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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Abstract

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

Keywords: Docosahexaenoic acid[1], Memory[2], B Vitamins[3], Physical Activity[4], Gait[5], Aging[6]

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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)

1 Introduction

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2 Research Aims

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

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

3 METHODS AND ANALYSIS

3.1 Design and Setting

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

3.2 Blinding Randomisation and Allocation

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

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

3.3 Participant Recruitment and Eligibility Criteria

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

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

3.4 Interventions

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

Dietary Supplement

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

Exercise Training

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

3.5 Screening

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

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

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

3.6 Demographic Information

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

3.7 Outcomes

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

3.7.1 Gait Analysis

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

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

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

3.7.2 Five Times Sit to Stand

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

3.7.3 Cognitive Function

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

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

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

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

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

3.7.4 Whole Blood Fatty Acids Analysis

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

3.7.5 Serum Homocysteine

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

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

3.7.6 Dietary Intake and Physical Activity Levels

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

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

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

3.8 Health Related Quality of Life

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

3.9 Data Management

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

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

3.10 Sample Size

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

3.11 Statistical analysis

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

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

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

3.12 Stepwise Procedure

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

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

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

3.13 Monitoring

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

3.14 Patient and Public Involvement

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

4 ETHICS AND DISSEMINATION

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

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

5 AUTHOR CONTRIBUTIONS

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

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8 CONFLICT OF INTEREST

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

9 REFERENCES

1. Soong, J., et al., *Quantifying the prevalence of frailty in English hospitals.* BMJ Open, 2015. **5**(10).
2. Prince, M.J., et al., *The burden of disease in older people and implications for health policy and practice.* Lancet, 2015. **385**(9967): p. 549-62.
3. Wittenberg, R., et al. *Care for older people*. 2012. 44.
4. Davis, J.C., et al., *Mobility and cognition are associated with wellbeing and health related quality of life among older adults: a cross-sectional analysis of the Vancouver Falls Prevention Cohort.* BMC Geriatr, 2015. **15**: p. 75.
5. Davis, J.C., et al., *Mobility predicts change in older adults' health-related quality of life: evidence from a Vancouver falls prevention prospective cohort study.* Health Qual Life Outcomes, 2015. **13**: p. 101.
6. Aguero-Torres, H., et al., *The impact of somatic and cognitive disorders on the functional status of the elderly.* J Clin Epidemiol, 2002. **55**(10): p. 1007-12.
7. Harada, C.N., M.C. Natelson Love, and K. Triebel, *Normal Cognitive Aging.* Clinics in geriatric medicine, 2013. **29**(4): p. 737-752.
8. Ko, S.-u., J.M. Hausdorff, and L. Ferrucci, *Age-associated differences in the gait pattern changes of older adults during fast-speed and fatigue conditions: results from the Baltimore longitudinal study of ageing.* Age and Ageing, 2010. **39**(6): p. 688-694.
9. Lara, J., et al., *Towards measurement of the Healthy Ageing Phenotype in lifestyle-based intervention studies.* Maturitas, 2013. **76**(2): p. 189-99.
10. Fuchs, J., et al., *Indicators for Healthy Ageing — A Debate.* International Journal of Environmental Research and Public Health, 2013. **10**(12): p. 6630-6644.
11. Rantakokko, M., M. Mänty, and T. Rantanen, *Mobility Decline in Old Age.* Exercise and Sport Sciences Reviews, 2013. **41**(1): p. 19-25.
12. Artaud, F., et al., *Decline in Fast Gait Speed as a Predictor of Disability in Older Adults.* J Am Geriatr Soc, 2015. **63**(6): p. 1129-36.
13. Verghese, J., et al., *Quantitative Gait Markers and Incident Fall Risk in Older Adults.* The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 2009. **64A**(8): p. 896-901.
14. Studenski, S., et al., *Gait Speed and Survival in Older Adults.* JAMA : the journal of the American Medical Association, 2011. **305**(1): p. 50-58.
15. Busch Tde, A., et al., *Factors associated with lower gait speed among the elderly living in a developing country: a cross-sectional population-based study.* BMC Geriatr, 2015. **15**: p. 35.
16. Savica, R., et al., *Comparison of Gait Parameters for Predicting Cognitive Decline: The Mayo Clinic Study of Aging.* J Alzheimers Dis, 2016.
17. Strike, S.C., et al., *A High Omega-3 Fatty Acid Multinutrient Supplement Benefits Cognition and Mobility in Older Women: A Randomized, Double-blind, Placebo-controlled Pilot Study.* J Gerontol A Biol Sci Med Sci, 2016. **71**(2): p. 236-42.
18. Montero-Odasso, M., et al., *Gait and Cognition: A Complementary Approach to Understanding Brain Function and the Risk of Falling.* Journal of the American Geriatrics Society, 2012. **60**(11): p. 2127-2136.

19. Hardy, S.E., et al., *Improvement in usual gait speed predicts better survival in older adults.* J Am Geriatr Soc, 2007. **55**(11): p. 1727-34.

20. Ayers, E.I., et al., *Walking While Talking and Falls in Aging.* Gerontology, 2014. **60**(2): p. 108-113.

21. Hausdorff, J.M., et al., *Dual-task decrements in gait: contributing factors among healthy older adults.* J Gerontol A Biol Sci Med Sci, 2008. **63**(12): p. 1335-43.

22. Chen, X., G. Mao, and S.X. Leng, *Frailty syndrome: an overview.* Clinical Interventions in Aging, 2014. **9**: p. 433-441.

23. Cesari, M., et al., *Pharmacological Interventions in Frailty And Sarcopenia: Report by The International Conference on Frailty And Sarcopenia Research Task Force.* J Frailty Aging, 2015. **4**(3): p. 114-120.

24. Liu, C.J. and N.K. Latham, *Progressive resistance strength training for improving physical function in older adults.* Cochrane Database Syst Rev, 2009(3): p. Cd002759.

25. Gomez-Pinilla, F. and C. Hillman, *The Influence of Exercise on Cognitive Abilities.* Comprehensive Physiology, 2013. **3**(1): p. 403-428.

26. Tan, M.S., et al., *Efficacy and adverse effects of ginkgo biloba for cognitive impairment and dementia: a systematic review and meta-analysis.* J Alzheimers Dis, 2015. **43**(2): p. 589-603.

27. Harber, M.P., et al., *Aerobic exercise training improves whole muscle and single myofiber size and function in older women.* Am J Physiol Regul Integr Comp Physiol, 2009. **297**(5): p. R1452-9.

28. Dysken, M.W., et al., *Effect of vitamin E and memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative randomized trial.* Jama, 2014. **311**(1): p. 33-44.

29. Reay, J.L., M.A. Smith, and L.M. Riby, *B Vitamins and Cognitive Performance in Older Adults: Review.* ISRN Nutrition, 2013. **2013**: p. 650983.

30. Barberger-Gateau, P., *Nutrition and brain aging: how can we move ahead?* Eur J Clin Nutr, 2014. **68**: p. 1245-1249.

31. Scheltens, P., et al., *Efficacy of a medical food in mild Alzheimer's disease: A randomized, controlled trial.* Alzheimers Dement, 2010. **6**(1): p. 1-10.e1.

32. Scheltens, P., et al., *Efficacy of Souvenaid in mild Alzheimer's disease: results from a randomized, controlled trial.* J Alzheimers Dis, 2012. **31**(1): p. 225-36.

33. Dyall, S.C. and A.T. Michael-Titus, *Neurological benefits of omega-3 fatty acids.* Neuromolecular Med, 2008. **10**(4): p. 219-35.

34. Hutchins-Wiese, H.L., et al., *The impact of supplemental n-3 long chain polyunsaturated fatty acids and dietary antioxidants on physical performance in postmenopausal women.* J Nutr Health Aging, 2013. **17**(1): p. 76-80.

35. Dyall, S.C., *Amyloid-beta peptide, oxidative stress and inflammation in Alzheimer's disease: potential neuroprotective effects of omega-3 polyunsaturated fatty acids.* International Journal of Alzheimer's Disease, 2010: p. vol. 2010, Article ID 274128, 10 pages, 2010. doi:10.4061/2010/274128.

36. Dyall, S.C., *Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA.* Frontiers in Aging Neuroscience, 2015. **7**(52).

37. Smith, G.I., et al., *Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial.* The American Journal of Clinical Nutrition, 2011. **93**(2): p. 402-412.

38. Rapoport, S.I., *In vivo approaches to quantifying and imaging brain arachidonic and docosahexaenoic acid metabolism.* J Pediatr, 2003. **143**(4 Suppl): p. S26-34.

39. Thomas, J., et al., *Omega-3 Fatty Acids in Early Prevention of Inflammatory Neurodegenerative Disease: A Focus on Alzheimer's Disease.* Biomed Res Int, 2015. **2015**: p. 172801.

40. Zhang, Y.P., et al., *Effects of DHA Supplementation on Hippocampal Volume and Cognitive Function in Older Adults with Mild Cognitive Impairment: A 12-Month Randomized, Double-Blind, Placebo-Controlled Trial.* J Alzheimers Dis, 2016.

41. Parihar, R., J.R. Mahoney, and J. Verghese, *RELATIONSHIP OF GAIT AND COGNITION IN THE ELDERLY.* Curr Transl Geriatr Exp Gerontol Rep, 2013. **2**(3).

42. Doi, T., et al., *Cognitive function and gait speed under normal and dual-task walking among older adults with mild cognitive impairment.* BMC Neurology, 2014. **14**(1): p. 67.

43. Sinn, N., et al., *Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: a 6-month randomised controlled trial.* Br J Nutr, 2012. **107**(11): p. 1682-93.

44. Yurko-Mauro, K., *Cognitive and cardiovascular benefits of docosahexaenoic acid in aging and cognitive decline.* Curr Alzheimer Res, 2010. **7**(3): p. 190-6.

45. Witte, A.V., et al., *Long-chain omega-3 fatty acids improve brain function and structure in older adults.* Cereb Cortex, 2014. **24**(11): p. 3059-68.

46. Hooper, C., et al., *Cognitive Changes with Omega-3 Polyunsaturated Fatty Acids in Non-Demented Older Adults with Low Omega-3 Index.* J Nutr Health Aging, 2017. **21**(9): p. 988-993.

47. Kim, H.Y., B.X. Huang, and A.A. Spector, *Phosphatidylserine in the brain: metabolism and function.* Prog Lipid Res, 2014. **56**: p. 1-18.

48. Richter, Y., et al., *The effect of soybean-derived phosphatidylserine on cognitive performance in elderly with subjective memory complaints: a pilot study.* Clinical interventions in aging, 2013. **8**: p. 557-563.

49. Mangialasche, F., et al., *Serum levels of vitamin E forms and risk of cognitive impairment in a Finnish cohort of older adults.* Exp Gerontol, 2013. **48**(12): p. 1428-35.

50. Farina, N., et al., *Vitamin E for Alzheimer's dementia and mild cognitive impairment.* Cochrane Database Syst Rev, 2012. **11**: p. Cd002854.

51. Stillwell, W., W. Ehringer, and S.R. Wassall, *Interaction of alpha-tocopherol with fatty acids in membranes and ethanol.* Biochim Biophys Acta, 1992. **1105**(2): p. 237-44.

52. Miller, A.L., *The methionine-homocysteine cycle and its effects on cognitive diseases.* Altern Med Rev, 2003. **8**(1): p. 7-19.

53. Wong, Y.Y., et al., *Homocysteine, frailty, and all-cause mortality in older men: the health in men study.* J Gerontol A Biol Sci Med Sci, 2013. **68**(5): p. 590-8.

54. McCaddon, A., et al., *Homocysteine and cognitive decline in healthy elderly.* Dement Geriatr Cogn Disord, 2001. **12**(5): p. 309-13.

55. Ganguly, P. and S.F. Alam, *Role of homocysteine in the development of cardiovascular disease.* Nutr J, 2015. **14**: p. 6.

56. Dangour, A.D., et al., *Effects of vitamin B-12 supplementation on neurologic and cognitive function in older people: a randomized controlled trial.* The American Journal of Clinical Nutrition, 2015. **102**(3): p. 639-647.

57. Swart, K.M.A., et al., *A Randomized Controlled Trial to Examine the Effect of 2-Year Vitamin B12 and Folic Acid Supplementation on Physical Performance, Strength, and Falling: Additional Findings from the B-PROOF Study.* Calcified Tissue International, 2016. **98**: p. 18-27.

58. de Jager, C.A., et al., *Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial.* Int J Geriatr Psychiatry, 2012. **27**(6): p. 592-600.

59. Durga, J., et al., *Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial.* Lancet, 2007. **369**(9557): p. 208-16.

60. Smith, A.D., et al., *Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial.* PLoS One, 2010. **5**(9): p. e12244.

61. Jerneren, F., et al., *Brain atrophy in cognitively impaired elderly: the importance of long-chain omega-3 fatty acids and B vitamin status in a randomized controlled trial.* Am J Clin Nutr, 2015. **102**(1): p. 215-21.

62. Selley, M.L., *A metabolic link between S-adenosylhomocysteine and polyunsaturated fatty acid metabolism in Alzheimer's disease.* Neurobiol Aging, 2007. **28**(12): p. 1834-9.

63. Huang, T., M.L. Wahlqvist, and D. Li, *Effect of n-3 polyunsaturated fatty acid on gene expression of the critical enzymes involved in homocysteine metabolism.* Nutr J, 2012. **11**: p. 6.

64. World Health Organization, *Global Recommendations on Physical Activity for Health*. 2010, WHO.

65. Cadore, E.L., et al., *Neuromuscular adaptations to concurrent training in the elderly: effects of intrasession exercise sequence.* Age, 2013. **35**(3): p. 891-903.

66. Sui, X., et al., *CArdiorespiratory fitness and adiposity as mortality predictors in older adults.* JAMA, 2007. **298**(21): p. 2507-2516.

67. Colcombe, S.J., et al., *Aerobic exercise training increases brain volume in aging humans.* J Gerontol A Biol Sci Med Sci, 2006. **61**(11): p. 1166-70.

68. Jonasson, L.S., et al., *Aerobic Exercise Intervention, Cognitive Performance, and Brain Structure: Results from the Physical Influences on Brain in Aging (PHIBRA) Study.* Frontiers in Aging Neuroscience, 2016. **8**: p. 336.

69. Barnett, A., et al., *Community-based group exercise improves balance and reduces falls in at-risk older people: a randomised controlled trial.* Age Ageing, 2003. **32**(4): p. 407-14.

70. Denison, H.J., et al., *The effects of aerobic exercise on muscle strength and physical performance among community dwelling older people from the Hertfordshire Cohort Study: a randomised controlled trial.* Journal of the American Geriatrics Society, 2013. **61**(6): p. 1034-1036.

71. Smith, P.J., et al., *Aerobic Exercise and Neurocognitive Performance: a Meta-Analytic Review of Randomized Controlled Trials.* Psychosomatic medicine, 2010. **72**(3): p. 239-252.

72. Rissel, C., et al., *Two pilot studies of the effect of bicycling on balance and leg strength among older adults.* J Environ Public Health, 2013. **2013**: p. 686412.

73. Wainwright, T.W., T. Immins, and R.G. Middleton, *A cycling and education programme for the treatment of hip osteoarthritis: a quality improvement study.* Int J Orthop Trauma Nurs, 2016. **23**: p. 14-24.

74. Barreto, P.S., et al., *Effects of multidomain lifestyle intervention, omega-3 supplementation or their combination on physical activity levels in older adults: secondary analysis of the Multidomain Alzheimer Preventive Trial (MAPT) randomised controlled trial.* Age Ageing, 2018. **47**(2): p. 281-288.

75. Kobe, T., et al., *Combined omega-3 fatty acids, aerobic exercise and cognitive stimulation prevents decline in gray matter volume of the frontal, parietal and cingulate cortex in patients with mild cognitive impairment.* Neuroimage, 2016. **131**: p. 226-38.

76. Da Boit, M., et al., *Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: a randomized controlled trial.* Am J Clin Nutr, 2017. **105**(1): p. 151-158.

77. Kelley, G.A. and K.S. Kelley, *Dropouts and Compliance in Exercise Interventions Targeting Bone Mineral Density in Adults: A Meta-Analysis of Randomized Controlled Trials.* Journal of Osteoporosis, 2013. **2013**: p. 250423.

78. Wu, A., Z. Ying, and F. Gomez-Pinilla, *DHA dietary supplementation enhances the effects of exercise on synaptic plasticity and cognition.* Neuroscience, 2008. **155**(3): p. 751-759.

79. Short, K.R., et al., *Age and aerobic exercise training effects on whole body and muscle protein metabolism.* Am J Physiol Endocrinol Metab, 2004. **286**(1): p. E92-101.

80. Serhan, C.N., et al., *Novel Anti-Inflammatory -- Pro-Resolving Mediators and Their Receptors.* Current topics in medicinal chemistry, 2011. **11**(6): p. 629-647.

81. Nicklas, B.J., et al., *Exercise training and plasma C-reactive protein and interleukin-6 in elderly people.* J Am Geriatr Soc, 2008. **56**(11): p. 2045-52.

82. Balagopal, P., et al., *Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise.* Am J Physiol Endocrinol Metab, 2001. **280**(2).

83. Bickel, C.S., J.M. Cross, and M.M. Bamman, *Exercise dosing to retain resistance training adaptations in young and older adults.* Med Sci Sports Exerc, 2011. **43**(7): p. 1177-87.

84. Suresh, K., *An overview of randomization techniques: An unbiased assessment of outcome in clinical research.* J Hum Reprod Sci, 2011. **4**(1): p. 8-11.

85. Witte, T.R., et al., *RBC and WBC fatty acid composition following consumption of an omega 3 supplement: Lessons for future clinical trials.* Lipids in Health and Disease, 2010. **9**: p. 31-31.

86. Villani, A.M., et al., *Fish oil administration in older adults: is there potential for adverse events? A systematic review of the literature.* BMC Geriatr, 2013. **13**: p. 41.

87. Cadore, E.L., et al., *Effects of Different Exercise Interventions on Risk of Falls, Gait Ability, and Balance in Physically Frail Older Adults: A Systematic Review.* Rejuvenation Research, 2013. **16**(2): p. 105-114.

88. Falck, R.S., et al., *How much will older adults exercise? A feasibility study of aerobic training combined with resistance training.* Pilot and Feasibility Studies, 2017. **3**: p. 2.

89. Lepretre, P.M., et al., *Impact of short-term aerobic interval training on maximal exercise in sedentary aged subjects.* Int J Clin Pract, 2009. **63**(10): p. 1472-8.

90. Petrella, R.J., et al., *Improving aerobic fitness in older adults: effects of a physician-based exercise counseling and prescription program.* Canadian family physician Medecin de famille canadien, 2010. **56**(5): p. e191-e200.

91. Farrance, C., F. Tsofliou, and C. Clark, *Adherence to community based group exercise interventions for older people: A mixed-methods systematic review.* Prev Med, 2016. **87**: p. 155-66.

92. Fried, L.P., et al., *Frailty in older adults: evidence for a phenotype.* J Gerontol A Biol Sci Med Sci, 2001. **56**(3): p. M146-56.

93. Tombaugh, T.N. and N.J. McIntyre, *The mini-mental state examination: a comprehensive review.* J Am Geriatr Soc, 1992. **40**(9): p. 922-35.

94. British Psychological Society, *Response to the UK National Screening Committee consultation: Appraisal Screening for Alzheimer's Disease* B.P. Society, Editor. 2010.

95. Bright, P., E. Jaldow, and M.D. Kopelman, *The National Adult Reading Test as a measure of premorbid intelligence: a comparison with estimates derived from demographic variables.* J Int Neuropsychol Soc, 2002. **8**(6): p. 847-54.

96. van Iersel, M.B., et al., *The effect of cognitive dual tasks on balance during walking in physically fit elderly people.* Arch Phys Med Rehabil, 2007. **88**(2): p. 187-91.

97. Hall, C.D., et al., *Cognitive and motor mechanisms underlying older adults' ability to divide attention while walking.* Phys Ther, 2011. **91**(7): p. 1039-50.

98. Ullmann, G. and H.G. Williams, *The relationships among gait and mobility under single and dual task conditions in community-dwelling older adults.* Aging Clin Exp Res, 2011. **23**(5-6): p. 400-5.

99. Perera, S., et al., *Gait Speed Predicts Incident Disability: A Pooled Analysis.* J Gerontol A Biol Sci Med Sci, 2016. **71**(1): p. 63-71.

100. McDowd, J.M., *The effects of age and extended practice on divided attention performance.* J Gerontol, 1986. **41**(6): p. 764-9.

101. Goldberg, A., et al., *The five-times-sit-to-stand test: validity, reliability and detectable change in older females.* Aging Clin Exp Res, 2012. **24**(4): p. 339-44.

102. Davidson, D.J., R.T. Zacks, and C.C. Williams, *Stroop interference, practice, and aging.* Neuropsychol Dev Cogn B Aging Neuropsychol Cogn, 2003. **10**(2): p. 85-98.

103. Mathôt, S., D. Schreij, and J. Theeuwes, *OpenSesame: An open-source, graphical experiment builder for the social sciences.* Behavior Research Methods, 2012. **44**(2): p. 314-324.

104. Leys, C., et al., *Detecting outliers: Do not use standard deviation around the mean, use absolute deviation around the median.* Journal of Experimental Social Psychology, 2013. **49**(4): p. 764-766.

105. Rey, A., *L'examen psychologique dans les cas d'encéphalopathie traumatique.(Les problems.).* Archives de psychologie, 1941.

106. Salthouse, T.A., et al., *Effects of aging on efficiency of task switching in a variant of the trail making test.* Neuropsychology, 2000. **14**(1): p. 102.

107. Tuck, M.K., et al., *Standard Operating Procedures for Serum and Plasma Collection: Early Detection Research Network Consensus Statement Standard Operating Procedure Integration Working Group.* Journal of proteome research, 2009. **8**(1): p. 113-117.

108. Hustad, S., et al., *Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism.* Clin Chem, 2012. **58**(2): p. 402-10.

109. Deer, R.R. and E. Volpi, *Protein Intake and Muscle Function in Older Adults.* Current opinion in clinical nutrition and metabolic care, 2015. **18**(3): p. 248-253.

110. Sublette, M.E., et al., *Validation of a food frequency questionnaire to assess intake of n-3 polyunsaturated fatty acids in subjects with and without major depressive disorder.* J Am Diet Assoc, 2011. **111**(1): p. 117-123.e1-2.

111. Stewart, A.L., et al., *CHAMPS physical activity questionnaire for older adults: outcomes for interventions.* Med Sci Sports Exerc, 2001. **33**(7): p. 1126-41.

112. Walters, S.J., J.F. Munro, and J.E. Brazier, *Using the SF-36 with older adults: a cross-sectional community-based survey.* Age Ageing, 2001. **30**(4): p. 337-43.

113. Brazier, J.E., et al., *Validating the SF-36 health survey questionnaire: new outcome measure for primary care.* Bmj, 1992. **305**(6846): p. 160-4.

114. Kwon, S., et al., *What is a meaningful change in physical performance? Findings from a clinical trial in older adults (the LIFE-P study).* J Nutr Health Aging, 2009. **13**(6): p. 538-44.

115. Chao, D., C.G. Foy, and D. Farmer, *Exercise adherence among older adults: challenges and strategies.* Control Clin Trials, 2000. **21**(5 Suppl): p. 212s-7s.

116. Picorelli, A.M., et al., *Adherence to exercise programs for older people is influenced by program characteristics and personal factors: a systematic review.* J Physiother, 2014. **60**(3): p. 151-6.

117. Clinicaltrials.gov. *ClinicalTrials.gov "Basic Results" Data Element Definitions*. 2016 [cited 2016 5/12/16]; Available from: https://prsinfo.clinicaltrials.gov/results_definitions.html#AdverseEventsDefinition.

118. Boutron, I., et al., *Extending the CONSORT statement to randomized trials of nonpharmacologic treatment: explanation and elaboration.* Ann Intern Med, 2008. **148**(4): p. 295-309.

119. Chan, A.W., et al., *SPIRIT 2013 statement: defining standard protocol items for clinical trials.* Ann Intern Med, 2013. **158**(3): p. 200-7.

120. Hoffmann, T.C., et al., *Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide.* Bmj, 2014. **348**: p. g1687.

121. Kobe, T., et al., *Combined omega-3 fatty acids, aerobic exercise and cognitive stimulation prevents decline in gray matter volume of the frontal, parietal and cingulate cortex in patients with mild cognitive impairment.* Neuroimage, 2015.

122. Da Boit, M., et al., *Sex differences in the response to resistance exercise training in older people.* Physiol Rep, 2016. **4**(12).

123. Radloff, L.S., *The CES-D scale: A self-report depression scale for research in the general population.* Applied psychological measurement, 1977. **1**(3): p. 385-401.

124. Washburn, R.A., et al., *The Physical Activity Scale for the Elderly (PASE): development and evaluation.* J Clin Epidemiol, 1993. **46**(2): p. 153-62.

Figure 1. Participant flow through the study.

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.			

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

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 (μg/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

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