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Role of polyunsaturated fatty acids in the management of Egyptian children with autism

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Abstract

Objective: Estimation of free polyunsaturated fatty acids (PUFAs) in blood and evaluation of behavior of autistic children before and after taking fish oil (Efalex®) were performed.
Design and methods: 30 autistic children (18 males and 12 females) aged 3–11 years and 30 healthy children as control group were included in this study. Tandem mass spectrometry and CARS were used to estimate the free PUFAs from dried blood spot and to evaluate the autistic behavior respectively.
Results: Before taking Efalex®, linolenic acid showed a significant reduction (71%), followed by docosahexaenoic acid (65%) and arachidonic acid (45%), while linoleic acid was the least affected PUFA (32%). After taking Efalex®, 66% of autistic children showed clinical and biochemical improvement, linolenic acid and docosahexaenoic acid showed the highest levels after Efalex® supplementation.
Conclusion: PUFA supplementation may play an important role in ameliorating the autistic behavior.

Keywords: Autism; Polyunsaturated fatty acids; Fish oil; Efalex®; Tandem mass spectrometry

Introduction

Autism is one of the pervasive developmental disorders that develop during the first three years of life. It is the most severe psychiatric disorder in childhood. Three main criteria are essential for its diagnosis: disturbed social interaction, lack of communication and restriction of normal variation in behavior and interests [1]. There are no available data on the prevalence of autistic disorders in Egypt. Prevalence of autistic spectrum disorders in Saudi Arabia is 6:1000 (unpublished data, personal communication) [2]. A study conducted in Haifa, Israel, showed an incidence rate of 1:1000 and a male to female ratio of 4.2:1 [3]. On the other hand, a survey was published in 2003 by the Canadian Committee on the Effectiveness of Early Education in Autism. They estimated the prevalence rate of the autistic spectrum disorders to be 60:10,000 [4].

Lipids constitute 60% of dry weight of human brain. Over 20% of the dry weight of the brain is made up of PUFAs, primarily docosahexaenoic acid (DHA) and arachidonic acid (AA) which are derived from essential fatty acids (EFAs). Those fatty acids are concentrated in the neuronal membranous phospholipids, in the myelin sheath [5].

Vancassel et al. [6] reported that PUFAs modulate membrane fluidity and hence functions of the neuronal cell. PUFAs are important in the regulation of many biochemical events including neurotransmitter release and uptake, receptor function in the central nervous system [7].

Postnatal DHA status has been found to correlate with visual acuity (retinal function) and neurodevelopment [5]. Deficiencies of n-3 PUFA lead to a loss of DHA from the brain and the retinal rod outer segment phospholipids, with a compensatory replacement by docosapentaenoic acid (22:5n-6). This minor change in membrane phospholipid structure is sufficient to lead to memory loss, learning disabilities, and impaired visual acuity [8]. Several studies have reported reduced plasma levels of PUFAs in neuropsychiatric

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disorders such as schizophrenia [9], attention deficit hyperactive disorder (ADHD) [10] and unipolar depression [11].

The aim of this study was to estimate the PUFA status of autistic children before and after taking Efalex® supplement for three months. Reevaluation of the autistic behavior after supplementation was done using Childhood Autism Rating Scale (CARS) [12] to detect the possible correlation between the blood fatty acid status and autistic behavior.

Methods

Participants

The present study included thirty autistic children (18 males and 12 females) from twenty-eight families ranging in age from 3–11 years, and thirty healthy children of the same age and sex as a control group. Diagnosis of autism was based on the Diagnostic and Statistical Manual for Mental Retardation (DSM IV) [13] and the CARS [12]. These children were selected from the attendants of the clinic of the Department of the Children with Special Needs (CSN), National Research Center. Careful pedigree construction, family history, prenatal history (history of taking drugs, exposure to X-ray, chronic diseases and smoking prior to and during pregnancy) and perinatal history were taken. Complete diagnostic workup including medical, neurological, psychiatric and psychological evaluations was done for all of the studied children with autism.

Inclusion criteria

(1) Selection of cases based on DSM IV of psychiatry and CARS.
(2) Good physical health.
(3) Candidates are not currently taking any medications or essential fatty acid supplements during the study.
(4) Agreement not to make any changes in treatments for autism (medical, nutritional, dietary, or behavioral) during the three months of the study.

Ethics approval and consent

A written consent was obtained from the parents of each individual case, according to the guidelines of the ethical committee of the National Research Center.

Product dosage

The supplement studied was Efalex® (supplied by Efamol Ltd., UK) a blend of high DHA fish oil and evening primrose oil. Each capsule contained:

– 60 mg of docosahexaenoic acid
– 12 mg of gamma-linolenic acid
– 13 mg of eicosapentaenoic acid
– 5 mg of arachidonic acid.

Efalex® was chosen as, compared to other preparations, it contains both omega 3 and omega 6 fatty acids which are important for the autistic children. It also contains antioxidant vitamin E. The autistic children received 2 capsules twice per day for three months, while the control children didn't receive either Efalex® or placebo.

Procedure

A venous blood sample was obtained by thumb prick and a drop of this sample was left to dry at room temperature on an absorbent pad. This whole blood sample (plasma and red cells) was used to estimate the free PUFAs.

Solvents, reagents, and internal standards

High-purity-grade methanol (Methanol Chroma) was obtained from Riedel-de-Haën (Belgium). Butanolic HCl (3 mol/L) was obtained from Regis Technologies (Belgium). Acetonitrile (LiChro solvent grade) and formic (98–100%) acid were obtained from Merck (Germany). Stable isotope used as internal standard was obtained from Cambridge Isotope Laboratories, UK, and included [$^2H_3$]palmitoylcarnitine (C16).

Sample preparation and analytical procedure

Lipid extraction was accomplished using HPLC grade methanol, acetonitrite, anhydrous butanol, acetyl chloride, butanolic-HCl and deuterium-labeled internal standard [$^2H_3$]palmitoylcarnitine (C16). Fatty acids were extracted and derivatized in the form of acylcarnitine butyl ester according to the method of Cavedon et al. [14].

Tandem mass spectrometry was used to estimate the extracted PUFAs in dried blood spot. The model of the mass spectrometer is LCQ Advantage Max and the manufacturer is Thermo Finnigan Company (Thermo Electron Corporation) in USA. Electrospray ionization was used for ionization of the sample, a process aided by a nitrogen nebulising gas that directs the spray emerging from the capillary tip toward the mass spectrometer. Quadrupole mass analyzer was used for analysis of ions, so only ions of a certain mass to charge ratio passed through the quadrupole filter and all other ions were thrown out of their original path [14].

The detector monitored the ion current, amplified it and the signal was then transmitted to the data system where it was recorded in the form of mass spectra. The *m/z* values of the ions were plotted against their intensities to show the number of components in the sample, the molecular weight of each component, and the relative abundance of the various components in the sample.

Acylcarnitine butyl ester undergoes fragmentation in multiple steps by a mechanism called collision induced fragmentation (CID) in which a collision between the analyte ions and neutral molecules results in the fragmentation of the analyte ions. Precursor ion scans of the acylcarnitines were accomplished by focusing MS 1 on the molecular mass (M+H) of the butanol ester of the acylcarnitine, and MS 2 was used to focus the (fragment) ion mass. The 3rd MS in all fatty acids ends with 85, but they differ in the 1st and 2nd MS according to the precursor ion. The precursor ions of linoleic, arachidonic, and docosahexaenoic are 480, 502 and 512 respectively (Table 1).

The precursor ion of the linolenic carnitine butyl ester is 478. This precursor ion is subjected to mass spectrometry (MSn) for

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Table 1
Molecular weight and MS/MS of PUFAs

PUFA	Precursor ion	MS 1	MS 2	MS 3
Linolenic acid	478	422	363	85
Linoleic acid	480	424	365	85
Arachidonic acid	502	446	487	85
Docosahexaenoic	512	456	397	85

3 times. The first MS causes fragmentation and releases the butanol (C4 H8), the second MS causes more fragmentation releasing [$(CH3)_3$ N], and the third MS releases the fatty acid (linolenic acid), producing *m*/*z* 422, 363 and 85, respectively.

Results

Over a 10 month period, about 652 patients attended the CSN clinic in the National Research Center. Only 80 patients from the 652 patients were diagnosed as autistic children and 30 of them were included in the study. During the present study, about 12% of patients who attended the CSN clinic were diagnosed as autistic children and the male to female ratio was 3.4:1.

The present study showed that all of the investigated PUFAs [linolenic acid, docosahexaenoic acid (22:6n-3), linoleic acid (18:2n-6) and arachidonic acid (20:4n-6)] were significantly reduced in the dried blood spot of autistic children before taking the Efalex® supplement compared to those in the control group (Table 2 and Fig. 1). The mean of AA/DHA was significantly higher in autistic children (2.77±0.84) compared to the control group (1.71±0.4) (Fig. 2).

The greatest difference in the content of an individual PUFA was that of linolenic acid which showed a significant reduction (71%), followed by docosahexaenoic acid (22:6n-3) which was significantly reduced by 65%. Arachidonic acid (20:4n-6), which represents a major intermediate component in the long chain derivatives of the (n-6) series was reduced by 45%. Linoleic acid (18:2n-6) which is the precursor of the (n-6) series, was the least affected PUFA and showed a significant reduction (32%), in comparison to levels in the normal healthy control children.

After taking the Efalex® supplement for three months, the mean levels of investigated PUFAs showed a significant improvement in comparison to the levels before taking the supplement (Fig. 3 and Table 3). Linolenic acid and docosahexaenoic acid showed the greatest response. Linolenic acid levels increased by 30%, while docosahexaenoic acid increased by 28%. The linoleic acid and arachidonic acid showed less marked increase. Linoleic acid increased by 21% while arachidonic acid increased by 15%. The AA/DHA ratio was significantly reduced in autistic children after taking Efalex®. After taking Efalex®, reevaluation was done for thirty autistic children using CARS (Table 4) [12]. Ten of them did not show a significant improvement in their autistic behavior. These 10 autistic children included two boys with sisters having autistic behavior, two autistic children with tuberous sclerosis and six sporadic cases. The other twenty autistic children showed a statistically significant improvement in their autistic behavior (Table 5).

Table 2
Comparison between PUFA levels in autistic children before treatment and the control group

PUFAs	Control group	Autistic children before treatment	*t*-value	*P*
	Mean±SD	Mean±SD		
Linolenic (μg/mL)	3.2±0.72	0.86±0.44	15.6	<0.0001**
DHA (μg/mL)	2.85±0.65	0.95±0.2	16.06	<0.0001**
Linoleic (μg/mL)	2.77±0.64	1.75±0.46	6.31	<0.0001**
Arachidonic (μg/mL)	4.65±0.5	2.5±0.5	16.34	<0.0001**
AA/DHA	1.71±0.4	2.77±0.84	6.82	<0.0001**

P>0.05 = nonsignificant, *P*<0.05* = significant, *P*<0.01, 0.001, 0.0001** = highly significant.
AA: arachidonic acid.
DHA: docosahexaenoic acid.

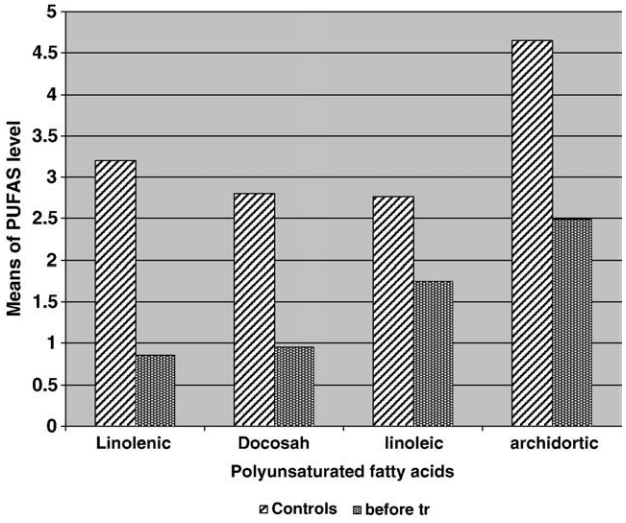

Fig. 1. Comparison between polyunsaturated fatty acid (PUFA) levels in autistic children before treatment and control group.

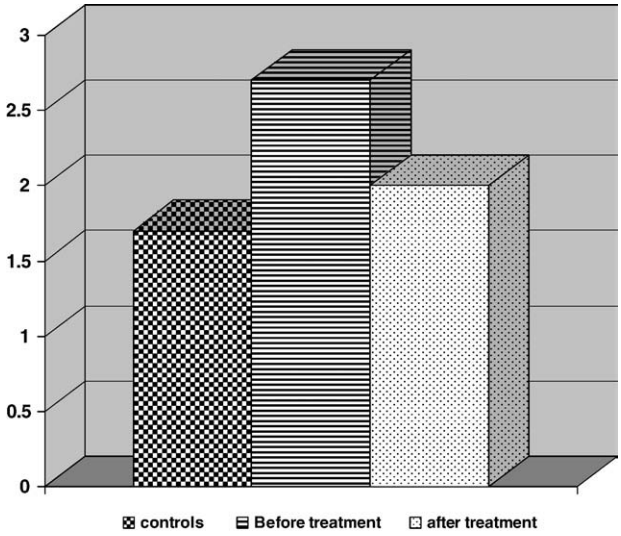

Fig. 2. Arachidonic/docosahexaenoic ratio in controls and autistic children before and after treatment.

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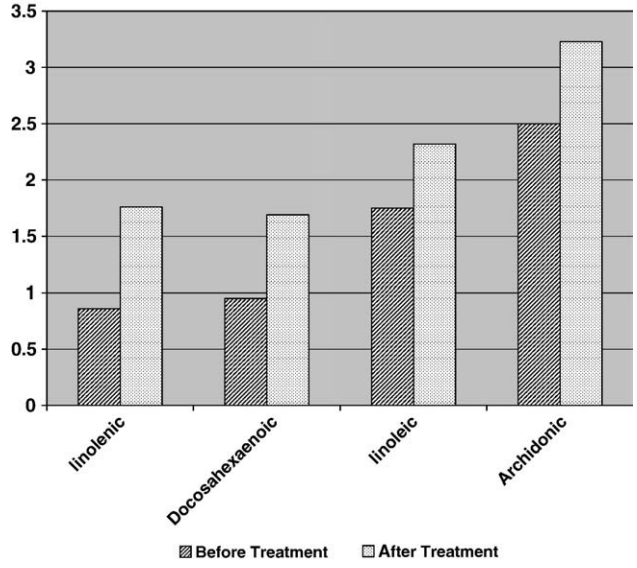

Fig. 3. PUFA levels in autistic children before and after treatment.

A statistically significant negative correlation was found between DHA level and CARS before treatment in the ten autistic children who didn't improve clinically.

Discussion

The present study is the first study in Egypt to estimate free PUFAs in the whole blood (plasma and red cells) using tandem mass spectrometry which is very sensitive, but a rather expensive technique [15]. Out of the 28 families included in the present study, 2 families had two affected siblings (son and daughter). The frequency of autism is much higher in the siblings of children with autism (6%) compared to the general population (2%), but the strongest evidence for the heritability of autism comes from twin studies [16].

The levels of the investigated fatty acids in the present study were similar to those previously reported. Vancassel et al. [6] studied the fatty acid content of total plasma phospholipids in 15 autistic subjects and 18 mentally retarded subjects as a control group. They reported a nonsignificant 18% reduction in AA and a 23% reduction in DHA levels in autistic children, compared to levels in controls. This reduction resulted in strong and significantly lower levels of total (n-3) PUFAs (20%) in autistic children and a significant increase in the (n-6)/(n-3) ratios (25%) in autistic subjects compared to controls. In the present work, PUFAs were estimated in the whole blood (plasma and RBCs) from a dried blood spot. Also the control group was different in both studies. The present study investigated normal healthy children, while Vancassel et al. [6] examined mentally retarded children.

Bell et al. [17] reported a significant decrease in docosapentaenoic acid (22:5n-3) and total n-3 in the red blood cells (RBCs) of children with regressive autism and Asperger's syndrome. In another study [18], the levels of free linolenic, docosahexaenoic and arachidonic acids in plasma were significantly reduced in autistic children. In their study, the free PUFAs were estimated in plasma using gas chromatography and the linoleic acid level was not reduced, while the present study used tandem mass spectrometry to measure the free PUFAs in a dried blood spot. Also the present study showed a significant reduction of linoleic acid (18:2n-6) by 37% before Efalex® supplement.

Bu et al. [19] reported increased levels of eicosaenoic acid (20:1n-9) and erucic acid (22:1n-9) in autistic subjects with developmental regression when compared with typically developing controls. In addition, a decrease in palmitoleic (16:1n-7) was observed in children with clinical regression compared to those with early onset autism. The reasons for the lower concentrations of (n-6) and (n-3) PUFAs are not well understood. Many hypotheses have been proposed. A primary deficiency may have occurred because of insufficient intake of PUFA or precursors [6]. A site linked to autism has been located on chromosome 11q22-23, in the vicinity of the gene for delta-6-desaturase, which is the enzyme first involved in the production of PUFA long chain derivatives of both the (n-3) and (n-6) series [20]. Because of their proximity on the chromosome, a concomitant impairment expression of these genes or of the product of these genes could

Table 3
Comparison between PUFA levels in autistic children before and after treatment

PUFAs	Autistic children before treatment	Autistic children after treatment	*t*-value	*P*
	Mean±SD	Mean±SD		
Linolenic (μg/mL)	0.86±0.44	1.76±0.56	9.3	<0.0001**
DHA (μg/mL)	0.95±0.2	1.69±0.42	14	<0.0001**
Linoleic (μg/mL)	1.75±0.46	2.32±0.41	7.6	<0.0001**
Arachidonic (μg/mL)	2.5±0.5	3.23±0.49	11.07	<0.0001**
AA/DHA	2.77±0.84	2.01±0.53	8.4	<0.0001**

P>0.05 = nonsignificant, *P*<0.05* = significant, *P*<0.01, 0.001, 0.0001** = highly significant.
AA: arachidonic acid.
DHA: docosahexaenoic acid.

Table 4
Comparison between CARS score before and after treatment using ANOVA

PUFAs	Before treatment	After treatment	*P*
	Mean±SD	Mean±SD	
CARS	39.5±3.86	32.7±3.37	<0.0001**

P>0.05 = nonsignificant, *P*<0.05* = significant, *P*<0.01, 0.001, 0.0001** = highly significant.

Table 5
Correlation between PUFAs and CARS in the 10 autistic children who didn't improve clinically before and after treatment

CARS and PUFA	Correlation	*P* value	Correlation	*P* value
	Before		After	
CARS and linolenic	0.471	>0.05	0.395	>0.05
CARS and DHA	−0.670	<0.05*	−0.541	>0.05
CARS and linoleic	−0.118	>0.05	−0.287	>0.05
CARS and AA	0.115	>0.05	0.262	>0.05

P<0.05* = significant.
AA: arachidonic acid.
DHA: docosahexaenoic acid.

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then represent a causative factor in lipid differences observed in autism. Horrobin [11]1998,) postulated an increase of phospholipase A_2 (PLA_2) activity in plasma, serum and platelet membranes in schizophrenic patients that enhanced the catabolism of PUFAs inserted into phospholipids [11]. A similar condition could occur in autism. In the present study, the four PUFAs were estimated again in dried blood from the autistic children after taking Efalex® for three months. There was a significant increase in all of them compared to their levels before taking Efalex®.

The increased level of DHA and linolenic after taking Efalex® could be explained by the presence of docosahexaenoic acid and linolenic acid in the supplement that was given to the autistic children. The increased level of linoleic acid could be explained by the increased activity of PLA_2 which acts on linoleic acid releasing it from the cell membrane to the blood [11]. Genetic sites linked to autism on chromosome 8q22 are in the proximity of the gene for secretory soluble (PLA_2) (8q24) [21].

After taking Efalex®, reevaluation was done for the 30 autistic children using CARS [12]. Twenty autistic children showed a statistically significant improvement in their autistic behavior according to the CARS. Their parents reported an improvement in their concentration, eye contact, language development and motor skills. However, they still had some repetitive movements and could not mingle and play with other children properly. The other ten autistic children did not show a significant improvement in their autistic behavior. This could be due to the possibility of improper dosage of Efalex® for them or short duration of Efalex® intake. Meanwhile, they showed statistically significant negative correlation between DHA level and CARS before Efalex®. These results may provide support for the value of PUFA intake on the autistic behavior.

Patrik and Salik [22] reported a significant increase in the language and learning skills of 18 children with autism who took a mixture of fish oil and borage oil for 90 days using Assessment of Basic Language and Learning Skills (ABLLS). Unfortunately, their study did not include CARS scoring for the subjects, so it is not possible to directly compare the results obtained in their study to those of the present trial. However, combined results of these studies provide substantial evidence linking abnormal fatty acid metabolism in autism and a possible dietary treatment that provides some benefit in this tragic condition. Amminger et al. reported an advantage of omega 3 fatty acids compared with placebo for hyperactivity and stereotyping using Aberrant Behavior Checklist (ABC) [23].

In conclusion, PUFA supplementation may play an important role in ameliorating the autistic features and improving their concentration ability, motor skills and language development. These results offer a new and additional approach in the investigation and management of autism.

References

[1] Wolanczyk T, Ostrowska-Galemba K, Mikulska J. Features of autism, autistic traits, autism: retrospective analysis of clinical symptoms in children treated in the pediatric clinic. Psychiatria Polska 2001;35(1):59–69.

[2] Talat A. National study of autism in Saudi Arabia 2008, King Abdulaziz City for Science and Technology; 2008.

[3] Michael D, Gabriela H, Emanuel T. Autism in the Haifa area — an epidemiological perspective. Isr Med Assoc J 2001;3(3):188–9.

[4] Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. J Autism Dev Disord 2003;33:365–83.

[5] Agostoni C, Riva E, Trojan S, Bellu R, Giovannini M. Docosahexaenoic acid status and developmental quotient of healthy term infants. Lancet Jun 1995;345(8963):1463–8 10.

[6] Vancassel S, Durand G, Barthelemy, Lejeune B, Martineau J, Guilloteau D, Andres C, Chalon S. Plasma fatty acids in autistic children. Prostaglandins Leuko Essen Fatty Acids 2001;65(1):1.

[7] Murphy MG. Dietary fatty acids and membrane protein function. J Nutr Biochem 1990;1:68–78.

[8] Lauritzen L, Hansen HS, Jorgensen MH. The essentiality of long-chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 2001;40:1–94.

[9] Khan MM, Evans DR, Gunna V, Scheffer RE, Parikh VV, Mahadik SP. Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. Schizophr Res 2002;58(1):1–10.

[10] Burgess RJ, Stevens L, Zhang W. Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. Am J Clin Nutr 2000;71(1):327–30.

[11] Horrobin DF. The membrane phospholipids hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. Schizophr Res 1998;30:193–208.

[12] Schopler E, Reichler RJ, Renner BR. The Childhood Autism Rating Scale (CARS) for diagnostic screening and classification of autism. New York: Irvington; 1993.

[13] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th edn (DSM-IV). Washington, DC: APA; 1994.

[14] Cavedon CT, Bourdoux P, Mertens K, Van Thi HV, Herremans N, De Laet C, Goyens P. Age-related variations in acylcarnitine and free carnitine concentrations measured by tandem mass spectrometry. Clin Chem 2005;51(4):745–52.

[15] Zytkovicz TH, Fitzgerald EF, Marsden D. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots. Clin Chem 2001;47:1945–55.

[16] Bailey A, Phillips W, Rutter M. Autism toward an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. J Child Psychol Psychiatry 1996;37:89–126.

[17] Bell J, MacKinlay E, Dick J, MacDonald D, Boyle R, Glen A. Essential fatty acids and phospholipase A2 in autistic spectrum disorders. Prostaglandins Leuko Essen Fatty Acids 2004;71:201–4.

[18] Mostafa GA, EL-Gamal HA, EL-Wakkad AS, EL-Shyorbagy OE, Hamza MA. Polyunsaturated fatty acids, carnitine and lactate as biological markers of brain energy in autistic children. Egyp J Child Neuropsych 2005;2(2):179–88.

[19] Bu B, Ashwood P, Harvey D, King IB, Water JV, Jin LW. Fatty acid compositions of red blood cell phospholipids in children with autism. Prostaglandins Leuko Essen Fatty acids 2006;74(4):215–21.

[20] Horrobin DF, Bennett CN. New gene targets related to schizophrenia and other psychiatric disorders: enzymes, binding proteins and transport proteins involved in phospholipids and fatty acid metabolism. Prostaglandins Leuko Essen Fatty acids 1999;60:141–67.

[21] Bolton P, Powell J, Rutter M, Buckle V, Yates JR, Ishikawa BY. Autism, mental retardation, multiple exostoses and short stature in a female with 46,x,t(X;8)(p 22.13;q 22.1). Psychiat Genet 1995;5:51–5.

[22] Patrick L, Salik R. The effect of essential fatty acid supplementation on language development and learning skills in autism and Asperger's syndrome. Autism — Asperger's Digest Jan/Feb 2005;0:36–7.

[23] Amminger GP, Berger GE, Schafer MR. Omega 3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study. Biol Psychiatry 2007;61(4):551–3.

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