Brief Report

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Effects of a Six-Month Multi-Ingredient Nutrition Supplement Intervention of Omega-3 Polyunsaturated Fatty Acids, vitamin D, Resveratrol, and Whey Protein on Cognitive Function in Older Adults: A Randomised, Double-Blind, Controlled Trial

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Abstract

OBJECTIVES: To investigate the impact of a six-month multi-ingredient nutrition supplement intervention (Smartfish®), containing omega-3 polyunsaturated fatty acids (PUFAs), vitamin D, resveratrol, and whey protein, on cognitive function in Irish older adults.

DESIGN: Double-blind, randomised controlled trial (ClinicalTrials.gov: NCT02001831). A quantitative, mixed-model design was employed in which the dependent variable (cognitive function) was analysed with a between-subjects factor of group (placebo, intervention) and within-subjects factor of testing occasion (baseline, three-months, six-months).

SETTING: Community-based intervention including assessments conducted at University College Dublin, Ireland.

PARTICIPANTS: Thirty-seven community-dwelling older adults (68-83 years; mean ($\bar{x}$)= 75.14 years; standard deviation (SD)= 3.64; 18 males) with normal cognitive function (>24 on the Mini Mental State Examination) were assigned to the placebo (n= 17) or intervention (n= 20) via a block randomisation procedure.

INTERVENTION: Daily consumption for six-months of a 200mL liquid juice intervention comprising 3000mg omega-3 PUFAs [1500mg docosahexaenoic acid (DHA) and 1500mg eicosapentaenoic acid (EPA)], 10µg vitamin D3, 150mg resveratrol and 8g whey protein isolate. The placebo contained 200mL juice only.

MEASUREMENTS: A standardised cognitive assessment battery was conducted at baseline and follow-ups. Individual test scores were z-transformed to generate composite scores grouped into cognitive domains: executive function, memory, attention and sensorimotor speed. Motor imagery accuracy and subjective awareness of cognitive failures variables were computed from raw scores.

RESULTS: A hierarchical statistical approach was used to analyse the data; first, by examining overall cognitive function, then by domain, and then by individual test scores. Using mixed between-within subjects, analyses of variance (ANOVAs), no significant differences in overall cognitive function or composite cognitive domains were observed between groups over time. The only significant interaction was for Stroop Color-Word Time ($p< 0.05$). The intervention group demonstrated reduced task completion time at three- and six-month follow-ups, indicating enhanced performance.

CONCLUSION: The present nutrition intervention encompassed a multi-ingredient approach targeted towards improving cognitive function, but overall had only a limited beneficial impact in the older adult sample investigated. Future investigations should seek to establish any potential clinical applications of such targeted interventions with longer durations of supplementation, or in populations with defined cognitive deficits.

Key words: Cognitive failures, executive function, aging, nutrition, supplementation.

Abbreviations and Symbols: ANOVA: Analysis of Variance; AVLT: Auditory Verbal Learning Test; BMI: Body Mass Index; CFQ: Cognitive Failures Questionnaire; COWA: Controlled Oral Word Association; C-W: Color-Word; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; INT: Intervention Group; MI: Motor Imagery; MMSE: Mini Mental State Examination; PI: Principal Investigator; PLAC: Placebo Group; PUFA: Polyunsaturated fatty acid; RCT: Randomised controlled trial; SD: Standard deviation; TMT: Trail Making Test; TUG: Timed Up and Go; UCD: University College Dublin; WAIS-III: Wechsler Adult Intelligence Scale III; $\bar{x}$: Mean.

Introduction

Cognitive function tends to decline with advancing age. Older adults may experience compromises in memory, attention and executive functioning that significantly impair their capacity to cope with daily social and occupational demands (1). In the quest to understand possible mechanisms, recent research has explored the role of modifiable risk factors, such as physical activity (2) and diet (3), in curbing age-related cognitive decline. Of the dietary factors investigated to date, omega-3 polyunsaturated fatty acids (PUFAs) has the highest evidence-based potential for clinical use (4). The precise nature of this impact, however, remains unclear. To illustrate, in some studies, high omega-3 PUFA consumption is associated with improved cognitive

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functioning or reduced risk of dementia; whereas in others, no such effect is evident (5-9). A Cochrane review (3) reported on three randomised controlled trials (RCTs) (10-12) in this field and found no benefit of omega-3 PUFA supplementation on cognitive function in healthy elderly. However, more recent RCTs have demonstrated enhanced executive functioning (13) and object location memory task performance (although no effect on the Auditory Verbal Learning Test; AVLT) (14) after omega-3 PUFA supplementation in healthy older adults.

Vitamin D insufficiency has been suggested as a potential modifiable risk for age-associated cognitive decline (15, 16). In this regard, two prospective population-based cohort studies (17, 18) examined this association, using the Mini Mental State Examination (MMSE) (19) and at least one version of the Trail Making Test (TMT) (20) in older adults at baseline and follow-up. Again, inconsistency of findings is apparent; whereas poorer cognitive function exists in participants who are vitamin D deficient (17), negligible evidence of a link between vitamin D and executive function or incident cognitive decline has also been observed (18). In addition, a 12-year population-based longitudinal study of 1058 adults (aged >50 years at baseline) found an association between vitamin D deficiency and poorer performance on a range of baseline cognitive assessments, but no association between vitamin D status and task performance or cognitive decline at follow-ups (21). As such, RCTs are warranted to causally determine the benefits, if any, of vitamin D supplementation in the treatment or prevention of cognitive decline.

Emerging research suggests that resveratrol, a polyphenol plant compound, may modulate mechanisms of neuronal aging (22-24). However, the complexity of the biological substrates of polyphenols in cells and animals represents a major challenge in extending this research to humans (25). In this regard, human studies evaluating the role of resveratrol on cognitive function are scant. The beneficial role of whey protein supplementation has also been examined; mostly regarding physiological health outcomes, including enhanced muscle mass (26), increased artery elasticity and decreased risk of heart disease and stroke (27). Despite the significant positive associations between these outcomes and brain function, interventional evidence is lacking on the specific role of dairy constituents in neurocognitive health over the lifespan (28).

In summary, evidence concerning the benefits of nutrition supplementation on cognitive processes in older adults remains inconclusive. Moreover, previous research has focused almost exclusively on the impact of individual ingredients on cognitive function. Against this background, the present study addresses this gap in the literature by experimentally evaluating a six-month multi-ingredient supplement intervention containing omega-3 PUFAs, vitamin D, resveratrol and whey protein on cognitive function in Irish older adults. It was hypothesised that the experimental intervention would improve overall cognitive functioning, executive function, memory, attention, sensorimotor speed, motor imagery (MI) accuracy and subjective awareness of cognitive failures, compared to the placebo condition.

Methods

Design

A double-blind RCT was employed to investigate the efficacy of a six-month, multi-ingredient nutrition supplement intervention for improving cognitive functioning in older adults; specifically, effects on executive function, memory, attention, sensorimotor speed, MI accuracy, and subjective awareness of cognitive failures were assessed. For this quantitative, mixed-model design, the dependent variable ("cognitive function") was analysed with respect to a between-subjects factor of "group allocation" (placebo or intervention group) and a within-subjects factor of "testing occasion" (baseline, three-months, and six-months).

Ethical approval

All study procedures were enacted in accordance with the ethical codes of conduct of the Psychological Society of Ireland and the guidelines of the Declaration of Helsinki (2008, 2013). The research protocol was reviewed under the broader Smartfish® project and granted ethical approval from the University College Dublin (UCD) Human Research Ethics-Sciences Board (reference: LS-13-28-Egan). Participants provided written informed consent prior to study enrolment. No animals were included in this research.

Sample size calculation and study power

To calculate an estimate for sample size, an alpha value of 0.05 and beta value of 0.2 was set to ensure Power would be 0.8. Given our two allocation groups and three testing occasions, this determined that a sample size of 28 participants would be required to detect a medium effect size (f= 0.25) (GPower v3.1). To account for potential drop-out rate, we aimed to recruit more participants prior to randomisation. A post-hoc calculation of our actual power based on 37 trial-completers was conducted and demonstrated a 0.914 power to detect medium effects, in line with our intended goal.

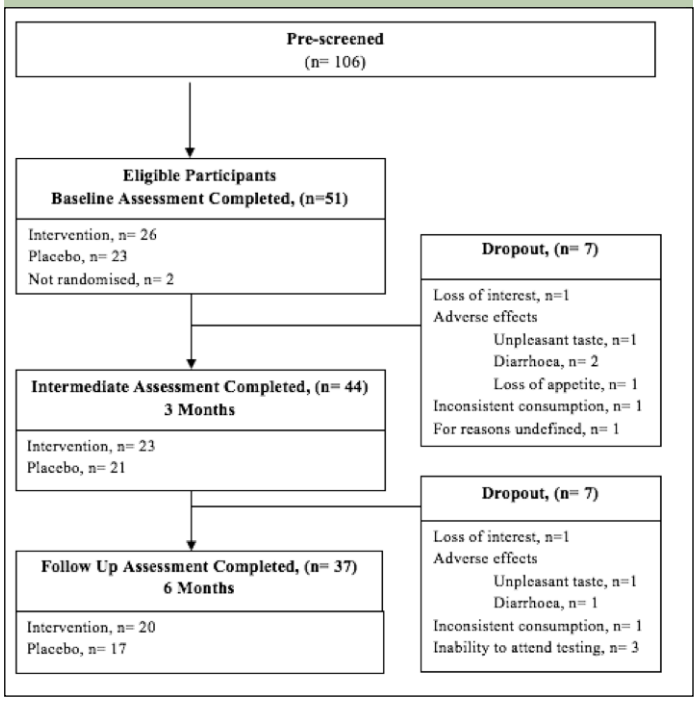
Participants

Participants were recruited via a combination of methods including an advertisement placed in a national newspaper (Irish Times), invitations issued on the UCD alumni website, and recruitment flyers distributed

to local elderly organisations and retirement homes. Individuals who expressed interest in the study were invited to UCD and provided with an information leaflet, which addressed issues of confidentiality, anonymity and data protection. At this point, a consent form was signed in the presence of the researcher. Eligibility for participation was then established from a pre-screening examination with a medical doctor. Participants aged 65 years or over, defined as 'healthy' (disease free) (29), who were independent, mobile and capable of completing the trial, and who scored above 24 on the MMSE (19) were considered eligible. Potential participants who concurrently fulfilled these inclusion prerequisites, and did not report current or recent (8-week) use of fish oil, or vitamin D or whey protein supplements, were subsequently selected for the trial.

Only participants who completed assessments at all three time-points were included in the statistical analysis (per protocol analysis). The total sample (N= 37) comprised 18 males and 19 females with an overall mean age of 75.14 years (SD= 3.64; range 68- 83 years). Of the 37 'trial completers', 17 had been randomised into the placebo group (PLAC) and 20 had been randomised into the intervention group (INT) (see Figure 1 for a consort diagram detailing study participation). The principal investigator (PI), blind to the assessments, conducted this random allocation procedure by means of a block randomisation. Envelopes were selected from an opaque container, which contained an equal distribution of placebo and supplement. Once an envelope had been drawn it was not returned prior to the subsequent randomisation.

Figure 1. Consort diagram detailing study participation



Intervention

In the active arm, the intervention liquid nutrient support (quantity 200mL per day; energy 200kcal per day) comprised 3000mg of long-chain omega-3 PUFAs [as 1500mg docosahexaenoic acid (DHA) and1500mg eicosapentaenoic acid (EPA)], 10µg of vitamin D3, 150mg of resveratrol, and 8g of whey protein isolate. The placebo nutrient support contained 200mL of juice only (energy 100kcal per day). Smartfish®, a Norwegian biotech company, provided both the supplement and placebo as ready-to-drink, palatable, pomegranate and apple flavoured juice formulations, presented in identically sealed TetraPak cartons. The formulations were indistinguishable in appearance and taste, and participants were required to consume their allotted formulation daily for a period of six-months. Research staff and participants were completely blind to group allocation until completion of the data collection. Participants received the juice cartons immediately following their baseline assessment, and these were replenished following their intermediate assessment. Compliance to the supplementation protocol was recorded using a daily tick-box diary completed by each participant.

Cognitive assessments

Between October 2013 and January 2015 data were collected in the Human Performance laboratory at the UCD Institute for Sport and Health. An extensive cognitive assessment battery comprising seven measures was conducted at three time points [baseline, intermediate (three-months) and follow-up (six-months)]. The battery was an English replication of that used in a previous investigation of omega-3 PUFA supplementation and cognitive function (13) with the addition of the Timed Up and Go (TUG) test (30) and the Cognitive Failures Questionnaire (CFQ) (31). The Trail Making Test (TMT) (20) was administered in two parts: Part A assessed sensorimotor speed and visual tracking and part B measured cognitive flexibility. The Auditory Verbal Learning Test (AVLT) (32) examined learning (immediate recall), retention (30-minute delayed recall) and retrieval (30-minute delayed recognition) of newly acquired verbal information. Alternate versions of the AVLT were used at follow-ups to prevent practice effects. The Stroop test (33) was administered as a measure of selective attention, processing speed, and susceptibility to cognitive interference. The version used consisted of two components, namely Color and Color-Word (C-W) tasks (34). The Controlled Oral Word Association test (COWA) (35) measured executive functioning and was administered in two parts to explore phonemic and categorical verbal fluency. The Digit Span test, taken from the Wechsler Adult Intelligence Scale III (WAIS-III) (36), comprised two different tests; the

Table 1. Descriptive and Test Statistics Comparing Baseline Group Characteristics

Variable	PLAC (n= 17)	INT (n= 20)	Statistic	p
Key Variables				
Gender	9 (8)	9 (11)	x^2= 0.02	.879
Age	74.76 (3.80)	75.45 (3.56)	t= -0.57	.575
Height (m)	1.70 (0.11)	1.67 (0.09)	t= 0.97	.338
Body Mass (kg)	71.15 (12.49)	71.37 (16.74)	t= -0.04	.966
Body Mass Index (BMI) (kg/m^2)	24.94 (5.44)	25.82 (6.50)	t= -0.44	.664
Education	15.18 (2.81)	15.72 (3.71)	t= -0.49	.628
Qualification:			x^2= 4.47	.215
None	0	1		
Leaving Certificate	4	1		
Undergraduate	8	13		
Postgraduate	5	3		

Note. Abbreviations: INT: Intervention group; PLAC: Placebo group. Gender data expressed as: n male (n female). Age, Height, Body Mass, BMI and Education data expressed as mean (SD). Qualification data expressed as frequency counts.

Table 2. Independent Samples t-Tests Comparing Baseline Cognitive Function Across Groups

Variable	PLAC	INT	Independent Samples t-test	
	Mean (SD)	Mean (SD)	t	p
Key Variables				
Phoneme Total	41.53 (15.97)	43.95 (13.47)	-0.50	.620
Category Total	39.00 (9.91)	43.60 (9.51)	-1.43	.159
TMT A Time	40.24 (11.19)	42.00 (14.99)	-0.40	.692
TMT B Time	85.81 (32.21)	84.55 (30.61)	0.12	.905
Stroop Color Time	60.75 (11.32)	59.53 (10.13)	0.34	.738
Stroop C-W Time	157.13 (39.21)	161.05 (38.51)	-0.30	.765
AVLT Total	42.82 (9.62)	44.95 (12.17)	-0.58	.564
AVLT Delay	7.82 (3.70)	8.45 (4.70)	-0.45	.659
AVLT Recognition	46.24 (3.36)	46.94 (2.99)	-0.66	.514
Digit Forward	11.71 (2.69)	11.50 (2.42)	0.25	.808
Digit Span Backward	7.71 (2.05)	6.60 (1.60)	1.84	.074
Digit Span Total	19.76 (4.74)	18.30 (4.11)	1.01	.320
TUG Real	9.53 (1.50)	9.10 (1.77)	0.79	.437
TUG Imagined	7.59 (1.97)	7.95 (2.98)	-0.44	.662
CFQ Total	36.76 (11.99)	36.95 (10.11)	-0.05	.960

Note. Abbreviations: AVLT: Auditory Verbal Learning Test; CFQ: Cognitive Failures Questionnaire; INT: Intervention group; PLAC: Placebo group; SD: standard deviation; Stroop C-W: Stroop Color-Word Time; TMT: Trail Making Test; TUG: Timed Up and Go.

digits forward task, which measured attentional capacity and digits backward, which assessed working memory performance.

The TUG (30) is a chronometric task designed to measure MI accuracy. MI is the mental simulation of an action in the absence of execution (37). The standard version of the task (TUG Real) (38) measures, in seconds, the time taken for participants to stand from a standard chair, walk a distance of three metres, turn around, return to the chair and sit down again. In the MI task version (TUG Imagined), participants perform this task in their imagination and then, signal 'stop' to terminate the task. This measure was added to the battery as recent research has focused on the interface between mental and physical functioning, namely MI, as a potential biomarker of cognitive decline (39). Finally, the CFQ (31) is a 25-item

self-report inventory that measures cognitive lapses in everyday life. It assesses frequencies of self-reported anomalies in perception, memory and motor function over the previous month. This aspect of cognitive function is often neglected in the literature, which focuses almost exclusively on subtle changes in performance as assessed by objective, lab-based measures. Few studies investigate the relative impact on real day-to-day functioning; and as impaired meta-awareness of cognitive failures has been demonstrated in early neurological conditions (40), this measure was included in the present study to fully establish the clinical utility of the intervention.

Data collection for each of the three testing occasions lasted approximately 45-minutes and was conducted by trained psychology Research Assistants under the supervision of a Clinical Neuropsychologist using scripted instructions and following standardised procedures. Each participant was issued a unique subject number at study entry. To ensure anonymity, only this subject number was used on the data recording forms; no other identifying information was linked to the assessments. Testing was conducted in the same quiet room at approximately the same time in the morning. Consumption of coffee and tea was not permitted before or during testing; participants were provided with a standard breakfast prior to commencement.

Statistical Analysis

IBM SPSS Statistics 20 (41) was used to analyse the data. Preliminary analyses were conducted to compare the placebo and intervention groups on demographics and baseline cognitive function variables (see Tables 1 and 2). Independent samples t-tests were used to compare the groups on continuous variables such as age, height, body mass, body mass index (BMI), number of years of full-time education and baseline cognitive test variables; while chi square tests compared the groups for gender and categorisation of highest qualification.

Following the protocol of previous research (12, 13), individual cognitive test scores were z-transformed and averaged to generate composite scores for each time point that were grouped for analysis in the following cognitive domains:

Executive function: $[Z_{\text{Phonemic Total}} + Z_{\text{Category Total}} - Z_{\text{TMT (part B-part A)/part A}} - Z_{\text{Stroop (part C-W – part C)}}]/4$

Memory: $(Z_{\text{AVLT Total}} + Z_{\text{15 AVLT Delay}} + Z_{\text{15 AVLT Recognition}} + Z_{\text{Digit Span Backward}})/4$

Attention: $Z_{\text{Digit Span Forward}}$

Sensorimotor speed: $(-Z_{\text{TMT A Time}} - Z_{\text{Stroop part C}} - Z_{\text{Stroop part C-W}})/3$

To establish a measure of MI accuracy that allowed for comparisons between groups, participants' durations when performing the TUG Real and TUG Imagined were entered in the following formula, yielding an objective index, namely 'TUG Delta' (30):

TUG Delta: [(TUGr – TUGi)/(TUGr + TUGi)/2]*100

Finally, the subjective awareness of cognitive failures variable comprised raw CFQ total scores.

Subsequently, a three-tier hierarchical approach was adopted to test the research hypotheses for a Group X Time interaction as evidence of change due to the intervention (see Table 3). Firstly, a mixed between-within subjects ANOVA investigated whether there was an effect of intervention on overall cognition at six-months using composite variables. The alpha coefficient used as the significance criterion was 0.05. Secondly, each composite variable (executive function, memory, attention, sensorimotor speed, MI accuracy, subjective awareness of cognitive failures) was explored separately using a number of individual mixed between-within subjects ANOVAs. Thirdly, each individual test variable was investigated for an effect of intervention compared to placebo using mixed between-within subjects ANOVAs.

Sensitivity analyses using intention-to-treat methods for dealing with dropout-missing data (last observation carried forward, imputing means of the group, imputing means of the other group) were also conducted, and the inferential analyses repeated. However, as there were no major differences in findings between methods, only the results of the per-protocol analysis of 37 'trial completers' are reported here.

Results

Cognitive function data for 37 participants, excluding the 14 dropout participants (27.45%), were available after six-months of the intervention. Seven participants withdrew from the trial before their intermediate assessment (1 male, 6 females; mean age 77.00 ± 5.60 years), and a further 7 withdrew before their final assessment (1 male, 6 females; mean age 73.86 ± 4.45 years). See Figure 1 for more detail on participant recruitment and retention. Chi-square and independent t-test analyses demonstrated that 'excluded' participants were not significantly different from 'included' participants regarding demographic characteristics or baseline cognitive function.

Data from the 37 trial completers were inspected for outliers using boxplots and any data points that extended above or below two standard deviations from the mean were excluded from further analysis. In total, 1.98% of data points were excluded as outliers and a further 0.66% of data points were counted as missing.

Continuous variables approximated normal distributions; thus, parametric statistics were utilised. At baseline, groups (PLAC, INT) were matched on demographic characteristics and cognitive function (Tables 1 and 2). Compliance to the supplementation protocol, using the self-report daily tick-box diary, was 95±5% for PLAC and 96±4% for INT.

Using mixed between-within subjects ANOVAs, no

Table 3. Mixed Between-Within Subjects ANOVAs for Composite and Individual Test Variables

Variable	PLAC Mean (SD)			INT Mean (SD)			Group (Main Effect)		Time (Main Effect)		Group X Time (Interaction)	
	0	3	6	0	3	6	F	p	F	p	F	p
Key Variables												
Overall Cognition	-	-	-	-	-	-	1.29	.301	3.27	.013*	.95	.499
Executive Functiona	-.08 (.83)	.01(.66)	.20 (.76)	.13 (.55)	-.00 (.49)	.16 (.58)	.06	.804	3.90	.032*	.91	.413
Memoryb	.15 (.73)	-.05 (.62)	-.10 (.71)	.23 (.74)	.14 (.71)	.01 (.75)	.31	.583	5.13	.012*	.18	.836
Attentionc	.09 (1.09)	.24 (1.03)	.26 (.98)	.01 (.98)	-.22 (.86)	-.30 1.05	1.49	.231	.16	.852	2.16	.131
Sensorimotor Speedd	-.06 (.63)	-.12 (.82)	-.07 (.84)	-.04 (.57)	.26 (.45)	.28 (.42)	1.46	.237	2.12	.138	3.19	.056
MI Accuracye	24.08 (22.65)	16.58 (26.15)	21.83 (24.53)	22.69 (29.59)	19.03 (35.45)	25.91 (29.80)	.05	.825	.84	.440	.14	.870
Subjective Awarenessf	36.00 (11.96)	37.19 (11.62)	38.50 (11.35)	36.95 (10.11)	37.30 (11.09)	38.80 (8.77)	.02	.894	3.03	.062	.05	.949
Phoneme Total	42.87 (16.59)	46.40 (16.33)	48.07 (18.84)	45.94 (12.05)	43.50 (8.93)	47.25 (8.43)	.00	.964	2.56	.086	1.93	.154
Category Total	39.93 (10.18)	39.00 (7.82)	41.20 (8.57)	45.06 (8.25)	43.31 (6.87)	47.31 (9.60)	3.77	.062	2.64	.080	.22	.802
TMT A Time	39.87 (11.89)	37.00 (9.76)	34.47 (8.38)	39.94 (7.72)	32.38 (7.72)	31.81 (8.46)	.73	.399	7.57	.001***	.84	.438
TMT B Time	86.27 (33.28)	75.73 (23.40)	68.33 (17.48)	81.44 (21.64)	83.81 (22.93)	82.13 (26.15)	.57	.456	2.30	.110	2.81	.069
Stroop Color Time	60.47 (11.65)	63.60 (10.58)	69.27 (23.01)	60.25 (10.38)	62.63 (12.12)	62.94 (10.10)	.35	.558	3.56	.035*	1.20	.309
Stroop C-W Time g	155.60 (40.10)	159.73 (57.20)	151.73 (49.71)	157.31 (33.67)	140.44 (28.50)	138.81 (25.69)	-	-	-	-	4.54	.015*
AVLT Total	42.82 (9.62)	38.82 (8.47)	39.41 (10.15)	46.61 (11.60)	46.22 (10.04)	44.17 (11.69)	2.86	.100	2.60	.082	.98	.381
AVLT Delay	7.82 (3.70)	6.88 (2.76)	6.24 (3.31)	9.39 (3.91)	7.94 (3.75)	7.56 (3.79)	1.48	.232	7.14	.002**	.15	.864
AVLT Recognition	46.24 (3.36)	45.76 (3.96)	44.53 (3.66)	46.94 (2.99)	46.72 (3.04)	45.94 (3.78)	1.19	.284	2.66	.078	.17	.842
Digit Forward	11.71 (2.69)	12.06 (2.54)	12.18 (2.42)	11.76 (2.19)	11.18 (2.04)	11.29 (2.34)	.55	.464	.09	.918	1.59	.212
Digit Backward	7.71 (2.05)	7.59 (1.91)	7.94 (1.89)	6.61 (1.69)	6.78 (1.66)	6.72 (1.32)	3.97	.055	.28	.753	.36	.703
Digit Total	19.76 (4.73)	19.65 (3.57)	20.06 (3.49)	18.44 (4.31)	17.67 (3.01)	17.44 (3.15)	2.92	.097	.53	.592	.99	.376
TUG Real	9.53 (1.50)	9.06 (2.05)	9.18 (2.01)	8.82 (1.70)	9.06 (1.56)	9.65 (1.84)	.03	.865	.52	.599	1.40	.253
TUG Imagined	7.59 (1.97)	7.65 (1.66)	7.35 (1.62)	7.35 (2.64)	7.82 (2.79)	7.65 (2.40)	.02	.903	.31	.733	.29	.752

Note. 0, 3 and 6 denote month of testing. Abbreviations: AVLT: Auditory Verbal Learning Test; INT: Intervention group; MI: Motor Imagery; PLAC: Placebo group; SD: standard deviation; Stroop C-W: Stroop Color-Word Time; TMT: Trail Making Test; TUG: Timed Up and Go; *Significance at the .05 level; **Significance at the .01 level; ***Significance at the .001 level; a. Calculated from the formula: [Z Phonemic Total + Z Category Total – Z TMT (part B-part A)/part A – Z Stroop (part C-W – part C)]/4. The resulting data for 'executive function' are based on z-scores; b. Calculated from the formula: (ZAVLT Total + Z15 AVLT Delay + Z15 AVLT Recognition + ZDigit Span Backward)/4. The resulting data for 'memory' are based on z-scores; c. Calculated from the formula: Zdigit span-forward. The resulting data for 'attention' are based on z-scores; d. Calculated from the formula: (-ZTMT A Time - ZStroop part C - ZStroop part C-W)/3. The resulting data for 'sensorimotor speed' are based on z-scores; e. Calculated from the formula: [("TUG" – "TUGi")/("TUG" + "TUGi"/2] x 100; f. Calculated from CFQ total scores; g. Significant effect of time for the intervention group, variance ratio F (2, 58) = 8.48; probability (p) <.05; No significant effect of time for the control group, F (2, 58) = 1.21; p >.05; No significant group effect at baseline, F (1, 58) = 0.12; p >.05; Significant group effect at 3-months, F (1, 58) = 14.57; p <.01; Significant group effect at 6-months, F (1, 58) = 6.53; p <.05.

statistically significant differences in overall cognitive function or cognitive function domain scores (executive function, memory, attention, sensorimotor speed, MI accuracy and subjective awareness of cognitive failures) were observed for either group over six-months (Table 3). There was no evidence to suggest that the groups differed; that is, there was no difference in the efficacy of the intervention compared with the placebo on these cognitive variables. The effect of time, regardless of group, was significant for overall cognitive function, executive function and memory. Inspection of the z-scored means demonstrated that participants improved on these variables over the multiple testing occasions.

This analytic procedure was repeated for all the individual cognitive test variables (Table 3). The only significant interaction between group and time was for 'Stroop Color-Word Time'. However, it should be noted that a Bonferroni adjustment would remove this effect. Tests of simple effects were conducted to explore the nature of this interaction (see 'Notes', Table 3 for exact statistics). Results revealed a significant effect of time (reduction in scores) for the intervention group; no such significant effect was observed in the control group. The tests revealed that groups did not significantly differ at baseline, but by 3-months, the intervention group demonstrated significantly lower time scores than the control group. These effects were also evident at 6-months. This suggests that Stroop Color-Word performance improved over time for the intervention group compared to the placebo group.

No other significant interactions or group effects occurred. However, significant effects for time were observed for TMT A Time, Stroop Color Time and AVLT Delay variables. Using tests of within-subjects contrasts these effects were observed to be linear. Irrespective of group, participants showed a pattern of dis-improvement on the Stroop Color Time and AVLT Delay variables, and a pattern of performance improvement on the TMT A Time, task over the three testing occasions.

Discussion

The present study investigated the effects of a six-month multi-ingredient nutrition supplement intervention on cognitive function in community-dwelling Irish older adults. Although some previous research has demonstrated beneficial effects of individual ingredients on cognitive function in interventions with nutrition supplementation, the present study employed a novel multi-ingredient approach with nutrients combined to target cognitive function in older adults. Importantly, the assessment of cognitive function was comprehensive, with only two previous studies in which a comparable range of cognitive outcomes was examined (12, 13). Overall, no statistically significant differences in cognitive functioning or in composite cognitive outcomes were observed between groups over time. Therefore, the hypotheses stating that overall cognitive function, executive function, memory, attention, sensorimotor speed, MI accuracy and subjective awareness of cognitive failures would improve in the intervention group compared to placebo group at six-months were not supported. However, with one exception, Stroop Color-Word performance did improve for participants receiving the intervention compared to the placebo at three- and six-month follow ups. However, it should be noted that a Bonferroni adjustment would remove this effect. Thus, the multi-ingredient nutrition intervention had only limited beneficial impact on cognitive functioning after six-months of supplementation in an Irish, community-dwelling older adult population.

When looking to studies exploring single-ingredient interventions, findings are mixed. Several studies have supported the clinical utility of omega-3 PUFAs for cognitive enhancement and reduced dementia risk (5, 7); while, other similarly designed studies have contradicted such purported benefits (8). These seemingly incompatible reported findings served as a point of departure for a more systematic investigation. To this end, a Cochrane review (3) assimilated data from three interventional studies investigating the impact of omega-3 PUFA (EPA-DHA) supplementation on cognitive function in healthy older adults. The results refuted the purported benefits of omega-3 PUFAs on cognitive function following supplementation of 700mg/day EPA-DHA over 24-months (10), 400mg/day EPA-DHA over 40-months (11), and 1800mg/day or 400mg/day EPA-DHA six-months (12). In contrast, Witte and colleagues (13) assessed the impact of 26-week supplementation of 2200mg/day EPA-DHA on cognitive function in healthy older adults and observed measureable enhanced executive functions in the treatment group. Moreover, a double-blind placebo-controlled proof-of-concept trial found a differential beneficial effect of 2200mg/day omega-3 PUFA supplementation over 26 weeks on recall in an object-location-memory task but not for AVLT performance (14). However, the present study used a similar research design, omega-3 PUFA intervention dose and duration, and comparable cognitive assessment battery, but did not yield concordant results. The present study provided limited evidence for the positive and prophylactic impact of the multi-ingredient intervention (including omega 3 PUFA, vitamin D, resveratrol and whey protein), for maintaining neuronal health in later life.

The effects of vitamin D are also unclear from the previous literature, while some recent research has claimed a beneficial role of vitamin D in neuronal function (17); in contrast, other research reports no association between vitamin D status and cognitive function (18) or decline in cognitive performance over time (21). Here, mixed findings may be attributed to the fact that these studies did not use an interventional design and featured limited cognitive assessment

batteries that may have lacked sensitivity for detecting subtle changes in cognitive function in healthy participants. The present study employed a prospective, longitudinal design with double-blind and placebo-controlled contrasts but reports negligible cognitive enhancement by the supplementation investigated.

Studies examining the impact of resveratrol on cognitive function remain in their infancy. Emerging animal and in vitro research suggests that dietary resveratrol may protect against cognitive decline in later life (22, 24). However, human clinical trials in this field are scarce. Moreover, evidence is lacking on the role of dairy constituents, such as whey protein, in cognition (28). Thus, the findings of the present study make an important contribution in this regard too; our results show that the combined omega 3 PUFA, vitamin D, resveratrol and whey protein supplementation did not yield benefits to cognitive function in this older adult sample.

A key challenge in the present study concerned retaining older adult participants in the longitudinal trial; seven participants dropped out before their intermediate assessment and a further seven, before their final assessment. Thus, it is possible that with a larger sample size, the associated increase in statistical power may have detected smaller effects. In addition, stringent recruitment procedures (namely, the pre-screening assessment of cognitive function via MMSE) may have favoured the selection of participants who were healthier than average i.e. the absence of a cognitive deficit. For instance, a recent investigation employing a broadly similar multi-ingredient nutrition supplement (omega-3 PUFAs, vitamin D, resveratrol and whey protein) reported improvements in cognitive function in older adults, albeit with a longer supplementation period and with participants with cognitive impairment ranging from mild to severe (43). Indeed, the majority of the present sample were university alumni. The recourse of educated participants is that the sample may have been unrepresentative of the wider older adult population. This may limit the generalizability of findings and raises issues from the standpoint of determining the efficacy of the intervention.

The study implemented a prospective, longitudinal design with double-blind and placebo-controlled contrasts to establish a causal effect of the intervention. In addition, standardised protocol was followed by trained and supervised researchers for data collection to reduce the potential impact of extraneous factors on cognitive performance. Participants were provided with a standard breakfast, and tested at the same time of day in the same room on both testing occasions. This allowed for the use of an extensive cognitive assessment battery comprising widely-used standardised measures with acceptable reliability and validity for use with the population under investigation. Finally, following previous research protocol, cognitive function was analysed by grouping crude individual test scores into a priori defined composite cognitive domains (12, 13, 30). The assimilation of cognitive measures in this way decreased variation associated with the individual tests, improved robustness of the outcomes and allowed for cross-comparison of findings with previous studies.

Although the present study reported no evidence elucidating the benefits of a combined omega-3 PUFA, vitamin D, resveratrol and whey protein intervention in this older adult sample, the results add to the large body of research in the field of nutrition, health and aging and extend the evidence base to an Irish context. From the perspective of identifying a suitable nutrition intervention to target age-related cognitive decline, current evidences are disappointing. Future researchers can build upon the current findings by conducting longer-term studies with larger more representative samples and incorporating diet and lifestyle measures, to more fully establish the prophylactic impact of the nutritional intervention on cognition.

In conclusion, the present study aimed to examine the impact of a targeted multi-ingredient nutrition supplement intervention, containing omega-3 PUFA, vitamin D, resveratrol and whey protein, on cognitive function. Overall, our findings suggest that the six-months of intervention had, with the exception of improved Stroop Color-Word performance, no beneficial impact on cognitive function in Irish community-dwelling older adults.

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*Ethical standards:*All study procedures were enacted in accordance with the ethical codes of conduct of the Psychological Society of Ireland and the guidelines of the Declaration of Helsinki (2008, 2013). The research protocol was granted ethical approval from the UCD Human Research Ethics-Sciences Board (reference: LS-13-28-Egan). Participants provided written informed consent prior to study enrolment. No animals were included in this research.

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