

Title: Combining a high DHA multi-nutrient supplement with aerobic exercise: protocol for a randomised controlled study assessing mobility and cognitive function in older women

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24 **Abstract**

25 There is a complex interplay between cognition and gait in older people, with declines in gait
26 speed coexisting with, or preceding cognitive decline. Omega-3 fatty acids, B vitamins, vitamin
27 E, phosphatidylserine, and Ginkgo Biloba show promise in preserving mobility and cognitive
28 function in older adults. Exercise benefits mobility and there is evidence suggesting positive
29 interactions between exercise and omega-3 fatty acids on physical and cognitive function in older
30 adults. Non-frail or pre-frail females aged ≥ 60 years are included in a randomized placebo
31 controlled study. Intervention groups are: high DHA multi-nutrient supplement and exercise,
32 placebo supplement and exercise, high DHA multi-nutrient supplement, and placebo supplement.
33 Dietary supplementation is 24 weeks. The exercise intervention, two cycle ergometer classes per
34 week, is for the final 12 weeks. The primary outcome is habitual walking speed, secondary
35 outcomes include gait variables under single and dual task, five times sit to stand, verbal and
36 spatial memory, executive function, interference control and health related quality of life. Blood
37 fatty acids, serum homocysteine, dietary intake, physical activity, and verbal intelligence are
38 measured to assess compliance and control for confounding factors. The study is registered at
39 www.clinicaltrials.gov (NCT03228550).

40 **Keywords:** Docosahexaenoic acid₁, Memory₂, B Vitamins₃, Physical Activity₄, Gait₅, Aging₆

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¹ **List of Abbreviations:** Dual task (DT), Template for Intervention Description and Replication (TIDieR), Mini Mental State Examination (MMSE), National adult reading test (NART), Habitual walking (HW), Fast walking (FW), Dual-task costs (DTC), Rey's Auditory Verbal Learning Test (RAVLT), Enzyme-linked immunosorbent assay (ELISA), Food frequency questionnaire (FFQ), Community health activities program for seniors (CHAMPS), Short form 36 questionnaire (SF-36)

45 1 Introduction

46 In Europe the proportion of adults aged ≥ 65 years is expected to rise from 16.1 to 22% by
47 2031[1]. Currently 23% of the total global burden of disease is attributable to disorders in people
48 aged 60 years and older [2]. The trend towards an ageing population and the expected continual
49 rise in age related disease will have profound implications for the health care systems for decades
50 to come. The UK spent £9.3 billion on health and social care for older adults in 2010 and this is
51 projected to increase to £12.7 billion by 2022 [3].

52 Mobility and cognitive function are two key functional domains upon which preventative
53 strategies should be targeted towards in older adults [4]. Mobility impairments are associated
54 with reduced health related quality of life [5], and cognitive decline is associated with both
55 increased risk for future inability to perform instrumental activities of daily living and loss of
56 independence in older adults [6]. It is normal to observe some decline in mobility and cognitive
57 function with age [7, 8], thus preventative interventions are aimed at reducing the trajectory of
58 this decline and promoting what is referred to as “healthy” or “successful” ageing[9]. Some of
59 the key domains to consider to achieve healthy ageing are autonomy in activities of daily living,
60 wellbeing, good quality of life, high social participation, only mild cognitive or functional
61 impairment, and little or no physical disability [10].

62 Mobility limitations increase with advancing age and are often a sign of further functional decline
63 [11]. Habitual and fast walking speeds are both examples of widely used performance based
64 indicators of mobility [12, 13]. Gait speed is an established clinically relevant marker in older
65 adults, it is associated with mortality[14], risk of falls[13] and functional capacity[15]. In addition
66 to its use as a measure of physical functioning, there is now strong evidence to suggest a
67 relationship between cognitive function and gait. Changes in several gait parameters including
68 speed, variability, cadence, stride length, and time spent in the double support phase coexist with
69 or precede onset of cognitive decline in older adults [16]. Interventions that can target cognition
70 improve mobility [17, 18], which can translate into increased survival rate [19]. The role of
71 cognitive function in relation to walking is increasingly important in older adults when they are
72 required to conduct a simultaneous secondary task (dual-task paradigm). Inability to maintain a
73 conversation while walking is a strong predictor of falls in older adults[20]. Consequently dual-
74 task (DT) gait protocols have become an established way to assess the relationship between
75 cognition and gait [21].

76 Most of the pharmacological approaches used in age related health conditions have been met with
77 limited success, and this is likely due to the multifactorial aetiology underlying these conditions,

78 for example varying physical and neurological pathologies as well as factors such as
79 inflammation, metabolism, and genetics [22]. Development of lifestyle interventions to reduce
80 the burden of age related health conditions would be highly advantageous considering the poor
81 efficacy of the current pharmaceutical options [23]. Dietary compounds and exercise have been
82 shown to separately act on a broad spectrum of health outcomes in older adults including gait
83 speed, cognition and muscle strength and function [17, 24, 25]. The capacity to act on multiple
84 outcomes makes these lifestyle interventions particularly valuable in the prevention of age related
85 cognitive and physical impairments. Some of the lifestyle interventions that have shown potential
86 promise in combatting age related declines in mobility and cognition include aerobic exercise and
87 dietary compounds such omega-3 polyunsaturated fatty acids (omega-3 PUFAs), vitamin E,
88 phosphatidylserine (PS), B vitamins, and *Ginkgo Biloba* [17, 26-29]. There is a growing
89 awareness of the importance of taking a more holistic approach to research into nutrition and
90 brain ageing, exploring potential synergies between nutrients and how these may influence both
91 cognition and mobility [30]. For example, work in our laboratory shows that the high DHA multi-
92 nutrient dietary supplement used in this protocol, which contains docosahexaenoic acid (DHA),
93 vitamin E, phosphatidylserine (PS), B vitamins, and *Ginkgo Biloba* increases habitual gait speed,
94 verbal memory, and processing speed in older women versus placebo [17]. Similarly, a multi-
95 nutrient supplements containing omega-3 polyunsaturated fatty acids (PUFAs), phospholipids, B
96 vitamins, and antioxidants shows promise in those with mild Alzheimer's disease [31, 32].
97 However, despite showing promise separately, there is currently no evidence available as to the
98 effects on mobility and cognition when combining these dietary factors with an exercise
99 intervention.

100 There is mounting evidence that the omega-3 PUFAs eicosapentaenoic acid (EPA) and DHA may
101 play a role in the prevention of age related cognitive decline [33] and mobility impairments [34],
102 through mechanism related to cell signalling, inflammation, enhancing neurogenesis, promoting
103 neuronal survival and increasing muscle protein synthesis [33, 35-37]. DHA is the predominant
104 omega-3 PUFA in the human brain [38], where it is concentrated in the phospholipid membranes,
105 particularly at the synapses[39]. Two recent trials have reported that DHA supplementation may
106 slow progression of brain atrophy and preserve cognitive function in older adults [40]. Control of
107 gait requires the appropriate integration of information from motor, sensory and cognitive
108 systems, and cognitive processes including executive function, attention and processing speed
109 share the strongest associations with habitual and DT gait outcomes [41, 42]. Several studies have
110 suggested that omega-3 PUFAs may benefit these cognitive processes. For example, omega-3

111 PUFA supplementation is associated with improved attention, verbal memory and immediate
112 recall [43, 44], as well as executive function and processing speed [17, 45, 46] in older adults.

113 PS is a major phospholipid class that accounts for 13-15% of the human cerebral cortex. PS is
114 essential for the activation of key signalling pathways that stimulate neuronal survival, neurite
115 growth and synaptogenesis [47]. There is currently limited data on supplementation trials with
116 PS; however, a small trial in older adults showed positive effects of 300 mg per day PS
117 supplementation for twelve weeks on memory, recall, executive functions, and mental flexibility
118 [48]. Since PS contains high levels of DHA it is unclear whether the benefits from PS
119 supplementation are due to intact PS or the release of DHA following hydrolysis [47].

120 Higher levels of vitamin E (α -tocopherol) are associated with lower risk for cognitive impairment
121 in older adults [49]; however, clinical trials have shown limited benefit of supplementation[50].
122 Vitamin E plays an important role in protecting cell membranes from damage by free radicals,
123 and protects the highly unstable polyunsaturated fatty acids, particularly DHA, from lipid
124 peroxidation [51], and it may be that beneficial effects of vitamin E are due to the role it plays in
125 protecting DHA in membranes.

126 B12 and folic acid act as cofactors for the methylation of homocysteine to methionine [52]. High
127 homocysteine levels are associated with physical frailty [53], cognitive decline [54], and
128 cardiovascular disease [55]. Dietary supplementation trials with B vitamins have shown mixed
129 results on cognitive function and physical outcomes [56, 57], with evidence suggesting those with
130 higher homocysteine levels are most responsive [58-60]. However, the effects may be dependent
131 on omega-3 PUFAs, since B vitamin supplementation in those with higher baseline plasma
132 omega-3 PUFAs resulted in a significant decrease in rates of brain atrophy versus placebo [61].
133 The mechanisms underpinning this interaction are currently not well understood, however
134 homocysteine has been shown to impact phospholipid and DHA metabolism by inhibiting
135 methylation reactions that convert phosphatidylethanolamine enriched with DHA to
136 phosphatidylcholine [62]. Furthermore DHA has been shown to influence gene expression of
137 enzymes that control homocysteine metabolism [63].

138 *Ginkgo Biloba* is one of the most widely used and studied herbal extracts for cognitive
139 impairment and dementia in older adults. Results from clinical studies have largely yielded
140 inconsistent results, however a recent meta-analysis indicates that supplementation with 240mg
141 standardized extract per day in patients with dementia and cognitive impairment can slow
142 cognitive decline over 22 to 26 weeks [26].

143 Regular physical activity and exercise are promoted by the World Health Organization (WHO) to
144 improve functional health and reduce the risk for non-communicable disease (WHO, 2010).

145 Exercise promotes adaptations to physiological systems that can in turn influence factors
146 associated with healthy ageing. This includes neuromuscular adaptations that influence strength
147 and the ability to coordinate movements [65], improvements to cardiorespiratory fitness [66] and
148 preservation of the brain [67]. Aerobic training has shown promise with regards to healthy ageing
149 due to its ability to act across a broad range of health related factors including both the physical
150 and cognitive domains [68-70]. Of particular importance to the older adult, aerobic exercise
151 interventions are shown to influence processing speed and executive function [71]. Cycling is a
152 form of aerobic exercise that can benefit muscle strength, cardiopulmonary fitness, balance and
153 proprioception in older adults [27, 72]. Furthermore cycling may be preferential for older adults
154 as it is non-weight bearing, has a low impact on joints and has been found to be suitable and
155 beneficial for those with joint pain [73].

156 Recent evidence suggests multi-domain approaches, such as combining omega-3 PUFAs with
157 exercise may provide additional benefits to both cognitive function and physical ability when
158 compared to either approach alone. For example, a recent multi-domain intervention, consisting
159 of omega-3 PUFA supplementation, nutritional and exercise counselling and cognitive training
160 was more effective than either omega-3 PUFA supplementation alone or placebo/usual care in
161 limiting long-term physical activity declines [74]. Similarly, a trial of older adults with mild
162 cognitive impairment compared the effects of daily supplementation with omega-3 PUFAs alone
163 or in combination with twice weekly stationary cycle training and a program of cognitive
164 stimulation for eight months [75]. The combined intervention led to an enhanced reduction of
165 brain atrophy in grey matter regions compared to supplementation alone. Interestingly, this effect
166 was associated with serum homocysteine levels, suggesting a potential important interaction with B
167 vitamin status. A further recent trial in older adults found that combining daily omega-3 PUFA
168 supplementation with resistance training over an 18 week period provided an additional benefit to
169 muscle strength (maximal isometric torque) in knee extensor muscles compared with the exercise
170 alone, although this effect was only observed in the female participants [76]. Therefore, since this
171 observation suggests that women are more amenable to the effects of omega-3 PUFA
172 supplementation and exercise, and females have been shown to have greater compliance to
173 exercise interventions[77], the study will restrict participation to female volunteers only. The
174 mechanisms underpinning the observed interaction between omega-3 PUFAs and exercise are not
175 clear however, both exercise and omega-3 PUFAs have been shown to share a number of similar
176 effects including increasing neurogenesis and neural plasticity, muscle protein synthesis and
177 reducing inflammation and homocysteine levels [37, 75, 78-81]. Furthermore it also cannot be
178 determined at this stage whether any interaction between the two interventions is additive or
179 synergistic. Overall, these results suggest that the addition of dietary supplementation with

180 omega-3 PUFA to exercise may enhance training adaptations in the older population, which is
181 important as older adults often display an attenuated response to exercise or require more regular
182 training stimulus to maintain muscle compared to younger adults [82, 83].

183 The present study extends our previous research by determining whether the preliminary
184 observations of positive effects of supplementation on cognition and mobility can be replicated
185 [17] and investigating whether the addition of an exercise intervention enhances these effects. By
186 using a unique blend of nutrients on their own and in combination with aerobic exercise, this
187 study will provide a novel insight into the efficacy of two promising lifestyle interventions, using
188 outcome measures that encapsulate healthy ageing. It is hypothesized that both the DHA multi-
189 nutrient supplement and the aerobic exercise will improve mobility and cognitive function versus
190 the placebo in older women and that combining the two interventions will produce a greater
191 benefit compared to each separately.

192 **2 Research Aims**

193 The aims of this semi-blinded randomised control trial on the effects a high DHA multi-nutrient
194 supplement alone and in combination with aerobic exercise in women aged 60 years and older are
195 as follows.

- 196 • To investigate the effects of each intervention in isolation and in combination on mobility,
197 cognitive function and health related quality of life, to establish whether there are
198 treatment effects as well as any additive or synergistic benefits.
- 199 • To investigate whether there are relationships between circulating DHA and serum
200 homocysteine with mobility and cognitive outcomes.

201 **3 METHODS AND ANALYSIS**

202 **3.1 Design and Setting**

203 The study is a randomised semi-blinded, placebo controlled trial in females aged 60 years and
204 above. The study is designed to examine the effects of a high DHA multi-nutrient dietary
205 supplement and aerobic exercise, both on their own and in combination, on outcomes related to
206 mobility and cognitive function. All measurements and data collection, as well as the aerobic
207 exercise intervention take place in the same study site (Bournemouth University, U.K.), with
208 participants being instructed to consume the dietary supplement at home.

209 **3.2 Blinding Randomisation and Allocation**

210 The dietary supplements are packed into identical containers and coded by the Principal
211 Investigator, who has no involvement in the data collection. Omega-3 PUFA capsules have a
212 distinct odour, therefore a small amount of fish oil is added to the placebo capsules to help maintain
213 blinding. Exercise class allocation is communicated through letters which are coded by the Principal
214 Investigator and distributed in sealed envelopes. A stratified block randomization design is
215 followed [84] with stratification based on frailty classification of non-frail or pre-frail (see section
216 3.5), followed by permuted block randomization. Randomization is achieved by creating a
217 computer-generated list of numbers consisting of four blocks for each strata referred to without
218 specification of intervention group (e.g., A, B, C and D). The list is generated and stored by the
219 Principal Investigator, who is not involved in the data collection. Due to the nature of the exercise
220 intervention participants are only blinded to the dietary intervention; however, the experimenters
221 are blinded to the group allocations.

222 In the event of a severe adverse effect being reported by a participant the Principal Investigator
223 will be able to gain access to the participant allocation so that appropriate action can be taken,
224 whilst maintaining the blinding of those involved with data collection and analysis.

225 **3.3 Participant Recruitment and Eligibility Criteria**

226 Participants are recruited through public advertisements and public engagements in Bournemouth,
227 U.K. The public advertisements include a brief study description as well as the contact details for
228 the research team. Interested individuals receive a participant information document including the
229 design, procedure, benefits, and risks of the trial. Before any data is collected all participants
230 provide signed written informed consent forms.

231 Females aged 60 years and above are recruited according to the following inclusion criteria: (1)
232 able to walk at least 50 m unaided, (2) classified as non-frail or pre-frail and community dwelling.
233 Exclusion criteria are: (1) vestibular impairments, (2) diagnosed neurological disorder, (3)
234 cognitive impairment (Mini Mental Status Examination score of 24 or below), (4) lower limb
235 surgery, (5) seafood allergy, (6) regular consumption of multivitamin or fish oil supplements
236 within six months prior to baseline measurements, and (6) previously received advice from a
237 health care professional not to undertake strenuous exercise.

238 **3.4 Interventions**

239 The study interventions are described in detail according to the Template for Intervention
240 Description and Replication (TIDieR) guidelines in table 1.

241 Dietary Supplement

242 All participants consume four capsules per day of their respective dietary supplement for the 24
243 weeks of the study, and are instructed to take them with their main meal of the day. The total
244 daily dose from the active capsules contains 1000 mg DHA, 160 mg EPA, 20 µg vitamin B12, 1
245 mg folic acid, 124 mg PS, 240 mg *ginkgo biloba* standardized leaf extract and 20 mg vitamin E.
246 The duration of 24 weeks and dose of supplementation has previously been shown to increase
247 tissue omega-3 PUFA levels and induce improvements in cognition and mobility [17]. The
248 placebo capsules contain an isocaloric oil blend typical of the U.K. diet including a small amount
249 of fish oil. The fatty acid content of the active and placebo capsules is analysed by gas
250 chromatography coupled to flame ionization detector, as detailed below. Active and placebo
251 capsules are kindly provided by Efamol Ltd. Compliance to the dietary supplement is measured
252 by changes in DHA levels compared to baseline, with a change of 5% being the threshold for
253 compliance [85], counting returned pills at 12 and 24 weeks, and exit questionnaire. A systematic
254 review concluded that the potential for adverse events with omega-3 PUFA supplementation
255 should be considered mild-moderate at worst and unlikely to be of clinical significance [86].

256 Exercise Training

257 The exercise intervention consists of two group sessions per week on a Spinner Fit stationary
258 bike, led by a qualified instructor. For the first six weeks classes last 30 min and in the second six
259 weeks session length increases to 45 min. All sessions consist of a 5 min warm up and cool down
260 at 7-8 on the Borg scale of rate of perceived exertion [87]. During the main part of the sessions
261 participants maintain intensity between 12 to 14 on the Borg scale. These intensity levels on the
262 Borg scale are considered moderate to vigorous, and similar intensity levels produce positive
263 responses in this population [88, 89]. Older adults are typically heterogeneous in terms of their
264 aerobic fitness [90] therefore using the Borg scale allows each participant to exercise at their own
265 level, whilst still being encouraged to maintain the moderate-vigorous intensity levels that are
266 desired. Compliance to the exercise intervention is monitored by recording attendances by each
267 participant and calculated as the percentage of classes attended, with 70% being the threshold for
268 compliance [91].

269

270 3.5 Screening

271 All participants are screened to assess frailty status, according to the criteria developed by Fried
272 and co-workers [92]. The criteria includes low muscle strength, self-reported exhaustion, slow

273 gait speed, low levels of physical activity, and unintentional weight loss, as shown in Table 2. A
274 score of zero out of the five indicates non-frail, one or two pre-frail, and three or above frail. As
275 well as a screening procedure non-frail and pre-frail status is used as a prognostic factor in the
276 randomisation.

277
278 The Mini Mental State Examination (MMSE) is performed to exclude participants with
279 undiagnosed cognitive impairment [93]. The test is performed according to British Psychology
280 Society guidelines (2010) and not used for diagnostic purposes, with individual results not
281 disclosed. Participants who score ≤ 24 are excluded from the trial.

282 **3.6 Demographic Information**

283 Information on the age, height, weight, verbal intelligence, and medication use are collected from
284 each participant. Information on medications is self-reported, with both type and number of
285 medications recorded. The national adult reading test (NART) is used to assess verbal
286 intelligence[95]. The test requires participants to read aloud 50 pre-prepared words, with a score
287 being calculated based on the number of correct pronunciations. Minor variations from the
288 pronunciations are not penalised as the aim of the test is to assess familiarity with the words
289 rather than exact pronunciation.

290 **3.7 Outcomes**

291 All measurements are performed at baseline and at the end of the study. The primary and
292 secondary outcomes are listed in Table 3.

293 **3.7.1 Gait Analysis**

294 Gait speed, stride length variability, stride length, cadence, and double support phase percentage
295 are measured using Opal inertial sensors and analysed using Mobility Lab™ software version 3.1
296 (APDM Inc, <http://apdm.com>). Sensors are placed on the feet over the shoes according to the
297 manufacturers' instructions. Acceleration and deceleration phases of the gait cycle are removed
298 from the analysis, and each test will take place over 13 m. Each tested condition is repeated five
299 successful times to obtain representative samples and the means of the trials are used for data
300 analysis for habitual and dual task gait with the maximum gait speed value being used for the fast
301 walking condition.

302 Participants are assessed under three gait conditions: habitual walking (HW), fast walking (FW),
303 and DT walking. Participants walk at a normal comfortable pace for the HW and DT protocols

304 and as fast as possible for the FW protocol. During the DT protocol participants count backwards
305 in integers of three from a randomly generated three digit number given three seconds before
306 commencement of the task. Although there is currently no standardised secondary task for dual
307 task gait protocols a backwards counting task in integers of three has been used in several prior
308 studies in similar demographics [21, 96-98]. Participants are not instructed to prioritize either
309 walking or counting backwards during the DT condition. The use of gait speed as a clinical
310 measure in older adults is well established due to its association with physical functioning, falls,
311 disability, and mortality [14, 99]. The relative dual-task costs (DTC) as percentage of loss relative
312 to the single-task performance is calculated based on the formula $DTC [\%] = 100 * (\text{single-task}$
313 $\text{score} - \text{dual-task score}) / \text{single-task score}$ [100].

314

315 **3.7.2 Five Times Sit to Stand**

316 The five times sit to stand tests is a valid measure of dynamic balance and functional mobility in
317 older adults that is commonly used in studies in geriatric populations [101]. To perform the five
318 times sit to stand participants start off seated on a standard chair 44 cm in height from the ground,
319 with arms folded across their chest and back against the chair. They stand up fully from the chair
320 and sit back down again five times, whilst keeping their arms in the same position. This task is
321 assessed by timing participants from the prompt to start until they reached a seated position on the
322 fifth repetition.

323

324 **3.7.3 Cognitive Function**

325 A Stroop test is used to assess interference control [102] using Open Sesame version 3.1.1.
326 software [103]. During this task a fixation point appears on screen for 500 ms followed by the
327 presentation of the names of one of four colours: blue, red, green, and white. These words are
328 presented in four different font colours varying between blue, red, green, and white. Participants
329 are instructed to identify, as quickly as possible without sacrificing accuracy, the colour of the
330 text rather than the word displayed on screen and press a designated key on the keyboard,
331 highlighted using coloured stickers. The test comprises 144 trials with half of trials having the
332 text and colour match (congruent trials) and half being a non-match (non-congruent trials).
333 Interference control is defined as the difference between the mean time taken to respond to the
334 congruent and non-congruent trials. Reaction times that are plus or minus 2.5 times the median
335 absolute deviation are excluded as anomalous results [104].

336 Spatial memory is assessed using a computerized task, run on Open Sesame version 3.1.1.
337 software, based upon work conducted by Nagamatsu, L. S. et al. (2013). The task requires

338 participants to recall the spatial location of dots presented on a screen. Each trial comprises a
339 presentation and a test phase. In the presentation phase three dots appear at randomly allocated
340 locations for 500 ms, this is followed by a fixation cross which appears for 3 s. After the retention
341 interval the test phase comprises presentation of a single red test dot on the screen, this can either
342 be in the same location as one of the previous black dots (match) or in a different location (non-
343 match). Participants are asked to identify if the red test dot was a match or a non-match to any of
344 the prior black dots by pressing an assigned key on the keyboard (“y” = match; “n” = non-match).
345 There is no time limit for the participants to respond as the focus of the task is on response
346 accuracy. The task consists of ten practice trials, followed by sixty recorded trials. Thirty of the
347 trials are matched and 30 are non-matched. The thirty non-matched are evenly split in three
348 degrees of difficulty, whereby they are placed at two (near), four (medium) and eight (far) degrees
349 visual angle. These angles were calculated based on the participant sitting 50 cm from the screen.
350 Accuracy for the task is recorded as the percentage of correct answers.

351 The Rey’s Auditory Verbal Learning Test (RAVLT) is an established cognitive testing tool that
352 requires participants to recall a list of 15 pre-set words and is used for assessing verbal
353 memory[105].

354 A trail making task is used to assess executive function[106]. In this task participants are asked to
355 draw lines between targets on a piece of paper, as rapidly as possible, in a grid of seven by seven
356 squares. There are four different conditions for the task: (1) a numbers condition where targets go
357 from one to 49 (numbers), (2) a letters condition where the targets go from A to Z (letters), (3) a
358 condition where participants alternate between numbers (1-25) and letters (A-X), (numbers-
359 letters), and finally, (4) a condition alternating between letters (A-Y) and numbers (1-24) (letters-
360 numbers). Scores are recorded as the total number of correct connections within the time limit.

361

362 **3.7.4 Whole Blood Fatty Acids Analysis**

363 Whole blood pin-prick samples from non-fasted participants are collected on Silica gel loaded
364 filter paper (Whatmantm) pre-treated with 2,6-di-tert-butyl-p-cresol (butylated hydroxytoluene,
365 BHT). Samples are collected and processed as described previously [17]. Pre- and post-
366 intervention fatty acid levels are compared to assess compliance and response to supplementation.

367 **3.7.5 Serum Homocysteine**

368 A non-fasted venous blood sample will be drawn to assess serum homocysteine. Samples are
369 collected using a Vacutainer Safety-Lok collection set fitted with a 10 mL serum collection tube
370 (Becton, Dickinson and Company). Each blood sample is allowed to clot and then immediately

371 centrifuged at 2000 x g for 10 minutes at 4°C and the serum extracted[107]. Serum samples are
372 stored at -80°C and analysed within three months[108]. Serum homocysteine levels are measured
373 using a competitive enzyme-linked immunosorbent assay (ELISA) kit (Cell Biolabs Inc.).
374

375 **3.7.6 Dietary Intake and Physical Activity Levels**

376 Differences in diet and physical activity habits between the groups, as well as changes in these
377 aspects within groups have the potential to influence the outcomes of the study, for example
378 increasing protein intake has been shown to maintain lean mass and physical function in older
379 adults [109]. Although participants are asked to maintain their current diet and lifestyle habits,
380 these aspects are also monitored at baseline and completion of the study.

381 Three day estimated food diaries are used to assess dietary intake. Written instructions are
382 provided alongside the food diaries. Participants record details of all foods and beverages
383 consumed at the time of consumption. They are asked to include brand names, cooking and
384 preparation methods and an accurate description of the portion size using standard household
385 measures or natural unit sizes. Results are analysed using computer dietary analysis software and
386 results expressed in grams or kilocalories for macronutrients and energy, respectively. A
387 previously validated seventeen item food frequency questionnaire (FFQ) is used to specifically
388 quantify omega-3 PUFA intake [110].

389 The community health activities program for seniors (CHAMPS) questionnaire is used to assess
390 physical activity levels [111]. The CHAMPS questionnaire is a validated and reliable measure of
391 physical activity in older adults, which covers a broad range of activities and has been shown to
392 be sensitive to change over six months [111].

393 **3.8 Health Related Quality of Life**

394 The short form (SF) 36 health questionnaire has been shown to be a practical and valid tool for
395 assessing health status [112]. The questionnaire is issued at baseline and end of the study.
396 Answers are divided into sub-categories: vitality, physical functioning, bodily pain, general health
397 perceptions, physical role functioning, emotional role functioning, social role functioning and
398 mental health. Each sub-category is scored on a scale of zero to one hundred with a higher score
399 indicating a more positive health status [113].

400 **3.9 Data Management**

401 The chief investigator will be responsible for all data collection, and has received training on all
402 collection and analysis techniques.

403 All data from participants will be assigned to a number to prevent results being tracked back to an
404 individual. The chief investigator will be responsible for all the storing and handling of data.
405 Digital data from the study will be stored on a password protected Bournemouth University staff
406 account only accessible by the research team. All paperwork including completed consent forms,
407 lifestyle questionnaires and raw data outputs will be locked in a filing system within a secure
408 building at Bournemouth University (U.K). Results from the study will be anonymised with
409 participants being assigned numbers. All data relating to the trial will be archived for 5 years after
410 the conclusion of the study

411 **3.10 Sample Size**

412 Sample size was determined based on the primary outcome of habitual walking speed. Using an
413 effect size based on previously published values, minimally significant changes in gait speed were
414 0.03 m/s and 0.05 m/s with substantial changes at 0.08 m/s[114]. The sample size calculation is
415 based on a difference of 0.08 m/sec with a power of 0.8 and α of 0.05 (two-tailed). A minimum
416 sample size of 25 participants per group is required to detect an effect size d of 0.8 between
417 experimental groups and the control. An overall recruitment target of 120 participants, 30 per
418 group has been set to allow for drop-outs over the 24 weeks of the trial.

419 **3.11 Statistical analysis**

420 Data analysis is performed at the conclusion of the study and includes data collected at baseline
421 and following the 24 week intervention. Data is tested for normal distribution using Shapiro-Wilk
422 test and Q-Q-plots. If data are normally distributed the following statistical methods will be used;
423 however, for data not fulfilling assumptions of normal distribution the non-parametric equivalent
424 will be substituted. A 2×2 -ANOVA test will be used to compare the two interventions over time
425 (from pre- to post-measurement) on changes on the dependent variables. Effect size calculation
426 (η^2 (Eta squared)) will also be calculated. Participants' demographic and health information, such
427 as age and NART score, in addition to changes in serum homocysteine and whole-blood PUFAs
428 will be examined in relation to the outcome measures to interpret the results in context. Analysis
429 will be carried out on an intention-to-treat basis and include any participants who decide to
430 discontinue treatment, but complete the intervention period and assessment at 24 weeks.

431 Associations between serum homocysteine, whole-blood DHA levels, and measures of mobility
432 and cognition will be examined at baseline using Pearson's partial correlations controlling for
433 age. NART score will also be included as a covariate in preliminary analysis. Correlation data
434 will be examined to ensure assumptions are not violated. In all analyses $P < 0.05$ will be
435 considered significant.

436 Baseline and 24 week results from the diet and physical activity assessments will be compared
437 within groups, using paired T-tests, to determine whether participants have made any significant
438 changes to their diet and physical activity habits during the study intervention. Diet and physical
439 activity data, along with data collected on medication use will be examined so that interpretation
440 of results can be made within the context of potential differences of other lifestyle related factors.

441 **3.12 Stepwise Procedure**

442 The stages of the study procedure are illustrated in Figure 1. Ethical approval for the study was
443 granted on 23/06/2016 with data collection commencing on 27/02/2017. Data collection is
444 expected to be completed by 07/10/2018. Measurements are undertaken at baseline and following
445 the 24 week intervention period. A mid-study appointment is given at 12 weeks to collect unused
446 dietary supplement capsules to monitor compliance, and to issue participants with the dietary
447 supplements required for the remainder of the trial. The baseline measurements consist of the
448 screening process assessing frailty and cognitive impairment, if eligible this will be followed by
449 the main testing battery, which includes the tests of mobility and cognitive function outlined
450 above. Upon completion of the data collection sessions at baseline and 24 weeks participants are
451 issued with a food diary, FFQ, CHAMPS and SF-36 questionnaires. These are fully explained by
452 a member of the research team and written instructions given, they will then be asked to fill these
453 out at home over the next week and return.

454 Participants' begin their dietary supplementation intervention on the same day their blood sample
455 is taken. Initially participants are given 12 weeks supply of their respective supplements, they are
456 asked to bring in remaining capsules at the 12 week point, before issuing them with the second
457 batch of supplements to be taken until the end of the study. The week following the 12 week data
458 collection the aerobic exercise intervention commences, this takes place twice a week for the final
459 12 weeks of the trial.

460 Adherence to exercise interventions can be problematic, an issue that has consistently been raised
461 in the literature [91, 115, 116]. To maximise adherence, the exercise intervention involves a
462 supervised programme, as these have been shown to be associated with higher adherence
463 rates[116]. Furthermore, the exercise sessions are scheduled at convenient times for participants,
464 and participants are given a phone call every two weeks to provide ongoing support and
465 encourage adherence to the exercise intervention and compliance with the dietary
466 supplementation.

467 **3.13 Monitoring**

468 The data and safety monitoring will be performed by the research team. The team will meet once
469 per month, to discuss any issue and check on the conduction of the study. Adverse events as
470 defined by Clinicaltrials.gov [117] will be monitored by participant self-reporting and exit
471 questionnaire. Adverse events will be reported by the Principal investigator to the institutional
472 research representative and sponsor. There is no independent data safety and monitoring board
473 made for this study due to the anticipated low risk nature of the intervention. There are no plans
474 for interim analyses due to the relatively low sample size.

475 **3.14 Patient and Public Involvement**

476 The primary and secondary outcomes for the study were chosen based on the latest
477 recommendations for clinically relevant measures in intervention trials on healthy ageing [9]. In
478 the design phase of the study older women without cognitive or mobility impairment were invited
479 to attend testing sessions, where they were asked to complete the cognitive testing and DT gait
480 protocols. These sessions allowed the research team to determine whether there were any floor or
481 ceiling effects of the testing. This meant that changes to the difficulty of the testing could be made
482 to ensure the validity of the testing as well as ensuring the safety and comfort of the participants.
483 Furthermore participants were invited to give their feedback during these sessions on how the
484 tasks were presented, to ensure that all tests had clear instructions and were well understood.
485 Upon completion of the trial all participants will receive a letter giving a full summary of the
486 study and the results.

487 **4 ETHICS AND DISSEMINATION**

488 Ethical approval for the study procedure has been granted by the Bournemouth University
489 Science Technology and Health research ethics panel (Ethics ID 10788) and conforms to the
490 declaration of Helsinki and guidelines for Good Clinical Practice. The trial protocol follows the
491 Consolidated Standards of Reporting Trials (CONSORT) statement on randomised trials of non-
492 pharmacological treatment [118] and the Standard Protocol Items: Recommendations for
493 Interventional Trials (SPIRIT) guidelines [119]. In the event of any important protocol
494 modifications, all investigators and trial participants will be notified, amendments will be made to
495 the clinical trials registry and a resubmission of the protocol will be made to the ethics panel. The
496 results of this study will be presented in a PhD thesis, at scientific conferences and submitted to
497 peer-reviewed journals. No data is collected until fully informed consent is given by participants.
498 Interested parties who meet the eligibility criteria are sent a copy of the participant information
499 sheet, which contains all the necessary information required to take part in the study, participants
500 are given an minimum of 24 hours before being asked to give their consent and are encouraged to

501 contact a member of the research team if they have any questions or concerns regarding
502 participation. Following completion of the trial all participants who received the placebo
503 supplement during the study will be offered 24 weeks supply of the active supplement,
504 furthermore all participants will be offered a written summary of the results.

505 **5 AUTHOR CONTRIBUTIONS**

506 SD developed the research question. SD, AJ and FT developed the study design. PF developed
507 the measurements of the protocol and SD and FT acted as methodological council. SD, FT and
508 AJ edited and revised the study protocol. SD was responsible for the final content of the paper
509 and all authors have read and approved the final manuscript.

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516 study.

517 **8 CONFLICT OF INTEREST**

518 The authors declare no conflicts of interest. The supporters have no role in the study design, data
519 collection, analysis, interpretation of the data, or the decision to publish the results.

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835 **Figure 1.** Participant flow through the study.

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856 **Table 1** Description of study intervention based on the Template for Intervention Description and
 857 Replication (TIDieR) checklist[120].

Item	Experimental Group	Experimental Group	Experimental Group	Control Group
1. Group	DHA Multi-nutrient Supplement and Exercise	Placebo Supplement and Exercise	DHA Multi-nutrient Supplement	Placebo Supplement
2. Why?	A high DHA multi-nutrient supplement formula has previously been shown to improve habitual gait speed, verbal memory and processing speed in older women [17]. Cycle ergometer training is a form of exercise that can benefit muscle strength and cardiopulmonary fitness in older adults [27]. There is some evidence for a positive interaction between omega-3 PUFA and exercise in older women on muscle strength and cognitive function [121, 122]			
3. What materials?	Participants receive containers with DHA multi-nutrient supplement capsules and instructions on daily intake. Exercise classes take place in a sport studio on spinning cycle ergometers.			
4. What procedure?	Participants take four capsules per day of their allotted supplement, alongside their main meal of the day. Those allotted to the exercise intervention attend two classes per week for the final 12 weeks of the study. Classes will initially last 30 min for the first 6 weeks and then increase to 45 min for the final 6 weeks.			
5. Who provides?	Principal Investigator issues participants with their dietary supplements. Exercise classes are carried out by a qualified instructor.			
6. How?	For the dietary supplements, all groups receive initial instructions about intake, duration and dosage by the Principal Investigator. The exercise classes will be performed in small groups.			
7. Where?	The participants take the dietary supplements at home. The aerobic exercise classes take place in sports studios at Bournemouth University U.K.			
8. When and how much?	For 24 weeks participants will take four capsules per day of their allotted supplement. After 12 weeks the participants will start their exercise classes, twice per week for the final 12 weeks of the study.			
9. Tailoring	Participants are told to maintain a specific revolution per minute on the cycle ergometer. They self-select a resistance to allow them to maintain 12-14 on the Borg scale. This method ensures participants maintain a similar and consistent intensity of exercise despite the likelihood of participants having mixed fitness levels.			

858 **Table 2** Frailty Screening assessment methods and defined cut of points[92]

Frailty Criteria	Assessment Method	Cut-off for Frailty
Unintentional Weight Loss	Self-reported	≥ 4.5 kg in the last year
Muscle Weakness	Grip Strength (dominant hand)	≤ 18 kg
Slow Gait Speed	Gait Speed over 13 meters	≤ 0.8 m/s
Exhaustion	Two questions from the Centre of Epidemiologic Studies Depression Scale [123]	Answering “much or most of the time” to the questions “I felt that everything I did was an effort” and “I could not get going.”
Low Levels of Activity	Physical Activity Scale in the Elderly [124]	≤ 56.4

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878 **Table 3** Summary of study outcomes.

Assessment Methodology	Outcome
Primary Outcome	
Habitual walking	Gait Speed (m/s)
Secondary Outcomes	
Habitual walking	Temporal and spatial parameters
Fast walking	Temporal and spatial parameters
Dual task walking	Temporal and spatial parameters
Five times sit to stand	Seconds
Spatial working test	Spatial working memory (percentage score)
Rey's Auditory Verbal Learning Test	Verbal memory (percentage score)
Trail making Task	Executive function (number of correct connections)
Stroop test	Interference control (ms)
Short form 36 questionnaire	Emotional role functioning, social role functioning, mental health, physical functioning, bodily pain, general health perceptions and physical role functioning
Other Measures	
Whole blood fatty acids	Fatty acid composition expressed as weight % of total fatty acids
Enzyme-linked immunosorbent assay	Serum homocysteine-bovine serum albumin ($\mu\text{g/ml}$)
Community health activities program for seniors questionnaire	Weekly caloric expenditure
Three Day Diet Diary	Mean daily calorie (kcal), carbohydrate (g), protein (g) and fat(g) intake
Omega-3 FFQ	Dietary omega-3 PUFA intake
National Adult Reading Test	Verbal intelligence