ORIGINAL ARTICLE

Omega-3 fatty acids improve psychomotor performance via mechanism not related to nitric acid production

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Omega-3 fatty acids (ω-3FAs) are essential polyunsaturated fats that protect the brain from cognitive impairment. It increases the activity of endothelial nitric oxide synthetase (eNOS) and thereby increases the nitric acid (NO) production. This study aimed to explore the effect of ω-3FAs on psychomotor performance and to relate this effect to the reactive nitrogen species. This study was conducted in Department of Pharmacology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq. Twenty healthy subjects, allocated randomly from medical college students, were participated in the single blind clinical trial. Participants were divided into two groups, each of ten subjects to receive either placebo or (ω-3FAs) (750 mg single oral dose daily for 5 days). They were asked to perform psychomotor performance before and after 5 days of treatment, and venous blood was obtained for determination of serum nitric oxide (NO) and peroxynitrite (ONOO). ω-3FAs treated group was significantly different from placebo-treated group in reducing choice and motor reaction times as well as the critical flicker frequency threshold. The serum levels of NO and ONOO in ω-3FAs-treated group did not significantly differ from placebo-treated group. Short term supplementation of ω-3FAs improves the psychomotor performance in young healthy subjects via a mechanism not related to the production of nitric oxide production. Inflorescence is a panicle few flowered and fruit is a capsule. The data of the results obtained were presented and discussed.

Key words: Nitrogen species, psychomotor performance, ω-3FAs

INTRODUCTION

Omega-3-fatty acids (ω-3FAs) are essential polyunsaturated fats, mainly eicosapentaenoic acid (EPA) and docosahexaenoic (DHA), cannot synthesize in mammalian cells and found primarily in fish. DHA is the major constituent of neuronal membranes, essential for brain development as well as normal brain functioning.[1,2] Dietary DHA is needed for the optimum functional maturation of the retina and visual cortex.[3] In the aged brain, ω-3FAs have neuro-protective effects *via* positive effects on neurogenesis and modulation of certain receptors that involved in learning and memory.[4] Experimentally, DHA supplementation reversed the progressive memory loss, cognitive alterations, and learning abilities change[5,6] and protected the brain against cognitive impairment.[7-9] Recently, McNamara *et al.* found that DHA supplementation for 8 weeks altered the functional cortical activity during attention in healthy boys aged 8-12 years.[10] The beneficial effect of ω-3FAs on endothelial function is mediated by increasing the activity of endothelial nitric oxide (NO) synthatase and thereby NO production.[11,12] Six week supplementation of EPA and DHA produced significant increase in serum NO level at rest and after a judo-training session.[13] *In vitro* using retinal microvascular endothelial cells, DHA improved NO bioavailability and decreased superoxide anion (Ö) production.[14] This study aimed to investigate the central effect of ω-3FAs supplementation in young healthy subjects by assessing the psychomotor performance and to link that effect on the serum reactive nitrogen species.

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.97102

MATERIALS AND METHODS

This study was conducted in Department of Pharmacology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq during 2010. Twenty healthy male volunteers (medical students) aged between 20 and 22 years (mean age 21 years) were allocated randomly from college by using randomized tables to participate in the study. They are grouped into placebo- and ω-3FAs-treated groups, each of 10 subjects. All participants were in good health, without any significant clinical history of physical or mental illness and not taking any concomitant medication that was likely to interfere with the study. Written informed

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Received: 01-10-2011; **Accepted:** 16-03-2012

consent was obtained from all participants. The study was approved by local scientific committee of the institution. This study was randomized, single-blind, where each subject acted as own control. The drug under investigation was ω-3FAs soft gelatin capsules of 750 mg weight (mixture of EPA and DHA) were purchased from local sources. All participants who entered the study were familiarized with the study procedures and trained on the battery of psychometric tests in order to preclude learning effects.

The test began with pre-treatment baseline assessment on the test battery and then the treatment dose of 750 mg/d ω-3FA was administered at 9.00 a.m. for 5 days. Performance, using Leeds Battery Psychomotor Instrument [choice reaction time (CRT) and critical flicker fusion (CFF)], was assessed daily up to 5 days.

The choice reaction time (CRT) task is used as an indicator of sensorimotor performance, assessing the ability to attend and respond to a critical stimulus. Participants are required to place the index finger of their preferred hand on a central starting button and are instructed to extinguish one of six equidistant red lights, illuminated at random, by pressing the response key in front of the light as quickly as possible. The mean of 15 consecutive presentations is recorded as a response measure of three components of reaction time: choice motor and total reaction time. Choice reaction time (CRT) is the time between stimulus (light) onset and the subject's lifting of the finger from the start button. Motor reaction time (MRT) indicates the movement component of this task and is the time between a participants' lifting of his finger from the start button and touching the response button. Total reaction time (TRT) is the sum of CRT and MRT. The critical flicker fusion (CFF) task assessed the integrative capacity of the central nervous system (CNS) and, more specifically, the ability to discriminate discrete 'bits' of sensory information. In this, the participants are required to discriminate flicker from fusion and *vice versa*, in a set of four light-emitting diodes arranged in a 1-cm square. The diodes are held in foveal fixation at a distance of one meter. Individual thresholds are determined by the psychophysical method of limits on five ascending (flicker to fusion) and five descending (fusion to flicker) scales. A decrease in the CFF threshold is indicative of a reduction in the overall integrative activity of the CNS.

Venous blood sample was obtained from each participant before intake of ω-3FA capsule and after 5 days treatment for determination of serum peroxynitrite, nitric acid.

Peroxynitrite ($ONOO^-$) mediated nitration of phenol was measured as described by others.[15,16] Briefly, 50 µl of serum was added to 5mM phenol in 50 mM sodium phosphate buffer, pH 7.4 in a final volume of 3 ml. After incubation for 2 hours at 37°C, 25 µl of 0.1 M NaOH was added, and the absorbance at 412 nm of the samples was immediately recorded. The yield of nitrophenol was calculated from ε=4400 M^1 cm^{-1}.

Nitric oxide donating activity was determined as described by Newaz and co-workers.[17] Briefly, 0.5 mL serum was added to 200 µl HCl (6.5M) and 200 µl sulfunalic acid (37.5 mM). After incubation for 10 min, 50 µl naphthylethylenediamine dihydrochloride (12.5 mM) was added and incubated for further 30 min, centrifuged for 10 min at 1000 g. The absorbance at 540 nm was immediately recorded.

Statistical Analysis

The results are expressed as mean±SD of the number of observations. The data were statistically analyzed by using one-way ANOVA, taking $P \leq 0.05$ as the lowest limit of significance.

RESULTS

Placebo did not show significant effect on TRT, CRT, MRT, and CFF frequency (flicker component) threshold [Table 1]. The baseline values of TRT, CRT, MRT, and CFF frequency threshold of participants received ω-3FA did not significantly differ from corresponding values of placebo-treated group. Five days treatment with ω-3FAs significantly reduced TRT, CRT, MRT by 18.2, 13.1, and 27.2%, respectively, from baseline values and by 20.1, 12.7, and 32.3%, respectively, from corresponding placebo-

Table 1: The effect of 5-days oral dose of 750 mg/day ω-3FA and its corresponding placebo on total reaction time, choice reaction time, motor reaction time and critical flicker-fusion threshold frequency

	Placebo		ω-3FA	
	Before	After	Before	After
Total reaction time (ms)	506±29.5	523.9±31.2	511.6±58.8	418.4±82.0*†
Choice reaction time (ms)	324.8±24.1	324.7±23.2	326.4± 80.2	283.6±58.5**††
Motor reaction time (ms)	188.2±30.9	199.2±31.8	185.2±53.2	134.9±80.7†††
Critical flicker frequency (Hz)	34.38±8.37	34.19±8.31	38.36±11.98	28.28±5.74***
Critical fusion frequency (Hz)	25.08±1.99	25.3±1.80	26.09±1.98	25.66±2.82

The results are expressed as mean±SD, n=10 for each group. *P=0.015, **P=0.025 ***P=0.038 in comparison with baseline value of ω-3FA treatment, †P=0.001, ††P=0.05, †††P=0.03 in comparison with placebo-treated value

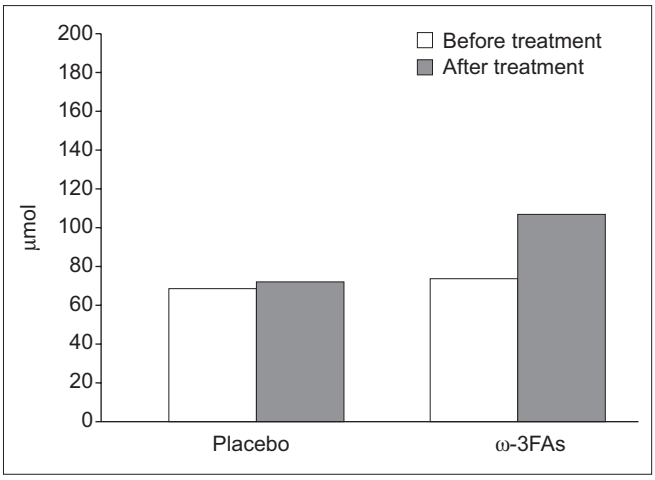

Figure 1: Effect of oral ω-3FA on the serum nitric oxide level

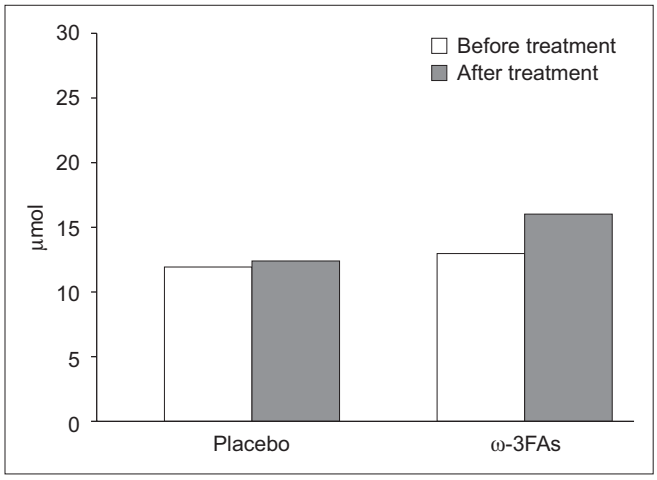

Figure 2: Effect of oral ω-3FA on the serum peroxynitrite level

treated values [Table 1]. ω-3FAs significantly decreased the CFF (flicker component) frequency threshold compared with the baseline value. After 5-days treatment with ω-3FAs resulted in a non-significant increase serum NO (107.34±69.4 µmol) compared with baseline (73.68±20.32 µmol) and placebo-treated group (72.0±18.2 µmol) [Figure 1]. Such changes were also observed with serum ONOO which non-significantly increased in ω-3FAs-treated group (15.73±13.16 µmol) compared with baseline value (12.82±8.09 µmol) or placebo-treated group (11.79±7.9 µmol) [Figure 2].

DISCUSSION

The results of this study show that short term supplementation of ω-3FAs improves significantly the psychomotor performance in young healthy individuals. This improvement is not associated with significant changes in nitrogen species levels. Previous report showed that 35 days supplementation of ω-3FAs reduced the choice reaction time utilising the Go/No Go test and the sustained attention time test.[18] Ferraz *et al.* studied experimentally the mechanism by which ω-3FAs involved in the cognitive function and found that ω-3FA counteracted the impairment in cognitive function induced by restraint stress via reducing corticosterone hormone level.[19] On the other hand, fish oil supplementation that contained high level of ω-3FAs prevented cognitive decline in mice, subjected to severe under-nutrition, *via* a mechanism involved normalisation of neurochemical systems in brain.[20] The effect of ω-3FAs in patients with decline cognitive function differed from that imposed on healthy subjects. In humans with mild-moderate Alzheimer's dementia, supplementation with docosahexaenoic acid (2g/d) for 18 months did not slow the rate of cognitive and functional decline.[21] The significant effect of ω-3FAs in decreasing the CFF (flicker component) frequency threshold compared with the baseline value is indicative of a reduction in the overall integrative activity of the CNS.[22] The non-significant changes in nitrogen species levels that reported in this study may be related to the dual effects of ω-3FAs on the recycling of nitrogen species. Previous reports showed that ω-3FAs activated the endothelial NO synthatase, which led to increase NO production.[11-13] Another report showed that all polyunsaturated fatty acids reduced the inducible NOS and nitrite accumulation *in vitro*.[23] The wide variation in serum levels of NO and peroxynitrite could be related to the dual effects induced by ω-3FAs on nitric oxide synthatase enzymes. It concludes that short term supplementation of ω-3FA improves the psychomotor performance *via* a mechanism not related to their effect on nitric oxide production. Further study is recommended to explore this beneficial effect in individuals complained from impairment in cognitive function.

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How to cite this article: Al-Nimer MS, Al-Gareeb AI, Al-Kuraishy HM. Omega-3 fatty acids improve psychomotor performance via mechanism not related to nitric acid production. Int J Green Pharm 2012;6:1-4.

Source of Support: Nil, **Conflict of Interest:** None declared.