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Evaluation of Cognitive Performance following Fish-Oil and Curcumin Supplementation in Middle-Aged and Older Adults with Overweight or Obesity

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ABSTRACT

Background: Obesity accelerates age-related cognitive decline, which is partly mediated by vascular dysfunction.

Objective: The aim was to test the hypothesis that supplementation with fish oil and curcumin can enhance cognitive performance by improving cerebral circulatory function in overweight or obese middle-aged to older adults.

Methods: In a 16-wk double-blind, placebo-controlled intervention trial, adults [50–80 y; BMI (kg/m^2): 25–40] were randomly assigned to either fish oil (2000 mg/d DHA + 400 mg/d EPA), curcumin (160 mg/d), or a combination. Effects on cerebrovascular function (primary outcome) and cardiovascular risk factors were reported previously. Effects on cognitive performance and cerebrovascular responsiveness (CVR) to cognitive stimuli are reported herein. One-factor ANOVA with post hoc analyses was conducted between groups in the whole cohort and in males and females separately. Two-factor ANOVA was conducted to assess independent effects of fish oil and curcumin and a potential interaction. Correlations between outcomes (those obtained herein and previously reported) were also examined.

Results: Compared with placebo, fish oil improved CVR to a processing speed test (4.4% ± 1.9% vs. −2.2% ± 2.1%; $P = 0.023$) and processing speed in males only (Z-score: 0.6 ± 0.2 vs. 0.1 ± 0.2; $P = 0.043$). Changes in processing speed correlated inversely with changes in blood pressure ($R = -0.243$, $P = 0.006$) and C-reactive protein ($R = -0.183$, $P = 0.046$). Curcumin improved CVR in a working memory test (3.6% ± 1.2% vs. −0.2% ± 0.2%, $P = 0.026$) and, in males only, performance of a verbal memory test compared with placebo (Z-score: 0.2 ± 0.1 vs. −0.5 ± 0.2, $P = 0.039$). Combining fish oil with curcumin did not produce additional benefits.

Conclusions: Improvements in processing speed following fish-oil supplementation in middle-aged to older males might be mediated by improvements in circulatory function. Mechanisms underlying the cognitive benefit seen with curcumin are unknown. As cognitive benefits were found in males only, further evaluation of sex differences in responsiveness to supplementation is warranted. This trial was registered at the Australian and New Zealand Clinical Trial Register at https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=370788 as ACTRN12616000732482p. *J Nutr* 2020;150:3190–3199.

Keywords: cognitive function, fish oil, curcumin, cardiovascular risk factors, cerebrovascular function, randomized controlled trial

Introduction

As life expectancy increases worldwide, it becomes increasingly important to maintain physical and especially cognitive health with aging. The normal aging process sees a gradual decline in cognitive abilities (1). However, the rate of decline varies between individuals and can be accelerated by certain lifestyle factors, such as obesity, which is associated with deficits in short-term memory and executive function (2). Moreover, being obese at midlife has been shown to increase the risk of developing dementia later in life by 74% (3). This accelerated cognitive decline is partly mediated by hastened endothelial dysfunction (impaired vasodilatation) in the peripheral and cerebral vasculature, caused by vascular risk factors, including low-grade chronic inflammation, that are associated with obesity (2). Studies have shown reduced cerebral perfusion and increased cerebrovascular resistance in overweight/obese older adults compared with normal-weight individuals (4–6) and chronic hypoperfusion has been linked to the initiation and progression of dementia (7). Since there is no current treatment for dementia, lifestyle strategies that are able to

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counteract the accelerated cognitive decline in our increasingly overweight/obese older population are urgently needed. One potential strategy might be supplementing the diet with bioactive nutrients that can improve vascular function.

Two such bioactive nutrients are the long-chain omega-3 PUFAs EPA and DHA, found in fish/seafood and fish oil, and the polyphenol curcumin (diferuloylmethane), the primary active ingredient of the curry spice turmeric (*Curcuma longa*) (8). Fish-oil and curcumin supplementation have independently been shown to enhance vascular function by improving endothelium-dependent vasodilatation (assessed by flow-mediated dilatation in the brachial artery) following 8–12 wk of supplementation (9–11). Moreover, fish oil and curcumin are known to have potent anti-inflammatory effects, which can further contribute to improvements in vascular function (12).

Fish oil and curcumin have also independently been shown to have cognitive benefits. If given in sufficiently high doses (DHA >500 mg/d) (13, 14), fish-oil supplementation can improve specific cognitive domains such as processing speed, executive function, and recall memory, especially in healthy older adults and those with mild cognitive impairment (15, 16). Curcumin's potential cognitive benefits have only recently gained attention and randomized controlled studies are limited in number and show inconsistent results, which might be partly explained by the poor bioavailability of curcumin (12). Nevertheless, more recent studies, using curcumin supplements with increased bioavailability, have found improvements in working memory (17, 18), visual memory, and attention in healthy older adults (19).

The mechanisms underlying the potential cognitive improvements following fish-oil or curcumin supplementation are still unclear and only suggestive in humans. Since cognitive benefits were observed following a relatively short time after supplementation (≤6 mo), we previously hypothesized they might be mediated, at least in part, by improvements in cerebral microcirculatory function, resulting from enhanced endothelium-dependent vasodilatation and reduced systemic inflammation, rather than effects on neuronal structure and function (12, 20). Furthermore, since fish oil and curcumin act via both similar and distinct pathways to improve vascular function and reduce inflammation [for detailed description see (12)], we hypothesized that the combination of both nutrients could exert additional circulatory and cognitive effects (12).

To test these hypotheses, we conducted a 16-wk dietary intervention trial examining the independent and combined effects of fish-oil and curcumin supplementation on cerebrovascular function, cardiovascular risk factors, and cognitive performance in overweight or obese middle-aged to older adults. We recently reported the effects on basal (steady state) cerebrovascular function, cerebrovascular responsiveness (CVR) to hypercapnia (primary outcome), and cardiovascular risk factors (21). We now report on the secondary outcomes, namely cognitive performance, CVR to cognition [reflecting neurovascular coupling capacity (NVC); i.e., the ability to increase local cerebral blood flow on demand to meet the metabolic needs of the neurons], and whether changes in cognitive function correlate with changes in cerebrovascular function and cardiovascular risk factors.

Methods

Study design and population

A 16-wk randomized, double-blind, placebo-controlled, 2 × 2 factorial dietary intervention trial was conducted at the University of Newcastle's Clinical Nutrition Research Centre according to International Conference on Harmonization Guidelines for Good Clinical Practice. The study was approved by the University of Newcastle's Human Research Ethics Committee (H-2016-0170) and registered with the Australian and New Zealand Clinical Trials Register (ACTRN12616000732482p).

From June 2017 to August 2018, community-dwelling adults residing in the Hunter region of New South Wales, Australia, were invited to participate in the study via approved media advertising if they were aged 50–80 y with a BMI (in kg/m^2) of 25–40 and a sedentary lifestyle (<150 min of planned physical activity/wk). Potentially eligible participants completed a health and lifestyle questionnaire to further determine suitability and were excluded if they were consuming >2 servings of fish/seafood per week or >300 mg/d of long-chain (LC) n–3 PUFAs from fish-oil supplements. Dietary curcumin intake from turmeric powder (e.g., in curries, smoothies etc.) was not limited due to the low bioavailability of curcumin. However, participants were asked to not consume curcumin in the form of health supplements for the duration of the trial. Further exclusion criteria were suspected dementia; diagnosed major depression; history of cardiovascular, kidney, or liver disease or neurological disorders; or currently being on insulin or warfarin therapy. Written consent was obtained prior to any assessments.

Investigational product and allocation

Participants were randomly assigned to 1 of the 4 treatment groups by Altman's minimization method (22) by an independent investigator based on their age, BMI, and sex:

1. Fish oil (FO): total dose of 400 mg EPA and 2000 mg DHA
2. Curcumin (CUR): total dose of 800 mg Longvida® containing 160 mg curcumin
3. Fish oil and curcumin (FO+CUR): 400 mg EPA, 2000 mg DHA, and 800 mg Longvida® containing 160 mg curcumin
4. Placebo (PL)

The fish oil (Blackmores Omega Brain™) and curcumin capsules (Blackmores Brain Active™) were supplied by Blackmores Institute (Sydney, Australia) and were identical in appearance to their respective placebos, identifiable only by code numbers. Each active fish-oil capsule consisted of 100 mg EPA and 500 mg DHA and the matching placebo was a mix of corn and olive oil with 20 mg fish oil to match the odor of the active capsules. Each active curcumin capsule comprised 400 mg Longvida® extract containing 80 mg curcumin and the matching placebo was maltodextrin and yellow food coloring. Blinding was maintained until all data analysis had been completed.

During the 16-wk intervention, participants were instructed to consume 6 capsules daily, of which 4 contained fish oil or matching placebo and 2 contained curcumin or matching placebo. The capsules were delivered in white opaque containers labeled A (fish oil or matching placebo) or B (curcumin or matching placebo) and participants were instructed to take 2 capsules from container A and 1 from container B in the morning and again in the evening with meals. The schedule of taking supplements twice a day was to *1*) ensure sustained concentrations of curcumin in the blood, as it has a relatively

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Supplemental Table 1 and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents available on the https://academic.oup.com/jn/.
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Abbreviations used: ACE-III, Addenbrooke's Cognitive Examination–III; BFV, blood flow velocity; BP, blood pressure; CRP, C-reactive protein; CUR, curcumin (group); CVD, cardiovascular disease; CVR, cerebrovascular responsiveness; FO, fish oil (group); LC, long-chain; MCA, middle cerebral artery; NVC, neurovascular coupling capacity; PL, placebo (group); RAVLT, Rey Auditory Verbal Learning Test; TCD, Transcranial Doppler; TMT, Trail Making Test.

short half-life [~7.5 h for Longvida® curcumin (23)] and 2) to minimize fishy burps from the fish-oil capsules, which are a common unpleasant side effect. The curcumin and fish-oil dose were based on previous literature (13, 17, 24). Furthermore, participants had to record each intake in an assigned supplement diary together with any changes in dietary supplement and/or medication intake to help with compliance.

Study procedures and outcome measures

Systemic and cerebral vascular function assessments.

Participants attended the research facility on 4 occasions, of which 2 were at the beginning and 2 at the end of the 16-wk intervention. The first visit was a screening/baseline visit and potentially eligible participants were instructed to refrain from medication, food, and beverages other than water for at least 2 h prior to their visit. After measurements of height, weight, and waist circumference, participants were screened for suspected dementia with Addenbrooke's Cognitive Examination–III (ACE-III). Those scoring >82/100 underwent further systemic and cerebral vascular function assessments, as described previously (21). Briefly, systemic vascular function was determined by averaging 3 consecutive measurements of clinic blood pressure (BP) and arterial compliance. Those with a BP >160/100 mm Hg were excluded. Furthermore, an overnight fasted venous blood sample was collected within 7 d of the screening visit for analysis of cardiometabolic (glucose, triglycerides, and LDL and HDL cholesterol) and inflammatory [high-sensitivity C-reactive protein (CRP)] biomarkers and analysis of the Omega-3 Index. Systolic BP, HDL cholesterol, and total cholesterol were used to calculate the Framingham Cardiovascular Disease (CVD) Risk Score (25). Cerebrovascular function was assessed by measuring bilateral cerebral blood flow velocities (BFVs) in the middle cerebral artery (MCA) with transcranial Doppler (TCD) ultrasound; participants with no detectable signal on either side were excluded.

Cognitive performance.

The neuropsychological test battery consisted of 7 tests from the NIH toolbox (26), in addition to 3 other validated tests: Rey Auditory Verbal Learning Test (RAVLT; immediate and delayed recall) (27, 28), Forward Spatial Span Test (29), and Trail Making Test (TMT) parts A and B (27, 30). Prior to each test, participants were able to familiarize themselves with the test in a practice round. At week 16, different test versions were used to avoid a practice effect (except for tests where versions were selected randomly by the computer). Each cognitive test was converted to a *Z*-score derived using the cohort's baseline mean and SD. At week 16, *Z*-scores for each cognitive test were normalized against the cohort's performance obtained at baseline. Individual tests were averaged and grouped by cognitive domains: processing speed, language, cognitive flexibility, and working, episodic, and verbal memory. An average of all cognitive domains yielded an overall cognitive performance score and a fluid cognition score, which excludes the domain of language. Fluid cognition refers to cognitive processes involved in assimilating and integrating critical information for decision making and problem solving and is known to decrease with age, in contrast to crystallized cognition, which refers to verbal ability and accumulated knowledge, and remains relatively stable during aging (31, 32). Furthermore, since cognitive performance was significantly different between males and females at baseline (overall cognitive performance, $P = 0.041$), *Z*-scores for all cognitive tests and domains were also derived for males and females separately at baseline and week 16.

Neurovascular coupling capacity (CVR to cognition).

During the neuropsychological tests, participants continued wearing the TCD headpiece to obtain continuous recordings of changes in mean BFV to assess CVR to cognitive stimuli during each cognitive test, reflecting NVC. Before giving test instructions, a 30-s baseline recording was taken to measure resting cerebral BFV in the MCA, with the left and right MCA values being averaged. CVR to cognitive stimuli was calculated as the peak increase in mean BFV expressed as a percentage of the mean BFV recorded under resting conditions. Individual CVRs to cognitive tests were also grouped into domains, following the same pattern as the cognitive tests. An overall CVR to cognition percentage was obtained by averaging all domains.

Follow-up.

At the end of the second visit (blood collection), participants received their allocated supplements and were instructed to maintain their habitual dietary and exercise regime during the intervention. Participants were contacted after 8 wk by a phone call to check for compliance and well-being. After 16 wk, participants returned to the clinic and baseline assessments were repeated in the same order. Participants remained on treatments until the day prior to their last visit, where another fasted venous blood sample was taken, and any remaining supplements were returned and counted to assess overall compliance.

Statistical analysis

The trial was powered to give 80% chance of detecting a statistically significant ($P < 0.05$) medium-sized difference (Cohen's $d = 0.7$) in the primary outcome (CVR to hypercapnia) between treatments. This required 136 datasets, as described in Kuszewski et al. (21).

Data collected were analyzed for normality using Shapiro-Wilk's test ($\alpha < 0.05$), and baseline data were checked for differences between groups using ANOVA or Kruskal-Wallis if assumption of normality was violated. Postintervention data (absolute changes) were analyzed using a per-protocol analysis and setting treatment compliance to a minimum of 80%. Missing data were not imputed. The raw scores of the cognitive tests from the NIH toolbox were adjusted for age and years of education (by the NIH toolbox) before being converted into *Z*-scores. For the remaining cognitive tests (RAVLT, TMT, and Forward Spatial Span Test), *Z*-scores were adjusted during the statistical analysis when looking at individual tests, with age and education as covariates.

One-factor ANOVA with post hoc (Tukey's) comparison was used to examine treatment (fixed variable) effects on cognition and CVR to cognition (dependent variables) between groups (IBM SPSS version 24). For significant findings, Cohen's *d* was calculated to assess the effect size between group differences in changes from baseline. Furthermore, a 2 × 2 factorial design was adopted and analyzed by 2-factor ANOVA to assess fish oil (yes/no) and curcumin (yes/no) treatments independently as separate fixed effects, with an interaction term to test for synergy (fish oil × curcumin interaction). Groups were divided as follows for the factorial analysis:

1. FO and FO+CUR group vs. no fish oil (CUR and PL group)
2. Curcumin (CUR and FO+CUR group) vs. no curcumin (FO and PL group)

Pearson's correlation analysis (or Spearman's correlation if data skewness >2) was used to determine whether changes in cognitive performance were related to changes in cerebrovascular function (basal hemodynamics and CVR to cognition) and cardiovascular risk factors [BP, serum lipids (triglycerides, LDL, HDL, CRP), and Framingham CVD risk score]. To allow for multiple comparisons, false discovery rate was applied according to the Benjamini-Hochberg procedure (33) (corrected significance level remained at 0.05). The literature suggests potential sex differences in response to fish oil (34); therefore, additional post hoc analyses were performed (1-factor ANOVA) to look at treatment effects in males and females separately. All results are presented as mean ± SEMs.

Results

Participant disposition and characteristics

We enrolled 152 participants, of whom 134 (71 females, 63 males) completed the intervention [PL, $n = 34$; FO, $n = 32$; CUR, $n = 34$; FO+CUR, $n = 34$; for CONSORT diagram, see Kuszewski et al. (21)]. Of the 18 participants who withdrew during the intervention, 4 participants experienced gastrointestinal side effects (digestive problems: PL, $n = 1$;

TABLE 1 Baseline demographics and cognitive test scores of participants included in the analysis[1]

	Placebo ($n = 32$)	Fish oil ($n = 32$)	Curcumin ($n = 31$)	Fish oil + curcumin ($n = 31$)
Demographics				
Sex, % female	56	53	52	55
Age, y	65.8 ± 1.4	65.8 ± 1.4	65.7 ± 1.4	66.1 ± 1.4
BMI, kg/m^2	31.2 ± 0.7	31.0 ± 0.7	30.6 ± 0.8	30.6 ± 0.7
Waist circumference, cm	105.3 ± 2.2	105.7 ± 1.8	103.8 ± 2.3	104.6 ± 2.0
Education, y	13.4 ± 0.4	14.4 ± 0.4	14.7 ± 0.4[2]	14.7 ± 0.4[2]
ACE-III score, %	91.8 ± 0.9	92.5 ± 0.9	92.4 ± 0.8	93.1 ± 0.8
Cognitive performance				
Processing speed				
Pattern Comparison Test, computed score	46.7 ± 2.3	50.4 ± 2.7	48.9 ± 2.2	48.9 ± 2.0
Trail Making Test part A time, s	38.7 ± 1.5	45.8 ± 2.5[2,3,4]	38.5 ± 1.6	37.9 ± 1.5
Language				
Picture Vocabulary Test, theta score	6.4 ± 0.2	6.9 ± 0.2	6.5 ± 0.3	7.0 ± 0.2[2,3]
Oral Reading Recognition, theta score	7.7 ± 0.3	7.6 ± 0.3	7.5 ± 0.3	7.7 ± 0.3
Working memory				
List Sorting Working Memory Test, raw score	16.9 ± 0.3	17.7 ± 0.5	16.8 ± 0.4	17.6 ± 0.4
Forward Spatial Span, raw score/10	5.6 ± 0.2	5.7 ± 0.2	5.4 ± 0.2	5.8 ± 0.2
Episodic memory				
Picture Sequence Memory Test, theta score	−1.0 ± 0.2	−0.6 ± 0.1	−0.5 ± 0.2	−0.8 ± 0.2
Verbal memory				
RAVLT immediate recall	12.4 ± 0.5	13.3 ± 0.3	13.0 ± 0.4	12.7 ± 0.4
RAVLT delayed recall	11.3 ± 0.5	12.4 ± 0.4[4]	12.0 ± 0.5	10.9 ± 0.6
Cognitive flexibility				
Dimensional Change Card Sort Test, computed score	7.7 ± 0.1	7.6 ± 0.2	7.8 ± 0.1	7.8 ± 0.1
Flanker Inhibitory Control and Attention Test, computed score	7.6 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.6 ± 0.1
Trail Making Test performance, ratio part B:part A	2.4 ± 0.2	2.3 ± 0.1	2.4 ± 0.2	2.3 ± 0.1

[1] Values are means ± SEMs. Cognitive scores are before conversion into *Z*-scores. ACE-III, Addenbrooke's Cognitive Examination–III; RAVLT, Rey Auditory Verbal Learning Test.
[2] Different from placebo, $P < 0.05$.
[3] Different from curcumin, $P < 0.05$.
[4] Different from fish oil + curcumin, $P < 0.05$.

CUR, $n = 1$; FO+CUR, $n = 1$; reflux: PL, $n = 1$) and 7 reported unrelated health issues, of which 1 occurred in the placebo group (mental health problems) and 1 occurred before supplementation was commenced (FO+CUR group: lung cancer diagnosis). Of the remaining 5 health issues, which were unlikely to be related to supplementation, 2 occurred in the FO group (pneumonia), 2 in the CUR group (vein thrombosis, knee operation), and 1 in the FO+CUR group (heart attack).

Average treatment compliance was 94% and similar across groups. However, setting compliance to a minimum of 80% excluded 8 participants (3 females, 5 males), leaving 126 participants for analysis (PL, $n = 32$; FO, $n = 32$; CUR, $n = 31$; FO+CUR, $n = 31$).

Participants were, on average, marginally obese and had high ACE-III scores, indicating normal cognitive function (**Table 1**). The mean Omega-3 Index was 6.4%, as previously reported (no sex differences) (21). CVR to cognition was measured in 131 of the 152 participants at baseline (**Supplemental Table 1**). There were no significant differences in participant demographics between groups at baseline, except for years of education, which was lower in the PL group compared with the CUR ($P = 0.022$) and FO+CUR ($P = 0.019$) groups (in participants included in the analysis; Table 1). As reported previously, there were no significant differences in BP or cardiometabolic or inflammatory biomarkers between groups at baseline (21).

Cognitive performance

Supplementation with DHA-rich fish oil and/or curcumin for 16 wk did not significantly improve performance of individual cognitive tests, cognitive domains, or the overall cognitive score compared with the placebo treatment. Fish-oil supplementation tended to improve processing speed ($P = 0.071$, Cohen's $d = 0.42$) and the improvement was significant when compared with curcumin supplementation ($P = 0.043$, Cohen's $d = 0.49$) or with the combined treatment ($P = 0.036$, Cohen's $d = 0.53$) (**Figure 1**A). However, factorial analysis did not reveal any significant main effect of fish oil or curcumin on changes in individual cognitive tests, cognitive domains, or overall cognitive scores; neither was there a significant fish oil × curcumin interaction on cognitive outcomes.

Sex differences

Secondary analysis of the influence of sex on responsiveness to treatment revealed that, in males ($n = 58$), processing speed was significantly improved following fish-oil supplementation compared with all groups (PL: $P = 0.043$, Cohen's $d = 0.66$; CUR: $P = 0.008$, Cohen's $d = 0.96$, FO+CUR: $P = 0.012$, Cohen's $d = 0.97$) (Figure 1B). Moreover, males showed trends towards improvement in cognitive flexibility ($P = 0.074$, Cohen's $d = 0.73$) as well as overall fluid cognition ($P = 0.091$, Cohen's $d = 0.71$) following curcumin supplementation compared with placebo. The combination of fish oil and curcumin had no additional effect.

Of the 12 individual cognitive tests, 2 were significantly improved following supplementation in males. Fish-oil supplementation significantly improved performance during the Pattern Comparison Test (assessing processing speed) compared with placebo ($P = 0.027$, Cohen's $d = 0.72$; **Figure 2**A), and

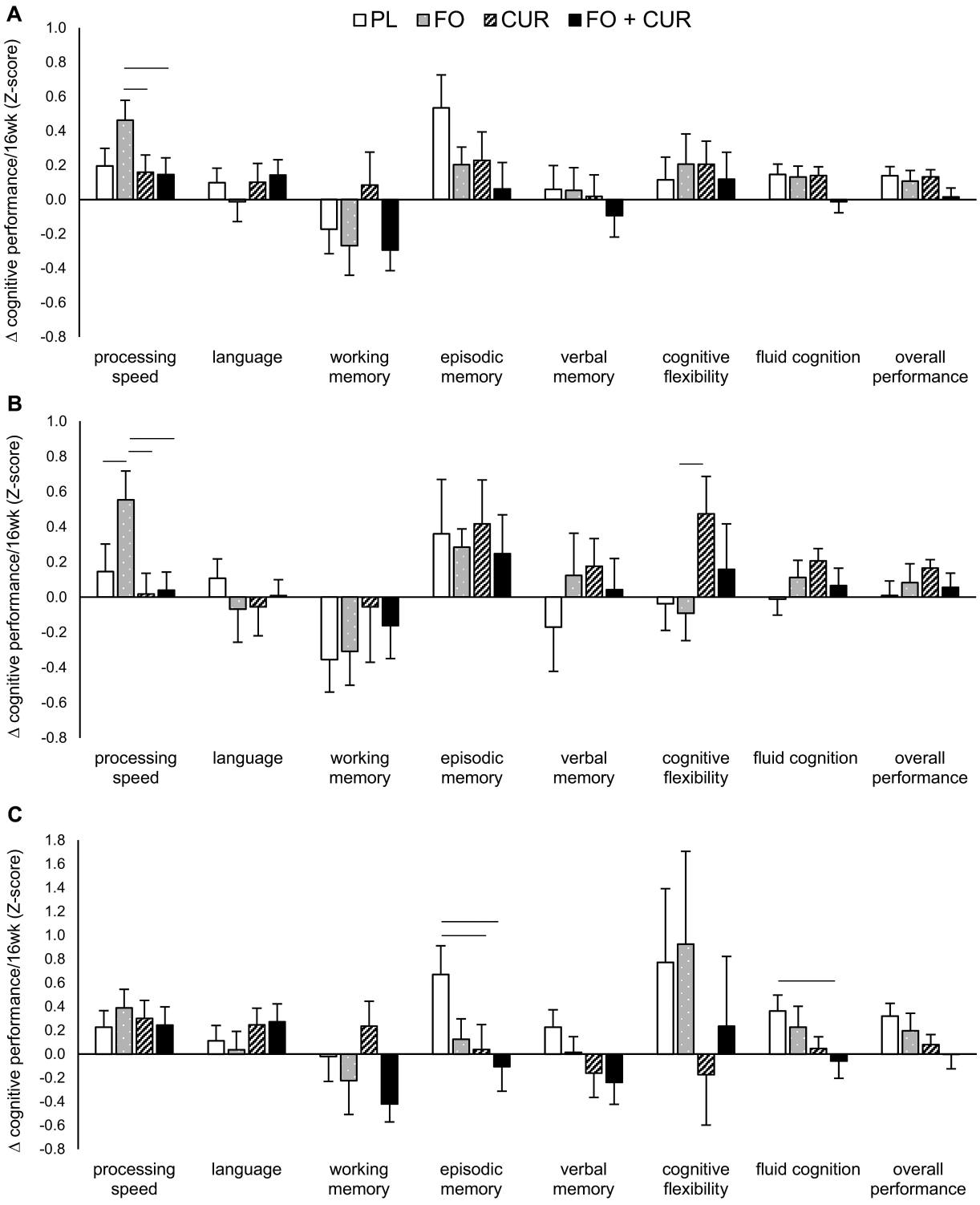

FIGURE 1 Changes in cognitive domains in the whole study cohort, $n = 125$ (A); males only, $n = 58$ (B); and females only, $n = 67$ (C) following supplementation with PL, FO, CUR, or both (FO+CUR) for 16 wk. Values are means (*Z*-score) ± SEMs. Bars indicate significant differences between groups. CUR, curcumin; FO, fish oil; PL, placebo.

curcumin supplementation significantly improved performance during the RAVLT immediate recall test (assessing verbal memory) compared with placebo ($P = 0.039$, Cohen's $d = 0.84$) (Figure 2B).

Females did not show significant improvements in any cognitive tests or domains following fish-oil and/or curcumin supplementation compared with placebo supplementation (Figure 1C). Unexpectedly, the placebo group showed the greatest increase in episodic memory performance, which was significant compared with curcumin ($P = 0.036$, Cohen's $d = 0.71$) and the combined treatment ($P = 0.011$, Cohen's $d = 0.87$), as well as in overall fluid cognitive performance, which was significant compared with the combined treatment ($P = 0.038$, Cohen's $d = 0.73$).

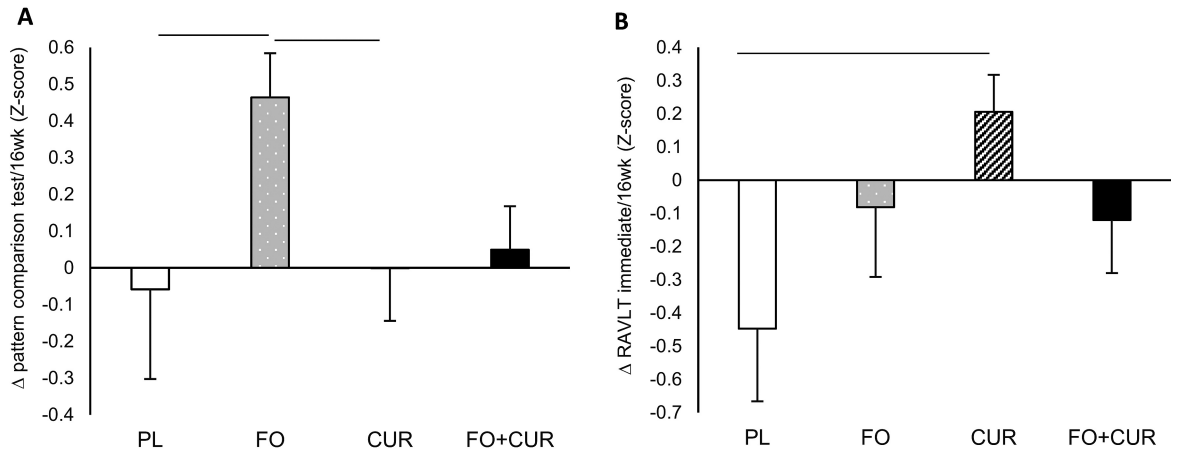

FIGURE 2 Changes in Pattern Comparison Test (A) and RAVLT immediate recall (B) in middle-aged to older men with overweight or obesity who were supplemented with PL, FO, CUR, or both (FO+CUR) for 16 wk. Values are means (*Z*-score) ± SEMs, $n = 58$. Bars indicate significant differences between groups. CUR, curcumin; FO, fish oil; PL, placebo; RAVLT, Rey Auditory Verbal Learning Test.

Neurovascular coupling capacity

At baseline, higher BMI correlated with lower CVR to processing speed ($R = -0.224$, $P = 0.010$, age adjusted) and lower overall CVR to cognition ($R = -0.193$, $P = 0.027$, age adjusted). However, BMI was not correlated with treatment change in CVR to processing speed or overall CVR to cognition.

Of the 12 cognitive tests in which NVC was assessed, CVR to 2 tests was significantly affected by treatment (Figure 3). Fish-oil supplementation improved CVR to TMT part A (part of processing speed; compared with PL: $P = 0.023$, Cohen's $d = 0.69$), while curcumin supplementation significantly improved CVR to List Sorting Working Memory (part of working memory; compared with PL: $P = 0.026$, Cohen's $d = 0.76$). NVC during each of the 6 cognitive domains was not significantly affected following treatment (Supplemental Table 1). The combined treatment did not significantly affect CVR to either individual cognitive tests or cognitive domains. Factorial analysis did not reveal any significant main effect of fish oil or curcumin on CVR to individual cognitive tests or CVR to cognitive domains; neither was there a significant fish oil × curcumin interaction on CVR to cognitive outcomes.

Sex differences.

There was no significant influence of sex on the responsiveness to treatment regarding CVR to cognitive tests.

Correlations

Fish-oil supplementation improved cognitive function in the domain of processing speed, increased CVR to processing speed (CVR to TMT-A; as reported above), and as previously reported (21), significantly reduced cerebral vessel stiffness in males and improved cardiovascular risk factors [significant reduction in triglycerides; trends towards reductions in systolic BP, the inflammatory biomarker CRP (in males) and Framingham

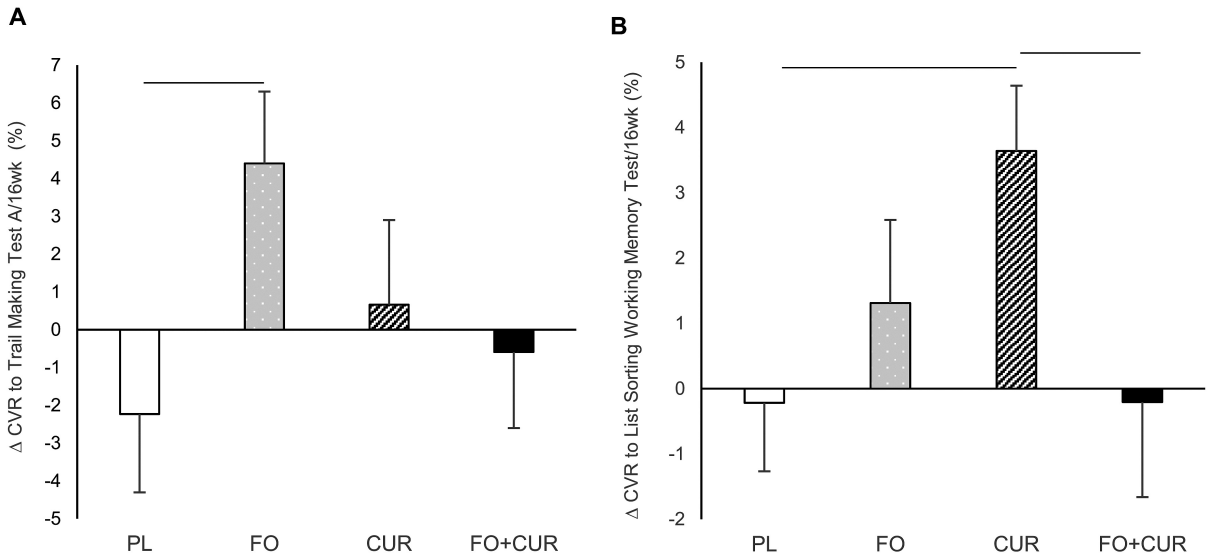

FIGURE 3 Changes in CVR to Trail Making Test A ($n = 91$) (A) and List Sorting Memory Test ($n = 98$) (B) in middle-aged to older adults with overweight or obesity who were supplemented with PL, FO, CUR, or both (FO+CUR) for 16 wk. Values are means (%) ± SEMs. Bars indicate significant differences between groups. CUR, curcumin; CVR, cerebrovascular responsiveness; FO, fish oil; PL, placebo.

TABLE 2 Correlations between changes in cardiovascular risk factors, cerebrovascular function, and processing speed[1]

	Processing speed (*Z*-score)	
	R	*P*
Cerebrovascular function		
Pulsatility index	−0.023	0.805
CVR to processing speed, %	0.166	0.116
Cardiovascular risk factors		
Systolic blood pressure, mm Hg	−0.243	0.006*
Pulse pressure, mm Hg	−0.206	0.021*
Heart rate, beats/min	0.104	0.250
Serum triglycerides, mmol/L	−0.131	0.146
Serum HDL cholesterol, mmol/L	0.060	0.507
Serum C-reactive protein, mg/L	−0.183	0.046*

[1] $n = 125$. *Significant as determined by Pearson's or Spearman's correlation, $P < 0.05$. CVR, cerebrovascular responsiveness.

CVD Risk Score]. We tested whether these changes were correlated (**Table 2**). Changes in processing speed performance did not correlate with changes in either cerebral vessel stiffness (pulsatility index) or in CVR to processing speed (CVR to TMT-A), although the latter tended to correlate with processing speed in males ($R = 0.261$, $P = 0.087$). However, changes in systolic BP, pulse pressure, and CRP were significantly correlated with treatment changes in processing speed (Table 2, **Supplemental Figures 1–3**). Furthermore, the overall reduction in CVD risk (Framingham score) was significantly correlated with increased CVR to processing speed ($R = -0.246$, $P = 0.019$) (**Supplemental Figure 4**).

Curcumin supplementation significantly improved verbal memory (RAVLT immediate recall) and tended to improve cognitive flexibility and fluid cognition (as reported above). However, curcumin did not significantly improve any cerebrovascular or cardiovascular biomarkers, except for CVR to working memory (CVR to List Sorting Working Memory Test), which did not correlate with changes in cognitive performance in this cognitive domain.

Discussion

We tested whether supplementation for 16 wk with DHA-rich fish oil and curcumin, independently or in combination, could improve cognitive performance and whether any improvements were related to changes in cerebrovascular function and cardiovascular risk factors in a population at risk of premature cognitive decline. There were no significant treatment-related effects on cognitive performance in the whole study population. We found that, in males only, fish-oil supplementation significantly improved processing speed, while supplementation with curcumin significantly improved RAVLT immediate recall (verbal memory). Unexpectedly, females in the PL group showed the greatest increase in cognitive performance (episodic memory and fluid cognition). As previously reported, females in the PL group also showed anomalous changes in CVR to hypercapnia and systemic inflammation (21). Further investigation of possible differences among females in the placebo group revealed a trend towards reduction in LDL cholesterol in those whose CVR to hypercapnia improved, suggesting that they may have inappropriately modified their diet or lifestyle during the intervention. Participants had been instructed to maintain their habitual diet, but potential dietary changes were not formally monitored, which is a limitation of this study. The anomalous improvement in cognitive performance in only females in the PL group possibly undermined our ability to detect cognitive responses to active treatments in the whole population.

Another possible explanation as to why cognitive benefits were observed in males only might be related to sex differences in cognitive function and age-related cognitive decline. In line with previous studies, we observed that females performed better in all cognitive domains except for working memory at baseline (32). Furthermore, studies have shown that males have a steeper cognitive decline in early adulthood (32) (age range in study: 18–45 y) as well as later in life (35) (age range in study: >50 y), indicating that males might benefit more than females from treatments that can slow down cognitive aging. For fish oil, the response rate to supplementation was the same in males and females; 87% showed improvements in processing speed. Nevertheless, the extent of improvement was smaller in females, possibly due to higher baseline performance, and the improvements in the PL group further masked any potential benefits. For curcumin, however, males seem to be more responsive than females with regard to changes in cognitive function following supplementation. The response rate to curcumin supplementation in females was low and there were no changes in cognitive performance. Thus, further investigation of potential sex differences in responses to curcumin supplementation is warranted.

Fish-oil supplementation: potential mechanisms underlying cognitive benefits in males

LC n–3 PUFAs, especially DHA, are known to be important for brain health through all stages of life, from supporting brain development in infants to maintaining cognitive function in older adults (36). The observed improvement in processing speed in our study following supplementation with DHA-rich fish oil confirms previous findings (16) and the literature suggests several mechanisms underlying fish oil's cognitive benefits (36). Next to direct neuronal effects such as stimulation of neurogenesis and reduction in amyloid plaque aggregation (37, 38), we hypothesized that fish oil's cognitive benefits are partly mediated by improvements in cerebral circulatory function resulting from reduced systemic inflammation and enhanced endothelial vasodilatation (12, 21). There is growing evidence that shows that cardiovascular and cerebrovascular impairments are associated with cognitive deficits and increased dementia risk (39). While fish oil's cardiovascular benefits are well known and there is increasing evidence on its cerebrovascular benefits, no prior human study has directly linked fish oil's beneficial circulatory effects to its cognitive benefits. This is the first study to explore potential associations in a population at increased risk of accelerated cognitive decline.

Cardiovascular risk factors.

We have reported elsewhere the effects of treatments on cardiovascular risk factors in this study (21). As briefly mentioned in the Results section, fish-oil supplementation resulted in reductions in systolic BP, CRP (in males) and the Framingham CVD score.

The reductions in systolic BP and pulse pressure were significantly correlated with improved processing speed. Vascular risk factors, such as hypertension, which are associated with endothelial dysfunction, have been shown to be related to poorer cognitive performance and increased dementia risk in longitudinal studies (40). A reduction in BP serves as an indirect marker of improved endothelial function, which plays

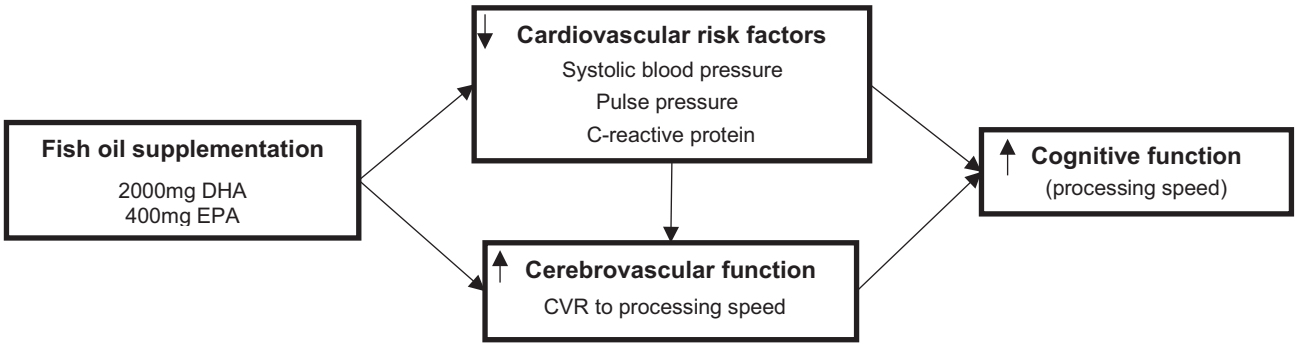

FIGURE 4 Illustration of potential mechanisms, and their interaction with each other, through which fish-oil supplementation may improve the cognitive domain of processing speed. CVR, cerebrovascular responsiveness.

an important role in regulating cerebral vascular resistance and cerebral blood flow on demand (20). An adequate cerebral perfusion during demand is crucial for optimal cognitive performance.

The reduction in the biomarker of systemic inflammation, CRP, was significantly correlated with improved processing speed. Increasing evidence shows an association between heightened systemic inflammation and poorer cognition (41). Hence, reducing inflammation with fish oil in overweight/obese older adults, who are likely to suffer from chronic low-grade inflammation, might help to counteract accelerated cognitive decline.

Regular fish-oil supplementation not only improved individual cardiovascular risk factors but also tended to reduce the Framingham CVD Risk Score (fish oil vs. no fish oil: $-2.2\% \pm 0.4\%$ vs. $-1.2\% \pm 0.4\%$; $P = 0.059$) (21). Reduction in Framingham CVD Risk Score following supplementation correlated significantly with improvement in CVR to processing speed (CVR to TMT-A), supporting the growing evidence of a relation between cardiovascular and cerebrovascular function (**Figure 4**).

Cerebrovascular function.

Previous studies have demonstrated poorer cerebrovascular function in overweight/obese adults compared with lean individuals (4–6). Within our overweight/obese study population, we observed an inverse correlation between BMI and CVR to processing speed and overall CVR to cognition at baseline, indicating limitations in meeting local perfusion demands during mental activation in those with a higher BMI. The improvement in cognitive performance during tests assessing processing speed following fish-oil supplementation was accompanied by a reduced cerebral vessel stiffness and an improved CVR to processing speed (TMT-A; independent of BMI; i.e., an improved ability to increase blood flow during that cognitive test). The latter tended to be correlated with improvements in processing speed performance in males. Thus, fish oil might be able to help maintain processing speed with aging, at least in overweight/obese older men, partly by restoring impaired NVC (Figure 4).

Of note, it is possible that the correlation between CVR to cognition (NVC) and cognitive performance did not reach significance due to a lower precision of the NVC measurement and a reduced number of participants included in this correlation (missing NVC data as the signal was not stable enough to last for the duration of the cognitive test battery of 1.5 h in some participants) compared with the more robust measurements of systemic vascular function and their correlation with cognitive performance.

Curcumin supplementation

Curcumin supplementation significantly improved performance during the RAVLT immediate recall, assessing verbal memory, in males. Previous studies by Cox et al., which used the same curcumin supplement, found some improvements in working memory after 4 wk (17) and 12 wk (18) of supplementation. Interestingly, Cox et al. excluded adults with impaired cardiovascular function and high BP and when we examined the effect of curcumin on normotensive participants only, we also observed a significant improvement in working memory ($P = 0.023$, compared with placebo). Further studies are warranted to see if potential benefits of curcumin on working memory might be limited to normotensive adults.

Curcumin seems to affect different cognitive domains compared with fish oil and its cognitive benefits appear not to be mediated by improvements in cardiovascular risk factors or cerebrovascular function. Thus, further studies are required to understand the mechanisms underlying the cognitive benefits of curcumin.

Combination of fish oil and curcumin

Contrary to our hypothesis, the combination of fish oil and curcumin did not result in any additional benefits. Since this is the first study to evaluate combined effects on cerebrovascular function, cardiovascular risk factors, and cognitive performance, further investigations are warranted to see whether there might be potentially unknown negative interactions between fish oil and curcumin in humans. Furthermore, there might be an optimal dose for the combination to elicit synergistic effects and any potential beneficial effects might be limited to specific population groups. Future studies might also want to consider the potential of other nutrient combinations in overweight/obese older adults.

Study limitations

Improvements in cognitive performance were limited to certain cognitive domains, with no changes observed in overall cognitive performance. Despite being overweight or obese, which increases the risk of having cerebrovascular dysfunction and cognitive impairments, our study cohort had high cognitive function, evident by their high ACE-III score (average, 92.4 ± 0.4/100). Thus, the ability to further improve cognitive performance was limited. Nevertheless, fish oil was able to improve processing speed, which is known to affect other cognitive domains (42), and curcumin was able to

improve short-term verbal memory (RAVLT immediate recall), which has been shown to be impaired in overweight/obese individuals (2). Another limitation of this study is the relatively short intervention period, as a longer intervention may have resulted in further cognitive improvements. The improvement in processing speed might indicate that it is more sensitive than other cognitive domains to early cerebrovascular/inflammatory changes following fish-oil supplementation. Tokuda et al. (43) also found improvements in processing speed after only 4 wk of fish-oil supplementation in elderly Japanese men. Thus, the choice of cognitive tests will influence whether improvements will be detected following shorter durations of fish-oil supplementation.

However, those cognitive improvements were limited to males. Since the influence of sex on the response to fish-oil and curcumin supplementation was a post hoc analysis and our study might have been confounded by the anomalous changes seen in the female PL group, further studies designed and powered to test for potential sex differences in cognitive responses to fish-oil and curcumin supplementation should be undertaken.

Another limitation of our study was the relatively high Omega-3 Index at baseline, which was unexpected as participants were limited to a maximum of 2 fish and/or seafood servings/wk and mostly reported fish intake of once/twice per month or once a week (21). Nevertheless, participants' Omega-3 Index in the FO and the FO+CUR groups nearly doubled at the end of the 16-wk intervention (21). However, as previous studies have shown an association between low Omega-3 Index and cognitive impairments in older adults (44), individuals with a low Omega-3 Index [<4% as classified by Harris (45)] might be more susceptible to the potential cognitive benefits of fish-oil supplementation.

Conclusions

In a population at increased risk of vascular dysfunction and accelerated cognitive decline (i.e., overweight or obese older adults), fish-oil supplementation was able to improve processing speed performance in males, which might be mediated by its beneficial effects on circulatory function. Curcumin supplementation had independent cognitive benefits for males, but the underlying mechanisms remain unclear. Adding curcumin to fish oil did not result in additional circulatory or cognitive benefits. Further research is needed to identify characteristics (e.g., sex, age, cardiovascular risk factors) of populations who would benefit from supplementation with these nutrients.

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