26
27 613 Both are fully configured, i.e. they are loaded with their personal dietary advice. After the 6-
28
29 614 month follow-up visit, participants will be asked to return the equipment and fill out
30
31 615 questions on the feasibility and user experience of the provided persuasive technology.

32
33 616 The secondary objectives are 1) to investigate to what extent participants perceive
34
35 617 messages of which the style and tone are adapted to their personal characteristics as
36
37 618 personalized and adequate, and 2) to determine the effect of gamification on the effectiveness
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39 619 and feasibility of the persuasive technology.

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43 621 2. Microbiota study

44
45 622 In the microbiota study, the effect of personalized dietary advice aiming at increasing protein
46
47 623 intake in community-dwelling older adults with lower habitual protein intake on both the oral
48
49 624 and gut microbiota is investigated. The study will be conducted at both study sites.

50
51 625 The human microbiota consists of the 4×10^{13} micro-organisms that inhabit the body
52
53 626 (74). The emergence of next generation DNA sequencing techniques at the start of the 21st
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3 627 century has allowed more detailed study of the microbiota and since then, the microbiota
4
5 628 composition has been associated with both health and disease (75), as well as aging itself (76,
6
7 629 77). Moreover, several interventional studies proved that dietary changes also affect the gut
8
9 630 microbiota, with the first microbial shifts being evident within 48 hours (78). The altered
10
11 631 microbiota in turn, can differentially affect the human host metabolism through the
12
13 632 production of metabolically active metabolites. Less is known about the oral microbiota. It
14
15 633 was found to be associated with oral health and function and even nutritional status (79, 80),
16
17 634 but its possible role in undernutrition in older adults has not been investigated.

18
19 635 A fresh frozen fecal sample and tongue swab is collected at baseline and 6-month
20
21 636 follow-up visit once written informed consent is provided. Participants from either the control
22
23 637 group or intervention group 1 can be included in this study. Participants from the intervention
24
25 638 group 2 are excluded to limit the number of groups and parameters in this exploratory study.
26
27 639 Additional exclusion criteria are: use of systemic antibiotics in the three months prior to the
28
29 640 first sampling visit, diagnosis with inflammatory bowel disease and prolonged
30
31 641 institutionalization (> 4 weeks) in the three months prior to the first sampling visit. There is
32
33 642 no restriction other than consent rate to the number of PROMISS participants that will be
34
35 643 included in this side study.

36
37 644 Once all samples from all participants are collected, fecal samples are shipped to the
38
39 645 Wallenberg Laboratory of Cardiovascular and Metabolic research (at the University of
40
41 646 Gothenburg, in Sweden) for 16S rRNA sequencing using sequencing methods previously
42
43 647 described (81). The tongue swabs will be send to the Netherlands Organisation for Applied
44
45 648 Scientific Research for 16S rRNA sequencing as is previously described (82).

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47 649

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49 650 3. fMRI study

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3 651 In the fMRI study, we will investigate the effect of personalized dietary advice aiming at
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5 652 increasing protein intake in community-dwelling older adults with lower habitual protein
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7 653 intake on central brain circuits involved in the regulation of appetite. Several studies
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9
10 654 demonstrated that increasing protein intake affects appetite (83) and the gut microbiota (84).
11
12 655 However, none have studied the effects on both simultaneously, or the interaction. A
13
14 656 functional MRI (fMRI) scan will be used to measure the brain responses to visual or actual
15
16
17 657 food cues. Brain activity in response to food cues will also be related to (shifts) in the gut
18
19 658 microbiota. Therefore, only participants from the microbiota side study can be included in
20
21 659 this study, with additional exclusion criteria: being claustrophobic, being diagnosed with a
22
23 660 mental disorder (e.g. depression or addiction), being uncorrectable visually or hearing
24
25 661 impaired, or having a contra-indication for MRI-scans (e.g. having a pacemaker). Up to 50
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27
28 662 participants will be included in this side study. This side study will only be conducted at the
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31 663 Dutch study site.

32
33 664 Once written informed consent is provided, participants who are included in this side
34
35 665 study will be asked to visit the Amsterdam University Medical Centre, location VUmc, for an
36
37 666 fMRI scan twice during the study period: at baseline and at 6-month follow-up visit. Prior to
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39
40 667 the fMRI-scan, additional salivary and blood samples will be collected for determination of
41
42 668 additional nutritional and microbial biomarkers. The protocol for the fMRI experiments have
43
44 669 been previously described (85, 86).
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48 49 671 **Discussion**

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51 672 There is an ongoing discussion whether the EFSA RDA of 0.8 g protein /kg BW/d is
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53 673 sufficient for older adults and whether it should be increased to at least 1.0-1.2 g protein/kg
54
55 674 BW/d to support muscle health and functioning. National guidelines of some European
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58 675 countries already increased their RDA, i.e. the RDA of the German-speaking countries (D-A-
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3 676 CH) is increased to 1.0 g/kg BW/d (87), and the Nordic Nutrition Recommendation has
4
5 677 increased their RDA to 1.2 g/kg BW/d (88). The PROMISS trial is the first RCT which will
6
7 678 investigate the effect of personalized dietary advice aiming at increasing protein intake and
8
9 679 the combined effect of personalized dietary advice aiming at increasing protein and the
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11 680 timing of protein intake in close proximity of usual physical activity, on change in physical
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13 681 functioning after 6 months among community-dwelling older adults (≥ 65 y) with a habitual
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15 682 protein intake of < 1.0 g/kg adjusted (a)BW/d. The PROMISS trial will therefore provide
16
17 683 additional insight to the question whether the current EFSA RDA for protein for older adults
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19 684 should be increased to 1.2 g/kg aBW/d, and whether optimal timing of protein intake will
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21 685 additionally benefit physical functioning.
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28 687 A strong and unique aspect of the PROMISS trial is that we will include participants with a
29
30 688 habitual protein intake < 1.0 g/kg aBW/d, excluding those with a BMI < 18.5 and > 32.0
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32 689 kg/m². This will allow us to examine the effects of increasing protein intake from < 1.0 to at
33
34 690 least 1.2 g/kg aBW/d. An innovative component of our study is that we will investigate the
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36 691 combined benefit of increasing protein intake and timing of protein intake with usual physical
37
38 692 activity on physical functioning and other health related outcomes. Another strength is that in
39
40 693 our study the intervention is based on personalized dietary advice which is likely more
41
42 694 feasible in the long term to maintain in everyday life, compared to providing custom-prepared
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44 695 meals (19) or protein supplements (89, 90), as done in most other studies. Finally, we will be
45
46 696 able to investigate the effect of persuasive technology on adherence to the dietary advice
47
48 697 strategy, and the effect of the dietary advice on the microbiota composition and on central
49
50 698 responses to food-cues in brain areas involved in appetite regulation. One limitation of this
51
52 699 study is that the biological value of the total protein intake (i.e. type of amino acids) is
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54 700 unknown. Another limitation is that the duration of the trial might not be long enough to
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3 701 observe a sufficient amount of incident cases of e.g. risk of malnutrition, frailty or risk of
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5 702 sarcopenia.

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8 703
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10 704 In summary, this randomized controlled trial will demonstrate the effectiveness of
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12 705 personalized dietary advice aiming at increasing protein intake to at least 1.2 g protein/kg
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14 706 BW/d on physical functioning in older adults with a lower habitual protein intake, with or
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16
17 707 without the advice to consume protein in close proximity of usual physical activity.

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21 709 **Ethics and disseminations**

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23
24 710 The study has been approved by the Ethics Committee of the Helsinki University Central
25
26 711 Hospital, Finland (ID of the approval; HUS/1530/2018) and The Medical Ethical Committee
27
28 712 of the Amsterdam UMC, location VUmc, Amsterdam, the Netherlands (ID of the approval;
29
30 713 2018.399). Oral informed consent will be obtained from each participants before the
31
32
33 714 screening procedure and written informed (please see appendix II) consent will be obtained
34
35 715 from each participant before any measurement take place. Personal data were not identifiable
36
37 716 during the analysis.

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41
42 718 Results will be send to national and international conferences and will submitted for
43
44 719 publication in peer-reviewed journals. In addition, lay abstracts will be made available for
45
46 720 participants and the public. Links to research output and dissemination activities will be made
47
48
49 721 available on the PROMISS website, available at www.promiss-vu.eu and social media
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51 722 channels.

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3 1002 **Author Contributions**
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5 1003 *IR, HAHW, IAB, MRO* and *MV* obtained funding for the PROMISS project. *IR* and *HAHW*
6
7 1004 coordinate the trial center at the Vrije Universiteit Amsterdam, the Netherlands. *SKJ* and
8
9 1005 *MHS* coordinate the trial center at the Helsinki University, Finland. All authors contributed to
10
11 1006 conception of and designing the trial. *IR* drafted the manuscript. *JB* provided cost-
12
13 1007 effectiveness expertise in clinical trial design. *LDK* provided statistical expertise and will
14
15 1008 conduct the primary statistical analysis. *KSF* drafted the sections for the microbiota and fMRI
16
17 1009 ancillary studies. *MCAK* and *LML* drafted the section for the persuasive technology study.
18
19 1010 *HAHW, SKJ, MHS, RN, IAB, MRO, KHP, RV* and *MV* critically reviewed the manuscript. All
20
21 1011 authors approved the final version.
22
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27
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31
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34 1016 did not participate in the study design, collection, management, analysis and interpretation of
35
36 1017 data; or writing of the manuscript. They did not participate in the decision to submit the
37
38 1018 report for publication, nor had ultimate authority over any of these activities.
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44 1020 **Competing interests**
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46 1021 The authors declare that they have no competing interests.
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54
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3 1027 **Consent for publication**
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5 1028 Not applicable. Personal data were not identifiable during the analysis.
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10 1030 **Availability of data and materials**
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12 1031 Datasets from this research will be stored at the repository of the Vrije Universiteit
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14 1032 Amsterdam, the Netherlands and potentially available for other researchers after submitting a
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16 1033 research proposal.
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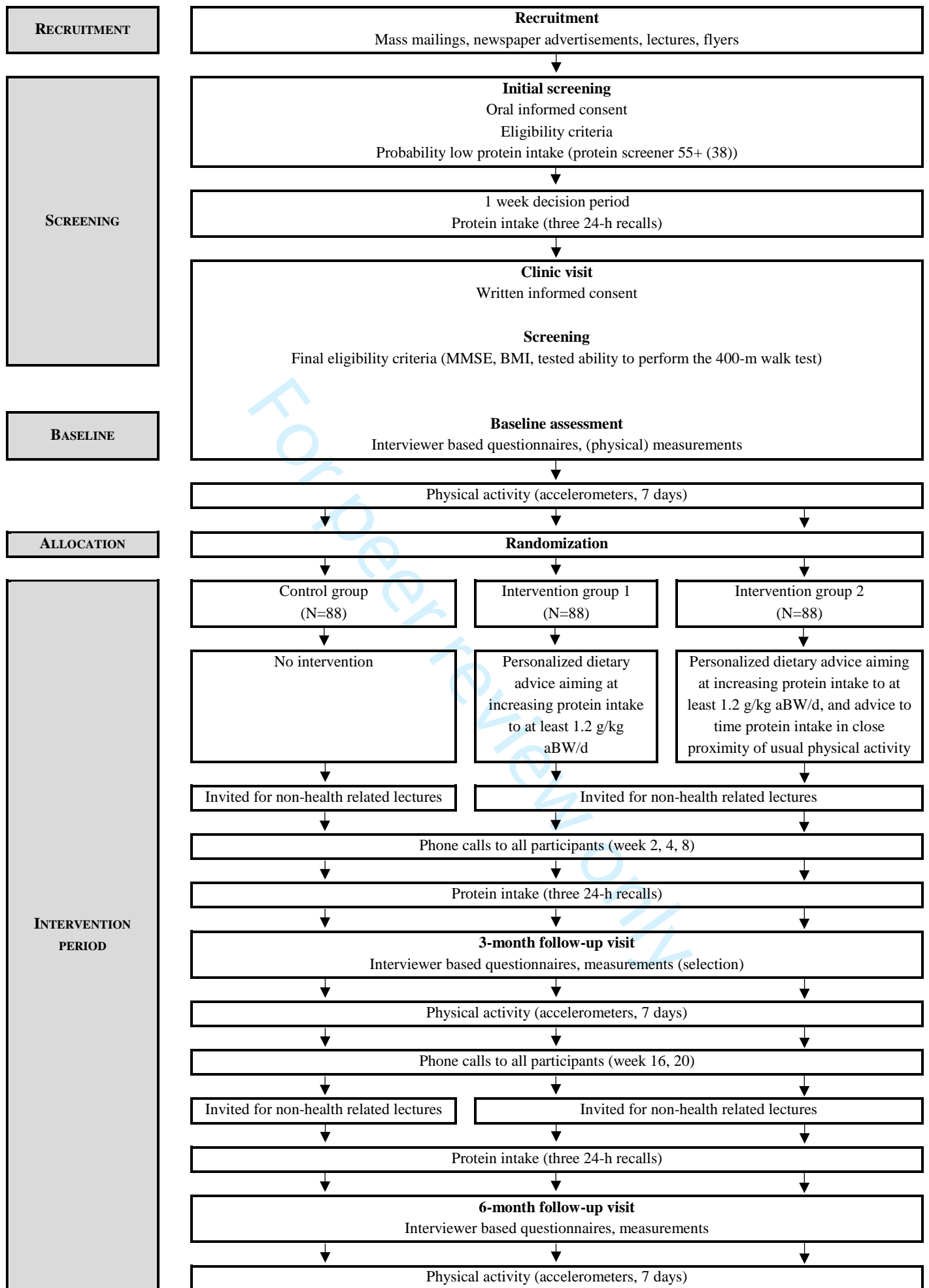
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3 1039 **Figure legends**
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8 1041 **Figure 1.** Study timeline of the PROMISS trial
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Appendix

Appendix I

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Margreet Olthof, PhD – Financial manager of the PROMISS project

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Toestemmingsformulier proefpersoon

Effectiviteit van het verhogen van eiwitname op fysiek functioneren

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.
- Ik geef toestemming voor het informeren van mijn huisarts dat ik meedoe aan dit onderzoek.
- Ik geef toestemming voor het informeren van mijn huisarts over eventuele afwijkingen die tijdens het onderzoek gevonden worden.
- Ik geef toestemming voor het opvragen van informatie bij mijn huisarts over mijn nierfunctie, mochten er twijfels over de effecten op mijn gezondheid bestaan.
- Ik weet dat sommige mensen mijn gegevens kunnen inzien. Die mensen staan vermeld in deze informatiebrief.
- Ik geef toestemming voor het verzamelen en gebruiken van mijn gegevens op de manier en voor de doelen die in de informatiebrief staan.
- Ik geef toestemming om mijn gegevens op de onderzoekslocatie nog 15 jaar na dit onderzoek te bewaren.
- Ik wil meedoen aan dit onderzoek.

Naam proefpersoon:

Handtekening:

Datum : __ / __ / __

Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker (of diens vertegenwoordiger):

Handtekening:

Datum: __ / __ / __



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

Section/item	ItemNo	Description	Page number, section
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	4; end of abstract, 9; study design
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	4; trial status
Funding	4	Sources and types of financial, material, and other support	49; funding
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 49; author contributions
	5b	Name and contact information for the trial sponsor	-
	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	49; funding
	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)	Appendix II

Introduction

1					
2	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	6, 7, 8, 9	
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7		6b	Explanation for choice of comparators	8	
8	Objectives	7	Specific objectives or hypotheses	8; second paragraph	
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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)	9; study design	
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18	Methods: Participants, interventions, and outcomes				
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20	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	9-11; eligibility criteria, 12-14; recruitment and screening	
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23	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)	9-11; eligibility criteria	
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26	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	16, 21-22; intervention	
27					
28		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)	-	
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31		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	22; compliance	
32					
33		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	-	
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36	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	23; primary outcome, 23-26; secondary outcomes, 26-27; other measures	
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2	Participant	13	Time schedule of enrolment, interventions (including	15-16; study
3	timeline		any run-ins and washouts), assessments, and visits	timeline, Figure 1
4			for participants. A schematic diagram is highly	
5			recommended (see Figure)	
6				
7	Sample size	14	Estimated number of participants needed to achieve	27; sample size
8			study objectives and how it was determined,	
9			including clinical and statistical assumptions	
10			supporting any sample size calculations	
11				
12				
13	Recruitment	15	Strategies for achieving adequate participant	12-14;
14			enrolment to reach target sample size	recruitment and
15				screening
16				

Methods: Assignment of interventions (for controlled trials)

Allocation:

21				
22	Sequence	16a	Method of generating the allocation sequence (eg,	14;
23	generation		computer-generated random numbers), and list of	Randomization,
24			any factors for stratification. To reduce predictability	allocation and
25			of a random sequence, details of any planned	masking
26			restriction (eg, blocking) should be provided in a	
27			separate document that is unavailable to those who	
28			enrol participants or assign interventions	
29				
30				
31	Allocation	16b	Mechanism of implementing the allocation sequence -	
32	concealment		(eg, central telephone; sequentially numbered,	
33	mechanism		opaque, sealed envelopes), describing any steps to	
34			conceal the sequence until interventions are	
35			assigned	
36				
37				
38	Implementation	16c	Who will generate the allocation sequence, who will	15; second
39			enrol participants, and who will assign participants to	paragraph of
40			interventions	study time line
41				
42	Blinding	17a	Who will be blinded after assignment to interventions	14;
43	(masking)		(eg, trial participants, care providers, outcome	Randomization,
44			assessors, data analysts), and how	allocation and
45				masking, 27;
46				statistical
47				analyses plan
48				
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51		17b	If blinded, circumstances under which unblinding is	-
52			permissible, and procedure for revealing a	
53			participant's allocated intervention during the trial	
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Methods: Data collection, management, and analysis

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2	Data collection	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	15-16; study timeline, 22-23, intervention fidelity, 23; primary outcome, 23-26; secondary outcomes, 26-27; other measures
3	methods			
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12		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	-
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18	Data	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	29-30; Data quality assurance and data management
19	management			
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26	Statistical	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	27-29; Statistical analyses plan
27	methods			
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32		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	27-29; Statistical analyses plan
33				
34				
35		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)	27-29; Statistical analyses plan
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41	Methods: Monitoring			
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43	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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52		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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2	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	29; Participants' safety
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7	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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12	Ethics and dissemination			
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14	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	36; Ethics and dissemination
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16				
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18	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-
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25	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12-14; recruitment and screening, 15-16; study timeline, 36; Ethics and dissemination
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34		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	31-32; Persuasive technology study, 32-33; Microbiota study, 33-34; fMRI study.
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42	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	29-30; Data quality assurance and data management
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48				50; Consent for publication
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51	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	49; Competing interests
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54	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	50; Availability of data and materials
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2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care,	N/A
3	post-trial care		and for compensation to those who suffer harm from	
4			trial participation	
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6	Dissemination	31a	Plans for investigators and sponsor to communicate	-
7	policy		trial results to participants, healthcare professionals,	
8			the public, and other relevant groups (eg, via	
9			publication, reporting in results databases, or other	
10			data sharing arrangements), including any	
11			publication restrictions	
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14		31b	Authorship eligibility guidelines and any intended	-
15			use of professional writers	
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18		31c	Plans, if any, for granting public access to the full	-
19			protocol, participant-level dataset, and statistical	
20			code	
21				
22	Appendices			
23				
24	Informed consent	32	Model consent form and other related	Appendix I
25	materials		documentation given to participants and authorised	
26			surrogates	
27				
28				
29	Biological	33	Plans for collection, laboratory evaluation, and	N/A
30	specimens		storage of biological specimens for genetic or	
31			molecular analysis in the current trial and for future	
32			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 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.