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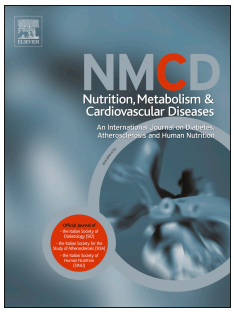

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Julia C. Kuszewski, Rachel H.X. Wong, Lisa G. Wood, Peter R.C. Howe

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Effects of fish oil and curcumin supplementation on cerebrovascular function in older adults: a randomized controlled trial

Julia C. Kuszewski[1], Rachel H.X. Wong[1,2], Lisa G. Wood[1,3] and Peter R.C. Howe[1,2]

[1] Clinical Nutrition Research Centre, School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, Australia

[2] Institute for Resilient Regions, University of Southern Queensland, Springfield Central, Australia

[3]Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, University of Newcastle, NSW, Australia

Corresponding author: Peter Howe, University of Newcastle, School of Biomedical Sciences and Pharmacy, Callaghan, New South Wales 2308, Australia, Email: peter.howe@newcastle.edu.au, +61 249217309

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Abbreviations

AC: arterial compliance, ACE-III: Addenbrooke's Cognitive Examination-III, BMI: body mass index, BP: blood pressure, CBF: cerebral blood flow, CRP: C-reactive protein, CVR: cerebrovascular responsiveness, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, FMD: flow-mediated dilatation, HDL: high-density lipoprotein, LCn-3 PUFA: long-chain omega-3 polyunsaturated fatty acids, LDL: low-density lipoprotein, MBFV: mean blood flow velocity, O3I: Omega-3 Index, PI: pulsatility index, TCD: Transcranial Doppler

Abstract

Background and Aims: Chronic conditions such as obesity, which contribute to endothelial dysfunction in older adults, can cause impairments in cerebrovascular perfusion, which is associated with accelerated cognitive decline. Supplementing the diet with bioactive nutrients that can enhance endothelial function, such as fish oil or curcumin, may help to counteract cerebrovascular dysfunction.

Methods and Results: A 16-week double-blind, randomized placebo-controlled trial was undertaken in 152 older sedentary overweight/obese adults (50-80 years, body mass index: 25-40kg/m^2) to investigate effects of fish oil (2000mg docosahexaenoic acid + 400mg eicosapentaenoic acid/day), curcumin (160mg/day) or a combination of both on cerebrovascular function (measured by Transcranial Doppler ultrasound), systemic vascular function (blood pressure, heart rate and arterial compliance) and cardiometabolic (fasting glucose and blood lipids) and inflammatory (C-reactive protein) biomarkers. The primary outcome, cerebrovascular responsiveness to hypercapnia, was not affected by the interventions. However, cerebral artery stiffness was significantly reduced in males following fish oil supplementation (P=0.007). Furthermore, fish oil reduced heart rate (P=0.038) and serum triglycerides (P=0.006) and increased HDL cholesterol (P=0.002). Curcumin did not significantly affect these outcomes either alone or in combination with fish oil.

Conclusion: Regular supplementation with fish oil but not curcumin improved biomarkers of cardiovascular and cerebrovascular function. The combined supplementation did not result in additional benefits. Further studies are warranted to identify an efficacious curcumin dose and to characterize (in terms of sex, BMI, cardiovascular and metabolic risk factors) populations whose cerebrovascular and cognitive functions might benefit from either intervention.

Clinical Trial Registration: ACTRN12616000732482p

Introduction

Maintaining adequate cerebral blood flow (CBF) to ensure a stable supply of nutrients and oxygen is crucial for optimal brain functioning [1]. With increasing age, mean CBF gradually declines and cerebral vascular reactivity becomes impaired, due to increasing arterial stiffness and endothelial dysfunction [2]. Certain conditions, such as obesity, have been shown to accelerate this age-related decline in CBF and vascular reactivity, leading to premature cerebrovascular dysfunction [3, 4]. This is due to the hastened endothelial dysfunction in obese individuals, partly caused by chronic low-grade inflammation, which leads to a reduced bioavailability of nitric oxide, a potent endothelium-derived vasodilator [3, 5]. Impaired cerebral perfusion has been shown to be predictive of cognitive decline and linked to initiation and progression to dementia [6, 7]. Therefore, it is crucial to identify lifestyle strategies to preserve adequate cerebral perfusion and vascular reactivity. One potential approach might be supplementing one's diet with bioactive nutrients that can counteract chronic inflammation and enhance endothelial function, such as the long-chain omega-3 polyunsaturated fatty acids (LCn-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and curcumin (diferuloylmethane), a polyphenolic compound.

Fish and fish oil are the primary dietary sources of LCn-3 PUFA, while curcumin is the main active component of the curry spice turmeric (Curcuma Longa) [8]. Both have been shown to independently exert beneficial effects on systemic endothelial function. In a previously published review, we concluded that fish oil supplementation tended to improve flow-mediated dilatation (FMD), a measure of systemic endothelial function, especially in individuals with endothelial dysfunction [9]. This may be attributable to increased nitric oxide (NO) production and a shift in the balance of vasaoactive eicosanoids [10]. Only three human trials have examined effects of regular curcumin supplementation on FMD; improvements were seen in younger (average age 21 years) adults [11] as well as healthy older (average age 62 years) adults [12, 13], which may be attributable to AMPK-mediated enhancement of endothelial NO production [10]. Both fish oil and curcumin have also been shown to have potent anti-inflammatory effects, which are mediated by changes in expression of inflammatory cytokines and, in the case of LCn-3 PUFA, by lipid mediators including eicosanoids and resolvins, and are associated with the improvements in systemic endothelial function [8, 10, 14]. Since both fish oil and curcumin can beneficially affect systemic endothelial function and inflammation by similar as well as distinct pathways and preclinical studies indicate additional benefits when combining both supplements [15-17], we have hypothesized that a combination of fish oil and curcumin could elicit additive or even synergistic effects on vascular function and systemic inflammation [10].

While it is unknown if curcumin's beneficial effects on systemic endothelial function extend to the cerebral microcirculation, there is some evidence for cerebrovascular benefits of fish oil. Jackson *et al.* and Konagai *et al.* showed improved regional blood flow during mental tasks in young (average age 21) [18] and healthy older adults (average age 67) [19] following fish oil supplementation. Our pilot study, conducted in older hypertensive adults (45-80 years) supplemented with DHA-rich fish oil for 20 weeks, was the first to show improvements in cerebrovascular responsiveness (CVR) to hypercapnia (reflecting cerebral endothelial vasodilator function) and a reduction in cerebral artery stiffness in women only [20]. Due to the limited number of studies, more research is needed to confirm these results in different population groups and to explore potential sex differences in response to fish oil.

The current study sought to determine whether regular supplementation with fish oil and curcumin, independently or in combination, could improve cerebrovascular function in sedentary, overweight/obese older adults and whether this is accompanied by improvements in cardiovascular risk factors. This population was chosen as they are likely to suffer from chronic low-grade inflammation, impaired systemic arterial function and reduced regional CBF [3, 5].

Methods

Subjects

Adults aged 50-80 years with a body mass index (BMI) of 25-40kg/m^2 and a sedentary lifestyle (<150 min of planned exercise per week) were recruited from the general public residing in the Hunter region of New South Wales, Australia via media advertising and giving presentations to local communities. Interested volunteers were sent detailed information about the study and completed a health and lifestyle questionnaire to determine suitability. Participants were excluded if they were consuming more than 2 meals of fish/seafood per week or more than 300 mg/day of LCn-3PUFA from fish oil supplements, had suspected dementia, had been diagnosed with major depression, had a history of cardiovascular, kidney or liver disease or neurological disorders, were on insulin of warfarin therapy or were likely to change pre-existing medication/supplements during the intervention. Written consent was obtained from each participant prior to any assessments.

Study design

This 16-week randomized, double-blind, placebo-controlled, 2x2 factorial dietary intervention trial was undertaken at the University of Newcastle's Clinical Nutrition Research Centre in accordance with International Conference on Harmonization Guidelines for Good Clinical Practice. The study protocol 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).

Allocation to one of the four treatment groups was performed by an independent investigator according to Altman's allocation by minimization method based on participant's age, BMI and sex:

- Fish oil + curcumin placebo (FO)
- Curcumin + fish oil placebo (CUR)
- Fish oil and curcumin (FO+CUR)
- Placebo (PL)

Participants were instructed to consume six capsules daily (two fish oil and one curcumin or matching placebo, both morning and evening with meals). The fish oil (Blackmores Omega Brain^{TM}) and curcumin capsules (Blackmores Brain Active^{TM}) 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 contained 100mg EPA and 500mg DHA, yielding a total dose of 400mg EPA and 2000mg DHA per day. Placebo oil capsules contained a mix of corn and olive oil and 20mg fish oil to match the odour of the active capsules. Each active curcumin capsule contained 400mg Longvida® Optimised Curcumin (80mg curcumin), yielding a total dose of 160mg curcumin per day, with the placebo comprising maltodextrin and yellow food colouring. Blinding was maintained until all data analysis had been completed.

Study procedures and outcome measures

Potentially eligible volunteers were invited to the clinic for a screening/baseline visit to further determine eligibility. They were instructed to refrain from medication, food and beverages other than water for at least 2 hours prior to their visit. Height, weight and waist circumference were measured and, if BMI criteria were met, they were then screened for dementia using Addenbrooke's Cognitive Examination-III (ACE-III). Those scoring above 82/100 underwent further assessments of systemic and cerebral vascular function.

Cerebral haemodynamics and CVR to hypercapnia (primary outcome)

Cerebrovascular function was measured non-invasively with Transcranial Doppler (TCD) ultrasound by fitting the participants with a headpiece supporting an ultrasound probe on each temporal region (DopplerBox X; Compumedics DWL, Singen, Germany). Basal mean blood flow velocity (MBFV) and pulsatility index (PI) were recorded for 30s in the middle cerebral artery (MCA) and the last 10s were used to determine MBFV and PI at rest. Those with no detectable signal on either side were excluded.

Afterwards, participants breathed carbogen gas (5% CO_2 and 95% O_2) for 180s and the peak increase in MBFV was recorded. Increases in MBFV in response to the hypercapnic stimulus reflect the endothelium-dependent vasodilator responsiveness of the distal cerebral microvasculature. CVR to hypercapnia was calculated as follows: [(peak MBFV during hypercapnia – resting MBFV) / resting MBFV x100].

Clinic blood pressure and arterial compliance

Participants were seated for at least 10 min before blood pressure (BP), heart rate and arterial compliance (AC) readings were taken at 2 min intervals using a Cardiovascular Profiler (Cardiovascular Profiler CR2000, Hypertension Diagnostics Inc.). An appropriate size blood pressure cuff was placed over the brachial artery of the non-dominant arm. After an initial reading, the dominant hand was stabilised by a rigid arterial wrist support and a tonometer was placed perpendicularly over the radial artery to assess the arterial elasticity index of the large and small arteries using artery pulse waveform analysis. Three consecutive BP and AC measurements were then taken and used for analysis. Those with blood pressures above 160/100 mmHg were excluded.

Cardiometabolic/inflammatory biomarkers and Omega-3 Index

Eligible participants returned to the clinic within 7 days for a second visit, having fasted overnight, for collection and analysis of a venous blood sample by a commercial pathology centre for fasting glucose, serum lipids (low-density lipoprotein (LDL cholesterol), high-density lipoprotein (HDL cholesterol) and triglycerides) and high-sensitivity C-reactive protein (CRP). After centrifuging and separating the plasma which was stored in aliquots, the red blood cell pellet was re-suspended in saline, re-centrifuged and stored frozen at -80□C for subsequent analysis of erythrocyte fatty acid profiles by gas chromatography [21]. Fatty acid standards were used as a reference to identify peaks for EPA and DHA by retention time, from which the Omega-3 index (O3I, combined percentage of EPA and DHA in erythrocytes) was determined.

Framingham Cardiovascular Disease Risk score

The 10 year risk of a cardiovascular event was calculated at baseline and after intervention using a Framingham risk score calculator (West Hertfordshire Cardiology, West Hertfordshire Hospital NHS Trust) which took into account changes in total cholesterol, HDL cholesterol and systolic blood pressure.

Intervention

At the end of the second visit, participants were given their allocated supplements and were instructed to record each intake in the assigned supplement diary together with any changes in dietary supplement and/or medication intake. They were asked to maintain their diet and exercise habits throughout the intervention and were followed up by a phone call after eight weeks to enquire about their well-being, any side effects and changes of medication.

After 16 weeks, participants returned to the clinic and all baseline assessments were repeated in the same order. A fasting blood sample was collected within seven days (generally within three days) of this visit. Participants remained on treatments until the day their blood was taken and they were instructed to return any remaining supplements which were counted to assess overall compliance.

Statistical Analysis

We estimated that 136 participants in this 2x2 factorial study design would give 80% power to detect a 0.7 effect size (Cohen's d) difference between treatments in CVR to hypercapnia (primary outcome) at alpha = 0.05. To allow for 15% attrition, we aimed to recruit a total of 160 participants.

Using a per-protocol analysis (excluding participants with compliance <80%), ANOVA (IBM SPSS version 24, New York, NY, USA) was applied to the primary and secondary outcomes to determine the significance of differences between each treatment and placebo group. Furthermore, to assess the effects of fish oil and curcumin treatment independently, a 2 x 2 factorial design was adopted:

- Fish oil (FO and FO+CUR group) vs. no fish oil (CUR and PL group)
- Curcumin (CUR and FO+CUR group) vs. no curcumin (FO and PL group)

Pearson's correlation analysis was used to see whether changes in O3I were related to changes in the primary and secondary outcomes. To account for multiple comparisons in the secondary outcomes, false discovery rate was applied (significance level remained at P=0.05). Since our pilot study suggested potential sex differences in responses to fish oil [20], additional posthoc analyses were undertaken for male and female participants. All results are presented as mean ± SEM.

Results

Participants

Of the 167 potentially suitable participants that were invited for a screening visit, 152 participants met the selection criteria and were enrolled (**Figure 1**). Eighteen participants withdrew prior to the end of the intervention; four experienced gastrointestinal side effects (digestive problems: PL n=1, CUR n=1, FO+CUR n=1; reflux: PL n=1). There were no serious adverse events to report. Overall compliance with supplementation was 94% based

on capsule counts and was similar across treatment groups. Eight participants failed to achieve 80% compliance (5 males, 3 females), leaving 126 participants (58 males, 68 females) for analysis. At week 16, a measurable TCD signal in the MCA could not be detected in 6 participants, leaving 120 participants for the analysis of changes in the primary outcome, CVR to hypercapnia, and basal cerebral haemodynamics.

Participants' baseline characteristics are detailed in **Table 1**. They were elderly, sedentary (averaged 47min planned physical activity per week) and marginally obese (high waist circumference indicating abdominal obesity), with 14 years of formal education and high ACE-III scores, indicating normal cognitive function. Most participants ate fish/seafood once or twice per month or once a week and 21 participants were taking fish oil capsules (4 infrequently and 17 daily) but, importantly, none exceeded the study exclusion limit (>300mg LCn-3 PUFA/day). Except for basal MBFV (FO only vs. FO+CUR group P=0.018), there were no significant differences between groups at baseline.

Cerebrovascular function

There was no change in the primary outcome, CVR to hypercapnia, with either fish oil or curcumin or the combined treatment (unadjusted values with non-compliers (n=128): PL: 4.67 ± 3.25%, FO: -0.75 ± 4.22%, CUR: -0.24 ± 3.68%, FO+CUR: 2.62 ± 3.59%) (**Table 2**). MBFV and PI were also unaffected by the separate or combined supplementation with fish oil and curcumin. Neither the ANOVA nor the 2x2 factorial analysis revealed any significant differences in treatment changes in CVR, MBFV or PI between groups (**Table 2, 3**).

Secondary analyses of the influence of sex on indices of cerebral vascular function showed no significant difference within or between sexes for CVR to hypercapnia (**Figure 2, A**). However, for PI (reflecting cerebral artery stiffness), we found a significant sex difference in response to fish oil supplementation (males: -0.09 ± 0.05, females: +0.02 ± 0.02, P=0.05). In males, fish oil supplementation significantly reduced PI by 9% (P=0.007, compared to PL). Furthermore, supplementation with fish oil on its own was significantly better than the combination with curcumin (P=0.017) in males (**Figure 2, B**).

Systemic vascular function and cardiometabolic biomarkers

While curcumin had no effect on indices of systemic vascular function or cardiometabolic markers, the combination of fish oil and curcumin significantly increased HDL cholesterol (P=0.001). Fish oil alone decreased serum triglyceride levels (P=0.018, compared to PL), but did not significantly affect other measures (**Table 2**). However, the factorial analysis (**Table 3**) showed that, compared to no fish oil, fish oil supplementation not only reduced serum triglycerides by 24% (P=0.006) but also reduced heart rate by 3% (P=0.038) and increased

serum HDL cholesterol by 8% (P=0.002). Furthermore, fish oil tended to reduce the Framingham Cardiovascular Disease risk score (P=0.059).

Examination of the influence of sex on these effects of fish oil revealed that the change in HDL cholesterol was significantly higher in females than males (males: +0.05 ± 0.04mmol/L, females: +0.16 ± 0.04mmol/L, P=0.046).

Systemic inflammation

Plasma CRP, a measure of systemic inflammation, tended to decrease in all groups; however, there were no significant differences between groups (**Table 2**). Analysis of the influence of sex on changes in CRP revealed a difference in response to fish oil supplementation (males: -1.14 ± 0.45mg/L, females: 0.04 ± 0.25mg/L; P=0.022). In males only, fish oil supplementation tended to decrease CRP (FO: -1.14 ± 0.45mg/L, PL: 0.04 ± 0.38mg/L, P=0.078). Combination with curcumin did not result in any further decreases.

Omega-3 Index

At baseline, the O3I averaged 6.4±0.1%; fish oil supplementation for 16 weeks nearly doubled omega-3 levels (P<0.001 compared to placebo and curcumin groups; **Table 2**). There were no significant sex differences in the treatment change in EPA, DHA or O3I.

Neither baseline EPA, DHA and O3I levels nor the treatment changes in EPA, DHA and O3I correlated with changes in cerebral or systemic vascular function indices. However, treatment changes in EPA, DHA and O3I significantly correlated with improvements in cardiometabolic biomarkers, viz. decrease in triglyceride levels (EPA: R= -0.190, P=0.039; DHA: R= -0.200, P=0.029; O3I: R= -0.202, P=0.028) and increase in HDL cholesterol (EPA: R=0.337, P<0.001; DHA: R= 0.282, P=0.002; O3I: R=0.302, P=0.001).

Discussion

In this placebo-controlled trial, supplementation with fish oil, curcumin or their combination for 16 weeks elicited no significant change in the primary outcome measure, CVR to hypercapnia. However, in males only, a slight trend towards improvement in CVR to hypercapnia with treatment was observed and cerebral artery stiffness, which is associated with cognitive impairment [22, 23], was significantly reduced following fish oil supplementation. Females in contrast showed no change in CVR to hypercapnia in the treatment groups, but an anomalous increase in the placebo group. This was unexpected, as our previous pilot study found improvements of CVR only in females following a similar level of fish oil supplementation (500mg/day EPA + 1600mg/day DHA) for 20 weeks [20]. Of the 18 females in the placebo group, eight showed an increase of 16-33% in CVR following intervention. Although participants had been instructed and reminded not to change their

habitual diet or exercise regime, it is evident that some might have altered their lifestyle sufficiently to elicit the observed improvements in CVR. While there were no changes in self-reported physical inactivity, there was a trend towards a reduction in LDL cholesterol after 16 weeks in those female participants who had an increase in CVR, suggesting changes in their diet quality. Thus, a limitation of this study was the lack of dietary monitoring to detect any unanticipated changes in diet.

A further limitation is that the magnitude of change in CVR to hypercapnia following fish oil supplementation was smaller than predicted by our previous pilot study, which would have necessitated a considerably larger sample size to discern whether changes were treatment mediated or coincidental. While the intervention period was 20 weeks in the pilot study, it was only 16 weeks in the present study, which might have been too short to detect significant changes. Moreover, the pilot study was performed in overweight hypertensive adults (average BMI: 27.6kg/m^2; BP: 141/80mmHg), while this study was conducted in marginally obese but normotensive adults. Thus, the effects of fish oil on cerebrovascular function may depend on not only the sex but also the blood pressure and BMI of an individual, necessitating further investigation of factors determining who will benefit most from fish oil supplementation. Regarding curcumin, this is the first study to explore its effects on cerebrovascular function and future studies are required to identify the optimal dose of curcumin and investigate effects in different population groups.

Assessments of systemic vascular function revealed abnormally low small artery elasticity at baseline (normal >5mL/mmHg x100), reflecting endothelial dysfunction [24]. However, neither fish oil nor curcumin supplementation significantly improved systemic arterial compliance, despite the improvement seen in cerebral arteries following fish oil supplementation. Fish oil supplementation has previously been shown to help lower elevated blood pressure [25, 26], and we observed a tendency towards reduction of systolic blood pressure, even though our participants were normotensive at baseline.

Supplementation with fish oil lowered heart rate and serum triglyceride levels and increased HDL cholesterol, all of which are well-recognized cardiovascular benefits of fish oil [27-29]. The improvement of HDL cholesterol and slight reduction in systolic blood pressure translate to a 12% reduction of Framingham Cardiovascular Disease Risk Score in the fish oil group (2x2 factorial design) relative to baseline. Curcumin supplementation, on the other hand, did not affect any of the measured cardiometabolic biomarkers. It should be noted that the participants' average baseline triglyceride (1.5mmol/L) and HDL cholesterol (1.4mmol/L) concentrations were within normal ranges (<1.7mmol/L for triglycerides; >1.0mmol/L for HDL cholesterol). A meta-analysis by Simental-Mendia *et al.* [30] showed a significant overall reduction in triglycerides following curcumin supplementation, with no change in LDL cholesterol. However, the individual studies showing significant reductions were performed in

adults with high baseline triglyceride levels due to diabetes, metabolic syndrome or hyperlipidemia. This suggests that, whereas fish oil supplementation can improve blood lipids even in metabolically normal individuals, curcumin might only be effective in adults with elevated lipid levels.

We were anticipating a reduction in CRP after fish oil and curcumin supplementation, especially since baseline CRP levels were indicative of chronic low-grade inflammation. CRP tended to be lower after fish oil supplementation, particularly in males. Once again, results may have been confounded by an apparent reduction of CRP in the placebo group, primarily in females. Previous studies showing reductions in CRP levels after fish oil [31, 32] or curcumin [33, 34] supplementation were undertaken in populations with much higher baseline CRP levels, suggesting that the baseline CRP levels in our study cohort might have still been too low to see a significant reduction.

The initial levels of EPA and DHA were higher than expected; cumulative data from previous studies indicated a typical O3I of ~5% in overweight/obese but healthy middle-aged Australian adults [35]. This may reflect increased use of fish oil supplements in the current study population. However, the baseline O3I did not correlate with changes in cerebral or systemic vascular function, yet we still observed a substantial increase in O3I following fish oil supplementation which correlated with improvements of cardiometabolic biomarkers. Hence it is unlikely that the high baseline O3I can account for the lack of improvement in cerebral and systemic vascular function in the fish oil groups.

The combination of fish oil and curcumin did not, as hypothesized, result in additional or synergistic effects. While our trial was ongoing, another study examining the combined effects of fish oil and curcumin on glycaemic indices and blood lipids also failed to find any synergistic effects [36]. It is important to note that investigations into the combined effects of fish oil and curcumin in humans are novel and limited, with our study being the first to examine effects on cerebrovascular function and cardiovascular risk factors. The lack of additional effects might possibly be due to suboptimal dosages or unknown interactions between fish oil and curcumin.

Conclusions and future directions

We have confirmed the cardiovascular benefits of fish oil supplementation, i.e. reductions of heart rate and serum triglycerides, increased HDL cholesterol and a trend toward lower BP. However, potential benefits of fish oil on cerebrovascular health require further investigation, especially as there appear to be sex differences in response to fish oil. Curcumin did not affect cerebrovascular function or cardiovascular risk factors in older overweight/obese adults and future dose-response studies are required to identify an efficacious curcumin dose. Further investigations are needed to elucidate underlying mechanisms of interaction

between fish oil and curcumin, identify optimal doses when combining these bioactives and characterize those individuals who would benefit most from either intervention.

Author contributions

R.H.X. and P.R.C.H. conceived the study design, J.C.K. recruited the participants, collected and analysed the data and drafted the manuscript under supervision from R.H.X. and P.R.C.H. L.G.W provided the facilities and supervision for the Omega-3 analysis. All authors read and approved the final manuscript. The authors declare no conflict of interest.

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Figure Legends

Figure 1. Consort diagram showing flow of participants from initial expression of interest until final assessment and analysis.

Figure 2. Treatment changes in indices of cerebral vascular function in males (n=55) and females (n=65) per group. A: cerebrovascular responsiveness to hypercapnia, B: pulsatility index, a negative value indicates a reduction in cerebral artery stiffness.*Significant difference between males and females ($P < 0.05$). PL: Placebo, FO: fish oil, CUR: curcumin, FO+CUR: fish oil and curcumin combination.

Tables

Table 1. Participant's baseline characteristics per group.

Characteristics	PL (n=36)	FO (n=39)	CUR (n=38)	FO + CUR (n=39)
Demographics				
Males/females (n)	18/18	17/22	17/21	17/22
Age (years)	65.4 ± 1.3	65.4 ± 1.2	65.4 ± 1.2	66.2 ± 1.3
Education (years)	13.5 ± 0.4	14.1 ± 0.3	14.2 ± 0.4	14.6 ± 0.4
BMI (kg/m^2)	31.0 ± 0.7	31.0 ± 0.7	30.5 ± 0.7	30.9 ± 0.6
Waist circumference (cm)	105.4 ± 2.0	105.5 ± 1.6	103.8 ± 2.0	104.4 ± 1.8
Physical activity (min/week)	33.8 ± 9.7	55.1 ± 12.8	44.4 ± 13.0	54.6 ± 14.3
ACE-III score (%)	91.8 ± 0.9	92.7 ± 0.7	92.4 ± 0.8	93.2 ± 0.7
Cardiovascular risk factors				
Systolic blood pressure (mmHg)	133.5 ± 2.6	132.0 ± 2.0	129.5 ± 2.1	132.5 ± 2.4
Diastolic blood pressure (mmHg)	77.1 ± 1.6	74.7 ± 1.5	74.6 ± 1.2	74.2 ± 1.5
Pulse pressure (mmHg)	56.4 ± 1.7	57.3 ± 1.6	54.9 ± 1.6	58.2 ± 1.9
Heart rate (bpm)	72.1 ± 2.2	67.9 ± 1.7	65.4 ± 1.5	65.8 ± 1.8
Large artery elasticity (mL/mmHg x10)	12.0 ± 0.7	13.2 ± 0.9	12.8 ± 0.7	12.4 ± 0.7
Small artery elasticity (mL/mmHg x100)	3.9 ± 0.3	4.7 ± 0.5	4.3 ± 0.4	4.8 ± 0.5
EPA (%)	0.97 ± 0.07	1.05 ± 0.06	1.08 ± 0.07	1.08 ± 0.06
DHA (%)	5.03 ± 0.19	5.52 ± 0.23	5.38 ± 0.16	5.47 ± 0.19
Omega-3 Index (%)	6.00 ± 0.25	6.56 ± 0.28	6.46 ± 0.21	6.54 ± 0.23
CRP (mg/L)	2.16 ± 0.35	2.28 ± 0.27	2.62 ± 0.49	2.32 ± 0.23
Glucose (mmol/L)	5.64 ± 0.28	5.43 ± 0.19	5.57 ± 0.16	5.71 ± 0.27
Triglycerides (mmol/L)	1.49 ± 0.09	1.54 ± 0.09	1.57 ± 0.10	1.34 ± 0.08
LDL cholesterol (mmol/L)	3.17 ± 0.16	3.49 ± 0.17	3.51 ± 0.17	3.38 ± 0.20
HDL cholesterol (mmol/L)	1.38 ± 0.06	1.31 ± 0.04	1.40 ± 0.06	1.44 ± 0.06
Framingham CVD risk score (%)	18.41 ± 1.76	18.19 ± 1.35	18.13 ± 2.00	19.82 ± 2.17
Cerebrovascular function				
Basal mean blood flow velocity (cm/s)	45.02 ± 1.83	47.74 ± 2.36	46.51± 1.83	41.37 ± 1.40
Pulsatility index	0.84 ± 0.02	0.91 ± 0.04	0.86 ± 0.02	0.89 ± 0.03
CVR to hypercapnia (%)	44.56 ± 2.78	47.35 ± 3.09	43.64 ± 2.90	47.56 ± 3.59

Characteristics	PL	FO	CUR	FO + CUR
Cardiovascular risk factors	**n=32**	**n=32**	**n=31**	**n=31**
Systolic blood pressure (mmHg)	-2.4 ± 1.3	-6.8 ± 1.9	-3.0 ± 2.3	-3.9 ± 2.1
Diastolic blood pressure (mmHg)	-2.0 ± 1.0	-4.4 ± 1.1	-2.5 ± 1.2	-2.0 ± 1.2

ACE-III: Addenbrooke's Cognitive Examination-III, BMI: body mass index, CRP: C-reactive protein, CVD: Cardiovascular Disease. Treatment groups = PL: Placebo, FO: fish oil, CUR: curcumin, FO+CUR: fish oil and curcumin combination.

Table 2. Treatment changes in systemic and cerebral vascular function indices and cardiovascular biomarkers.

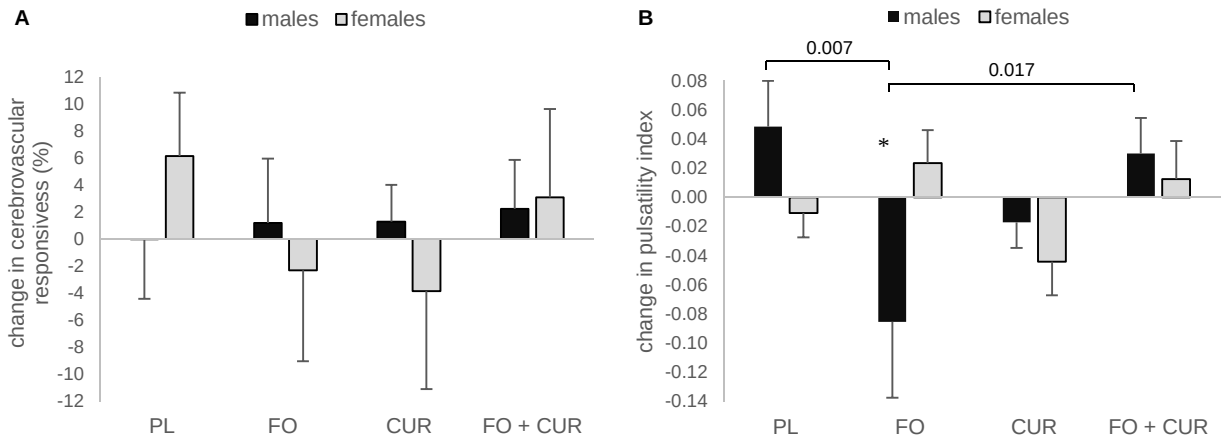
Pulse pressure (mmHg)	-0.4 ± 1.1	-2.4 ± 1.2	-0.6 ± 1.6	-1.9 ± 1.7
Heart rate (bpm)	-1.2 ± 1.1	-2.6 ± 1.3	2.9 ± 1.0	-1.1 ± 1.6
Large artery elasticity (mL/mmHg x10)	-0.5 ± 0.6	0.6 ± 0.9	0.5 ± 1.2	0.3 ± 0.6
Small artery elasticity (mL/mmHg x100)	0.2 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.6 ± 0.4
EPA (%)	-0.07 ± 0.05	**1.07 ± 0.08***	-0.08 ± 0.06	**1.28 ± 0.09***
DHA (%)	-0.01 ± 0.10	**4.28 ± 0.30***	0.07 ± 0.09	**4.64 ± 0.23***
Omega-3 Index (%)	-0.08 ± 0.14	**5.35 ± 0.37***	-0.01 ± 0.14	**5.92 ± 0.26***
CRP (mg/L)	-0.41 ± 0.28	-0.49 ± 0.26	-0.22 ± 0.32	-0.23 ± 0.24
Glucose (mmol/L)	0.04 ± 0.07	0.08 ± 0.08	0.08 ± 0.10	0.25 ± 0.22
Triglycerides (mmol/L)	-0.15 ± 0.06	**-0.40 ± 0.09***	-0.14 ± 0.07	-0.30 ± 0.07
LDL cholesterol (mmol/L)	-0.09 ± 0.15	-0.07 ± 0.09	-0.13 ± 0.09	0.04 ± 0.12
HDL cholesterol (mmol/L)	0.01 ± 0.03	0.06 ± 0.03	-0.00 ± 0.03	**0.16 ± 0.04***
Framingham CVD risk score (%)	-1.09 ± 0.60	-2.33 ± 0.44	-1.15 ± 0.63	-2.15 ± 0.69
Cerebrovascular function	**n=30**	**n=29**	**n=31**	**n=30**
Basal mean blood flow velocity (cm/s)	-1.53 ± 1.50	-0.35 ± 2.15	0.52 ± 1.44	-1.02 ± 1.74
Pulsatility index	0.01 ± 0.02	-0.03 ± 0.03	-0.03 ± 0.01	0.02 ± 0.02
CVR to hypercapnia (%)	3.48 ± 3.26	-0.75 ± 4.22	-1.36 ± 3.93	2.69 ± 3.82

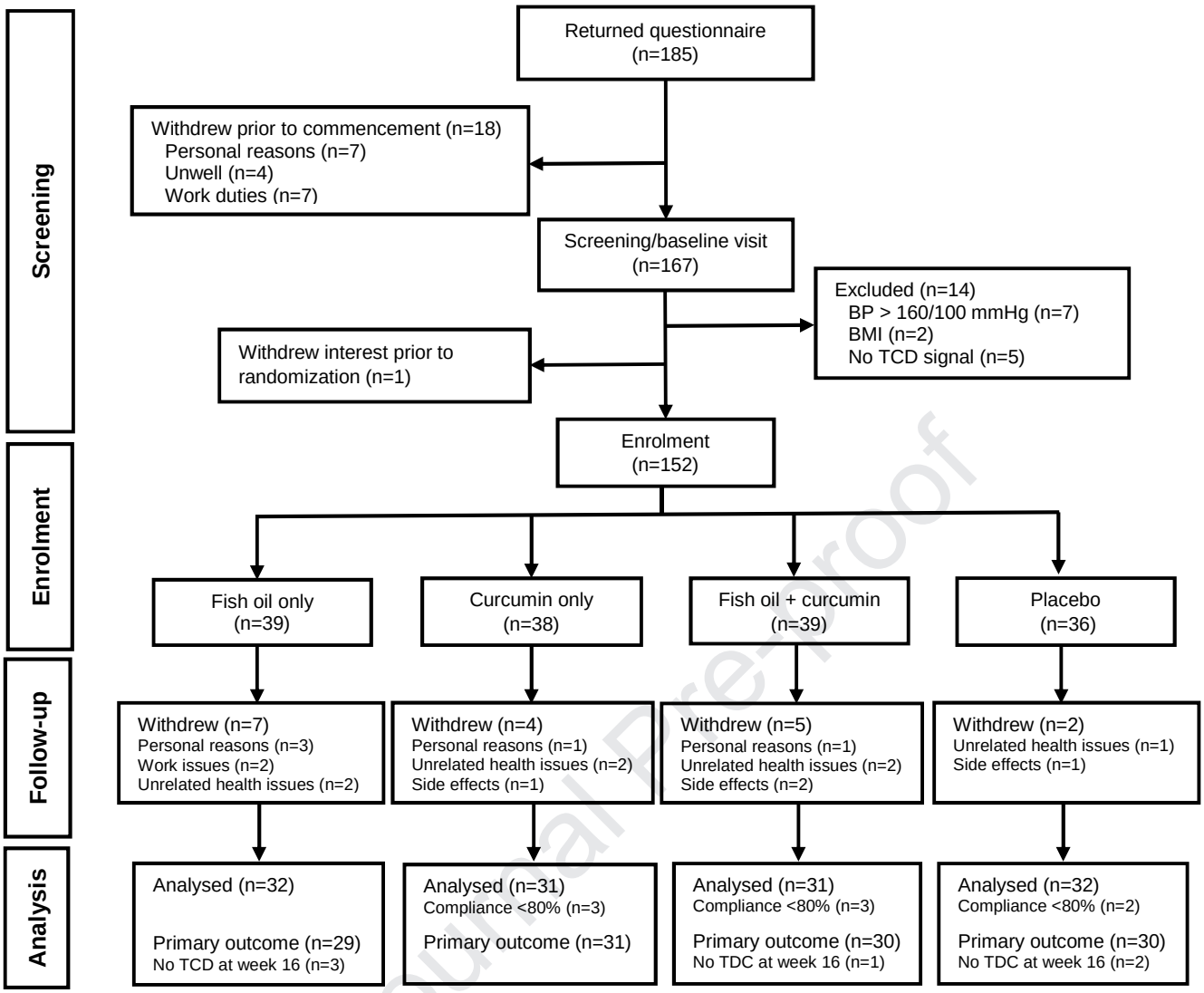
*Significantly different from placebo (P<0.05).

Table 3. Factorial analysis of treatment changes in systemic and cerebral vascular function indices and cardiometabolic biomarkers.

	Fish oil			Curcumin		
	no FO	**FO**	**P**	**no CUR**	**CUR**	**P**
Cardiovascular risk factors	**n=63**	**n=63**		**n=64**	**n=62**	
Systolic blood pressure (mmHg)	-2.7 ± 1.3	-5.4 ± 1.4	0.164	-4.6 ± 1.2	-3.5 ± 1.5	0.556
Diastolic blood pressure (mmHg)	-2.3 ± 0.8	-3.2 ± 0.8	0.382	-3.2 ± 0.8	-2.3 ± 0.8	0.389
Pulse pressure (mmHg)	-0.5 ± 1.0	-2.1 ± 1.0	0.235	-1.4 ± 0.8	-1.2 ± 1.2	0.906
Heart rate (bpm)	0.8 ± 0.8	-1.9 ± 1.0	**0.038***	-1.9 ± 0.8	0.9 ± 1.0	**0.031***
Large artery elasticity (mL/mmHg x10)	0.0 ± 0.6	0.5 ± 0.6	0.577	0.1 ± 0.6	0.4 ± 0.6	0.680
Small artery elasticity (mL/mmHg x100)	0.2 ± 0.2	0.4 ± 0.2	0.509	0.2 ± 0.2	0.4 ± 0.2	0.504
CRP (mg/L)	-0.32 ± 0.21	-0.37 ± 0.18	0.847	-0.45 ± 0.19	-0.23 ± 0.20	0.418
Glucose (mmol/L)	0.06 ± 0.06	0.16 ± 0.11	0.428	0.06 ± 0.05	0.16 ± 0.12	0.461
Triglycerides (mmol/L)	-0.14 ± 0.04	-0.35 ± 0.06	**0.006***	-0.27 ± 0.05	-0.22 ± 0.05	0.439
LDL cholesterol (mmol/L)	-0.11 ± 0.09	-0.02 ± 0.07	0.419	-0.08 ± 0.09	-0.04 ± 0.07	0.753
HDL cholesterol (mmol/L)	0.00 ± 0.02	0.11 ± 0.03	**0.002***	0.03 ± 0.02	0.08 ± 0.03	0.179
Framingham CVD risk score (%)	-1.11 ± 0.43	-2.24 ± 0.40	0.059	-1.71 ± 0.38	-1.65 ± 0.47	0.919
Cerebrovascular function	**n=61**	**n=59**		**n=59**	**n=61**	
Basal mean blood flow velocity (cm/s)	-0.50 ± 1.04	-0.69 ± 1.36	0.913	-0.95 ± 1.29	-0.25 ± 1.12	0.683
Pulsatility index	-0.01 ± 0.01	-0.00 ± 0.02	0.752	-0.01 ± 0.02	-0.01 ± 0.01	0.979
CVR to hypercapnia (%)	1.02 ± 2.56	1.00 ± 2.83	0.996	1.40 ± 2.65	0.63 ± 2.73	0.840

CVD: Cardiovascular Disease. Treatment groups = FO: fish oil, CUR: curcumin. *Significant compared to no treatment (P<0.05).





Highlights

Kuszewski et al: Effects of fish oil and curcumin supplementation on cerebrovascular function in older adults: a randomized controlled trial

- Impaired cerebrovascular function contributes to cognitive decline in the elderly.
- Bioactive nutrient supplementation might counteract cerebrovascular dysfunction.
- Fish oil improved several cardiovascular biomarkers in overweight/obese adults.
- Fish oil also reduced an index of cerebral artery stiffness in males only.
- Curcumin did not affect measured outcomes, either alone or combined with fish oil.