

Plasma, red blood cells phospholipids and clinical evaluation after long chain omega-3 supplementation in children with attention deficit hyperactivity disorder (ADHD)

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Abstract

Omega-3 and omega-6 long-chain polyunsaturated fatty acids (LCPUFAs), are crucial to brain development and function. Increasing evidence indicates that deficiencies or metabolic imbalances of these fatty acids might be associated with childhood developmental and psychiatric disorders including attention-deficit/hyperactivity disorder (ADHD). Omega-3 are often lacking on modern diets. Moreover preliminary evidences suggest that supplementation with omega-3 LCPUFAs, might help in the management of the ADHD linked behavioural and learning difficulties. However, few studies published to date have involved different populations, study designs, treatments and outcome results. Thus, further researches are required to assess the durability of the treatment effects, to determine optimal composition and dosages of the supplement and to develop reliable ways to identify patients that might have some benefits from this kind of treatment, also because the study of LCPUFAs and their metabolism might offer new approaches to the early identification and management of ADHD.

In this paper, we provide new insight on the lipid pattern in plasma and red blood cells (RBC) phospholipids, together with evaluation of the arachidonic acid (AA)/eicosapentaenoic acid (EPA) ratio which seems to correlate with the improvement of the patients both from a biochemical and clinical point of view.

Keywords: ADHD, omega-3, clinical improvement, cell membrane, AA/EPA, fatty acid

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of most common neurodevelopmental syndrome of childhood.

Recent reviews estimate ADHD prevalence between 2 and 18% (Goldman et al. 1998; Elia et al. 1999; Brown et al. 2001). The 2002 National Health Survey of the CDC indicates that 7% of children between the ages of 5 and 11 have been diagnosed with ADHD (Dey et al. 2002).

The diagnosis of the syndrome is complicated by the frequent occurrence of comorbid conditions such as learning disability, behaviour and anxiety disorder.

In the diagnostic and statistical manual for mental disorders DSM-IV (American Psychiatric Association, 1994) three specific subtypes of ADHD are identified:

1. ADHD, combined type: if the following criteria, i.e. (a) “often fails to give close attention in tasks or play activities” and (b) “often fidgets with hands or feet or squirms in seat” were together for the past six months.
2. ADHD, predominately inattentive type: if criterion (a) is met but criterion (b) is not met during the past six months.

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3. ADHD, predominately hyperactive-impulsive type: if criterion (b) is met but criterion (a) is not met for the past six months.

Therefore, the symptoms must have been present for at least six months and accompanied by “clinically significant” impairment (American Psychiatric Association 1994). DSM-IV requires that symptoms and impairment should be present before the age of seven years Mugnaini et al (2005).

Measurement of catecholamines or metabolites in plasma and urine of ADHD patients has been implemented in order to investigate the possibility to have a laboratory test, but with mixed success (Halperin et al. 1993; Pliszka et al. 1994). The current consensus (as stated in DSM-IV) is that no biochemical tests reliably predict ADHD. Therefore, teacher and parent rating scales or interviews about the children’s behaviour continue to be the most important diagnostic procedures available.

As already stated, ADHD often is not present in isolation, but rather with another disorder (comorbidity), including learning disabilities, oppositional defiant disorder, conduct disorder, Tourette syndrome, depression, anxiety, and bipolar disorders (Barkley 1998).

Three main areas, i.e. neuroimaging studies, genetic studies and other etiologic studies were investigated to find evidence suggesting a biologic basis for ADHD.

Some findings generally converge on “dysfunction and deregulation of cerebellar-striatal/adrenergic-pre-frontal circuitry” (Castellanos 2001) and abnormal right prefrontal anatomy and function have been found in several studies.

A genetic feature of ADHD is strongly suggested because the syndrome clusters in families (Thapar et al. 2005). Thanks to studies at molecular level, two polymorphisms in the dopamine transporter and receptor genes that seem to influence the risk of ADHD have been identified (Swanson et al. 2001). The genetic links involving these two polymorphisms have been replicated many times but the general consensus is that many other genes are probably involved in the transmission of the disorder (Swanson et al. 2001).

A suggestive evidence that an environmental toxicant might be an etiologic risk factor for ADHD is for lead. The literature on this element is important because it creates a paradigm for understanding how an environmental agent might increase the risk of ADHD, e.g. cigarette smoking during pregnancy has been reported to increase the risk (Millberger et al. 1996).

Diet too seems to be an ethiological factor for ADHD; it is known that children with ADHD have lower levels of long-chain omega-3 fatty acids in their blood (Stevens et al. 1995; Burgess et al. 2000).

This is thought to be due to lack of dietary intake in conjunction with a more rapid metabolism (Ross et al. 2003).

Two fatty acids, i.e. linoleic acid (LA, omega-6) and α -linolenic acid (ALA, omega-3) cannot be synthesized in our body and must be introduced with food stuffs.

From these EFA, long chain polyunsaturated fatty acids (LCPUFAs) derive: arachidonic acid (AA, 20:4 omega-6), eicosapentaenoic acid (EPA, 20:5 omega-3) and docosahexaenoic acid (DHA, 22:6 omega-3).

LCPUFAs of the omega-6 and omega-3 families are incorporated into membrane phospholipids primarily into phosphatidylethanolamine (PE) and phosphatidylserine (PS) (Anderson and Sperling 1971). AA accounts for the 5–15% of the membrane phospholipid fatty acids, while DHA is present in high concentration in retina, testes and sperm and it is very important for the growth of retina and brain (Uauy et al. 2001; Singh 2005)

Moreover, the omega-3 polyunsaturated fatty acids have a wide range of beneficial effects in several human health conditions. Studies *in vitro*, in animal models and in humans indicate that omega-3 LCPUFAs concentration affects blood lipid profile, cardiovascular health, membrane lipid composition, cell signaling cascades and gene expression (Wainwright 2002; SanGiovanni and Chew 2005; Damsgaard et al. 2006).

LCPUFAs released from phospholipids by phospholipase 2 are the precursors of hormonelike substances called “eicosanoids”.

The oxidation of AA by cyclooxygenase, lipoxygenase, and epoxygenase produces prostaglandins, leukotriens, lipoxines, and P-450 compounds of the group 2.

EPA can compete with AA for the same enzymes to form a different class of eicosanoids (group 3) which counteract the effects of those derived by AA (Calder 2003; Harbige 2003).

A number of reports demonstrates that phospholipids containing DHA can have an impact upon membrane properties, e.g. increasing membrane permeability (Huster et al. 1997), membrane fusion (Kafrawy et al. 1998) and vesicle formation (Williams et al. 1998), phospholipid flip-flop (Armstrong et al. 2003), membrane elasticity (Koenig et al. 1997) and possibly promoting lateral segregation of lipids into domains (Litman et al. 2001; Stillwell and Wassal 2003).

DHA is the most prevalent fatty acid in cerebral grey matter phospholipids and constitutes 45–65% of fatty acids in the nervous tissues (Hamilton et al. 2000) and in brain is involved in the regulation process of cognitive function (Stillwell and Wassal 2003).

As previously mentioned, some studies have highlighted a deficiency of LCPUFAs in the membrane phospholipids in the patients affected with ADHD

(Stevens et al. 1995; Burgess et al. 2000). Furthermore, the rate of arachidonic acid to eicosapentaenoic acid in blood and red blood cell (RBC) membrane phospholipids seems to be elevated in children with ADHD. The ratio AA/EPA is indicative of increased upstream inflammatory potential. However, the data reported in the literature are controversial mainly for the gaps in the used protocols (e.g. the lack of adequate control, the used dosage, the type of supplement, the age of onset) (Richardson 2004a,b).

Moreover, some studies reported no benefit at all from supplementation with DHA alone (Voigt et al. 2001; Hirayama et al. 2004).

The purposes of this study were to verify the hypothesis whether:

1. the supplementation with EPA and DHA with enough high doses, related to the patient weight, might result in attention level improvement and/or a decrease of the hyperactivity levels/impulsiveness;
2. in patients affected by ADHD presenting an high ratio AA/EPA, the supplementation with EPA and DHA would determine in blood and in cell membrane phospholipids a new balance of the rates of these fatty acids;
3. there is a correlation between the dose of omega-3 LCPUFAs, the decrease of the AA/EPA ratio and/or the entity of the clinical improvement (score); and
4. the change in the AA/EPA ratio due to the increased intake of omega-3 LCPUFAs is accompanied in RBC membranes by a change in the cholesterol amount and in the phospholipids profile.

Materials and methods

Subjects

The study was a regional pilot study and was conducted by the service of NPI of the Hospital "Casa Sollievo della Sofferenza" of S. Giovanni Rotondo (Foggia, Italy), where patients were addressed from their own physicians when a suspected diagnosis for ADHD was posed.

Criteria of inclusion: age comprised between 3.5 and 16 years; hyperkinesias—impulsiveness and inattention. Informed consent was signed by the parents/tutors.

Criteria of exclusion: medium-serious mental insufficiency; serious pathologies or encephalic malformations; epilepsy; presence of chronic or recidivant inflammatory pathologies; known intolerance to the product or similar; refusal of the informed written consent.

The patient enrolled (31) were screened and confirmed for ADHD, after psychology evaluation

with the help of diagnostic and statistical manual (DMS IV) criteria (American Psychiatric Association 1994).

Parents were trained to record children behaviour following the scale for the location of the inattention behaviours and hyperactivity and the scale of evaluation by Conners, short version.

Patients case history, medical condition and assessment of hyperactivity scores was also done, at the time of recruitment and at control after supplementation, using pre-validated parent rating scale (Savani and Nadkarni 1998) that includes different variables (such as restlessness, inattention, impulsivity, self-control, social problems, learning problem).

Supplementation with omega-3

Long chain omega-3 supplementation was given to selected patients for eight weeks.

The fish oil assumed was standardized in long-chain omega-3 (at least 75%) through a procedure of molecular distillation, with a relationship between EPA and DHA about 2–1 (other ingredients: lemon aroma—anti-oxidants: vitamin E and ascorbilpalmitate—acidifying agent: citric acid). The supplement is commercially available and was kindly provided by Also-Enervit.

The analysis of the supplement was conducted before the study in our laboratory and the percentage of fatty acid composition found was: C16:0 1,120; C16:1 0,399; C18:0 3,439; C18:1 5,159; C18:2 0,770; C18:3 α 0,691; C18:3 γ 0,772; C20:3 0,862; C20:4 3,382; C20:5 52,481; C22:5 3,226; C22:6 27,700. Total omega-6 found were 5.79% and total omega-3 84.10%; the EPA/DHA ratio found was 1.89.

The prescribed dose of supplement was of 2.5 g/die/10 kg and was defined by the physician after careful evaluation of the literature with the aim to have an intake related to body weight.

During supplementation, patients were asked to entry in the hospital every 15 days to collect the supplements and to have a check up by a pediatrician. No other treatments like behavioural therapy or psycho stimulant or other medication was given to these children.

A placebo group was not included; instead, the pre- (before) and post- (after) supplementation, measures within the subjects were used to assess the change in the psychopathology with parallel changes in the AA/EPA ratio in plasma and RBC membrane phospholipids.

Normal controls (NC, $n = 36$), consisting of healthy children from the general population, were matched for age and had similar lifestyle and dietary habits; a control group of children that follows omega-3 supplementation was also included. Blood from

ADHD as well as NC subjects (with and without supplementation) were processed and analyzed for AA/EPA blood ratio, before and after omega-3 supplementation.

Hematochemical parameters were also recorded (hemochrome, glycemia, BUN, creatininaemia, transaminases, total cholesterol, triglycerides, glycosylated haemoglobin, insulinaemia at fast, T3, T4, TSH).

The effectiveness of the supplement was estimated from the improvement of the clinical psychological condition for inattention and hyperactivity behaviours, through the Conners scale, short version, by the parents and teaching. The improvement was evaluated by the difference at basal values and after eight weeks. There were no adverse effects or allergies due to the supplement; the patients that refused to assume the therapy and the volunteers who dropped out the study were excluded from the final evaluation.

Blood and red blood cells analysis

Fasting venous blood was collected in tubes containing EDTA at the time of enrolment of the subjects including patients and NC after clinical assessment. An aliquot of total blood was immediately analyzed for fatty acid content and AA/EPA ratio assessment with the same procedure described below for RBC membrane phospholipids.

RBCs were separated by centrifugation and stored at -70°C until used for analysis. The analysis was carried out blind to the subject status.

Cell membranes of RBC (ghosts) were prepared by lysis with hypotonic buffer (phosphate 5 mM pH 8, EDTA 0.5 mM) and washed several times to eliminate haemoglobin residues.

Ghost lipids were extracted with chloroform/methanol according to Folch et al. (1957) and fractionated by silicic acid chromatography (200–400 mesh BIORAD) into non polar lipids, glycolipids and phospholipids (Vance and Sweeley 1967). Cholesterol was assayed according to Pearson et al. (1953). RBC membrane phospholipids were analyzed by quantitative thin-layer chromatography to identify each species (Rizzo et al. 1995).

Lipids from purified membrane phospholipids and from total blood fatty acids were determined by gas-chromatographic analysis. The fatty acid methylesters were obtained after derivatization with sodium methoxide in methanol 3.33% w/v and injected into gaschromatograph (Agilent Technologies 6850 Series II) equipped with a flame ionization detector (FID) under the following experimental conditions: capillary column: AT Silar length 30 m, film thickness 0.25 μm . Gas carrier: helium, temperature: injector 250°C , detector 275°C , oven 50°C for 2 min, rate of $10^{\circ}\text{C min}^{-1}$ until 200°C for 20 min. A standard mixture containing methyl ester fatty acids was injected for calibration.

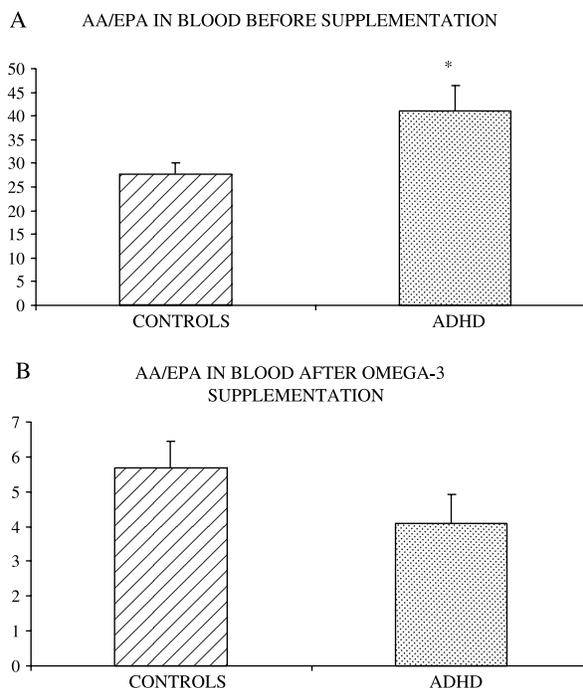


Figure 1. AA/EPA ratio in blood of control and ADHD children before (A) and after (B) omega-3 supplementation. (Mean \pm SE; controls $n = 31$ ADHD $n = 16$; * $p < 0.05$).

Statistical analysis

All data were expressed as the mean \pm SD or SE. Data were analyzed by student *t*-test to determine the variance between subjects with ADHD against controls, and between ADHD children before and after supplementation. Psychological results are expressed by delta between values before and after supplementation and analyzed utilizing parametric and non parametric tests.

Results and discussion

Thirty-one patients were recruited (28 males and 3 females); the general data of the subjects are reported in Table I.

Twelve children dropped out the study; among these, 10 did not come back for the first control and 2 were excluded from the study as they refused to assume fish oil.

Table I. General data of the patients included in the study ($n = 31$).

	Mean \pm SD
Age	8.41 \pm 2.75
Weight	33.97 \pm 12.94
Height	129 \pm 23.52
Inattention before	19 \pm 4.2
Hyperactivity before	20 \pm 4.18

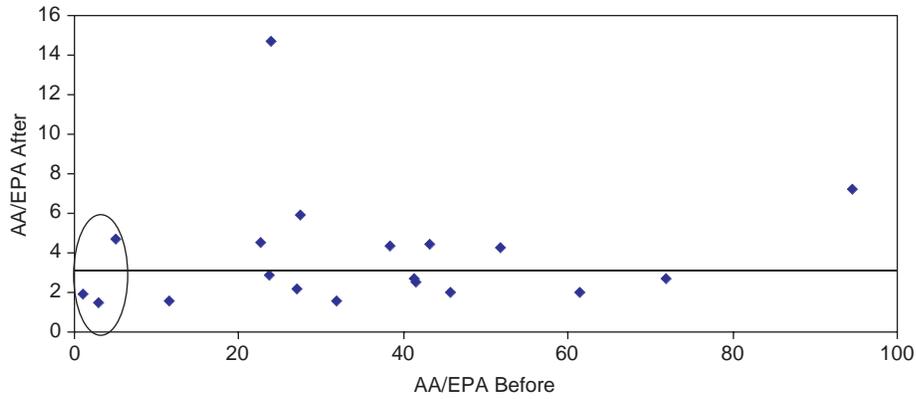


Figure 2. Correlation of AA/EPA ratio in blood of ADHD children before and after omega-3 supplementation. (Surrounded dots represents children that already assumed omega-3 when the study started).

Three out of the 19 patients kept in the study (indicated as n.3, n.10 and n.18 in Figures 2–4) declared of assuming fish oil before entering the study; therefore, only 16 patients were considered for the evaluation of the supplement (before vs. after comparison).

The mean dose of fish oil consumed by these 16 patients was 0.234 g/kg/die.

Among the patients which dropped out, 2 referred of sickness, 1 of sickness and increasing pimples, 1 of lack of amelioration after one month and 2 found fish oil too expensive.

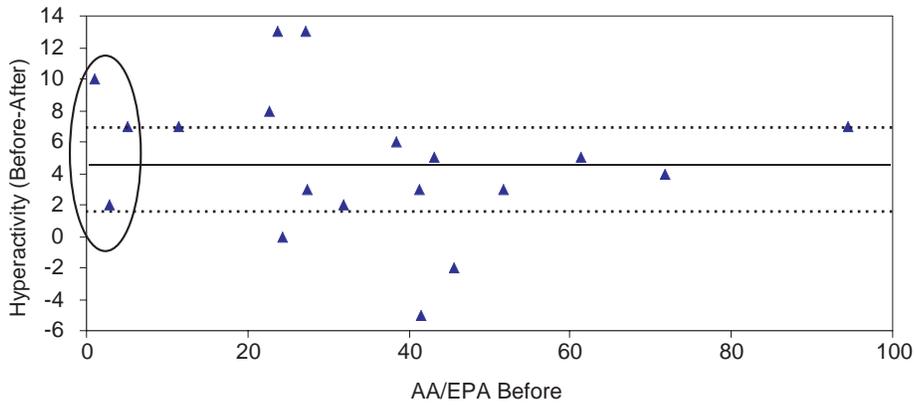


Figure 3. Correlation of AA/EPA ratio in blood of ADHD children before omega-3 supplementation with hyperactivity improvement. (Surrounded dots represents children that already assumed omega-3 at start).

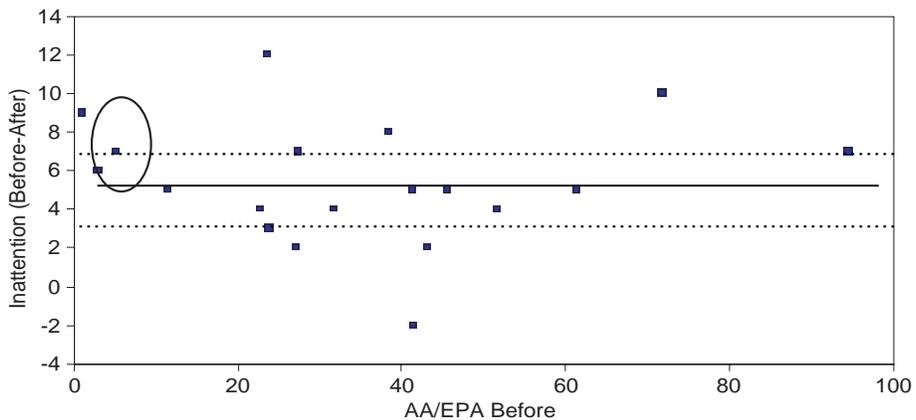


Figure 4. Correlation of AA/EPA ratio in blood of ADHD children before omega-3 supplementation with inattention improvement. (Surrounded dots represents children that already assumed omega-3).

Body mass index (BMI) of all the recruited subjects was compared to BMI international (Cole et al. 2000) and Italian centiles (Luciano et al. 1997; Cacciari et al. 2002) (Table II).

It is possible to observe that the ADHD children considered at the beginning of the study have an higher percentage of overweight and obesity compared to the Italian paediatric population, even if the number of patients does not allow statistical analysis Lobstein and Frelut (2003).

However, it is to point out that many parents of patients affected by ADHD refer that children asked frequently for sweet food and eat excessively. To our opinion, the excessive food consumption could be a mechanism to fight with the anxiety or a state of psychic uneasiness (carbohydrate craving). This aspect would deserve to be further investigated.

Four patients out of the 19 patients have shown an excessive increase of the BMI, even after the supplementation. The parents of these children have reported an increased ingestion of food for unknown causes.

The overweight condition and/or obesity do not seem to contribute to the higher levels of omega-6 fatty acid as shown from the AA/EPA ratio (Table III). Some authors in fact have recently reported increased values of the AA/EPA ratio in overweight/obese subjects with parallel well known increase of PCR (Mayman et al. 2005). Worth to note, as demonstrated in Figure 1(A), the ADHD children have an higher AA/EPA ratio compared with control with the same median age; this observation can be responsible for the absence of relationship between weight and AA/EPA ratio in these subjects. The AA/EPA ratio returns to values similar to controls using long-chain-omega-3 as supplement (see Figure 1(B)).

The average values of the hematochemical analysis appear normal without differences in the subjects with different BMI.

Table IV shows that the inattention score mean value (\pm SD) decreases from the basal one of 19 ± 4.2 to 13.9 ± 4.01 (difference: 5.06 ± 3.32 , Wilcoxon signed rank text $p = 0.002$; score maximum value for the short scale is 27).

The hyperactivity score mean value (\pm SD) decreases from the basal data of 20 ± 4.18 to a value

Table II. Distribution of slightness, normality, overweight and obesity according to the referring tables.

	Subjects number according to international centiles (%)	Subjects number according to Italian centiles (%)
Thin subjects	3/31 = 9.67	3/31 = 9.67
Normal subjects	10/31 = 32.2	14/31 = 45.2
Overweight subjects	14/31 = 45.2	11/31 = 35.48
Obese subjects	4/31 = 12.9	3/31 = 9.67

Table III. AA/EPA mean distribution at recruitment* in the subjects grouped according to BMI.

Classification according to Italian centiles	AA/EPA
Thin subjects	32.99
Normal subjects	41.43
Overweight subjects	31.34
Obese subjects	18.43

* Five patients were excluded because of their assumption of fish oil before the first determination of AA/EPA.

of 15.5 ± 4.41 (difference: 4.5 ± 4.74 , Wilcoxon signed rank text $p = 0.007$; score maximum value for the short scale is 27).

In parallel, the AA/EPA ratio mean value (\pm SD) decreases from the basal one of 41.1 ± 21.05 to 4.1 ± 3.26 (difference: 37.01 ± 21.29 , Wilcoxon signed rank text $p = 0.001$).

Therefore, the dosage of LCPUFAs seems to be useful both to reduce the high values of AA/EPA ratio and to improve the clinical conditions of the patients.

Clinical improvement entity (score difference) seems not to be linearly related to fish oil dose or AA/EPA ratio decrease.

Figure 2 shows the effects on the AA/EPA ratio with a supplement medium dosage of 0.234 g/kg weight/die; AA/EPA ratio values, high and scattered before the treatment, converge to the value of 4.1, next to 3 which is the one reported by the literature for populations showing high longevity rate, low coronary risk and low depression disposition (Nemets et al. 2002; Peet and Horrobin 2002; Sinopoulos 2002; Kang 2003).

This grouping relates to a general improvement of general clinical conditions (Figures 3 and 4) showing a mean score difference of 5 for inattention and of 4.5 for hyperactivity.

Omega-3 dose assumed by patient n. 4 was lower when compared with the other patients and patient n. 7 ended the assumption after four weeks because of diarrhoea and pimples arising.

Table IV. Statistical analysis of psychological evaluation before and after supplementation with omega-3 (dose 2.5 g/10 kg/die $n = 16$).

	Mean \pm SD (N = 16)	Wilcoxon signed rank test
AA/EPA before	41.1 \pm 21.05	
AA/EPA after	4.1 \pm 3.26	
AA/EPA (before-after)	37.01 \pm 21.29	$p = 0.001$
Inattention before	19 \pm 4.2	
Inattention after	13.9 \pm 4.01	
Inattention (before-after)	5.06 \pm 3.32	$p = 0.002$
Hyperactivity before	20 \pm 4.18	
Hyperactivity after	15.5 \pm 4.41	
Hyperactivity (before-after)	4.5 \pm 4.74	$p = 0.007$

Table V. Fatty acid composition of RBC membrane phospholipids before and after supplementation with omega-3 ($n = 16$)^{*}.

RBC PL FA	Before	After
16:0	26.23 ± 0.92	26.55 ± 1.45
16:1	0.75 ± 0.08	0.75 ± 0.07
18:0	15.92 ± 0.77	16.08 ± 0.93
18:1	19.46 ± 0.78	19.85 ± 1.15
18:2 ω -6	18.18 ± 1.14	17.00 ± 1.49
18:3 ω -3	1.30 ± 0.30	1.35 ± 0.36
20:3 ω -6	1.71 ± 0.12	1.63 ± 0.19
20:4 ω -6	11.38 ± 0.81	10.89 ± 1.19
20:5 ω -3	0.99 ± 0.11	1.83 ± 0.28 [‡]
22:5 ω -3	1.09 ± 0.09	1.45 ± 0.15 [†]
22:6 ω -3	1.99 ± 0.16	2.62 ± 0.28 [†]
Total ω -6	31.26 ± 1.76	29.53 ± 2.69
Total ω -3	5.38 ± 0.53	7.24 ± 0.88 [†]
ω -6/ ω -3 ratio	6.27 ± 0.42	4.36 ± 0.29 [‡]

* Mean ± SE *t*-test; [†] $p < 0.05$; [‡] $p < 0.06$.

The fatty acid composition of RBC phospholipids is shown in Table V; after the supplementation, there is a statistical significant enrichment for all the LC-PUFA omega-3 (C20:5, C22:5, C22:6) paralleled with a small decrease of C20:4 omega-6. Therefore, the omega-6/omega-3 ratio results in a statistically significant decrease.

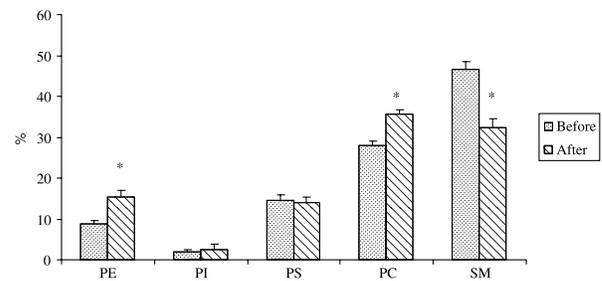
In RBC membranes, we analyzed also the amount of the main lipid components (i.e. total phospholipids and cholesterol). It is possible to observe a slight increase of phospholipid content after supplementation, while the cholesterol amount decreased (Table VI). Due to these differences, the phospholipids/cholesterol ratio increases significantly. To our opinion, these modifications could be related to changes in membrane architecture and fluidity due to the increased omega-3 incorporation. This hypothesis deserves further investigation; however other AA reported the changes in phospholipids pattern (Yehuda et al. 1998; Hashimoto et al. 2005) (Figure 5). Moreover, we observed a significant increase of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) with a parallel decrease of sphingomyelin (SM). This pattern is in agreement with data of the literature even if obtained in different cell model (Fan et al. 2003).

Work are in progress in our laboratory to analyze the lipid raft isolated from RBC membranes, due to their

Table VI. Lipid composition of RBC membranes before and after integration with omega-3 (dose 2.5 g/10 kg/die $n = 16$)^{*}.

	Before	After
Cholesterol μ g/mg Hb	1.783 ± 0.363	1.426 ± 0.213
Phospholipids μ g/mg Hb	4.675 ± 0.825	5.186 ± 0.728
Phospholipids/cholesterol	2.724 ± 0.286	3.798 ± 0.397 [†]

* Mean ± SE *t*-test; [†] $p < 0.05$.

Figure 5. RBC membrane phospholipids composition of ADHD children before and after omega-3 supplementation. (Mean ± SE; $n = 16$; * $p < 0.05$).

possible involvement in the etiopathogenesis of degenerative diseases in nervous system (Chauhan 2003; Vigh et al. 2005).

All together, the data reported show a beneficial effect of omega-3 supplementation in children with ADHD; because of the parallel improvement of AA/EPA ratio our working hypothesis is to connect the clinical condition of ADHD with a modification of cell membrane fluidity and architecture. We assume also that the AA/EPA ratio might be a biochemical marker to support the ADHD diagnosis even if further analysis are needed to confirm this assumption.

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References

- American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders. 4th ed., Washington, DC: American Psychiatric Press.
- Anderson RE, Sperling L. 1971. Positional distribution of the fatty acids in the phospholipids of bovine retina rod outer segments. *Arch Biochem Biophys* 144:673–677.
- Armstrong VT, Brzustowicz MR, Wassal SR, Jenki LJ, Stillwell W. 2003. Rapid flip-flop in polyunsaturated (docosahexaenoate) phospholipid membranes. *Arch Biochem Biophys* 414:74–82.
- Barkley RA. 1998. Attention-deficit hyperactivity disorder: A handbook for diagnosis and treatment. 2nd ed., New York: Guilford.
- Brown RT, Freeman WS, Perrin JM, Stein MT, Amler RW, Feldman HM, Pierce K, Wolraich ML. 2001. Prevalence and assessment of attention-deficit/hyperactivity disorder in primary care settings. *Pediatrics* 107:43–54.
- Burgess JR, Stevens L, Zhang W, Peck L. 2000. Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. *Am J Clin Nutr* 71(suppl): 327S–330S.
- Cacciari E, Milani S, Balsamo A, Dammacco F, De Luca F, Chiarelli F, Pasquino AM, Tonini G, Vanelli M. 2002. Italian cross-sectional growth charts for height, weight and BMI (6–20 y). *Eur J Clin Nutr* 56:171–180.

- Calder PC. 2003. N-3 polyunsaturated fatty acids and inflammation: From molecular biology to the clinic. *Lipids* 38:343–352.
- Castellanos FX, et al. 2001. Neuroimaging studies of ADHD. In: Solanto MV, editor. *Stimulant drugs and ADHD: Basic and clinical neuroscience*. New York: Oxford University Press. p 243–258.
- Chauhan NB. 2003. Membrane dynamics, cholesterol homeostasis, and Alzheimer's disease. *J Lipid Res* 44:2019–2029.
- Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. 2000. Establishing a standard definition for child overweight and obesity worldwide: International survey. *BMJ* 320:1–6.
- Damsgaard CT, Schack-Nielsen L, Michaelsen KF, Fruekilde MB, Hels O, Lauritzen L. 2006. Fish oil affects blood pressure and the plasma lipid profile in healthy Danish infants. *J Nutr* 136: 94–99.
- Dey AN, Shiller JS, Tai DA. 2002. Summary health statistics for U.S. children: National health interview survey. *National Center for Health Statistics Vital and Health Stat* 10:221.
- Elia J, Ambrosiani PJ, Rappoport JL. 1999. Treatment of attention-deficit-hyperactivity-disorder. *New Engl J Med* 11:780–788.
- Fan YY, McMurray DN, Ly LH, Chapkin RS. 2003. Dietary (n-3) polyunsaturated fatty acids remodel mouse T-cell lipid rafts. *J Nutr* 133:1913–1920.
- Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509.
- Goldman LS, Genel M, Bezman RJ, Slanetz PJ. 1998. Diagnosis and treatment of attention-deficit/hyperactivity-disorder in children and adolescents. *JAMA* 279:1100–1107.
- Halperin JM, Newcorn JH, Schwartz ST, et al. 1993. Plasma catecholamine metabolite levels in ADHD boys with and without reading disabilities. *J Clin Child Psychol* 22:219–225.
- Hamilton L, Greiner R, Salem N, Jr. 2000. N-3 fatty acid deficiency decrease phosphatylserine accumulation selectively in neuronal tissues. *Lipids* 35:863–869.
- Harbige LS. 2003. Fatty acids, the immune response, and autoimmunity: A question of n-6 essentiality and the balance between n-6 and n-3. *Lipids* 38:323–341.
- Hashimoto M, Hossain S, Agdul H, Shido O. 2005. Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid β -infused rats relates to the decreases of amyloid β and cholesterol levels in detergent-insoluble membrane fractions. *Biochim Biophys Acta* 1738:91–98.
- Hirayama S, Hamazaki T, Terasawa K. 2004. Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder: A placebo-controlled double-blind study. *Eur J Clin Nutr* 58:467–473.
- Huster D, Albert JJ, Arnold K, Gawrish K. 1997. Water permeability of polyunsaturated lipid membranes measured by ^{17}O NMR. *Biophys J* 73:856–864.
- Kafrawy O, Zerouga M, Stillwell W, Jensi LJ. 1998. Docosahexaenoic acid in phosphatidylcholine mediates cytotoxicity more effectively than other omega-3 and omega-6 fatty acids. *Cancer Lett* 132:23–29.
- Kang JX. 2003. The importance of omega-6/omega-3 fatty acid ratio in cell function: The gene transfer of omega-3 fatty acid desaturase. *World Rev Nutr Diet* 92:23–36.
- Koenig BW, Strey HH, Gawrisch K. 1997. Membrane lateral compressibility determined by NMR and X-Ray diffraction: Effect of acyl chain polyunsaturation. *Biophys J* 73:1954–1966.
- Litman BJ, Niu SL, Polozova A, Mitchell DC. 2001. The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: Visual transduction. *J Mol Neurosci* 16:237–243, discussion: 279–284.
- Lobstein T, Frelut ML. 2003. Prevalence of overweight among children in Europe. *Obes Rev* 4:195–200.
- Luciano A, Bressan F, Zoppi G. 1997. Body mass index reference curves for children aged 3–19 years from Verona, Italy. *Eur J Clin Nutr* 51:6–10.
- Mayman GA, Evans W, Restrepo H, Hutchins HL, Sears B. 2005. Effect of fish oil supplementation on metabolic syndrome and inflammatory parameters during the use of low glycemic load diets in the treatment of pediatric obesity. Communication at annual scientific meeting of American Diabetes Association.
- Millberger S, Biederman J, Faraone SV, Chen L, Jones J. 1996. Is maternal smoking during pregnancy a risk factor for attention deficit hyperactivity disorder in children? *Am J Psychiatry* 153:1138–1142.
- Mugnaini D, Masi G, Brovedani P, Che lazzi C, Matas M, Romagnoli C, Zuddas A. 2005. Teacher reports of ADHD symptoms in Italian children at the end of first grade. *Eur Psychiatry*, [Epub ahead of print] PMID: 15961290 [PubMed—as supplied by publisher].
- Nemets B, Stahl Z, Belmaker RH. 2002. Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. *Am J Psychiatry* 159:477–479.
- Pearson S, Stern S, Mc Gavack TH. 1953. A rapid accurate method for the determination of total cholesterol. *Anal Chem* 25: 313–314.
- Peet M, Horrobin DF. 2002. A dose-ranging study of the effects of ethyl-eicosapentanoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch Gen Psychiatry* 59:913–919.
- Pliszka SR, Maas JW, Javors MA, Rogness GA, Baker J. 1994. Urinary catecholamines in attention-deficit hyperactivity disorder with and without comorbid anxiety. *J Am Acad Child Adolesc Psychiatry* 33:1165–1173.
- Richardson AJ. 2004a. Clinical trials of fatty acid treatment in ADHD, dyslexia, dyspraxia and the autistic spectrum. *Prostaglandins leukot Essent Fatty Acids* 70:383–390.
- Richardson AJ. 2004b. Long-chain polyunsaturated fatty acids in childhood developmental and psychiatric disorders. *Lipids* 39: 1215–1222.
- Richardson AJ, Montgomery P. 2005. The Oxford–Durham study: A randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. *Pediatrics* 115:1360–1366.
- Rizzo AM, Galli CF, Montorfano G, Berra B. 1995. Phospholipid distribution and fatty acid composition in different brain regions during chick embryo development. *J Neurochem* 64: 1728–1733.
- Ross BM, McKenzie I, Glen I, Bennett CP. 2003. Increased levels of ethane, a non-invasive marker of n-3 fatty acid oxidation, in breath of children with attention deficit hyperactivity disorder. *Nutr Neurosci* 6:277–281.
- SanGiovanni JP, Chew EY. 2005. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 24:87–138.
- Savani A, Nadkarni A. 1998. Attention deficit disorder, the ADD puzzle & some cognitive behavioral solutions Asian. *J Pediatr Pract* 2(2):45–48.
- Singh M. 2005. Essential fatty acids, DHA and human brain. [Review]. *Indian J Pediatr* 72:239–242.
- Sinopoulos AP. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 56:365–379.
- Stevens LJ, Zentall SS, Deck JL, Abate ML, Watkins BA, Lipp SR, Burgess JR. 1995. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. *Am J Clin Nutr* 62: 761–768.
- Stillwell W, Wassal SR. 2003. Docosahexaenoic acid: Membrane properties of a unique fatty acid. *Chem Phys Lipids* 126:1–27.
- Swanson J, Deutch C, Cantwell D. 2001. Genes and attention-deficit hyperactivity disorder. *Clin Neurosci Res* 1:207–216.
- Thapar A, O'Donovan M, Owen MJ. 2005. The genetics of attention deficit hyperactivity disorder. [Review]. *Hum Mol Genet* 14(Spec No. 2):R275–R282.

- Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE. 2001. Essential fatty acids in visual and brain development. [Review]. *Lipids* 36:885–895.
- Vance DE, Sweeley CC. 1967. Quantitative determination of the neutral glycosyl ceramides in human blood. *J Lipid Res* 8: 621–630.
- Vigh L, Escriba' PV, Sonnleitner A, Sonnleitner M, Piotto S, Maresca B, Horvath I, Harwood JL. 2005. The significance of lipid composition for membrane activity: New concepts and ways of assessing function. *Prog Lipid Res* 44:303–344.
- Voigt RG, Llorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC. 2001. A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. *J Pediatr* 139:189–196.
- Wainwright PE. 2002. Dietary essential fatty acids and brain function: A developmental perspective on mechanisms. [Review]. *Proc Nutr Soc* 61(1):61–69.
- Williams EE, Jensky LJ, Stillwell W. 1998. Docosahexaenoic acid (DHA) alters the structure and composition of membrane vesicles exfoliated from the surface of a murine leukemia cell line. *Biochim Biophys Acta* 1371:351–362.
- Yehuda S, Rabinovitz S, Mostofsky DI. 1998. Modulation of learning and neuronal membrane composition in the rat by essential fatty acid preparation: Time-course analysis. *Neurochem Res* 23:627–634.

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