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Effects of a high DHA multi-nutrient supplement and exercise on mobility and cognition in older women (MOBILE): A randomised semi-blinded placebo controlled study

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Abstract

There is a complex interplay between mobility and cognition in older adults. We have previously shown that a high DHA multi-nutrient supplement improves habitual walking speed, verbal memory, and psychomotor response latency in older women. Exercise also improves mobility and cognition in older adults, and omega-3 fatty acids and exercise share a range of overlapping biological effects. This study examined for the first time the effects of the high DHA multi-nutrient supplement and aerobic exercise on mobility and cognition in older women. Women (mean age 67 y, SD 8) were assigned to the following groups: multi-nutrient (1 g DHA, 160 mg EPA, 240 mg *Ginkgo biloba*, 60 mg phosphatidylserine, 20 mg d-α tocopherol, 1 mg folic acid, and 20 µg vitamin B12 per day, N=13), multi-nutrient and exercise (spin class twice per week, N=14), exercise and placebo (N=12), or placebo (N=12). The multi-nutrient was given for 24 weeks, and exercise for 12 weeks. Trial registration: NCT03228550. No treatment effects were observed for the primary outcome, habitual walking speed. Improvements in verbal memory and executive function were seen for all treatments groups versus placebo (all, $p<0.05$). Significant improvements in self-reported emotional wellbeing were seen with multi-nutrient and exercise groups versus placebo ($p=0.03$). The results suggest that the high DHA multi-nutrient supplement produces similar improvements in cognitive function to aerobic exercise, offering the intriguing prospect that supplementation may be able to mitigate some of the effects of low physical activity on cognitive function in the elderly.

Introduction

Ageing is associated with a progressive decline in both cognitive and physical function, which can lead to a number of age-related health conditions including frailty and dementia [1]. Walking is a complex task with a significant cognitive aspect, and changes in several gait parameters including speed often coexist with or precede the onset of cognitive decline in older adults [2]. Gait speed is a clinically relevant marker in older adults, due to strong associations with physical functioning and disability [3]. Previous work in our laboratory suggests a high docosahexaenoic acid (DHA) multi-nutrient supplement providing the omega-3 polyunsaturated fatty acids (PUFAs), DHA and eicosapentaenoic acid (EPA), and supporting nutrients phosphatidylserine (PS), d-α tocopherol, folic acid, vitamin B12, and *Ginkgo biloba*, improves cognition and mobility in older females [4].

Providing omega-3 PUFAs in combination with other compounds indicated to support brain function may provide greater efficacy than if supplemented in isolation, although results are so far mixed. For example, the same multi-nutrient supplement as used in our previous and present studies did not improve cerebral hemodynamic or cognitive function in healthy older adults [5]; however, the authors acknowledge that the cognitive tests were selected on the basis that they are able to activate the prefrontal cortex, and may not be sensitive to the components in the supplement. Similarly, an omega-3 PUFA multi-nutrient supplement showed no significant effects on a battery of cognitive tests of participants with prodromal Alzheimer's disease, although improvements were seen in secondary outcomes of cognitive function and hippocampal atrophy [6].

Aerobic exercise has consistently been shown to improve both mobility and cognition in older adults [7]. Omega-3 PUFAs and exercise share a range of overlapping biological effects, including enhancing neurogenesis, neural plasticity, and reducing inflammation [8, 9]. Indeed, preliminary evidence suggests combining omega-3 PUFAs with exercise may provide additional benefit when compared to either approach alone. For example, omega-3 PUFA supplementation combined with twice weekly stationary cycle training and cognitive stimulation led to an enhanced reduction of brain atrophy in grey matter regions compared to supplementation [10]. Similarly, combining omega-3 PUFA supplementation with resistance training in older females provided an additional benefit to muscle strength compared with the exercise alone [11]. In addition, an omega-3 PUFA multi-nutrient supplement combined with exercise was recently shown to improve verbal recall and executive function in older men, more than supplementation alone [12]. The mechanisms underpinning these interactions are currently unclear, as the studies were not been designed to elucidate these effects. However, decreasing Hcy levels may be a potential factor, as omega-3 PUFAs regulate the expression of genes encoding enzymes involved in Hcy metabolism [13] and exercise decreases Hcy levels [14].

The aim of this study is to further our initial observations and examine for the first time the effects of the multi-nutrient supplement and aerobic exercise on mobility and cognition in older women. The study was restricted to female participants to enable comparison with our previous work [4], and it has been suggested that the additive effects of combining exercise and omega-3 PUFA supplementation may be limited to women only [11]. Women have also been shown to have a greater compliance to exercise interventions [15]. Habitual gait speed was the primary outcome, with secondary outcomes related to mobility, cognition, and quality of life [16].

Methods

The study is a randomised semi-blinded, placebo controlled trial in women aged 60 years and above with a factorial design. Detailed information of the study procedure has been previously published [17], in line with guidelines of the Template for Intervention Description and Replication [18]. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Bournemouth University Science Technology and Health research ethics panel (Ethics ID 10788). Prior to data collection participants provided written informed consent. The trial was listed on www.clinicaltrials.gov (NCT03228550), and follows the Consolidated Standards of Reporting Trials (CONSORT) statement on randomised trials [19]. All data was collected and analysed at Bournemouth University, U.K.

Participants

Females aged 60 years and above were recruited according to the following inclusion criteria: (1) able to walk at least 50 m unaided, (2) classified as non-frail or pre-frail [20], and (3) community dwelling. Exclusion criteria were: (1) vestibular impairments, (2) diagnosed neurological disorder, (3) cognitive impairment (Mini Mental Status Examination score <24), (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. A stratified block randomisation design was followed based on frailty classification of non-frail or pre-frail, followed by permuted block randomisation. Participant demographics can be found in Table 1. For further information please refer to the published protocol [17].

Interventions

Participants were allocated to one of four groups: multi-nutrient supplement and exercise (MS+EX), placebo supplement and exercise (P+EX), multi-nutrient supplement only (MS), or placebo supplement only (P).

Dietary supplement

Participants received four capsules per day of their respective dietary supplement for 24 weeks. The daily dose from the active capsules contained 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 placebo capsules contained an isocaloric oil blend typical of the U.K. diet including a small amount of fish oil. A small amount of fish oil was added to the placebo capsules to aid with participant blinding, which provided a daily dose of 21.6 mg EPA and 14.4 mg DHA, an amount unlikely to produce any therapeutic benefit (Supplementary Table 1). Participants were told to take their capsules with the largest meal of the day. The capsules were coded by the Principal Investigator (PI), who had no involvement in the data collection, and all subsequent operational oversight and interpretation of the data was undertaken with the PI blinded to specific participant codes. Compliance to the dietary supplement was measured by changes in whole-blood DHA levels compared to baseline, counting returned pills at 12 and 24 weeks, and exit questionnaire. Adverse events were monitored by subject self-reporting and exit questionnaire.

Exercise

The exercise intervention consisted of two group sessions per week on a Spinner Fit stationary bike. Classes lasted 30 min for the first six weeks and increased to 45 min for the

second six weeks, with five min warm up and cool down at 7-8 on the Borg scale of rate of perceived exertion. During the main body of the sessions participants maintained an intensity of 12-14 on the Borg scale. Compliance to the exercise intervention was monitored by recording attendances by each participant and calculated as the percentage of classes attended.

Outcome measures

The outcome measures were based on changes in mobility, cognitive function and health related quality of life, with the primary outcome measure change in habitual walking speed. Information on the age, Body Mass Index (BMI), verbal intelligence, and medication use were collected from each participant. Information on medications was self-reported, with both type and number of medications recorded. The National Adult Reading Test (NART) was used to assess verbal intelligence. All measurements were performed at baseline and end of the study.

Mobility

Participants were assessed under three gait conditions: habitual walking, fast walking, and dual task (DT) walking. During the DT participants were required to count backwards in integers of three from a randomly generated three digit number, and were not instructed to prioritise either walking or counting backwards during the task. Gait speed was measured using Opal inertial sensors and analysed using Mobility Lab™ software version 3.1 (APDM Inc, http://apdm.com). Sensors were placed over the shoes. Each tested condition was repeated five times and the mean value for each trial used for habitual and DT gait with the maximum gait speed value being used for fast walking. The five times sit to stand (5TSTS) test was used to assess dynamic balance and functional mobility.

163

164 **Cognition**

165 A Stroop test was used to assess interference control using Open Sesame version 3.1.1.
166 software [21]. Participants were presented names of one of four colours: blue, red, green, and
167 white, in four different font colours varying between these colours. Participants were required
168 to identify the colour of the text. Interference control was defined as the difference between
169 the mean time taken to respond to the congruent and non-congruent trials. Spatial memory
170 was also assessed using Open Sesame software, based upon Nagamatsu and colleagues [22].
171 Each trial comprised presentation of three dots at randomly allocated locations for 500 ms,
172 followed by a fixation cross for three seconds. The test comprised presentation of a single red
173 dot on the screen, either in the same location (match) or a different (non-match) location, and
174 participants had to identify if the test dot was match or non-match. The Rey's Auditory
175 Verbal Learning Test (RAVLT) was used to assess verbal memory, where participants were
176 required to recall a list of 15 pre-set words [23]. The trail making task was used to assess
177 executive function [24], where participants drew lines between targets on a piece of paper, as
178 rapidly as possible, under four different conditions: (1) numbers condition (one to 49), (2)
179 letters condition, (A to Z), (3) alternated numbers (one to 25) and letters (A to X), and (4)
180 alternated letters (A to Y) and numbers (one to 24). Scores were recorded as the total number
181 of correct connections.

182

183 **Health related quality of life**

184 The short form 36 questionnaire (SF-36) was used to asses health related quality of life [25].

185

186 **Biochemical measures**

Biochemical measures were conducted to assess compliance to the supplementation and to better understand how changes may contribute towards any therapeutic effects on the measured outcomes. Finger pin-prick blood samples were collected and total fatty acid content analysed, as described previously [26]. Individual fatty acids were quantified using a gas chromatograph with flame ionisation detector (Agilent Technologies, U.S.A), and expressed as % of total fatty acids. A non-fasted venous blood sample was drawn to assess serum Hcy. Each sample was allowed to clot and immediately centrifuged at 2000 x *g* for 10 min at 4^0C and serum extracted. Samples were stored at -80°C and analysed within three months. Hcy levels were measured by competitive enzyme-linked immunosorbent assay (ELISA) (Cell Biolabs Inc.) according to manufacturer's instructions.

Diet and lifestyle

Participants' diet and lifestyle habits were monitored. Three day food diaries were used and results analysed using Nutritics dietary analysis software (www.nutritics.com). A validated food frequency questionnaire (FFQ) was used to quantify omega-3 PUFA intake [27]. The community health activities program for seniors (CHAMPS) questionnaire was used to assess physical activity levels. These measures were taken so that baseline diet and physical activity habits could be included as covariates and to monitor whether there were any significant changes in diet and lifestyle during the course of the intervention period.

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics version 21 (Chicago, USA). 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 [28]. The sample size calculation was based on a difference of 0.08 m/sec with the level of variability set at 0.1 based on our previous work [4] with a minimum sample size of 13 participants per group required to detect an effect size d of 0.5. In our original protocol a large effect size was suggested; however, this was subsequently changed to a medium effect size based on our previous pilot study [4, 17]. Data were tested for normal distribution using Shapiro-Wilk test and Q-Q-plots and Levenes test of equality of error variances to check for assumptions of homogeneity. Correlations between mobility, cognitive function, and health related quality of life, exercise, circulating omega-3 PUFAs and Hcy were examined using partial correlations, controlling for age, BMI and dietary protein. For any variables that did not meet the assumptions of normality for partial correlations a syntax was used to produce a non-parametric partial correlation. This method consisted of using the NONPAR CORR and /MATRIX OUT commands to produce a Spearman Rho matrix, which could then be read by the /MATRIX IN and PARTIAL CORR command whilst factoring in the appropriate covariates. For all correlation analysis a sample size of 46 was required to achieve a power of 0.80 for a correlation coefficient of 0.40 and an α of 0.05 [29]. Evidence suggests a non-linear relationship between Hcy and health of older adults [30], therefore non-parametric correlation analysis was performed. NART score was included as a covariate in preliminary analysis, but was excluded in final models.

Baseline and 24 week results from the diet and physical activity assessments were compared within groups, using paired T-tests. A general linear model was used to compare the active intervention groups versus the placebo over time (from pre- to post-measurement) on changes on the dependent variables on an intention to treat basis. Effect size calculation (η^2 (Eta squared)) was performed. Demographic and health information, such as age, and BMI were included as covariates in the analysis if they were significantly correlated with the dependent

variable. The Benjamini-Hochberg procedure was used to decrease the false discovery rate and probability of type I errors, with a Q value of <0.25 accepted as significant, and all P values are expressed as raw values [31]. Linear regression we used to identify whether a difference in whole-blood DHA was associated with a change in outcome score. All results are expressed as means (SD). Pre-trial registration plans dictated that a 2 x 2 ANOVA would be conducted to examine treatment effects, however due to the potential interaction between the supplementation and exercise independence of these variables could not be guaranteed, thus examining treatment effects based on the assigned groups was determined to be a more robust method. Correlation analysis was used to identify whether a difference in whole-blood DHA was associated with a change in outcome score. All results are expressed as means (SD).

Results

Participant flow is shown in Fig 1. The study had three separate intakes of participants, starting in March 2017, May 2017, and February 2018, with data collection taking place between March 2017 and January 2019. In total 60 participants were allocated to intervention groups and 51 completed the study. Analyses were conducted on all who completed the study with no exclusions irrespective of compliance or protocol violations. Participants who withdrew during the study were invited to attend follow up assessment; however, the invitations were declined. Role limitations due to physical health problems, role limitations due to emotional health problems, and social functioning from the SF-36 questionnaire showed clear signs of ceiling effects, therefore these were omitted from analysis. Hcy data was only available for 48 baseline values. Upon inspection four results of the baseline whole-blood DHA levels were identified as unreliable and were subsequently excluded from the analysis.

The group baseline characteristics are shown in Table 1. There were no reported adverse effects in any group over the study. The multi-nutrient supplementation led to significant increases in DHA and EPA compared to baseline levels in the MS (28% and 190%, respectively) and MS+EX (43% and 140%, respectively) groups, and significant decreases in the omega-6 PUFA, arachidonic acid levels of -28% and -26%, respectively, all $p < 0.05$, Table 2. Compliance to the supplementation measured via changes in DHA and was 91% for MS+EX, and 80% for MS, 100% for P+EX and 100% for P, which was corroborated by capsule counting. Compliance to the exercise intervention was 55% for MS+EX and 56% for P+EX, which is consistent with other exercise intervention trials in a similar demographic [22]. There were no significant changes in reported calorie, macronutrient or omega-3 PUFA intake between baseline and follow up, Table 2. Participants maintained their current use of medications and there were no reported changes health status throughout the duration of the study with the exception of two participants in the MS+EX who discontinued their use of analgesic and anti-inflammatory medications.

Primary outcome measure

There was a non-significant decrease of 0.8% in the mean habitual walking speed by the P group over the study; whereas, the other groups increased their mean walking speed by 0.05 m/s (0.07 m/s) (4.0%) for the MS group, 0.03 m/s (0.09 m/s) (2.5%) for the P+EX group, and 0.01 m/s (0.12 m/s) (0.8%) for the MS+EX group. However, these changes were not statistically significant for supplementation ($p = 0.25$), exercise ($p = 0.50$) or for the combined intervention ($p = 0.79$), Fig 2.

285 **Secondary outcome measures**

286 The results for the effects of the interventions on mobility, cognition, and health related
287 quality of life are summarised in Table 3. There was a significant effect on verbal memory
288 compared with the P group, by the MS [$F(1,46) = 7.59$, $p = 0.008$, partial η^2 0.144)], P+EX
289 [$F(1,46) = 7.70$, $p = 0.008$, partial η^2 0.144] and MS+EX interventions [$F(1,46) = 15.82$, $p <$
290 0.001, partial η^2 0.256], all with large effect sizes, Fig 3. Significant effects versus placebo
291 were also observed for executive function for the MS [$F(1,47) = 8.02$, $p = 0.007$, partial η^2
292 0.146)], P+EX [$F(1,47) = 8.37$, $p = 0.006$, partial η^2 0.151)], and MS+EX groups [$F(1,47) =$
293 8.60, $p = 0.005$, partial η^2 0.155)] all with large effect sizes, Fig 4. The MS+EX group also
294 reported significant improvements in emotional wellbeing, compared to the P group [$F(1,47)$
295 $= 8.07$, $p = 0.03$, partial η^2 0.146], with a large effect size. No significant treatment effects
296 were observed for the other outcomes.

297

298 **Correlations of outcomes with DHA status and Serum Hcy**

299 Significant baseline relationships between DHA and DT gait speed $r(53) = 0.32$, $p = 0.018$,
300 bodily pain $r(51) = 0.35$, $p = 0.013$ and emotional wellbeing $r(51) = 0.30$, $p = 0.032$ from the
301 SF-36 questionnaire were identified. These relationships were moderate to weak in strength.
302 There were no significant associations between DHA and any cognitive outcomes. Following
303 the interventions there was a direct relationship between changes in verbal memory and DHA
304 levels, $r(18) = 0.66$, $p = 0.001$, Fig 5, however no relationship was detected for changes in
305 executive function. At baseline there were no significant associations between serum Hcy and
306 any of the outcome measures (Supplementary Table 2).

307

Discussion

The MOBILE study explores for the first time the effects of a high DHA multi-nutrient supplement and aerobic exercise on mobility and cognition in older women. Although the supplementation led to significant increases in DHA levels, there were no significant improvements in the primary outcome measure of habitual walking speed. However, significant improvements in verbal memory and executive function were seen following the interventions, with the improvements in verbal memory in direct relation to increases in whole-blood DHA levels. Furthermore, lower circulating DHA was associated with poorer DT performance, and measures of pain and emotional wellbeing at baseline.

Significant improvements in habitual walking speed were previously shown by us following treatment with the same multi-nutrient supplement, reflecting clinically relevant improvements within a similar sample size [4]. These improvements were driven by both improvements in the treatment group and decreases in the placebo group. However, in the present study although there were increases following the multi-nutrient supplementation of a similar magnitude to our previous study, the decline in the placebo group was much less than predicted, with a mean decline of 0.8%, as opposed to 2% in our previous study [32], which may reflect a high functioning nature of the participants. Indeed, the lack of improvement following the exercise intervention supports this hypothesis, as aerobic exercise has consistently been shown to improve walking speed [33, 34]. Interestingly the effect size for the combined supplementation and exercise group was modest in comparison to each intervention on their own however this may be partly explained by the aforementioned participants who discontinued daily use of analgesic and anti-inflammatory for joint pain who both observed small declines in gait speed of 0.01 m/s.

The individual and combined interventions led to improvements in verbal memory, with the total recall across the trials representing eight to twelve more words remembered than the placebo group. These findings corroborate previous observations showing positive effects on verbal memory following treatment with the same multi-nutrient supplement [4]. Similarly, the improvements following exercise are consistent with previous research [7]. The individual and combined interventions also resulted in significantly improved executive function. The observation of exercise improving this cognitive domain is consistent with previous experimental work [7]. There is currently no clear consensus in the literature as to whether the factors in the multi-nutrient supplement improve executive function in older adults. For example, 900 mg DHA for 24 weeks had no effect on executive function [35], whereas 1320 mg EPA and 880 mg DHA for 26 weeks produced significant improvements [36]. Similarly, although B vitamins [37] and *Ginkgo biloba* [38] do not appear to improve executive function, preliminary evidence suggests PS has some beneficial effects [39]. A recent study suggested an omega-3 PUFA multi-nutrient supplement combined with exercise improves executive function in older men [12]; however, as there was no exercise only control group the contribution of omega-3 PUFA multi-nutrient supplement to these effects cannot be identified. Indeed our analysis found no significant relationship between changes in blood level of DHA and the executive function suggesting that other factors may have contributed towards the change in this outcome.

Due to the small sample size analysis to detect superiority of the active interventions over one another was not possible. It may be hypothesised that the effects of the multi-nutrient supplement and exercise operate via overlapping biological mechanisms. This includes decreasing inflammation, for example, interleukin 6 (IL-6) levels are linked to declines in verbal memory [40], and both omega-3 PUFAs and aerobic exercise have both been shown to decrease IL-6 levels [41]. Alternatively, exercise and factors in the multi-nutrient supplement,

such as DHA and *Ginkgo biloba* increase brain-derived neurotrophic factor (BDNF) levels, which may contribute to enhancing synaptic plasticity and cognitive function [42-44]. However, further work is needed to explore these potential mechanisms, if they mediate any of the observed effects and if indeed the combination of the two interventions can provide an additional benefit to the older adult.

Due to the range of compounds in the multi-nutrient supplementation it is not possible to ascribe the effects to any single factor, and the present results further support the use of a combination of dietary factors in aging [4, 45]. Unfortunately, due to methodological issues we were unable to monitor changes in serum Hcy levels over the study and so cannot identify the potential role of folic acid and vitamin B12 in the treatment effects. However, a major unifying factor in the multi-nutrient intervention is DHA, and the supplementation increased circulating DHA and EPA levels. To identify the potential contribution of DHA to the treatment effects correlations between changes in DHA and changes in outcome measures were performed. The increases in DHA levels over the study were in direct relation with improvements in verbal memory, suggesting increasing DHA was an important contributor to the treatment effects on this outcome. At baseline, significant relationships were seen with DHA and DT gait speed, although no other measures of mobility or cognition. Previous observations suggest circulating DHA levels correlate with gait and mobility outcomes [4, 46] , although erythrocyte DHA and EPA was not shown to be associated with a slower decline in gait speed over three years in a fully adjusted model [47]. The results from the present study do provide some further support for DHA status as a potential blood biomarker for monitoring physical performance in aging, although further work is needed to establish this link more clearly.

The strengths of this study are that it was randomised, placebo controlled semi-blinded design, with high retention rate, thereby increasing the reliability of the data. The study used

a range of detailed and sensitive mobility and cognitive tests to assess treatment effects. It is important in this type of study to monitor participant's dietary intake and physical activity; these were assessed by diet diary and FFQ, and CHAMPS questionnaire, respectively. This meant it could be confirmed that the participants diet and physical activity outside of the interventions remained consistent. The only notable, although not statistically significant, change in diet was a decrease in fat intake across all four groups; however we have no reason to believe this decrease across the groups would have influenced the results of the analysis. Furthermore, the trial design was reviewed and made publicly available on a clinical trials registry and was subject to peer review during the protocol publication process. Additionally, blood fatty acid levels were taken at the start and end of the study, to monitor absorption and incorporation of the omega-3 PUFAs. Limitations were the low recruitment, and high functional ability of the participants. A further limitation was that ApoE genotype was not assessed, as this may affect participant's response to the supplementation [48], and is associated with gait speed decline in aging [49].

Conclusions

Overall, these results suggest that the multi-nutrient supplement produces similar improvements in verbal memory and executive function to aerobic exercise, offering the intriguing prospect that supplementation may be able to mitigate some of the effects of low physical activity on cognitive function in the elderly. These improvements are clinically relevant and were identified in able female adults. Treatment effects were not identified in the primary outcome, nonetheless the improvements in verbal memory and executive function does provide some promising insight into the benefits of dietary supplementation and exercise for the promotion of healthy ageing. The study identified for the first time

406 relationships between DHA and DT ability. Further work should seek to explore the effects
407 of the supplement on participants who may be most likely to respond, i.e. those with low
408 DHA and high Hcy levels, and also explore supplementation for a longer period, or in a more
409 frail population.

410

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414

415

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421

422 **Conflict of Interest**

423 The authors declare no financial conflicts of interest.

424

425 **Declaration of Ethical Standards**

Ethical approval was granted by Bournemouth University Science Technology and Health research ethics panel (Ethics ID 10788) and the study conformed to the declaration of Helsinki and guidelines for Good Clinical Practice.

Author Contributions

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

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562

563

564 Figure 1 Participant flow through study

565

566 Figure 2 Effects of multi-nutrient supplement and exercise on the primary outcome of
567 habitual gait speed. There were no significant effects for any intervention on changes in gait
568 speed.

569

570 Figure 3 Effects of multi-nutrient supplement and exercise by general linear model on Rey's
571 auditory verbal learning test. Significant effects for multi-nutrient supplement ($p = 0.008$)
572 placebo supplement and exercise ($p = 0.008$) and combination of multinutrient and exercise
573 ($p < 0.001$). See text for further detail.

574 * Indicates a significant effect of the intervention on changes in verbal memory versus
575 placebo.

576

577 Figure 4 Effects of multi-nutrient supplement and exercise by general linear model on
578 executive function. Significant effects for multi-nutrient supplement ($p = 0.007$) placebo
579 supplement and exercise ($p = 0.006$) and combination of multinutrient and exercise ($p <$
580 0.005). See text for further detail.

581 * Indicates a significant effect of the intervention on changes in executive function versus
582 placebo.

583

584 Figure 5 Scatter plot for changes in DHA and change in verbal memory for MS and P groups,

585 $r(18) = 0.66$, $p = 0.001$.

Table 1 Participant characteristics at baseline.

Parameter	PL (N = 12)	PL + EX (N = 12)	MS (N = 13)	MS + EX (N = 14)
Age (years)	67 (4)	67 (4)	69 (4)	68 (5)
Height (m)	1.63 (0.1)	1.64 (0.1)	1.62 (0.1)	1.61 (0.1)
Weight (kg)	69.5 (14.9)*	77.1 (33)*	75.6 (17.4)*	68.6 (11.1)*
BMI (kg/m^2)	26.8 (5.2)*	27.8 (12.0)*	27.4 (5.3)*	28.6 (5.9)*
Comorbidities	Hypertension (2) Hypercholesterolemia (1) Osteoarthritis (2)	Hypertension (3) Osteoarthritis (1)	Hypertension (2) Hypercholesterolemia (1) Osteoporosis (1)	Hypertension (3) Hypercholesterolemia (2) Diabetes (1) Osteoarthritis (2)
NART Score	36 (8)	36 (5)	37 (5)	36 (7)
MMSE score	30 (1)*	30 (0)*	30 (1)*	30 (2)*
Hand Grip Strength (kg)	20.5 (3.4)	21.0 (5.9)	19.3 (5.4)	19.0 (4.0)
PASE Score	114 (97)*	113 (30)*	111 (73)*	100 (55)*

*Indicates value is the median (IQR), otherwise means (SD) presented.

Abbreviations: Mini Mental Sate Exam, MMSE; National Adult Reading Test, NART; Physical Activity Scale in the Elderly, PASE

Table 2: Participant compliance

Parameter	PL (N = 12)[a]		PL+EX (N = 12)		MS (N = 13)[b]		MS+EX (N = 14)	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
				Fatty acid levels (% total fatty acids)				
ARA	6.6 (1.2)	6.3 (1.5)	6.7 (2.0)	6.9 (1.1)	7.1 (3.4)	5.1 (1.2)*	6.9 (2.0)	5.1 (1.4)*
EPA	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4 (0.3)	0.3 (0.1)	0.9 (0.2)*	0.4 (0.2)	0.8 (0.4)*
DHA	1.7 (0.4)	1.6 (0.4)	1.7 (0.6)	1.5 (0.4)	1.8 (0.6)	2.3 (0.5)*	1.5 (0.4)	2.1 (0.6)*
				Dietary intake per day				
Kcal	1869 (440)	1772 (518)	1530 (386)	1507 (310)	1642 (464)	1489 (439)	1738 (293	1565 (339)
Carbohydrate (g)	190 (41)	180 (51)	156 (45)	154 (43)	175 (49)	168 (51)	175 (33)	164 (39)
Protein (g/kg bodyweight)	1.12 (0.38)	1.11 (0.34)	0.91 (0.40)	0.91 (0.36)	1.00 (0.26)	0.93 (0.28)	1.09 (0.19)	0.96 (0.21)
Fat (g)	84 (25)	76 (31)	68 (23)	62 (10)	67 (27)	63 (25)	73 (19)	66 (23)
				Physical activity				
CHAMPs (kcal)	3619 (2159)	3390 (1686)	3454 (2009)	4011(2622)*	4314 (2328)	3945(2098)	3289 (1537)	3650 (1523)*

* Indicates a significant change versus baseline, assessed using paired T-test or Wilcoxon signed-rank test where appropriate

[a] N = 10 and [b] N = 10 for whole-blood fatty acid value

Table 3 Secondary outcome measures displayed as mean (SD)

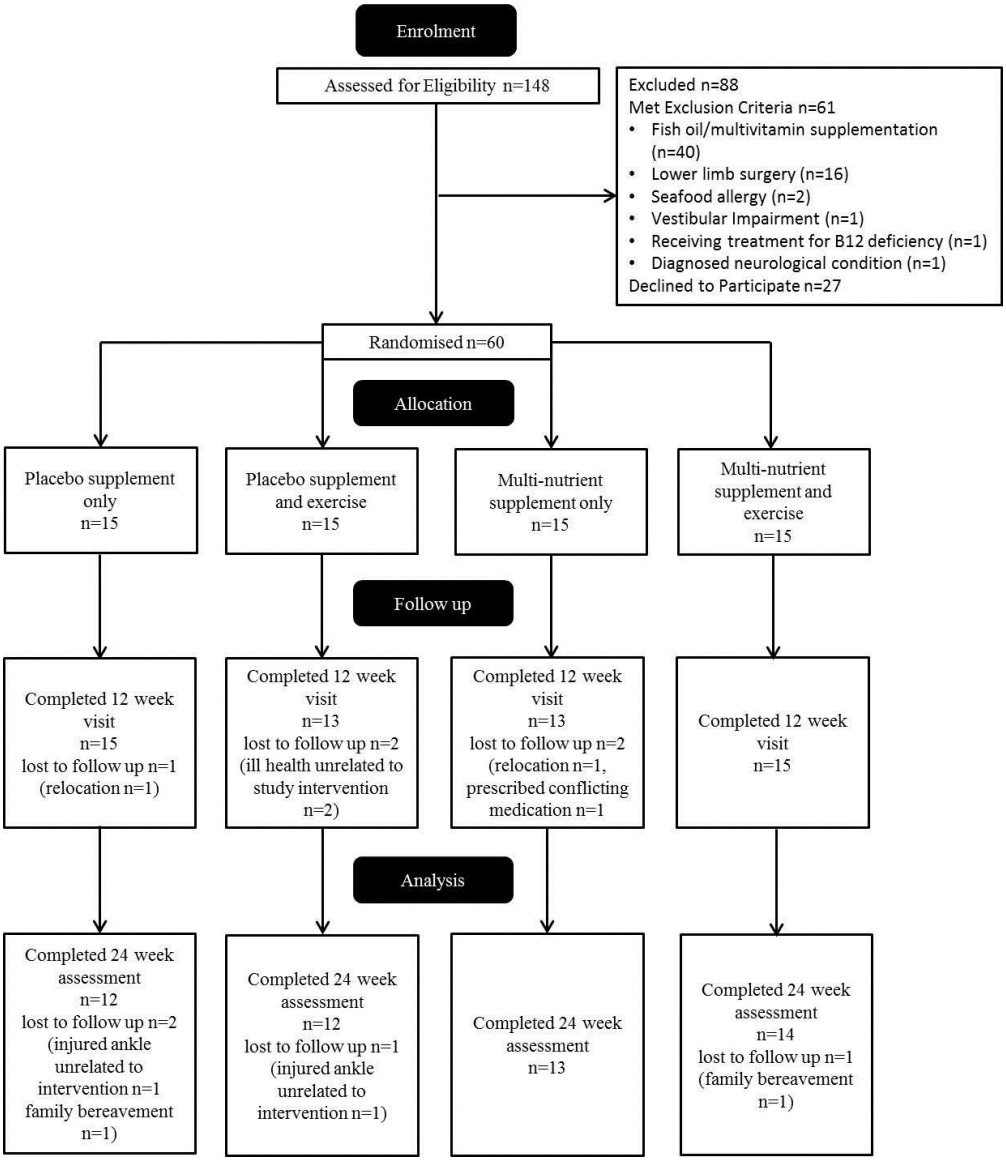
Parameter	PL (N = 12)		PL + EX (N = 12)		MS (N = 13)		MS + EX (N =14)	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
				Mobility				
Fast Walking Speed (m/s)	1.8 (0.3)	1.7 (0.2)	1.6 (0.3)	1.6 (0.2)	1.6 (0.2)	1.6 (0.2)	1.7 (0.2)	1.7 (0.2)
DT Gait Speed (m/s)	0.9 (0.3)	1.0 (0.2)	1.1 (0.2)	1.1 (0.2)	1.0 (0.3)	1.0 (0.3)	1.2 (0.3)	1.2 (0.3)
Five Times Sit to Stand (s)	11.8 (2.1)	11.7 (2.3)	13.9 (3.9)	13.0 (3.0)	11.9 (2.1)	11.9 (2.2)	13.9 (3.9)	12.3 (2.6)
				Cognition				
Verbal Memory (%)	58 (11)	54 (16)	61 (14)	67 (13)	54 (13.0)	60 (13)	58 (13)	67 (15)
Spatial Memory (%)	75 (7)	75 (6)	77 (5)	75 (6)	77 (7)	73 (7)	75 (7)	72 (10)
Executive Function (Correct Connections)	80 (18)	75 (19)	68 (21)	76 (23)	60 (15)	67 (21)	68 (18)	75 (15)
Interference Control (ms)	159 (90)	152 (77)	149 (96)	118 (104)	210 (106)	189 (104)	205 (198)	169 (148)
				SF-36 Questionnaire				
Physical Function	79 (24)	78 (26)	75 (21)	79 (19)	82 (15)	83 (16)	75 (26)	77 (22)
Bodily Pain	75 (24)	73 (25)	75 (22)	80 (13)	74 (19)	77 (17)	66 (21)	66 (16)
Emotional Wellbeing	75 (23)	72 (24)	82 (17)	80 (14)	79 (14)	79 (13)	69 (18)	74 (17)
Energy / Fatigue	57 (25)	55 (25)	65 (26)	67 (24)	62 (22)	63 (19)	58 (25)	58 (24)
General Health	66 (23)	70 (23)	75 (18)	76 (18)	66 (16)	66 (19)	62 (24)	62 (24)

Supplementary Table 1 Fatty Acid Composition of Placebo Capsules.

Fatty Acid Profile		% Fatty Acid Methyl Ester	Milligram triglyceride per Capsule
14:0	Myristic	0.4	3.3
16:0	Palmitic	7.3	67.8
16:1	Hexadecenoic	0.5	4.2
16:3	Heptadecatrienoic	0.1	0.5
16:4	Hexadecatetraenoic	0.1	0.8
17:0	Heptadecanoic	0.1	0.5
18:0	Stearic	6.6	60.3
18:1 (n-9)	Oleic	72.4	656.5
18:1 (n-7)	CIS-vaccenic	0.8	6.8
18:2 (n-7)	Linoleic	8.2	74.1
18:3 (n-3)	Alpha-linoleic	0.1	1.1
18:4 (n-3)	Octadecatetraenoic	0.1	1.2
20:0	Icosanoic	0.3	2.7
20:1	Icosenoic	0.3	3.0
20:4 (n-6)	Arachidonic	0.0	0.4
20:5 (n-3)	Icosapentaenoic	0.8	6.8
22:0	Docosanoic	0.9	7.6
22:1 (n-11)	Cetoleic	0.1	0.8
22:5 (n-3)	Docosapentaenoic	0.1	0.7
24:0	Tetracosanoic	0.3	2.5
22:6 (n-3)	Docosahexaenoic	0.5	4.3

Supplementary Table 2 Correlation matrix plotting serum homocysteine against primary and secondary outcome measures.

Outcome Measure	Homocysteine Correlation
Habitual Gait Speed (m/s)	R=0.190 (*p*=0.197)
Fast Walking Speed (m/s)	R=0.256 (*p*=0.079)
Dual Task Gait Speed (m/s)	R=-0.005 (*p*=0.975)
DTE on Gait Speed (%)	R=-0.209 (*p*=0.155)
Dual Task Variability (%)	R=0.040 (*p*=0.788)
FTSTS (s)	R=
Verbal Memory (%)	R=-0.093 (*p*=0.528)
SWM (%)	R=-0.194 (*p*=0.187)
EF (number of correct connections)	R=0.218 (*p*=0.136)
Interference control (ms)	R=-0.158 (*p*=0.283)
Physical Function (SF-36 score)	R=0.083 (*p*=0.591)
Emotional Wellbeing (SF-36 score)	R=-0.289 (*p*=0.057)
Energy/ Fatigue (SF-36 score)	R=0.042 (*p*=0.787)
Bodily Pain (SF-36 score)	R=-0.050 (*p*=0.749)
General Health Perceptions (SF-36 score)	R=-0.138 (*p*=0.373)



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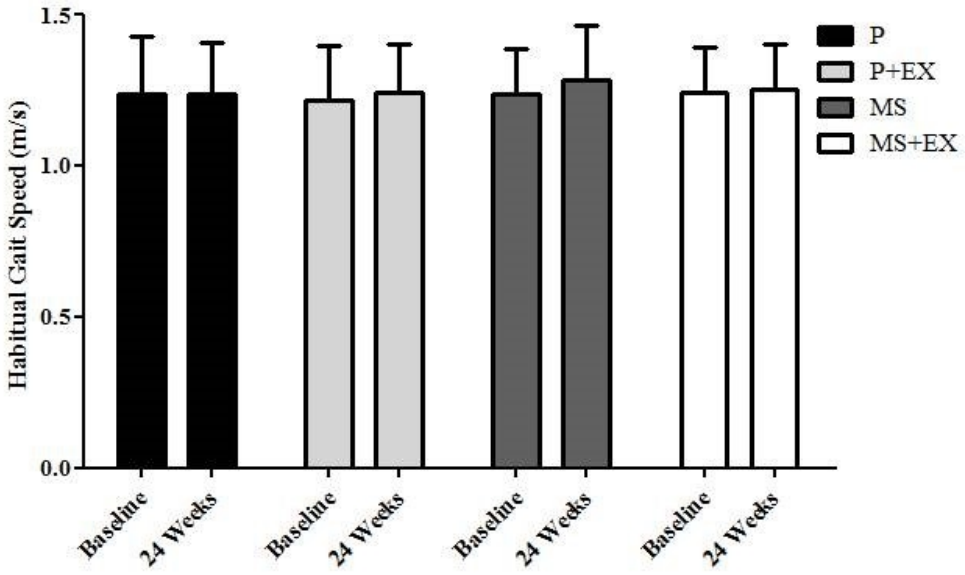

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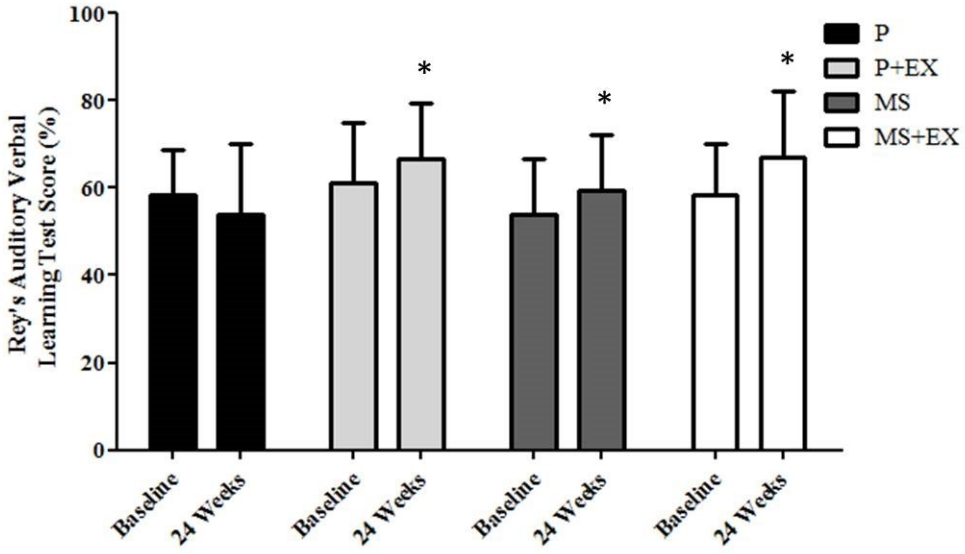

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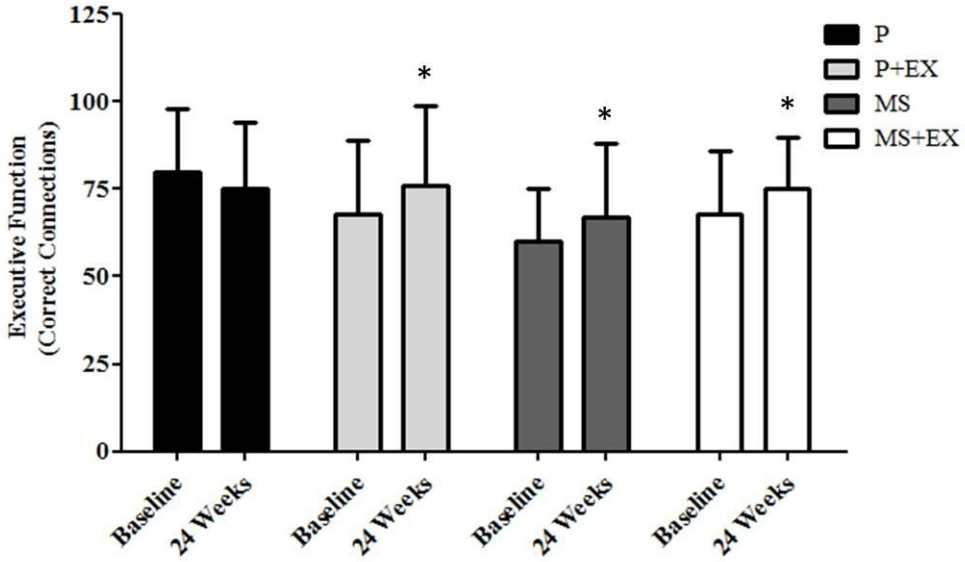

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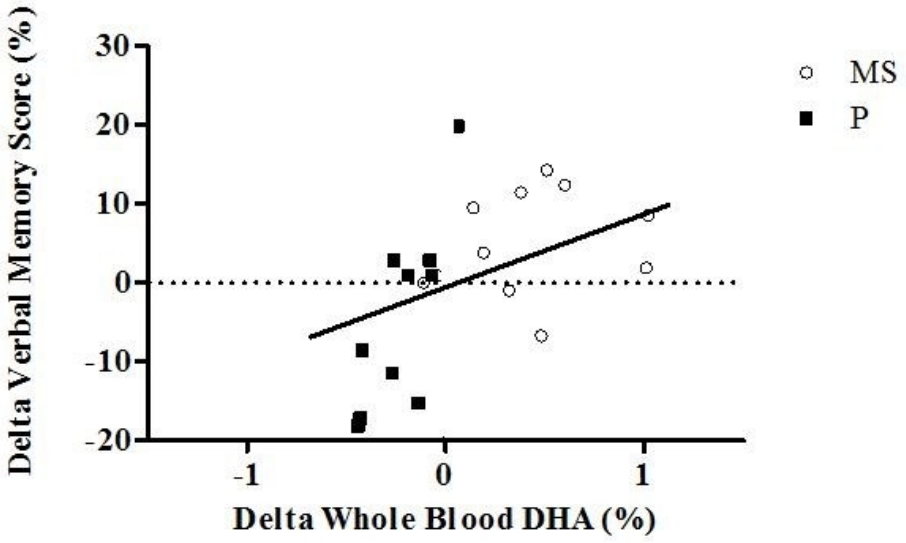

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