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Omega-3 supplementation improves cognition and modifies brain activation in young adults

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Objective The current study aimed to investigate the effects of eicosapentaenoic acid (EPA)-rich and docosahexaenoic acid (DHA)-rich supplementations on cognitive performance and functional brain activation.
Design A double-blind, counterbalanced, crossover design, with a 30-day washout period between two supplementation periods (EPA-rich and DHA-rich) was employed. Functional magnetic resonance imaging scans were obtained during performance of Stroop and Spatial Working Memory tasks prior to supplementation and after each 30-day supplementation period.
Results Both supplementations resulted in reduced ratio of arachidonic acid to EPA levels. Following the EPA-rich supplementation, there was a reduction in functional activation in the left anterior cingulate cortex and an increase in activation in the right precentral gyrus coupled with a reduction in reaction times on the colour–word Stroop task. By contrast, the DHA-rich supplementation led to a significant increase in functional activation in the right precentral gyrus during the Stroop and Spatial Working Memory tasks, but there was no change in behavioural performance.
Conclusions By extending the theory of neural efficiency to the within-subject neurocognitive effects of supplementation, we concluded that following the EPA-rich supplementation, participants' brains worked 'less hard' and achieved a better cognitive performance than prior to supplementation. Conversely, the increase in functional activation and lack of improvement in time or accuracy of cognitive performance following DHA-rich supplementation may indicate that DHA-rich supplementation is less effective than EPA-rich supplementation in enhancing neurocognitive functioning after a 30-day supplementation period in the same group of individuals. Copyright © 2014 John Wiley & Sons, Ltd.

KEY WORDS—omega-3 fatty acid; fMRI; eicosapentaenoic acid; docosahexaenoic acid; arachidonic acid; Stroop task

INTRODUCTION

Although the beneficial effects of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) on cardiovascular health (Psota *et al*., 2006), mood (Hibbeln, 2009) and neuroinflammation (Pascoe *et al*., 2011; Gillies *et al*., 2012) have often been reported in the literature, it is still unclear whether omega-3 fatty acids alter the cognitive functionality of the brain and whether the benefits, if any, are specific to a particular omega-3 fatty acid. This is particularly interesting as although both EPA and DHA cross brain membranes with equal ease, both brain and retinal DHA levels exceed EPA by several hundredfold (Arterburn *et al*., 2006). Chen has suggested that this extreme difference in brain concentrations may be because EPA is more vulnerable than DHA to β-oxidation and degradation and hence less likely to be incorporated into membranes (as EPA) in the long term (Chen *et al*., 2009; Chen *et al*., 2011).

The first functional magnetic resonance imaging (fMRI) study in the omega-3 fatty acid research field (Mcnamara *et al*., 2010b) revealed that an 8-week DHA supplementation led to an increase in functional activation in the dorsolateral prefrontal brain regions during a sustained visual attention task (a simple continuous performance task) compared with pre-supplementation in healthy children aged 8 to 10 years. However, these cortical activation changes were not accompanied by a corresponding change in either accuracy or reaction times comparing pre-supplementation and post-supplementation testing sessions, a finding supported by other DHA intervention studies using near-infrared spectroscopy (Dullemeijer *et al*., 2007;

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Dangour *et al*., 2010; Stough *et al*., 2011; Jackson *et al*., 2012a; Benton *et al*., 2013).

Less well-investigated are the effects on brain function of supplementation with EPA. EPA-rich supplementation has been reported to improve speed of mental processing, to decrease electromyographic onset latencies, and to alter the ratio of the theta-to-alpha band frequency on Go/No-Go and sustained visual attention tasks in young volunteers (Fontani *et al*., 2005; Fontani *et al*., 2009). Our recent publication has also shown that a 30-day EPA-rich supplementation improved performance on a choice reaction time task in young adults and enhanced neural recovery for high-contrast multifocal visual evoked potentials (mfVEPs) (Bauer *et al*., 2011).

Extending the principles of neural efficiency of Haier *et al*. (Haier *et al*., 1988; Haier *et al*., 1992a; Haier *et al*., 1992b) to neurocognitive effects of supplementations, using the results of our psychophysical and mfVEP study (Bauer *et al*., 2011), we predicted that 30-day EPA-rich supplementation would show a decrease in functional activation relative to cognitive performance (Colour/Word Stroop, Spatial Working Memory), whereas the DHA-rich supplementation would show a lesser effect compared with EPA-rich supplementation.

MATERIALS AND METHODS

Subjects

The analyses presented in this paper are based on data from a subset of the participants who took part in our previous psychophysical and mfVEP study (Bauer *et al*., 2011). Thirteen of these participants (four men and nine women) aged 20 to 34 years (23.84 ± 3.53 (M ± SD)) gave written informed consent prior to taking part in the present fMRI investigation. Eleven participants completed all three scanning sessions, and two participants withdrew from the study before the final testing session. The protocol was approved by the Swinburne University Human Research Ethics Committee and conformed to the Declaration of Helsinki. Inclusion criteria comprised normal or corrected to normal achromatic vision, no known neurological or psychiatric conditions, and no fish oil supplementation in the 4 weeks prior to testing.

Study design

The study procedures were approved by the Swinburne University Human Ethics Committee (SUHREC approval number 0607/138). A repeated measure counterbalanced crossover design was employed (Figure 1). Participants were tested prior to supplementation at baseline (No Diet),

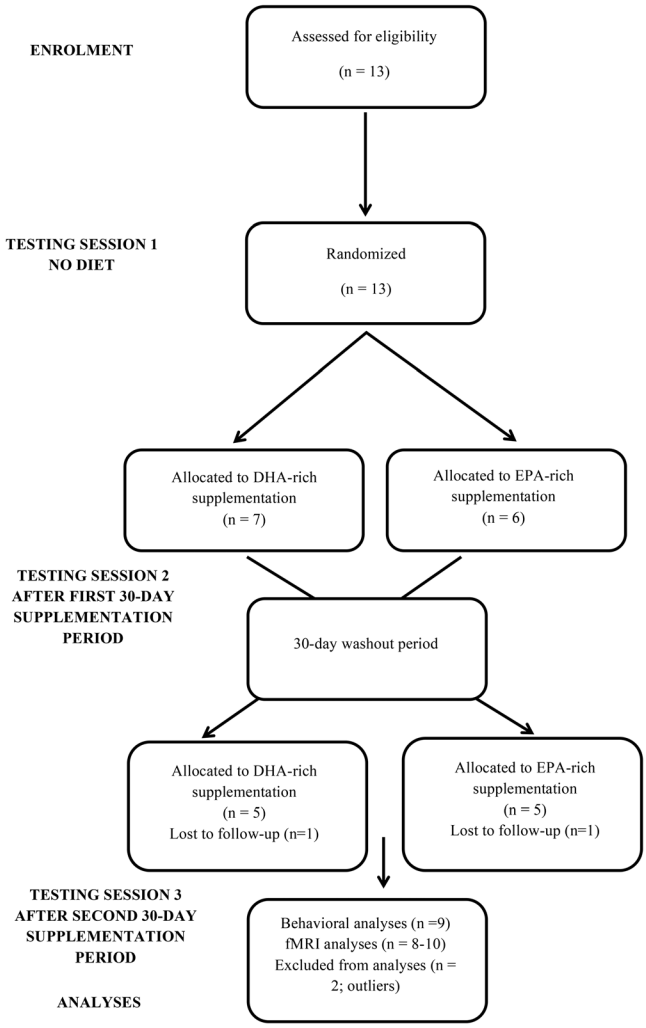

Figure 1. CONSORT diagram showing the flow of participants through each stage of the fMRI data collection and analysis

after a 30-day supplementation period (time T1), and again at completion of the second supplementation (time T2), with a 30-day washout period between the two formulations (on expectation that fatty acid levels would be insignificantly different from baseline; Cao *et al*., 2006). The order of supplementation was counterbalanced across the participants (Randomization and Blinding). Plasma phospholipid blood tests were conducted at Baseline (No Diet), T1 and T2.

Randomization and blinding

The researchers involved in this study were blinded in terms of supplementation allocation. Novasel Australia Pty Ltd provided the investigators with unlabelled bottles numbered 1 or 2 corresponding to the two different treatments. For example, participant 1 was initially supplemented with bottle 1 and then (after washout) with bottle 2. Participant 2 was initially supplemented with bottle 2 and (after washout) with

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bottle 1. Novasel Australia Pty Ltd provided the investigators with a code identifying the contents of bottles 1 and 2, only after analysis had been completed.

Supplementation

The study utilised two different fish oil diets. The first diet (Eye-Q™, Novasel) was a high EPA: DHA formulation (3:1) (400 mg of natural fish oil) with added evening primrose oil (100 mg), whereas the second diet (Efalex™, Efamol) was a high DHA : EPA (4:1) formulation (365.7 mg of natural fish oil) with added D-alpha-tocopherol (7.5 mg), evening primrose oil (142.2 mg) and thyme oil (1.3 mg). Participants supplemented with 6 capsules daily (3 morning and 3 night), for both fish oil diets (a detailed description of the ingredients contained in each supplementation is in Table 1).

Testing procedure

As reported by Bauer *et al.* (2011), participants attended three 2-h testing sessions at the Brain Sciences Institute, Swinburne University, Melbourne, Australia. On the first testing session, participants read and signed the consent form and completed a demographic questionnaire. They then underwent a 30-min cognitive testing session including the Swinburne University Computerized Cognitive Ageing Battery (SUCCAB) cognitive testing battery (Pipingas *et al.*, 2008). Multifocal mfVEPs were recorded at the end of the testing session. Participants underwent three (No Diet, T1, T2) additional 1-h fMRI brain scanning session at the Brain Research Centre (Austin Hospital, Heidelberg, Australia) (Figure 1 and Table 2). Supplementation batches were provided at the end of the first and second testing sessions.

Table 1. Daily amount of EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), GLA (gamma-linolenic acid) and LA (linoleic acid) provided by six capsules of each fish oil formula

Supplement	EPA (mg)	DHA (mg)	GLA (mg)	LA (mg)
EPA-rich	590	137	53	456
DHA-rich	159	417	97	450

SUCCAB cognitive battery

Task protocols. Participants performed the colour–word Stroop and the Spatial Working Memory tasks of the SUCCAB (Pipingas *et al.*, 2008). These tasks were selected because previous supplementation studies administering treatments rich in flavonoids, C vitamin and multivitamins (Pipingas *et al.*, 2008; Macpherson *et al.*, 2011) have shown the sensitivity of the Spatial Working Memory task of the SUCCAB to nutrition-related cognitive changes. Further, omega-3 supplementation studies of Fontani *et al.* have shown beneficial effects on response inhibition tasks (e.g. Go/No Go) comparable with the colour–word Stroop task (Fontani *et al.*, 2005; Fontani *et al.*, 2009).

Outside the scanner, the colour–word Stroop and Spatial Working Memory tasks were presented on a 17-in. colour CRT monitor using a DOS-based computer software package to ensure precise timing to 1 ms for stimulus exposure and to guarantee synchronisation to the screen refresh signal. Each task was preceded by a short-practice trial, and participants were given opportunities to ask questions.

In the colour–word Stroop task, participants were presented with names of colours (red, blue, green and yellow) and were instructed to respond with a button press corresponding to the colour of the word. The task included a Congruent and an Incongruent condition. In the Congruent condition, the word meaning matches the colour of presentation of the word, while in the Incongruent condition, the word meaning identified a different colour from the colour of presentation of the word. The Stroop interference index was calculated by subtracting reaction times on correct trials for the Congruent condition from reaction times for the Incongruent condition. During the fMRI investigation, each scanning run of the colour–word Stroop task comprised 60 volumes and involved four active blocks: two 24-s blocks of Congruent stimuli and two 36-s blocks of Incongruent stimuli. Congruent blocks contained eight stimuli (trials), whereas Incongruent blocks contained 12 stimuli (trials). During Congruent and Incongruent blocks, stimuli were presented for 1000 ms followed by a 2000-ms black fixation cross on a white screen,

Table 2. Study design

No diet 1st session	No diet 2nd session	T1 First 30-day supplement period—1st session	T1 First 30-day supplement period 2nd session	T2 Second 30-day supplement period 1st session	T2 Second 30-day supplement period 2nd session
Consent Form Demographic Questionnaire SUCCAB mfVEP Blood Test	fMRI	SUCCAB mfVEP Blood Test	fMRI	SUCCAB mfVEP Blood Test	fMRI

SUCCAB, Swinburne University Computerised Cognitive Ageing Battery; mfVEP, multifocal visual evoked potentials.

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during which participants gave their response. All active blocks were preceded and followed by a 12-s rest block during which a black fixation cross was presented on a white screen (Figure 2). The length of the Congruent and Incongruent blocks of the colour–word Stroop task was unequal because the functional response to Congruent stimuli is more variable than that during Incongruent stimuli.

In the Spatial Working Memory task, participants were initially shown a 4 × 4 grid of 16 small black squares, six of which were white. They were then presented with four empty grids, with a white square located in random positions. Participants were instructed to remember the location of the white squares on the original grid, and press on a YES or NO button to determine if the location of the white squares presented on the four subsequent grids matched its location on the first grid (Figure 3). The Spatial Working Memory task comprised 111 volumes and involved nineteen 9-s active blocks. Each active block included a 1000 ms 4 × 4 grid made of black squares, immediately followed by four 500-ms empty grids, during which participants gave their response. Each block of stimuli was separated by a 9-s black fixation cross (Rest block) on a white screen (Figure 3).

Functional magnetic resonance imaging

Imaging procedures. Participants were scanned using a 3 Tesla Tim Trio MRI scanner (Siemens, Erlangen, Germany) fitted with a 12-channel head coil at the Brain Research Institute, Heidelberg, Australia. In the first session, a high-resolution T1-weighted image was acquired (axial slice acquisition), using a 3D MPRAGE sequence (TR = 1900 ms, TE = 2.6 ms, 192 slices, 0.9 × 0.9 × 0.9 voxel, field of view (FOV) 230 mm). In each of the subsequent testing sessions, 66 functional images were acquired using a T2*-weighted gradient-echo echo-planar image (EPI) pulse sequence (TR = 3000, TE = 30 ms, FOV = 216 mm, voxel size 3 × 3 × 3 mm) while participants performed the cognitive tasks. Participants were asked to minimise head movements. The use of foam padding inserted around the participant's head and neck aided this. The participant was provided with a microphone to enable communication with the researcher and MRI technician while in the scanner. Stimuli were presented on an MRI-compatible screen positioned behind the scanner. A mirror enabled subjects to see the screen. Participants held an MRI-compatible button box in their right hand.

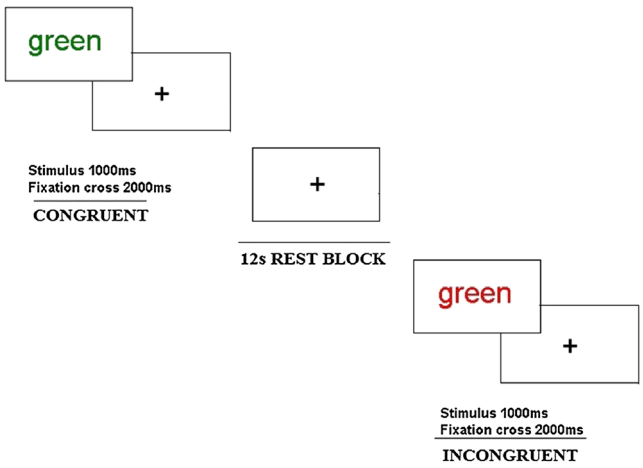

Figure 2. fMRI protocol for the colour–word Stroop task of the SUCCAB

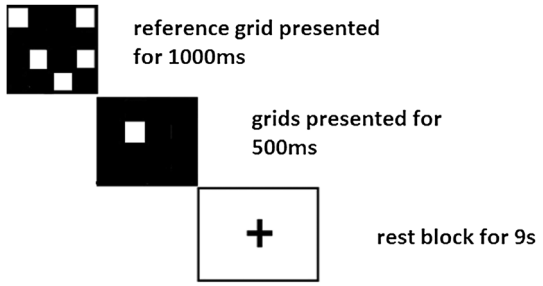

Figure 3. fMRI protocol for the Spatial Working Memory task of the SUCCAB

Imaging analyses

Preprocessing. Preprocessing and statistical analysis of image data was performed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Prior to preprocessing, the first six volumes of each functional sequence were discarded to reduce T1 saturation effects in image time-series. 'ArtRepair' (Mazaika *et al*., 2007) routines were used to minimise voxel noise and repair aberrant image slices on the remaining image time-series.

For each session, the repaired images were realigned to the first image in the first session and a mean realigned image was created. The high-resolution T1-weighted structural image was then coregistered to the mean realigned image. After visually inspecting the quality of this co-registration, the co-registered T1 image was spatially normalised to the T1 template supplied with SPM8. The parameters describing this transformation (i.e. T1 spatial normalisation) were applied to the realigned EPI images, and subsequently, these spatially normalised EPIs were spatially smoothed using a Gaussian kernel (6 mm full-width at half maximum). ArtRepair was then used to detect and replace using an interpolation algorithm, any spatially smoothed volumes exhibiting highly variant signal intensity.

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Modelling: participant level analysis. At the first level of analysis, the preprocessed functional images for each dietary condition were modelled in two ways: (1) all sessions were modelled together (separately for each participant) to obtain statistical parametric maps that were not biased toward any particular session, and (2) image data were modelled separately for each testing session (three separate models per participant) using the same modelling parameters. In both models, the time-series was first high-pass filtered (150 s) and then entered into a multiple regression model. Each condition was modelled separately by an explicit box-car regressor that was defined by the onset and duration of the blocks of stimuli representing each condition. These box-car regressors were convolved with the canonical haemodynamic response function supplied with SPM8. Rest periods were not modelled explicitly and contributed to the implicit baseline. To control for fluctuation in the BOLD signal arising from head movement during scanning, the six parameters describing the realignment of each image (representing translational and rotational movement) were added to the model as regressors of no interest for each testing session. After estimating the beta parameters for each task condition (Congruent Stroop, Incongruent Stroop, Spatial Working Memory), contrast maps depicting the Interference effect (contrast: Stroop Incongruent Stroop Congruent) were computed (absolute values). Next, the image data were remodelled separately for each session (i.e. three separate models per participant) using the same modelling parameters.

Modelling: group level analysis. The contrast maps for the larger model representing the difference between the beta parameter estimates over the three sessions were entered into a one-sample *t*-test. Statistical thresholding for the resultant group activation map was $p < .001$ (uncorrected) at the voxel level, and only those clusters that were significant after correcting for multiple comparisons ($p \leq .05$, family wise error (FWE) corrected) at the cluster level were considered significant.

These clusters were used as the basis for a regions of interest (ROI) analysis that was performed using the Marsbar Region of Interest toolbox for SPM (Brett *et al*., 2002) to compare the effects of EPA-rich and DHA-rich supplementation on functional activation. Initially, a sphere of 10-mm radius was constructed around the peak coordinates of significant activation clusters. Then, the mean contrast estimate for each contrast, within each ROI, was extracted from each session-specific contrast map, yielding three values per ROI for each participant corresponding to No Diet, EPA-rich and DHA-rich supplementation groups.

Statistical analyses

Tests of normality assumptions (tests of homogeneity: Levene's statistics $> .05$, and sphericity tests) were conducted, and a Greenhouse–Geisser correction was utilised when the data distribution did not approach normality. Outliers were deleted if values were two standard deviations above or below the mean of the variable (Hill and Lewicki, 2006). Missing data were handled by using a list-wise deletion approach thus excluding participants who missed one or two testing sessions (Howell, 2010). Changes from No Diet for behavioural and fMRI measures of the EPA-rich and DHA-rich supplementation groups were compared using paired *t*-tests with SPSS Statistics (IBM, version 19) (Howell, 2010). Given the small sample size and the high risk of type II errors, the statistical threshold was not corrected for multiple comparisons and results were considered statistically significant with $p \leq .05$.

RESULTS

Plasma fatty acid profile

Mean levels of omega-3 and omega-6 fatty acids in plasma phospholipids at baseline and after supplementation are shown in Table 3. Repeated measures MANOVA revealed a significant decrease in omega-6 fatty acids ($F(2,18) = 4.25$; $p = .03$), and *post hoc* (Bonferroni) analyses showed a significant decrease in omega-6 fatty acids following EPA-rich supplementation compared with No Diet, and a smaller decrease following DHA-rich supplementation (DHA-rich < No Diet: $p = 0.06$). Further, arachidonic acid (AA) levels decreased ($F(2,18) = 4.38$, $p = .02$) after DHA-rich supplementation compared with No Diet, and marginally decreased following EPA-rich supplementation ($p = .06$). Similarly, the AA to EPA ratio decreased ($F(2,18) = 4.64$, $p = .024$), after both EPA-rich and DHA-rich supplementations compared with No Diet (Table 3).

Behavioural Results

Supplementation rich in EPA was shown to decrease the reaction times of the Congruent condition of the colour–word Stroop task compared with supplementation rich in DHA using *t*-tests ($t(9) = 2.37$, $p = .04$). No effect of supplementation on reaction times and accuracy of the Spatial Working Memory task was found. Reaction times and rates of accuracy on these tasks can be found in Tables 4 and 5.

Brain activation after supplementation

Colour–word Stroop task. Functional activation was observed in the prefrontal dorsolateral, fronto-parietal

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Table 3. Plasma fatty acid mean levels (%) (±SEM) of omega-3 and omega-6 fatty acids in plasma phospholipids at baseline and after supplementation. Results are expressed as percentiles of the total fatty acid contained in plasma phospholipids. *F* and *p*-values are provided

Fatty acids	*N*	No diet	EPA-rich	DHA-rich	*F*	*p*
Total omega-3	10	4.97 ± 0.45	5.30 ± 0.70	4.83 ± 0.35	.29	.74
EPA	10	1 ± 0.12	1.44 ± 0.24	1.04 ± 0.08	2.77	.08
DHA	10	2.92 ± 0.33	2.81 ± 0.39	2.83 ± 0.39	.04	.96
Omega-6	10	35.98 ± 0.66	32.26 ± 0.91**	33.59 ± 0.94	4.25	.03
AA	10	9.13 ± 0.63	7.76 ± 0.58	7.79 ± 0.34**	4.38	.02
Omega-3/6	10	0.13 ± 0.01	0.16 ± 0.02	0.14 ± 0.09	1.38	.27
AA/EPA	10	10.38 ± 1.30	6.97 ± 1.30*	7.52 ± 0.40*	4.64	.02

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid.
*$p \leq .05$.
**$p \leq .01$.

Table 4. Response times (ms) and accuracy (%) (±SEM) at No Diet and mean change from No Diet for DHA-rich and EPA-rich supplementations (±SEM) for the colour–word Stroop task. The minus sign refers to faster reaction times and accuracy responses

	No diet	DHA-rich	EPA-rich
	(M ± SEM)	Difference from baseline	
Stroop Congruent RT (ms)	634.85 ± 29.17	2.53 ± 0.44	−35.82 ± 4.37*
Stroop Congruent (% Accuracy)	98 ± 0.72	−0.2 ± 0.52	−1.2 ± 0.52
Stroop Incongruent RT (ms)	706.39 ± 30.28	−15.32 ± 1	−16.26 ± 0.46
Stroop Incongruent (% Accuracy)	95.25 ± 1.76	−0.2 ± 0.48	0.9 ± .054
Interference RT (ms – absolute values) (Incongruent minus Congruent)	71.54 ± 18.33	17.84 ± 21.96	19.56 ± 0.18

*$p < .05$.

Table 5. Response times (ms) and accuracy (%) (±SEM) at No Diet, and mean change from No Diet for DHA-rich and EPA-rich supplementations (±SEM) for the Spatial Working Memory task. The minus sign refers to faster reaction times and accuracy responses

	No diet	EPA-rich	DHA-rich
	(M ± SEM)		
Spatial Working Memory RT (ms)	744.96 ± 34.12	−38.20 ± 27.04	−26.54 ± 29.50
Spatial Working Memory (% Accuracy)	91.55 ± 2.39	−1.32 ± 0.08	−2.77 ± 0.16

regions and anterior cingulate cortices (for more details on whole brain activations associated with the cognitive tasks (see supporting information Tables 1 and 2). Only clusters of activation with a minimum extent threshold of 10 contiguous voxels were considered for further analyses. *t*-Tests revealed an increase in activation in the right precentral gyrus during the Congruent condition of the colour–word Stroop task, following EPA-rich supplementation, compared with No Diet ($t(8) = 2.8$, $p = .02$) (Figure 4). On the other hand, in the Incongruent condition, there was an increase in activation in the left precentral gyrus following DHA-rich supplementation compared with No Diet ($t(8) = 2.34$, $p = .04$) (Figure 4). The Interference contrast was characterised by a reduction in activation in the left anterior cingulate cortex (ACC) following EPA-rich supplementation ($t(8) = 2.3$, $p = .05$) compared with No Diet (Figure 5).

Spatial Working Memory task

Statistical analyses showed a significant increase in activation in the right precentral gyrus following DHA–rich supplementation compared with No Diet ($t(10) = 2.13$, $p = .05$) (Figure 6).

DISCUSSION

This study assessed the effects of omega-3 supplementation on neural activity during the performance of the colour–word Stroop task and the Spatial Working Memory tasks of the SUCCAB. On the basis of a neural efficiency interpretation of our previous publication showing the positive effects of EPA-rich supplementation on visual neural recovery and choice reaction times in the group of participants from which a subset was selected for the present study (Bauer

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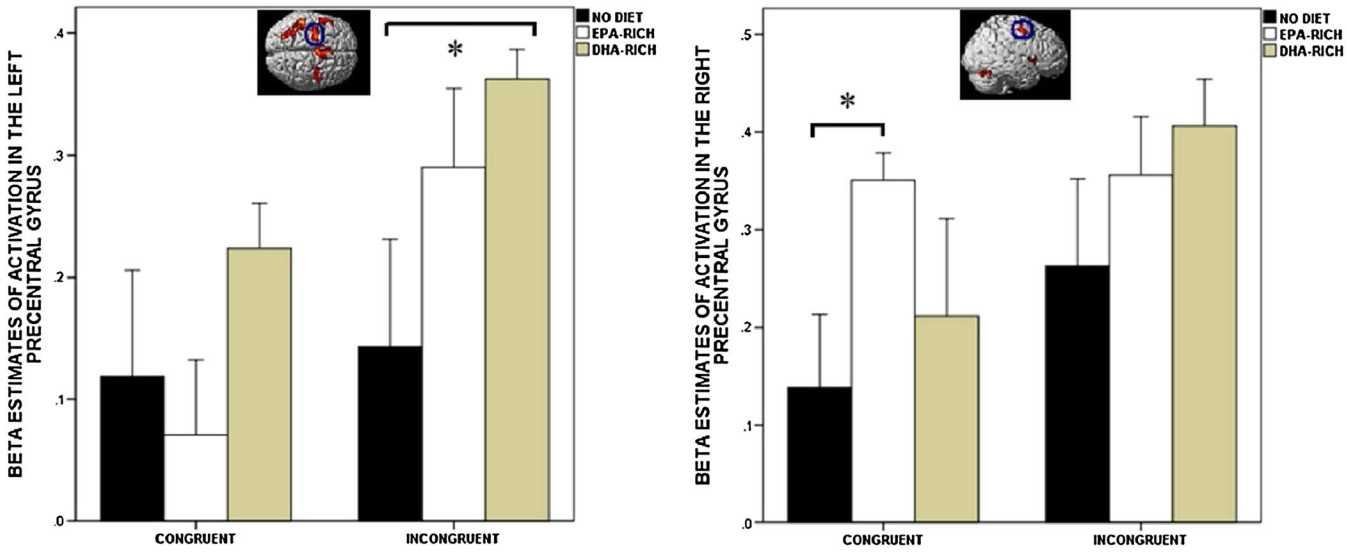

Figure 4. Beta estimates of functional activation in the left precentral gyrus (graph on the left) and the right precentral gyrus (graph on the right) during the Congruent and Incongruent conditions of the colour–word Stroop task at No Diet, and after EPA-rich and DHA-rich supplementations (means ± SEM). *t*-Tests were performed. A significant difference ($p < .05$) compared with No Diet was indicated with an asterisk (*)

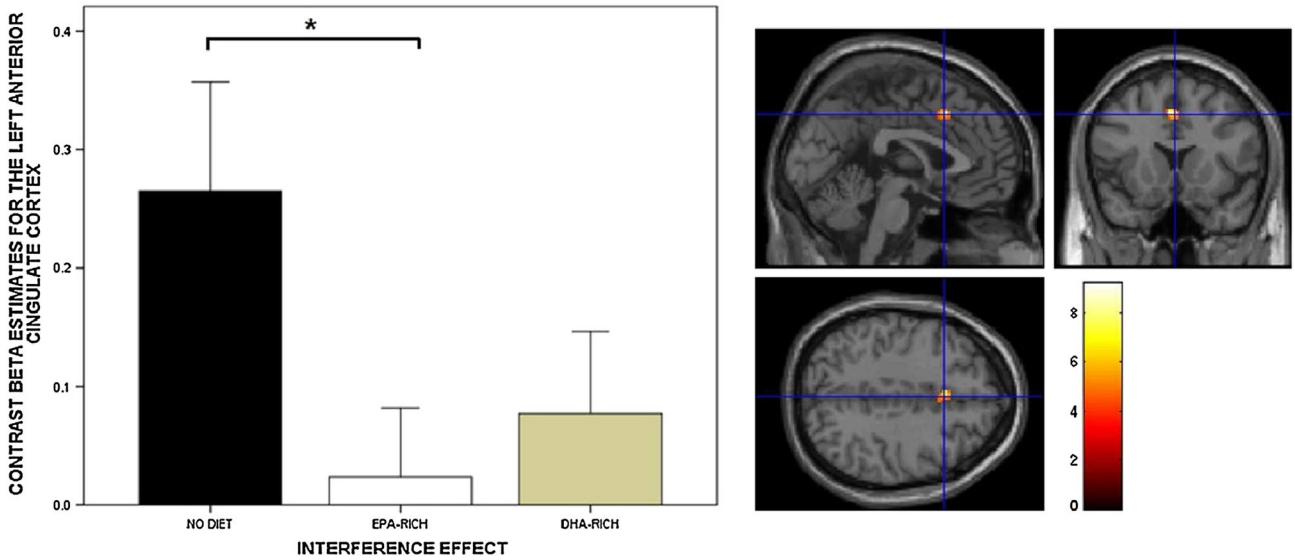

Figure 5. Contrast estimates of functional activation in the left anterior cingulate cortex (means ± SEM) during the Interference contrast of the colour–word Stroop task, at No Diet, and after EPA-rich and DHA-rich supplementations. *t*-Tests were performed. A significant difference ($p \leq .05$) compared with No Diet was indicated with an asterisk (*)

et al., 2011), it was predicted that EPA-rich supplementation would reduce neural activity relative to cognitive performance to a greater extent than DHA supplementation during higher order cognitive tasks. Indeed, following EPA-rich supplementation, there was an improvement in cognitive performance during the Stroop task associated with a strong reduction in functional brain activation in the left ACC, and an increase in activation in the right precentral gyrus that was not observed following DHA-rich supplementation.

The decrease in reaction times associated with a reduction in functional activation in the ACC following EPA-rich supplementation could also be interpreted by extending Haier *et al*.'s theory of neural efficiency to the within-subject investigation of the neurocognitive effects of omega-3 supplements (Haier *et al*., 1988; Haier *et al*., 1992a; Haier *et al*., 1992b). On the basis of the findings of their 1988 and 1992 studies, Haier and colleagues hypothesised that brains of individuals with higher intellectual quotient (IQ) may require less

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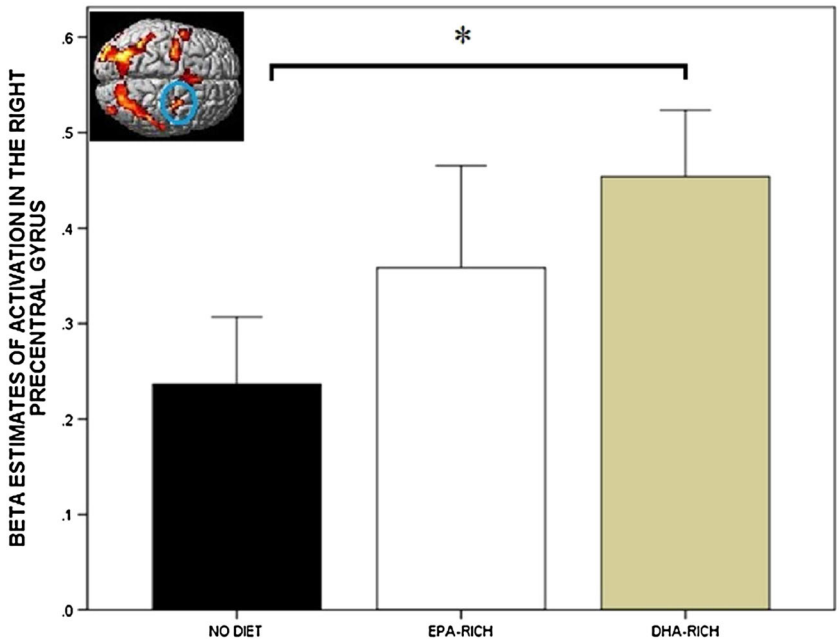

Figure 6. Beta estimates of functional activation in the precentral gyrus (means ± SEM) for the Spatial Working Memory task at No Diet, and after EPA-rich and DHA-rich supplementations. *t*-Tests were performed. A significant difference ($p \leq .05$) compared with No Diet was indicated with an asterisk (*)

neural energy resources than those of individuals with average IQ to perform higher order cognitive tasks. A supplementation that increases neural efficiency would be one that shows a relative increase in cognitive performance with a relative reduction in neural activity. Conversely, an increase in brain activation associated with unaltered cognitive performance may indicate that the brain needs to recruit more neural resources to maintain the same cognitive performance—a sign of reduced neural efficiency.

Hence, the reduction in functional activation associated with an improvement in cognitive performance following EPA-rich supplementation observed in this study together with previous identification of a beneficial role of EPA on VEP recovery (Bauer *et al*., 2011) may therefore indicate a more efficient utilisation of neural resources and/or consumption of oxygen and glucose in the brain. Additional evidence that EPA-rich supplementation increases neural efficiency comes from the electroencephalography (EEG) studies of Fontani *et al*. (2005, 2009) that showed that a 4-week EPA-rich supplementation resulted in improved attentional performance together with a reduction of higher frequency EEG spectral bands (beta) and an increase in low-frequency band strength (theta).

Compared with EPA-rich supplementation, our results, following DHA-rich supplementation of the same duration, are less supportive of an improvement in neural efficiency. This conforms with three 8 to 12-week DHA-rich supplementation studies that were accompanied by either an increase in functional MRI brain activation (Mcnamara *et al*., 2010a) or a rise in neural tissue oxygenation (Jackson *et al*., 2012c; Jackson *et al*., 2012b) during high-order cognitive tasks without a concomitant behavioural improvement.

In agreement with the earlier fMRI study by Mcnamara *et al*. (2010a), this study showed an increase in activation during the Stroop and the Spatial Working Memory tasks following DHA-rich supplementation. Furthermore, as with the earlier studies of Mcnamara *et al*. (2010a) and Jackson *et al*. (2012b, 2012c), this study did not find any effect of DHA-rich supplementation on behavioural accuracy or timing measures. It is also relevant that the near-infrared spectroscopy studies of Jackson *et al*. (2012b, 2012c) following DHA-rich supplementation observed an increase in the levels of oxyhaemoglobin in the prefrontal areas of the brain. A reduction in reaction times on the Stroop task was also found following the 12-week DHA-rich supplementation period; however, given the lack of statistical significance after correcting for multiple comparisons, the authors concluded that there was no substantial evidence of the beneficial effects of the DHA-rich supplementation on neurocognition (Jackson *et al*., 2012a). By contrast, Mcnamara *et al*. (2010b) interpreted the increase in functional activation (measured using fMRI) after supplementation with DHA as suggesting that dietary

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DHA intake is a robust modulator of functional cortical activity. The neural efficiency hypothesis postulated in this paper provides an alternative framework for the interpretation of these results.

Interestingly, a recent magnetic spectroscopy ^{1}H-MRS study (Mcnamara *et al*., 2013) showed that children with low erythrocyte DHA levels (low DHA) have reduced concentrations of N-acetyl aspartic acid, creatine, myo-inositol and choline in the ACC. The low-DHA group also exhibited slower reaction times on a continuous performance task than the high-DHA group. By contrast, erythrocyte EPA levels correlated positively with cerebral metabolic parameters but were not associated with cognitive performance. These findings were interpreted as suggesting that DHA levels are the best positive predictors of neurochemical functioning in children. Indeed, N-acetyl aspartic acid is the precursor of N-acetyl-aspartyl-glutamate, a catalyst for the production of the excitatory neurotransmitter glutamate (Faull *et al*., 1999), whereas myo-inositol is synthesised from glucose-6-phosphate and is an important precursor of signalling and secondary messenger molecules down-regulated in psychiatric disorders such as unipolar and bipolar disorders (Kim *et al*., 2005). However, because the nervous system of children is still developing, the neurochemical effects of DHA in this population may not reflect those in a population with a mature central nervous system.

The theory of neural efficiency has been used for the interpretation of previous fMRI findings in diabetic and HIV populations. Bolo *et al*. (2011) interpreted an increase in fMRI signal intensity (in the dorsolateral, parietal and anterior cingulate brain regions) without behavioural improvement in a group of diabetic/hypoglycaemic patients compared with a healthy/hypoglycaemic population in terms of neural efficiency. They concluded that the (regular) depletion of cerebral glucose supply in the people with diabetes may have led the brain to 'hyperactivate' to maintain the same standard of cognitive performance, resulting in a reduction in neural efficiency. Similarly, Ernst *et al*. (2009) compared the functional activation of brains of a group of middle-aged individuals suffering from immunodeficiency (HIV) to a HIV-seronegative control group of the same age over a 12-month period. At the end of the 12-month period, the cognitive performance of the two groups did not change; however, the HIV group presented with increased activation in a greater number of brain regions of the attentional network during the two-ball, three-ball and four-ball tasks when compared with baseline measurements and to the control group (Ernst *et al*., 2009). The authors concluded that the brain of HIV patients coped with the decline in brain function and probable premature brain ageing induced by HIV by upregulating brain 'energy'.

We note that during the Stroop task, EPA-rich supplementation was associated with a decrease in functional activation in the ACC, which activates during conflict-generating tasks (Barch *et al*., 2009), and an increase in activation in the precentral gyrus, which is responsible for implementing corrective strategies (Carter *et al*., 2000; Barch *et al*., 2009). Because the ACC and the precentral gyrus are core regions of the attentional control network and work closely together (Barch *et al*., 2009), it could be hypothesised that the benefits of EPA-rich supplementation on neural efficiency are due to a positive action of EPA on the functional connectivity between the ACC and the precentral gyrus. In other words, participants focussed on working as fast and as accurately as they could and were less distracted by irrelevant stimuli compared with prior to supplementation.

The theory of neural efficiency lends itself well to a crossover design in which the same group of participants perform tasks under different supplementation conditions because their brain activity and cognitive performance can be easily equated. In other words, a change in functional activation in a certain brain region is likely to be due to an effect of omega-3 supplementation and indicate a change in the utilisation of neural resources, rather than be due to individual differences in IQ or differences in patterns of functional activation between participant groups as might occur in a parallel design.

That neural efficiency reflects a relationship between cognitive performance and brain effort perhaps helps us understand why previous reports on the effects of omega-3 supplementation on cognitive performance have been inconsistent. The essence of neural efficiency is an interplay between cognitive performance and brain effort. Humans tend to modify their brain activity to maintain an acceptable level of performance. Hence, when only cognitive function is measured, without taking into account brain activation levels, one might expect this inconsistent interaction between supplementation and performance (leaving aside differences in the literature in terms of age group and omega-3 content of food ingested). A handful of short-term EPA-rich supplementation studies using cognitive and neural measures claim benefit in healthy young adults (Fontani *et al*., 2005; Fontani *et al*., 2009). Longer term studies with DHA-rich and EPA-rich supplementations with equal to or greater quantity of EPA than the present study found limited effects of DHA-rich supplementation on cognitive response times, with DHA-rich supplementation increasing cerebral oxygenation and

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functional activation of the brain during higher order cognitive tasks (Mcnamara *et al*., 2010a; Stough *et al*., 2011; Jackson *et al*., 2012b; Benton *et al*., 2013). One problem with interpreting longer term studies is that the changes engendered in cerebral activation could be adaptive responses to maintain performance at the same standard as at times prior to supplementation, or alternatively, they could reflect an overall improvement change in brain function associated with general physiological response to potentially healthier diet.

The varied diets employed in the literature raise the question of the extent to which the EPA : DHA ratio alters neurocognitive effects. This issue has not been clearly addressed in previous studies. Indeed, only a small number of publications have simultaneously administered both EPA and DHA, and only this present study and the parallel VEP paper (Bauer *et al*., 2011) have investigated this issue in a crossover design, arguably more sensitive to neural changes associated with different diets. It is, however, notable that in our previous VEP publication (Bauer *et al*., 2011), where the total omega-3 fatty acid concentrations in both supplementations was very similar and two different EPA : DHA ratios were administered (over a short period of 4 weeks), electrophysiological measures of neural recovery showed stronger amplitude changes following the EPA-rich supplementation compared with the DHA-rich supplementation. Similarly, the changes in neural efficiency reported here were associated with the EPA-rich supplementation.

A possible confound for the difference observed between EPA-rich and DHA-rich supplementations in this study is that there was comparatively more EPA in the EPA-rich supplementation (590 mg) than DHA in the DHA-rich supplementation (417 mg). However, the DHA dose is similar to the low dose of McNamara *et al*. (2010), sufficient to cause significant upregulation of BOLD activation in the dorsolateral prefrontal cortex. A second potential confound comes from observed differences in the rates of incorporation into membranes (Cao *et al*., 2006). Indeed, whereas EPA is rapidly esterified into phosphatidylcholine phospholipids that are located in the outer layer of the cellular membrane, DHA is slowly incorporated into the phosphatidylethanolamine phospholipids in the inner cellular membranes (Neuringer and Connor, 1986; Stasi *et al*., 2004; Metherel *et al*., 2009). However, this could only be considered a confound if the mechanism of neurocognitive change requires membrane incorporation. Competing mechanisms could include the availability of fatty acids for glycolytic processes during times of metabolic stress (such as seen under conditions inducing the Warburg Effect; Vander Heiden *et al*., 2009).

Two further limitations of this study was the absence of a placebo treatment group given our crossover design and absence of blood tests after the 4-week washout period, therefore the absence of reliable information on the clearance rates of EPA and DHA from membrane and plasma phospholipids during washout. However previous studies indicate that a 4-week washout is probably adequate to reduce erythrocyte membrane EPA and DHA levels dramatically (Cao *et al*., 2006; Metherel *et al*., 2009). Cao *et al*. (2006) found that after 8 weeks of supplementation, the 4-week washout reduced EPA levels greatly though the new levels were still significantly greater than baseline for another 2 weeks, while plasma phospholipid levels were insignificantly different from baseline. Furthermore, Metherel *et al*. (2009) showed that a 4-week washout after a 4-week supplementation with a 2:1 EPA and DHA mixture can induce almost complete clearance of EPA and DHA from plasma phospholipids.

We randomly counterbalanced the diet order across the sample to control for order effects. However, there was insufficient sample size to include treatment order as a covariate in our analyses. In terms of statistical power, previous fMRI nutritional crossover designs (Smeets *et al*., 2005; Purnell *et al*., 2011; Smeets *et al*., 2011) employed a sample size of 9 or 10 participants (a little less than the 11 in this study) with repeated measurement across conditions considered sufficient to yield reliable results. The crossover design employed here has obvious benefits when measuring brain activation because it enables researchers to compare the effects of two dietary conditions using the brains of the same group of individuals, reducing variance when comparing dietary conditions on a voxel-by-voxel basis. Thus, as a rule of thumb, between group comparisons for fMRI experiments using parallel rather than crossover designs generally need 15–20 participants per group though, obviously, 11 was adequate for a significant result using the crossover design (Desmond and Glover, 2002; Wei *et al*., 2004).

In summary, this experiment used fMRI techniques to investigate the effects of 30-day EPA-rich and DHA-rich supplementations on neurocognitive functioning in young healthy volunteers. We demonstrated that the EPA-rich supplementation reduces reaction times and decreases functional activation in the ACC as compared with prior to supplementation. DHA-rich supplementation increased functional activation in the precentral gyrus but did not induce any behavioural

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improvement. We offer an alternative interpretation of the effects of EPA-rich and DHA-rich supplementations on cognition and brain measures by combining brain and behavioural findings in light of the theory of neural efficiency. It is concluded that a 30-day EPA-rich supplementation is more successful than a 30-day DHA-rich supplementation in improving neural efficiency during higher order cognitive tasks.

CONFLICT OF INTEREST

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