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ORIGINAL ARTICLE

Early monitoring of fatty acid profile in children with attention deficit and/or hyperactivity disorder under treatment with omega-3 polyunsaturated fatty acids

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ABSTRACT

BACKGROUND: Cognitive effects of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) might make them helpful in attention deficit/hyperactivity disorder (ADHD). However, the results derived from supplementation studies in children depend on the respective combinations and the study period. We aimed to investigate the serum fatty acid profile, attention scores and the tolerability in a group of ADHD children after receiving methylphenidate (MPH) and ω-3 PUFAs for 1 month.

METHODS: A combination of MPH (1 mg/kg/day) and eicosapentaenoic (EPA, 70 mg/day) + docosahexaenoic acids (DHA, 250 mg/day) was administered to 40 ADHD children (7-15 years). An analysis of serum fatty acids by gas chromatography and an assessment of attention by using the Magallanes Scale of Visual Attention (MSVA) were carried out before and after 1 month of treatment.

RESULTS: Our data revealed significant decreases of several ω-6 PUFAs, like arachidonic acid ($P<0.0259$). EPA and DHA concentrations increased by 27% and 3% respectively, and the ω-6/ω-3 index slightly decreased. The quality of attention significantly increased ($P<0.026$) and an improvement of ADHD core symptoms was reported both by parents and by teachers. No severe side effects occurred.

CONCLUSIONS: Results demonstrate that the combination of MPH and EPA+DHA at the tested doses has positive clinical effects and an adequate safety profile. Therefore, our study suggests that ω-3 PUFAs may represent a feasible and a safe adjuvant therapy in children with ADHD and might enhance the effects of MPH. Further long-term follow-up studies are required to confirm these initial findings.

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KEY WORDS: Attention deficit disorder with hyperactivity; Fatty acids, omega-3; Methylphenidate; Child.

Attention deficit and/or hyperactivity disorder (ADHD) is an important chronic disorder of childhood, with an overall pooled prevalence between 5% and 7% in school-aged children.[1] The etiology is diverse and based on neurobiological disturbances.[2] Clinically, it is characterized by levels of activity, attention span and impulsivity that do not correspond to the developmental age of the child.[3] These symptoms cause behavioral difficulties and problems to follow social norms

leading to maladjustment to family, school and work environments.[4, 5] The importance of providing an early optimal treatment (multimodal treatment[6]) is sustained by the possibility of persistence into adolescence and adulthood.[7] In addition, emotional or conduct disorders and other comorbidities are frequently observed, up to 60-80%.[8]

Along with the different aspects reflected in the multimodal treatment, dietary supplementation with long chain (LC) omega-3 (ω-3) and omega-6 (ω-6) polyunsaturated fatty acids (PUFAs) is being widely debated nowadays.

In the organism, LC-PUFAs are essential to maintain the fluidity of cell membranes, including in neurons. They are involved in energy transformation and information flow between cells.[9] In addition, they control the production of prostaglandins, the absorption of fat-soluble vitamins (vitamins A, D, E, K) and cholesterol metabolism.[10] The most important families of LC-PUFAs, in terms of extent of occurrence and human health, are the ω-6 and the ω-3 families. Linoleic acid (LA) (C18:2 ω-6) is the parent fatty acid of the ω-6 family. Similarly, the alpha-linolenic acid (ALA) (C18:3 ω-3) is the parent fatty acid of the ω-3 family. The diverse LC-PUFAs of the ω-6 and ω-3 families are synthesized from both essential fatty acids (LA and ALA, respectively) by the action of elongase and desaturase enzymes. In relation to the ω-6 PUFAs, arachidonic acid (AA) is the most important because it is the primary precursor for the ω-6 derived eicosanoids. Regarding the ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most important ω-3 fatty acids in human nutrition. The enzymatic conversion process is extremely slow, in fact it is estimated that only 1% of ingested ALA is converted into DHA (C22:6 ω-3).[10]

Neural, retinal and other nervous tissues are particularly rich in LC-PUFAs. Their concentrations in the brain account for approximately 50% of its dry weight, with a predominance of AA and DHA as basic components of cell membrane phospholipids.[11] In contrast to other body tissues where their relative concentrations are minor, AA and DHA levels in the brain are important not only from a structural perspective but also because they influence brain development and its function by means of a great variety of mechanisms (for example, production of signal mediators and reinforcement of synaptic neurotransmission). In addition, DHA participates in neuronal proliferation, gene expression and synthesis of neurotransmitters like dopamine and serotonin,[12] leading to an engagement in cognitive, behavioral and visual functions.[13] Regarding EPA, it has been suggested to have considerable neuroprotective effects through the production of eicosanoids that have anti-inflammatory, antithrombotic and vasodilatory properties.[14]

Recent meta-analyses have reported lower red blood cell (RBC) levels of DHA, EPA, AA and total ω-3 PUFAs,[15] as well as an increased ω-6/ω-3 ratio, in children and adolescents with ADHD compared to controls.[16] This reduced concentration of ω-3 PUFAs in patients with ADHD may be caused by either a decreased intake or an increased turnover. On the one hand, children with neurodevelopmental disorders often consume unbalanced diets.[17] On the other hand, they may have an excessive metabolism due to inflammatory processes, maintenance of neural function and stress-resistant feeding of ω-3 PUFAs.[18] In either case, higher levels of ω-6 in relation to ω-3 fatty acids may lead to an overproduction of pro-inflammatory cytokines,[19] apart from the presence of an association between ω-3 PUFA deficiency and dysregulations of monoamine neurotransmission at the mesocortical and mesolimbic levels.[20]

Randomized controlled trials of ω-3 PUFA supplementation in participants with ADHD and related neurodevelopmental disorders have generated mixed results. In a meta-analysis conducted by Bloch *et al.* in 2011,[21] supplementation with ω-3 PUFAs was shown to produce a small but statistically and clinically significant reduction of ADHD symptoms. In a Cochrane systematic review, Gillies *et al.*[22] obtained no statistically significant differences in parent-rated or teacher-rated ADHD symptoms when patients receiving PUFA supplements were compared with those who were administered placebo. Recently, Königs *et al.*[23] carried out a systematic review that included 25 clinical trials of ω-3 PUFA supplementation in ADHD children.

They reported some contradictory results but, in general, showed evidence for a successful treatment of ADHD symptoms.

The length of supplementation has been proposed as a potential reason for the lack of consistent results, together with large variations between studies in relation to the sample size, methodological design, dose and the choice of formula of PUFAs used.[14] The trials included in the critical appraisal conducted by Königs *et al.*[23] usually lasted 8 or 12 weeks based on the recovery time of the fatty acid profile both in the blood and in the brain.[24] However, to date there is no study that has analyzed possible effects derived from ω-3 supplementation on ADHD symptoms in a shorter period of time as the present study aims.

The objectives of this paper were: 1) to investigate the influence of an exogenous intake of ω-3 PUFAs (EPA and DHA) over the general serum lipid profile, the levels of fatty acid categories (saturated fatty acids or SFAs, monounsaturated fatty acids or MUFAs and PUFAs) and the values of PUFA/SFA and ω-6/ω-3 indices; 2) to report the tolerability and safety profile; 3) and analyze the short-term cognitive effects in children with ADHD.

Materials and methods

Study design

The study was an open-label clinical trial involving an early monitoring for 1 month.

Participants

Participants came from the Health Area of Granada (Spain). They received a suspected diagnosis of ADHD from their primary care pediatrician and were referred to the Neuropediatric, Neuropsychology and Early Intervention Unit of San Cecilio University Hospital (Granada) for subsequent monitoring and evaluation. Exclusion criteria included metabolic and endocrine disorders as well as medications able to modify the fatty acid profile.

The study protocol was approved by the Clinical Research Ethics Committee of Granada (San Cecilio University Hospital, Granada, Spain). Written informed consent signed by parents or tutors was required to participate in the study, as well as the assent of school aged children to their involvement in the research. Written informed consent was also required from patients over 12 years and sufficiently mature in relation to their developmental age. All procedures were carried out in accordance with the Helsinki Declaration as revised in 2013.[25]

The total study population consisted of 40 ADHD patients, 27 boys (67.50%) and 13 girls (32.50%), aged 7-15 years (10.45±2.34 years) at start of the intervention period.

Demographic characteristics and details on personal and family history of our patients are provided in Table I:

• perinatal history: the majority of patients were born at term. Three of them (7.50%) were diagnosed with mild perinatal asphyxia at birth. The remaining 6 patients (15%) were born preterm between 32 and 36 weeks of pregnancy, with an adequate birth weight.

• Psychomotor development: most of the patients showed an adequate psychomotor development in relation to the corrected age. However, 1 of them (2.50%) had been diagnosed with an autism spectrum disorder (ASD) and other 2 patients (5%) had shown a language delay.

• Previous neurological disorders: as can be observed in Table I, 7 patients (17.50%) were diagnosed with neurological disorders such as enuresis, dyslexia, ASD and epilepsy.

• Family history: neurological and psychiatric disorders had been diagnosed in the family members of 14 patients (35%). Specifically, a family history of ADHD was confirmed in 7 patients (17.50%).

Study protocol

Clinical procedures

The same study protocol was followed by all patients in order to reach the definitive diagnosis of the ADHD subtype and possible comorbidities. A complete medical record was taken and a careful physical examination was initially performed. The medical record was based on the interviews of the patient and parents, as well as information provided by the teachers. A neuro-

TABLE I.—*Baseline characteristics of our patients.*

Patient	Age (years)	Sex	Perinatal history	Psychomotor development	Previous neurological disorders	Family history
1	10	M	Preterm 36 weeks Adequate BW	Adequate	No	No
2	8	F	Born at term Adequate BW	Adequate	No	No
3	8	F	Born at term Adequate BW	Adequate	No	Sibling with ADHD
4	11	F	Preterm 32 weeks Adequate BW	Adequate	No	Sibling with ADHD
5	11	F	Preterm 32 weeks Adequate BW	Adequate	No	Sibling with ADHD
6	14	M	Born at term Adequate BW	Adequate	No	No
7	14	M	Born at term Adequate BW	Adequate	No	No
8	10	M	Preterm 33 weeks Adequate BW	Adequate	No	No
9	8	F	Born at term Adequate BW	Adequate	No	Sibling with epilepsy
10	15	M	Born at term Adequate BW	Adequate	No	No
11	15	M	Born at term Adequate BW	Adequate	No	No
12	8	M	Born at term Mild perinatal asphyxia	Adequate	No	No
13	7	M	Preterm 33 weeks Adequate BW	Adequate	No	Cousin with ADHD
14	12	M	Born at term Adequate BW	Adequate	No	No
15	11	M	Born at term Adequate BW	Adequate	No	No
16	11	M	Born at term Adequate BW	Adequate	No	No
17	8	M	Born at term Adequate BW	Adequate	No	Maternal grandfather with bipolar disorder
18	7	M	Born at term Adequate BW	Adequate	No	Maternal grandfather with schizophrenia
19	10	F	Born at term Adequate BW	Adequate	No	Cousin with ADHD
20	13	M	Born at term Adequate BW	ASD	ASD	No
21	13	M	Born at term Adequate BW	Adequate	No	Paternal grandfather with bipolar disorder
22	9	M	Born at term Adequate BW	Adequate	No	No
23	14	M	Born at term Adequate BW	Adequate	No	Cousin with ADHD
24	11	M	Born at term Mild perinatal asphyxia	Adequate	Enuresis	No
25	9	M	Born at term Mild perinatal asphyxia	Adequate	No	No
26	14	M	Born at term Adequate BW	Adequate	No	No
27	11	M	Born at term Adequate BW	Adequate	Enuresis	No
28	8	M	Born at term Adequate BW	Adequate	No	No
29	9	F	Born at term Adequate BW	Adequate	Dyslexia	Cousin with ADHD
30	13	F	Born at term Adequate BW	Adequate	No	No
31	11	M	Born at term Adequate BW	Adequate	No	No
32	13	M	Born at term Adequate BW	Adequate	No	Mother with depressive disorder

(To be continued)

TABLE I.—*Baseline characteristics of our patients (continues).*

Patient	Age (years)	Sex	Perinatal history	Psychomotor development	Previous neurological disorders	Family history
33	8	F	Born at term SGA	Adequate	Dyslexia	No
34	9	F	Born at term Adequate BW	Language delay	No	No
35	9	M	Born at term Adequate BW	Adequate	No	No
36	11	F	Born at term Adequate BW	Adequate	No	No
37	8	M	Born at term Adequate BW	Adequate	No	No
38	9	F	Preterm 34 weeks Adequate BW	Language delay	Enuresis	Mother with epilepsy
39	10	M	Born at term Adequate BW	Adequate	No	No
40	8	F	Born at term Adequate BW	Adequate	Epilepsy	Maternal grandfather with epilepsy

M: male; F: female; BW: birth weight; SGA: small for gestational age; ASD: autism spectrum disorder; ADHD: attention deficit and/or hyperactivity disorder.

psychological assessment was carried out in order to complement the diagnostic process, which included the following questionnaires: 1) the NICHQ Vanderbilt Parent Assessment Scale[26] and the NICHQ Vanderbilt Teacher Assessment Scale;[27] 2) the Behavior Rating Inventory of Executive Functions (BRIEF) parent form;[28] 3) the Kaufman Brief Intelligence Test (K-BIT)[29] and the Wechsler Intelligence Scale for Children 4th edition (WISC-IV)[30] to assess intelligence and cognitive capacity. The total intelligence quotient (IQ) was divided in categories according to the final score obtained: low IQ (50-69); medium-low IQ (70-89); medium IQ (90-109); high-medium IQ (110-119); high IQ (over 120). It is worth mentioning that the global cognitive assessment was not only based on the specific IQ scores, but also on the impairments showed by the patients in the conceptual, social and daily life areas; and 4) the Magallanes Scale of Visual Attention (MSVA).[31] The purpose of this scale is to make a quantitative and qualitative assessment of the capacity of focusing, maintaining, coding and stabilizing attention to visual stimuli during a certain period of time (6 minutes in children under 9 years of age and 12 minutes in those older) while a simple motor task is performed. The patient has to identify the figure of a man described within a grid full of distracting stimuli in the form of human figures in other postures or rotations. The two measured variables are the sustained visual attention (SA) and the quality of attention (QA). While the SA represents the capacity to focalize and code visual stimuli during this time, the QA refers to the efficacy in focalizing and coding of visual stimulus.[32] The MSVA-associated computer program (TIPI-SOFT) provided the percentile scores of SA and QA by introducing the number of correct responses, commission errors and omission errors made by the patient. The final diagnosis of ADHD and the specific subtype was made according to the diagnostic criteria established by the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5).[33] Once the diagnosis of ADHD was confirmed, a multimodal treatment regimen was designed based on the joint decision made by family, teachers, psychologists and pediatricians of our unit. The decision was adapted to the characteristics of each patient. The pharmacological treatment consisted of a combination therapy with extended-release methylphenidate (MPH) (initial daily dose of 1 mg/kg/day) and EPA+DHA supplement (daily dose of 70 mg EPA+250 mg DHA). Both medications started at the same time, and they were administered orally in the morning in one single dose.

Serum fatty acid profile and MSVA results of each patient were analyzed before starting treatment and after 1 consecutive month receiving MPH and EPA+DHA at indicated doses.

Analytical procedures

Fasting venous blood samples were drawn by using a fine needle and were immediately centrifuged. After centrifugation, fresh plasma samples obtained were frozen at -80°C until further analysis. Fatty acid analysis was performed after extraction of serum lipids with chloroform-methanol mixture (7:3, v/v) according to the technique described by Haan *et al.*[34] and subsequent methylation with boron trifluoride-methanol.[35, 36] Gas chromatography was necessary for the identification of fatty acids by the use of a Focus GC chromatograph model (ThermoScientific, Milan, Italy) equipped with a silica gel capillary column 100 m x 0.25 mm x 0.2 µm (TR-CN100 Teknokroma, Barcelona, Spain) and a flame ionization detector. The standard mixture FAME mix 47885-U provided by the company Sigma-Aldrich (Milan, Italy) was used to identify methyl esters in accordance with their retention times. Results were expressed as percentages of the total fatty acids obtained in the serum sample.

Statistical analysis

Analyses were conducted using Statgraphics Centurion version XVII (Statpoint Technologies, Inc., Warrengton, VA, USA). Descriptive data were presented as mean±standard deviation (SD). The two-tailed Student-t test for paired samples was performed to compare means of fatty acid values. In the case of MSVA results, the comparisons were made by using the nonparametric Wilcoxon Signed Rank test. The level of significance was set at the usual alpha (α)=5%.

Results

Study population

Clinical characteristics of our patients are shown in Table II:

• K-BIT and WISC-IV results: 15 patients (37.50%) obtained a medium-low IQ, 22 (55%) achieved a medium IQ, 2 patients (5%) obtained a high-medium IQ and 1 children achieved a high IQ (2.50%). The mean IQ of our population was 96 (± 12.63).

• ADHD subtype: according to the DSM-5 diagnostic criteria, 22 patients (55%) were diagnosed as being inattentive, 16 (40%) as combined subtype (inattentive/hyperactive) and 2 patients (5%) as predominantly hyperactive/impulsive.

• Comorbidities: comorbid disorders were diagnosed in 15 patients (37.50%). Among them, specific learning disorders, like dysgraphia, dyslexia and dyscalculia, were the most frequent comorbidities (40%), followed by enuresis (20%),

TABLE II.—*Clinical characteristics (IQ, subtype, comorbidities) of our patients.*

Patient	Total intelligence quotient (IQ)	ADHD subtype	Comorbidity
1	80	Inattentive	No
2	89	Inattentive	No
3	107	Combined	Motor tic disorder
4	105	Inattentive	Dysgraphia
5	104	Inattentive	No
6	106	Inattentive	No
7	85	Inattentive	Fine motor skills disorder
8	83	Inattentive	Dyscalculia
9	82	Combined	No
10	102	Inattentive	No
11	99	Combined	No
12	77	Inattentive	Dyscalculia
13	115	Combined	No
14	80	Inattentive	No
15	100	Inattentive	No
16	106	Combined	No
17	99	Combined	No
18	104	Combined	No
19	80	Inattentive	No
20	122	Inattentive	ASD
21	97	Inattentive	No
22	81	Combined	No
23	105	Inattentive	No
24	82	Inattentive	Enuresis
25	117	Combined	No
26	100	Inattentive	Sleep disorder
27	77	Hyperactive/ restrictive	Enuresis
28	75	Inattentive	Dysgraphia
29	104	Inattentive	Dyslexia
30	81	Inattentive	No
31	83	Combined	No
32	108	Combined	No
33	104	Combined	Dyslexia
34	106	Combined	No
35	102	Combined	No
36	108	Combined	No
37	100	Combined	Fine motor skills disorder
38	96	Inattentive	Enuresis
39	106	Hyperactive/ restrictive	No
40	86	Inattentive	Epilepsy

fine motor skills disorders (13.33%), sleep disorders (6.70%), motor tic disorders (6.70%), ASD (6.70%) and epilepsy (6.70%).

Serum fatty acid profile

The results of fatty acid profiles before starting treatment and one month later are presented in Table III. As can be observed, the total sum of SFAs significantly increased after treatment with EPA+DHA (from 32.80±0.60% to 35.38±0.36%, P<0.0005). This change was especially due to the increase of palmitic (C16:0) and stearic acid (C18:0) concentrations. By the same token, the diverse findings observed in the case of unsaturated fatty acid levels attracted our interest and invited us to reflect about their modifications. Some values barely changed before and after treatment, as occurred with MUFA and PUFA total values. However, two of ω-6 PUFAs significantly decreased by 25% after 1 month of EPA+DHA supplementation, AA (C20:4 ω-6, P<01.0259) and eicosadienoic acid (C20:2 ω-6, P<0.0156) (Table III). Regarding ω-3 PUFAs, differences were not statistically significant, although EPA and DHA concentrations slightly increased after supplementation (by 27% and 3%, respectively). In consequence, total ω-3 fatty acid levels increased by 13% and ω-6/ω-3 index decreased by 8% (P>0.05) (Table III).

TABLE III.—*Comparative analysis of serum fatty acid profile before and after 1 month of treatment.*

Fatty acids	Before treatment (mean±SD)	After treatment (mean±SD)	P
SFAs	32.80±0.60	35.38±0.36	0.0005
C12:0	0.082±0.010	0.147±0.047	0.1947
C14:0	0.782±0.040	0.843±0.071	0.4704
C16:0	21.86±0.417	23.13±0.263	0.0127
C17:0	0.305±0.011	0.300±0.015	0.7715
C18:0	9.393±0.240	10.54±0.250	0.0017
C20:0	0.200±0.125	0.250±0.143	0.7930
C22:0	0.091±0.009	0.093±0.006	0.9050
MUFAs	21.89±0.78	20.39±0.67	0.1483
C14:1	0.052±0.006	0.057±0.005	0.5254
C16:1	1.171±0.062	1.137± 0.063	0.7063
C18:1 cis 9	19.12±0.724	17.76±0.599	0.1519
C18:1 cis 11	1.366±0.053	1.281±0.042	0.2136
C20:1	0.180±0.011	0.151±0.007	0.0310
PUFAs	45.31±1.14	44.23±0.77	0.4371
ω-6	42.39±1.03	40.90±0.75	0.2457
C18:2 ω-6	27.02±0.824	28.55±0.753	0.1773
C18:3 ω-6	0.298± 0.019	0.341±0.027	0.2092
C20:2 ω-6	0.320±0.018	0.262±0.014	0.0156
C20:3 ω-6	1.738±0.059	1.900±0.088	0.1381
C20:4 ω-6 (AA)	12.44±1.287	9.249±0.573	0.0259
C22:2 ω-6	0.040±0.004	0.048±0.014	0.6225
C22:4 ω-6	0.276±0.016	0.291±0.014	0.4736
C22:5 ω-6	0.252±0.011	0.260±0.020	0.7161
ω-3	2.91±0.19	3.33±0.25	0.1892
C18:3 ω-3	0.210±0.014	0.173±0.011	0.0526
C20:3 ω-3	0.886±0.181	1.199±0.198	0.2492
C20:5 ω-3 (EPA)	0.342±0.044	0.436±0.059	0.2104
C22:6 ω-3 (DHA)	1.476±0.058	1.525±0.113	0.7071
PUFA/SFA	1.40±0.06	1.26±0.03	0.0280
ω-6/ω-3	15.62±0.79	14.28±0.23	0.3670

Values of individual fatty acids were calculated as percentages in relation to the total of fatty acids identified in the serum sample.
SD: standard deviation; SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; ω-6: omega-6; AA: arachidonic acid; ω-3: omega-3; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Attention scores

The scores of attention (QA and SA) obtained by the MSVA are shown in Table IV. As we can observe, the QA percentile score significantly increased after treatment (from 32±32 to 51±32, P<0.026). This change was mainly due to a decrease in the number of commission errors and, to a lesser extent, of omission errors. The SA percentile score increased by 20%, but this difference was not statistically significant (P>0.05).

Adaptation to the dose of medication was adequate in all patients after 1 month of pharmacological treatment with MPH and EPA+DHA. A relevant improvement of core symptoms was reported by parents and teachers despite the short clinical observation period.

Tolerability and adverse effects

No severe side effects were reported. Four patients (10.52%) experienced headache and gastric discomfort in the first week, which was transient.

TABLE IV.—*Attention scores obtained before and after 1 month of treatment.*

MSVA	Before treatment (mean±SD)	After treatment (mean±SD)	P
Correct responses	57.96±42.0	78.28±52.22	0.069
Commission errors	1.81±5.01	0.68±2.11	0.510
Omission errors	9.92±14.70	6.20±11.0	0.092
QA (percentile score)	32±32	51±32	0.026
SA (percentile score)	12±18	15±17	0.387

SD: standard deviation; QA: quality of attention; SA: sustained visual attention.

Discussion

To date, this is the first pilot study that investigates the biochemical and clinical effects derived from 1 month of treatment with MPH and ω-3 PUFAs in a group of children and adolescents with ADHD.

As we have previously mentioned, therapeutic administration of ω-3 fatty acids to children with ADHD remains a controversial aspect of the nutritional and therapeutic recommendations for these patients. In this initial study, we tested a combination of EPA+DHA based on our previous experience and on account of its safety and its relatively low dose. We considered beforehand that this formulation might be beneficial for ADHD patients. Obviously, the definitive estimation in relation to the possible beneficial effects of supplementation with ω-3 PUFAs will be reached in the future when the project is finished. Our next expectations are to completely evaluate the current patient cohort, continue the recruitment process and to modify the dose of supplementation throughout a 2-year follow-up. This is the reason why we firmly believe that obtaining preliminary data provides an initial reference regarding clinical effects (beneficial), tolerability (excellent) and possible serum changes of the different fatty acids.

Changes of serum fatty acid profile

Groups of experts recognize that individual fatty acids may have different biological properties and specific effects on human health.[10] In particular, the three wide categories of fatty acids (SFAs, MUFAs and PUFAs) are based on a chemical classification, without distinguishing individual characteristics. However, the majority of epidemiological evidences reviewed by experts are guided by this classification, which makes it difficult to separately analyze the diverse effects of the numerous fatty acids.

In this study, there was observed a slight but not statistically significant decrease of ω-6/ω-3 index. Apart from being a short-term study, this finding invites us to think about possible changes in the administered dose. As a matter of fact, the proportions of EPA+DHA have been modified throughout the subsequent follow-up of these patients in order to improve their protective functions against disease. On the other hand, there was found a significant increase of SFA concentrations especially due to higher levels of palmitic and stearic acids, as can be observed in Table III. It is somewhat unexpected to find an increase of SFA values, although it might be influenced by the general dietary patterns. In this regard, it is worth remembering that one of the common side effects produced by MPH is the loss of appetite and a lesser weight gain.[37] Given this effect, it is possible that parents of these children may have changed their dietary patterns accordingly (by increasing the intake of energy-dense foods and beverages, for instance). Carrying out dietary assessment methods through dietary records or food diaries would help to resolve this question. Although it is difficult to find a straightforward explanation for these differences, it would be interesting to follow their long-term trend.

A 12-week double-blind randomized placebo-controlled clinical trial was conducted by Matsudaira *et al.*[14] in a sample of 76 male adolescents with ADHD who were not taking concomitant medication to investigate the psychological and biochemical effects derived from PUFA supplementation. They reported a significant increase of DHA, EPA and total ω-3 fatty acid levels in the group receiving supplementation, with no significant changes of AA, total ω-6 fatty acid and SFA concentrations. Apart from differences in the duration trial and the recruited sample compared to our study, these authors administered a combination of EPA (558 mg/day), DHA (174 mg/day) and gamma-linolenic acid (GLA, 60 mg/day).

The proportions of EPA+DHA used in our investigation are in line with European recommendations. In accordance with the European Food Safety Authority (EFSA), the recommended intake for children and adolescents aged 2 to 18 years consists of 1-2 servings of oily fish per week or 250 mg of EPA+DHA per day.[38] However, a therapeutic combination of EPA+DHA+GLA in decreasing quantities might be the most appropriate one in patients with ADHD. Their beneficial results in reading and communication skills, behavior, attention levels and core symptoms of ADHD have been demonstrated by differ-

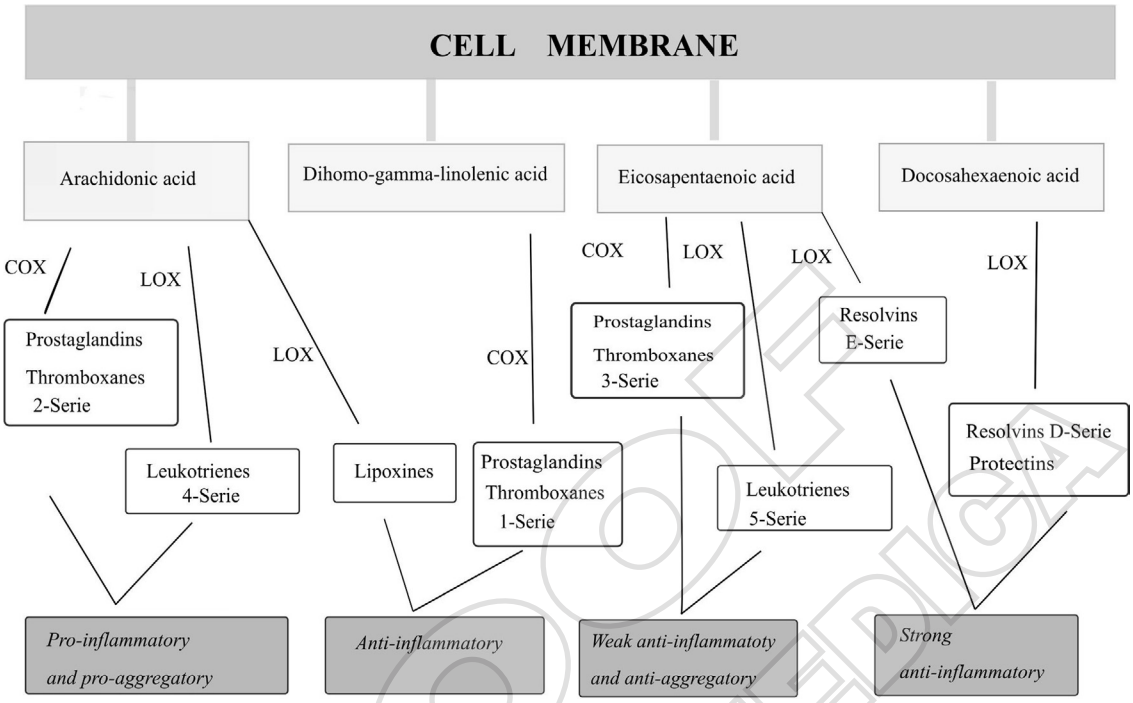

Figure 1.—Conversion of long-chain polyunsaturated fatty acids to eicosanoids and docosanoids.

ent studies.[39-41] GLA is further metabolized to dihomo-gamma-linolenic acid, which undergoes oxidative metabolism to produce anti-inflammatory eicosanoids[42] (Figure 1). Therefore, GLA complements the anti-inflammatory actions derived from omega-3 fatty acids. In addition, the combination of ω-6 and ω-3 fatty acids is useful to avoid possible adverse effects associated with long-term administration of GLA, which is able to cause an indirect increase of AA levels.[43] In a recent systematic review conducted by Derbyshire,[44] ω-3/ω-6 PUFA supplementation was reported to produce an improvement of ADHD symptoms, including favorable results in hyperactivity, attention, impulsivity, visual learning, word reading and working/short-term memory.

Clinical effects

In our investigation, the changes observed in the serum fatty acid levels were accompanied by an improvement of clinical symptoms, like was shown by the results obtained in the MSVA. One month after treatment with MPH and EPA+DHA, there was found a significant increase of the QA ($P<0.026$) as a consequence of a lower number of mistakes and omissions and an increase of correct responses. In this sense, the effects on cognition and ADHD symptoms derived both from MPH and EPA+DHA should be considered jointly.

On the one hand, MPH is able to normalize the performance deficits in omission errors observed in patients with ADHD, compared to placebo. The beneficial effects of MPH are more pronounced for inattention problems (omission errors) than impulsivity errors (reflected by commission errors). This can be explained by an enhanced brain activation caused by MPH in several areas that are underactivated during a selective attention task in children with ADHD, such as the cerebellum, precuneus, posterior cingulate, right premotor, inferior frontal and parietal regions. In addition, the psychostimulant medication is able to restore the inter-regional connectivity deficits in fronto-striatal, fronto-cerebellar and cerebello-striatal intercorrelations.[45] Additionally, DHA has been reported to reinforce the neuroplastic brain changes induced by repeated amphetamine

treatment in animal studies, although more trials are required to provide more solid evidence about this effect.[46]

On the other hand, Chang *et al.*[15] reported that ω-3 PUFAs significantly improved parental reports of total ADHD symptom scores, inattention and hyperactivity. In their systematic review, these authors included double-blind randomized placebo-controlled clinical trials of supplementation with DHA or EPA alone or in combination in children and adolescents with ADHD. The dosage of ω-3 PUFAs ranged from 2.7 mg to 640 mg of DHA and 80 mg to 650 mg of EPA, with only one study using EPA (560 mg) as the sole source of ω-3 PUFA supplementation. They also obtained significant improvements derived from ω-3 PUFA supplementation compared with placebo in terms of cognitive performance for omission errors and commission errors, but not for forward memory, backward memory or information processing. Another interesting finding reported in this systematic review was that only studies with EPA dosage of 500 mg/day or higher showed a significant effect on hyperactivity symptoms, but not those with smaller dosages. In another recent systematic review, Agostoni *et al.*[47] investigated the effect of combined EPA and DHA at different dose levels in children and adolescents aged 4-18 years and diagnosed with ADHD. Eleven studies were identified. Among them, 6 reported improvement in ADHD symptoms, whereas the other 5 studies did not find any significant benefit. The 6 trials that reported positive effects had a minimum duration of 12 weeks and a minimum sample size of 40. By contrast, the systematic review conducted by Rangel-Huerta *et al.*[48] revealed no significant cognitive benefits derived from ω-3 PUFA (EPA+DHA) supplementation during pregnancy or breastfeeding on later stages of development in healthy participants. In children and adolescents with ADHD, they did not find significant results compared with placebo, although increased RBC concentrations of EPA and DHA were associated with improved working memory, reading speed and behavior. It is worth mentioning a randomized clinical trial carried out by Barragán *et al.*[49] to evaluate the effects of ω-3/ω-6 fatty acids (EPA, DHA and GLA), MPH and combined MPH and ω-3/ω-6 in terms of symptom reduction and tolerability in a sample of children with ADHD. The combination of MPH and ω-3/ω-6 did offered no efficacy benefits over MPH monotherapy, but it allowed lower doses of MPH and showed a better tolerability profile.

Taking into consideration the previously mentioned evidence, it could be stated that ω-3 PUFAs seem to have clinical benefits for patients with ADHD and, therefore, they may be considered as a possible adjuvant therapy in medication-resistant cases or as an alternative therapy when parents refuse pharmacological treatment. However, it should be considered that overall results depend, to a large extent, on the combinations of LC-PUFAs and the relative concentration of each component.

The commentary above is essential for the discussion of this study, because it endorses the previously mentioned controversies in this area and raises the following reasonable questions: Which are the most appropriate doses and therapeutic combinations? How should LC-PUFA doses be modified according to the age and characteristics of patients? What specific changes should be considered in the case of some diseases, like ADHD?

Turning to our study, there was used a therapeutic combination of MPH and ω-3 fatty acids, with a higher proportion of DHA in comparison with EPA. The improvement of ADHD core symptoms observed in our patients after 1 month of treatment was correlated with favorable changes of the number of omission errors, as well as commission errors, during the visual attention task (MSVA). These results might be due to the combined benefits derived from both MPH and EPA+DHA supplementation. Clearly, a modification of the EPA/DHA proportions with the possible addition of ω-6 fatty acids (GLA), as well as studying different groups of treatment (only MPH and MPH + ω-3/ω-6) during the further follow-up of our patients, will provide stronger evidences in relation to the real effects produced by different combinations of PUFAs.

Tolerability and adverse effects

Only mild and transient side effects were reported by our patients. Headache and gastric discom-

fort are common adverse effects produced by the intake of MPH.[37] It was expected to find no severe adverse effects derived from EPA+DHA supplementation since it is considered that the doses used were relatively low in comparison with other studies, like the clinical trial conducted by Bos *et al.*.[50] Those authors reported a benign side-effect profile of EPA+DHA supplementation in spite of investigating higher doses of either ω-3 fatty acids, up to 650 mg per day.

Strengths and limitations of the study

Limitations of our study highlight directions for future research. It should be considered that our investigation represents a pilot study designed as an open-label clinical trial. Therefore, not conducting a double-blind randomized controlled trial and not having a control group that only received MPH made it difficult to know exactly the relative effect due to EPA+DHA supplementation. The sample size (40 patients) was also modest to obtain a great magnitude of differences. Additionally, the short study period (1 month) was probably not enough to observe total cognitive effects and long-term tolerability of ω-3 fatty acids.

Despite these limitations, the present study possessed several strengths: careful medical screening; sophisticated sample processing; and above all providing an initial reference in relation to short-term clinical effects and tolerability of ω-3 fatty acid supplementation in children with ADHD.

Throughout the further follow-up and the design of a randomized controlled trial with parallel groups of treatment (only MPH and MPH + (ω-3+ω-6)), we expect to obtain more information in relation to the dose adjustment, proportions of ω-3/ω-6 fatty acids, tolerability and medium and long-term therapeutic effects.

Conclusions

The results demonstrate that the combination of MPH and EPA+DHA at the tested doses has positive clinical effects and an adequate safety profile. Therefore, our study suggests that ω-3 PUFAs may represent a feasible and a safe adjuvant therapy in children with ADHD and might enhance the effects of methylphenidate. In addition, this combination could reduce the required dose of psychostimulant medications, decreasing the probability of adverse effects. Further long-term follow-up studies are required to confirm these initial findings.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions.—Antonio Muñoz-Hoyos designed the study, wrote the protocol, supervised the data collection and reviewed the manuscript; Ana Checa-Ros undertook the data collection and drafted the manuscript. Isabel Seiquer and Ana Haro-García conducted the data statistical analysis and assisted with the fatty acid data interpretation. Antonio Molina-Carballo and José Uberos-Fernández assisted with the data interpretation and reviewed the study protocol. All authors read and approved the final manuscript.

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