

Effect of n-3 Supplementation on Hyperactivity, Oxidative Stress and Inflammatory Mediators in Children with Attention-Deficit-Hyperactivity Disorder

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ABSTRACT

Background: Attention-deficit/hyperactivity disorder (ADHD) is associated with difficulties in learning, behaviour and psychosocial adjustment that persist into adulthood. Decreased omega-3 fatty acids and increased inflammation or oxidative stress may contribute to neuro-developmental and psychiatric disorders such as ADHD. The aim of this study was to determine the effect of n-3 supplementation on hyperactivity, oxidative stress and inflammatory mediators in children with ADHD. **Methods:** In this double blind study, 103 children (6-12 years) with ADHD receiving maintenance therapy were assigned randomly into two groups. The n-3 group received n-3 fatty acids (635 mg eicosapentaenoic acid (EPA), 195 mg docosahexaenoic acid (DHA)), and the placebo group received olive oil capsules which were visually similar to the n-3 capsules. The duration of supplementation was 8 weeks. Plasma C-reactive protein (CRP), interleukin-6 (IL-6) and the activity of glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) were determined before and after the intervention. Likewise the Conners' Abbreviated Questionnaires (ASQ-P) was applied. **Results:** After 8-week intervention, a significant reduction was observed in the levels of CRP ($P < 0.05$, 95% CI = 0.72-2.02) and IL-6 ($P < 0.001$, 95% CI = 1.93-24.33) in the n-3 group. There was also a significant increase in activity of SOD and GR ($P < 0.001$). A significant improvement was seen in the ASQ-P scores in the n-3 group ($P < 0.005$). **Conclusion:** Eight weeks of EPA and DHA supplementation decreased plasma inflammatory mediators and oxidative stress in the children with ADHD. These results suggest that n-3 fatty acid supplementation may offer a safe and efficacious treatment for children with ADHD.

Keywords: Eicosapentaenoic acid, docosahexaenoic acid, attention-deficit-hyperactivity disorder, oxidative stress, inflammatory mediators

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INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common behavioural childhood disorders. ADHD has a prevalence rate of 5.29% worldwide and is associated with difficulties in learning behaviour, and psycho-social adjustment (Barbaresi *et al.*, 2002)

Conners' Abbreviated Questionnaires (ASQ-P) is an abbreviated version of the Conners parent rating scale (CPRS) which contains 10 items and is known as the Hyperactivity Index (Goyette, Conners & Ulrich, 1978). The inter-correlation of ASQ-P and CPRS-R is a high 0.87 in the hyperactive factor, demonstrating the ability of ASQ-T to identify children's hyperactive behaviours.

Increased inflammation or oxidative stress is known to contribute to psychiatric and neuro developmental disorders such as bipolar disorder, schizophrenia, and depression (Kuloglu *et al.*, 2002; Bilici *et al.* 2001). Oxidative stress is a condition caused by impaired balance between oxidants and antioxidants (Azadbakht, Mirmiran & Azizi, 2004). Cytokines are inflammatory biomarkers which are considered as markers of glial cell function (Oades *et al.*, 2010). Circulatory levels of cytokines arise largely but not exclusively from astrocytes (Steiner *et al.*, 2007) It has been reported that with brain damage, major depression, psychosis and dementia, the level of cytokines tend to increase (Andrezza *et al.*, 2007).

Studies have suggested that n-3 fatty acids may improve behaviour in children with ADHD (Sorgi *et al.*, 2007; Gustafsson *et al.*, 2010). It has been found that n-3 fatty acids may exert its function through a reduction in inflammatory mediators and oxidative stress (Calder, 2006).

There are limited studies on oxidative stress and inflammatory biomarkers in ADHD children. Two studies on circulatory levels of cytokine in ADHD found a

significant correlation between inflammatory cytokines and behaviour problems in children with ADHD (Mittelman *et al.*, 1997; Oades *et al.*, 2010). One of the few studies on oxidative stress in ADHD reported high levels of oxidative stress in ADHD children (Ceylana *et al.*, 2010). The present study aimed to determine the effect of n-3 fatty acids on inflammation, oxidative stress and ASQ-P scores in children with ADHD.

METHODS

Study subjects

A randomised, double-blind, placebo-controlled clinical trial was conducted on 120 children (46 girls and 74 boys) with ADHD (6 to 11 years). Subjects were referred from EbneSina Hospital, Mashhad, Iran to participate in the study. Written informed consent was obtained from all parents. The study was approved by the Ethics Committee of Human Experimentation of Tehran University of Medical Sciences. Anthropometric indices including weight, height, body mass index (BMI) were obtained through comprehensive interviews and through physical examinations. The subjects were on Ritalin as medication and their Conners' Abbreviated Questionnaires (ASQ-P) scores for hyperactivity were greater than 14. Subjects' exclusion criteria were infectious diseases, diabetes, hyperthyroidism, convulsion, epilepsy and consumption of n-3 fatty acids supplements.

Supplementation

Soft gel capsules of n-3 fatty acids with a total daily dose of 900mg n-3 fatty acids (635mg EPA, 165mg DHA and 100mg other n-3 fatty acids) were provided to the subjects. Capsules were supplied by Minami Nutrition, Belgium. The placebo soft gel capsules, visually similar to n-3 capsules, contained 900 mg of olive oil (Gabbay *et al.*, 2012; Stevens *et al.*, 2003).

Study design

The trial was originally designed to test the therapeutic effects of n-3 fatty acids in patients with ADHD. In the present study, change in plasma IL-6 and CRP as well as the activity of GR, SOD and CAT were determined. Patients were randomly allocated into two groups according to pre-arranged balanced block randomisation to receive daily n-3 fatty acids or placebo for 8 weeks. Patients underwent psychiatric assessment by ASQ-P at the baseline and after 8 weeks. Blood samples were obtained at the baseline and after 8 weeks of intervention. Adherence to the study was estimated by counting pills. Patients were considered compliant if they consumed more than 90% of the medication.

Laboratory methods

Fasting blood samples were obtained and plasma and serum were isolated and frozen at -70°C until analysed. Serum concentrations of IL-6 were determined by enzyme-linked immunosorbent assay (ELISA) which were obtained from Bender Med System, Vienna, Austria. Validity for IL-6 was 0.92 and sensitivity was 0.84 pg/ml. CRP was measured using the immunoturbidometrical method. GR, SOD, CAT were also determined by spectrophotometry and kits were purchased from Randox,

Antrim, United Kingdom. Validity for GR, SOD and CAT were 0.87, 0.71 and 0.92 respectively. Sensitivity for GR, SOD and CAT was 0.89 ug/hb, 0.91 ug/hb and 0.76 ug/hb.

Statistical analysis

Differences between the two groups were determined by independent *t*-test using 11.5 SPSS software. All values are expressed as mean \pm SD. A value of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

One hundred and three out of the one hundred and twenty patients completed 8 weeks of study. In the n-3 group, two patients withdrew because of steatorrhoea after week 3; three were lost to follow up and two patients refused to give blood samples. In the placebo group, three patients dropped out because of skin rash, five due to non-compliance and two refused to give blood samples.

As shown in Table 1, there were no significant differences in body mass index (BMI), gender, age, duration of disease and age of disease onset among groups at the beginning. There were no significant differences in serum concentration of IL-6 and CRP as well as the activity of GR, SOD

Table 1. Initial demographic and disease characteristics of patient in study groups

	<i>N-3 group</i>	<i>Placebo group</i>	<i>P-value</i>
Age (year)	7.90 \pm 1.53	7.90 \pm 1.45	NS*
Weight (kg)	27.54 \pm 6.68	27.45 \pm 7.26	NS
Height (cm)	127.63 \pm 10.95	126.06 \pm 10.52	NS
BMI ** (kg/cm ²)	16.98 \pm 2.22	16.69 \pm 2.18	NS
Age (when disease was diagnosed)	8.20 \pm 1.20	7.90 \pm 1.10	NS
Duration of disease (months)	19.82 \pm 4.50	19.34 \pm 3.90	NS
Gender [no (%)]:			
Male	35(66%)	32(64%)	NS
Female	18(34%)	18(36%)	NS

* NS=not significant

** BMI=body mass index

and CAT at the beginning of the study between the two groups. However, there was a significant difference between two groups in terms of serum concentration IL-6 and CRP as well as the activity of GR and SOD ($P < 0.001$). There was no significant difference between two groups in terms of CAT activity at end of intervention ($P > 0.005$). There was no significant difference between groups for ASQ-P scores before intervention; however, there was a significant difference after intervention ($P < 0.001$) (Table 2).

This is probably the first randomised, controlled trial of its kind on children with ADHD. The study hypothesised that supplementation of n-3 fatty acid would lead to a significant improvement on ASQ-P scores, inflammation and oxidative stress. Our results showed that the supplementation of n-3 PUFAs could decrease the inflammation in ADHD patients. A significant decrease in IL-6 and CRP levels subsequent to supplementation of n-3 PUFAs, shown in this study, is confirmed by other studies (Hassan *et al.*, 2010).

N-3 PUFAs have been shown to decrease inflammation via (1) inhibiting the inflammatory pathway of arachidonic acid (AA); (2) decreasing production of pro-inflammatory cytokines; and (3) reducing immune cell function (Saedisomeolia *et al.*,

2009). Long chain fatty acids are the substrate for production of eicosanoids, which are inflammatory regulators in the human body. Eicosanoids produced from AA are more inflammatory than those produced by long chain n-3 PUFA (Goldman, Pickett & Goetzl, 1983).

Our results also showed that the supplementation of n-3 PUFAs could decrease the oxidative stress in ADHD patients. A significant increase in GR and SOD activity due to the supplementation of n-3 PUFAs, which is shown in this study, is confirmed by other studies (Bouzidi *et al.*, 2010). However, other studies have reported increased oxidative stress due to n-3 PUFAs supplementation (Simão *et al.*, 2010). Another study reported that the n-3 fatty acid supplements (1 gr 3 times a day) increases antioxidant enzymes including GPx and SOD (Tayyebi-Khosroshahi *et al.*, 2010).

It has been reported that modest dietary omega-3 fatty acid supplementation could reduce stimulated human monocyte-free-radical production. N-3 PUFAs impair the capability of macrophages derived from monocyte-free-radical production and might impair the capability of macrophages derived from monocytes to promote oxidation of low density lipoprotein

Table 2. Plasma inflammatory and oxidative stress mediators before and after 8 weeks of intervention

Parameter	Study groups	Week 0	Week 8	P-value
IL-6 (pg/ml)	N-3	41.83±45.11	23.46±21	0.001
	Placebo	37.53±32.60	36.60±34.60	0.231
CRP (mg/ml)	N-3	8.05±2.92	5.24±1.73	0.001
	Placebo	8.70±4.04	7.65±2.97	0.523
GR (u/g hb)	N-3	7.29±3.12	9.89±3.24	0.001
	Placebo	8.29±2.80	8.65±2.96	0.142
CAT (u/g hb)	N-3	236.22±46.88	240.83±52.53	0.654
	Placebo	234.72±42.84	226.34±43.52	0.413
SOD (u/g hb)	N-3	1277.08±24	1411.04±165.80	0.001
	Placebo	1243.36±174.22	1113.44±163.00	0.321
Conners' Abbreviated Questionnaires	N-3	24.45±4.95	21.03±3.98	0.001
	Placebo	24.12±4.86	24.02±4.22	0.251

cholesterol and associated cellular toxicity. Therefore, it appears to have a beneficial effect on prevention of oxidative stress in humans (Fisher, Levine & Weiner, 1990).

There is considerable evidence that n-3 PUFAs supplementation causes an improvement in educational and behavioural problems among children with ADHD (Sorgi *et al.*, 2007; Gustafsson *et al.*, 2010). This study showed that the ASQ-P is decreased from eight weeks n-3 PUFAs supplementation. Our result is in the same track with other studies reporting decreased hyperactivity of ADHD patients due to supplementation of n-3 PUFAs (Gustafsson *et al.*, 2010; Sorgi *et al.* (2007)).

N-3 PUFAs are essential for growth and function of the developing and mature brain particularly for brain gene expression and cell membrane structure and electrophysiological properties (McNamara & Carlson, 2006). A number of studies have also provided strong arguments in favour of the need for minimum amounts of n-3 PUFAs in the brain for normal neurotransmission functioning, particularly in relation to the dopaminergic and serotonergic systems that are very important in the pathogenesis of ADHD (Chalon, 2006).

Recently, there have been numerous reports about the association between cytokines with brain damage and psychiatric disorders (Tanaka *et al.*, 2008). No study has reported the association between hyperactivity and inflammation. However, many studies confirm the involvement of pro-inflammatory cytokines in cognitive performance both in animal models and human (Oades *et al.*, 2010).

We suggest that an increase in level of oxidants may play a role in the pathology of ADHD by impairing the structure and functions of dopamine (Tarazi, Kehong & Baldessari, 2002). Although no study has reported on the association between the effect of n-3 fatty acids and oxidative stress in

children with ADHD, a few studies have reported on the positive effects of n-3 PUFAs on oxidative stress. Neurotransmitters dopamine, serotonin and norepinephrine play a key role in the pathogenesis of ADHD (McGough, 2005). The oxidation of catecholamines such as dopamine and norepinephrine by monoamines may result in an increased radical burden (Tezcan, Atmaca & Kuloglu, 2003). As in other psychiatric disorders, higher MDA levels may play a role in the pathophysiology of ADHD. A remarkable increase in MDA levels suggests a strong association between ADHD and lipid oxidation (Mahmut *et al.*, 2007).

Inflammatory mediators affect the nature of tryptophan metabolism. Evidence suggests that increased inflammation causes 80 to 95% of brain levels of l-tryptophan being metabolised in the kynurenine pathway and the remainder contributing to the synthesis of 5-HT (Stone, 1993). Activity in this pathway merits attention for two reasons. First, there is good reason to believe that levels of 5-HT activity in ADHD are anomalous (Oades *et al.*, 2002). Second, catabolic products of kynurenine can be potentially neurotoxic (3-OH-Kynurenine, 3HK, a glutamate agonist) (Guillemin *et al.*, 2007). Clearly unusual levels of inflammatory mediators could be indicative of factors influencing the pathology of ADHD.

In conclusion, eight weeks of n-3 fatty acids supplementation decreased inflammation and increased the activity of antioxidant enzyme activity such as SOD and GR. This supplementation regimen also decreased the score of hyperactivity.

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