Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder[1–4]

Laura J Stevens, Sydney S Zentall, John L Deck, Marcey L Abate, Bruce A Watkins, Steven R Lipp, and John R Burgess

ABSTRACT Attention-deficit hyperactivity disorder (ADHD) is the term used to describe children who are inattentive, impulsive, and hyperactive. The cause is unknown and is thought to be multifactorial. Based on the work of others, we hypothesized that some children with ADHD have altered fatty acid metabolism. The present study found that 53 subjects with ADHD had significantly lower concentrations of key fatty acids in the plasma polar lipids (20:4n−6, 20:5n−3, and 22:6n−3) and in red blood cell total lipids (20:4n−6 and 22:4n−6) than did the 43 control subjects. Also, a subgroup of 21 subjects with ADHD exhibiting many symptoms of essential fatty acid (EFA) deficiency had significantly lower plasma concentrations of 20:4n−6 and 22:6n−3 than did 32 subjects with ADHD with few EFA-deficiency symptoms. The data are discussed with respect to cause, but the precise reason for lower fatty acid concentrations in some children with ADHD is not clear. *Am J Clin Nutr* 1995;62:761–8.

KEY WORDS Essential fatty acids (EFAs), attention-deficit hyperactivity disorder (ADHD), thirst, dry skin, frequent urination, arachidonic acid (20:4n−6), docosahexaenoic acid (22:6n−3)

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is the term used to describe children who are inattentive, impulsive, and hyperactive (1). These behaviors may severely affect school performance, family relationships, and social interactions with peers. ADHD is thought to affect 3–5% of the school-age population (1). Boys are more commonly identified with ADHD than are girls (2). Stimulant drugs such as Ritalin (CIBA, Woodbridge, NJ) or Cylert (Abbott Laboratories, Abbott Park, IL) are often used to calm children with ADHD and have an effectiveness rate of ≈75% (3). In most children with ADHD, the cause is unknown but is thought to be biological and multifactorial (4). For example, various studies suggest that genetic factors (5), neurotransmitter imbalances (6), lead toxicity (7), or food sensitivities (8, 9) may adversely affect behavior in some children with ADHD.

A few studies have focused on essential fatty acid (EFA) metabolism in children with ADHD. Fatty acids play important structural roles as components of all cell membranes, affecting their biological properties. EFAs serve as precursors to substrates in the biosynthesis of eicosanoids, which mediate a wide variety of functions in every cell in the body (10).

Colquhoun and Bunday (11) generated interest in the relation between EFA metabolism and hyperactivity when they surveyed a large population of children with hyperactivity, predominantly male, in West Sussex, UK. They found that children with hyperactivity seemed to be more thirsty than children without hyperactivity. Increased thirst is a primary symptom of EFA deficiency (12). Many of the children with hyperactivity studied had eczema, asthma, or other allergies that have been reported to be alleviated by EFA supplementation (13, 14). However, thirst, eczema, and asthma are nonspecific symptoms that may be related to EFA deficiency but can also be due to other factors. Mitchell et al (15) measured plasma fatty acids in 48 unmedicated children with hyperactivity and in matched control subjects. They found that concentrations of docosahexaenoic (22:6n−3; DHA), dihomogammalinolenic (20:3n−6; DGLA), and arachidonic (20:4n−6; AA) acids were significantly lower in the children with hyperactivity.

The present study measured plasma polar lipid and red blood cell (RBC) total fatty acid concentrations in control subjects and in boys with ADHD and compared the incidence and severity of symptoms indicative of EFA deficiency, as well as allergies, infections, and other somatic complaints. Furthermore, another goal was to identify a possible subgroup of subjects with ADHD who exhibited signs of EFA deficiencies and to compare their concentrations of fatty acids with those of subjects with ADHD who showed few symptoms of EFA deficiencies. A 3-d diet record for each subject was computer-analyzed to detect any differences in dietary intake between control subjects and children with ADHD that might account for differences in blood EFA concentrations.

[1] From the Department of Foods and Nutrition, the Department of Educational Studies, the Department of Statistics, and the Department of Food Science, Purdue University, West Lafayette, IN; and Arnett Clinic, Lafayette, IN.

[2] Paper no. 14498 of the Purdue University Agricultural Experiment Station, West Lafayette, IN.

[3] Supported by a Crossroads grant from the state of Indiana (to JRB).

[4] Address reprint requests to JR Burgess, Department of Foods and Nutrition, Purdue University, Stone Hall, West Lafayette, IN 47907-1264.

Received December 16, 1994.

Accepted for publication May 25, 1995.

SUBJECTS AND METHODS

Subject selection

One hundred healthy control subjects and boys with ADHD, ages 6–12 y, were volunteers from north central Indiana. Subjects were recruited for this study by television, radio, and newspaper announcements seeking both healthy boys with normal behavior and healthy boys who had been diagnosed as having or suspected of having ADHD. Investigators sent each family a pamphlet outlining the purpose of the study and the steps required for each subject: completion of questionnaires by parents and teachers, a 3-d diet record, and a blood test. Interested parents and children then completed consent forms, a screening questionnaire, and the Conners' Parent Rating Scale—the most widely used, standardized behavioral rating system for ADHD assessment (16). Parents and children gave written permission to send the child's primary teacher the Conners' Teacher Rating Scale. The protocol was approved by the Purdue University Human Subjects in Research Committee.

Behavioral assessment

Boys were designated as control subjects or subjects with ADHD based on their scores on the Parent and Teacher Conners' Rating Scales. Parents responded to 48 questions on a scale of 0 to 3 ("not at all," "just a little," "pretty much," and "very much"). Ten of these questions were used to calculate the hyperactivity index. Teachers responded similarly to 28 questions. If a subject scored $\geq$ 15 points (out of a possible 30) on either or both questionnaires he was considered to have ADHD. Control subjects scored $<$ 15. Four subjects were eliminated from the study because of large discrepancies in teacher and parent scores. Fifty-three subjects were defined as having ADHD and 43 as control subjects. Twenty-four subjects with ADHD were taking stimulant medication (Ritalin or Cylert) whereas 29 subjects with ADHD were taking no medication.

Health assessment

After each subject agreed to participate in this study, the child, parent, and investigator met at a local medical clinic. Additional consent forms for both parent and child were explained and signed. Parents completed questionnaires about age, race, adoption, and socioeconomic status (**Table 1**). Investigators recorded each subject's height and weight. Investigators asked parents questions about family history of allergies, hyperactivity, and learning disabilities, and parents responded ("yes," "no," or "don't know") and identified the specific family members. Parents also completed questions about their son's symptoms of allergies and frequency of infections: the frequencies of ear infections and antibiotic use since birth were rated on a scale of 1 to 5 (1 was 0–1 antibiotic prescriptions and/or infections and 5 was $>$ 10 antibiotic prescriptions and/or infections). Also, the number of colds in the past year was reported. Parents were asked if their son had ever had tubes surgically placed in his ears because of repeated ear infections. Parents evaluated seven signs of possible EFA deficiencies (thirst, frequent urination, dry skin, dry hair, dandruff, brittle nails, and follicular keratoses) and somatic complaints (headaches, stomachaches, diarrhea, and constipation) using a scale similar to that of the Conners' questionnaires of 0 to 3 ("not at all," "just a little," "pretty much," and "very much").

TABLE 1
Characteristics of boys with attention deficit hyperactivity disorder (ADHD) are control subjects[1]

	Control subjects (n = 43)	ADHD (n = 53)
Age (y)	9.1 ± 2.3[2]	9.1 ± 2.0
Race (% white)	100	92.4
Adopted (%)	0	7.6
Height (cm)	140.0 ± 17.3	137.4 ± 11.9
Weight (kg)	34.8 ± 12.7	32.1 ± 8.9
BMI (kg/m^2)	17.6 ± 3.9	16.5 ± 2.5
Breast-fed (%)	81.4	45.3[3]
Duration of breast-feeding (mo)	6.5 ± 5.3	2.5 ± 4.0[4]
Taking stimulant medication (%)[5]	—	43.4
Behavior assessment		
Conners' Parent Rating Scale-48	4.7 ± 3.3	20.1 ± 4.5[4]
Conners' Teacher Rating Scale-28	3.5 ± 3.8	15.6 ± 7.1[4]
Temper tantrums (%)[6]	7.0	54.7[4]
Problems getting to sleep (%)[6]	7.0	39.6[3]
Problems waking up (%)[6]	0	32.1[4]
Somatic complaints		
Headaches (%)[6]	7.0	18.9
Stomachaches (%)[6]	0	15.1[7]
Infections (%)		
>3 Colds in past year (%)	9.3	13.2
>10 Antibiotics since birth	32.6	56.6[8]
>10 Ear infections since birth	9.3	30.2[8]
Surgical tubes in ears (%)	7.1	28.3[9]
Allergy history (%)		
Any allergy	53.5	56.6
Asthma	9.3	32.1[10]
Hay fever	11.9	17.3
Eczema	7.0	9.0
Chronic stuffy nose	39.5	43.4
Hives	11.6	7.6

[1] Categorical variables expressed as percentages were analyzed by Fisher's exact two-tailed test. Continuous variables expressed as arithmetic $\bar{x}$ ± SD were analyzed by Student's two-tailed t tests.
[2] $\bar{x}$ ± SD.
[5] Ritalin or Cylert.
[6] Percent of control subjects or subjects with ADHD whose symptoms were rated as one of the two most severe categories. Each subject's symptoms were rated on a 4-point scale: 0 = not all all, 1 = just a little, 2 = pretty much, and 3 = very much.
[3,4,7–11] Significantly different from control subjects: [3] $P < 0.0003$, [4] $P < 0.0001$, [7] $P < 0.008$, [8] $P < 0.02$, [9] $P = 0.009$, [10] $P < 0.01$.

Blood collection

Ten milliliters of venous blood was drawn from each subject and immediately put on ice. The blood was then centrifuged at 1400 × g for 20 min at 4 °C and plasma reserved for fatty acid analyses. Plasma samples were stored in glass vials at −80 °C. RBCs were resuspended in phosphate-buffered saline and washed gently for 5 min. Samples were centrifuged and the upper layer discarded. The RBC pellet was resuspended to 50% of the original volume and dispensed into glass vials for analysis of RBC fatty acids. Samples were stored at −80 °C.

Fatty acid analyses

Lipids were extracted from plasma and RBCs by using the method of Bligh and Dyer (17) and evaporated to dryness

under nitrogen. Plasma samples were processed to separate neutral from polar lipids by solid-phase extraction using a modification of the method of Hamilton and Comai (18) as follows: lipids were dissolved in 2.0 mL hexane:methyltertiarybutylether (MTBE) (200:3, by vol) and applied to silica PrepSep (Fisher Scientific, Fair Lawn, NJ) cartridges. After being washed with 4.0 mL MTBE:acetic acid (100:0.2, by vol), triacylglycerols, monoacylglycerols, cholesterol, cholesterol esters, and fatty acids were eluted by using 8.0 mL MTBE: acetic acid (100:0.2, by vol) and were discarded. Polar lipids were eluted from the column by using 20 mL MTBE:methanol:1 mmol ammonium acetate/L (pH 8.6) (5:8:2, by vol). Methyl esters of RBC total fatty acids (FAMEs) and plasma polar lipids were prepared by using boron trifluoride (14%) and analyzed by capillary gas-liquid chromatography (19) in the Lipid Chemistry and Metabolism Laboratory in the Department of Food Science, Purdue University. The methyl esters were extracted in isooctane for chromatographic analysis by an HP 5890 Series II gas chromatograph equipped with a flame-ionization detector, HP Chemstation, and autosampler (model 7673; Hewlett-Packard Co, Avondale, PA). A DB 23 fused-silica capillary column (J & W Scientific Co, Rancho Cordova, CA; 30 m × 0.53 mm internal diameter, 0.5-μm film thickness) was used with helium as the carrier gas. The initial oven temperature of 175 °C was held for 10 min and increased at a rate of 1 °C/min until the final temperature of 210 °C was reached. The total gas-chromatographic run was 50 min. All samples were introduced by split injection (1:50). An external standard mixture prepared from known amounts of triacylglycerols and methylated fatty acids (Nu Check-Prep, Elysian, MN) was used to obtain retention times. Fatty acid values are presented as area percentages.

Dietary intake

The completion of 3-d diet records was explained in detail to parents by using food models to illustrate serving sizes. These records were analyzed by computer for macro and micro components with the computer software program FOOD ANALYST PLUS (Hopkins Technology, Hopkins, MN).

Statistical analysis

Statistical analyses of the data were performed on an IBM 3090 computer with the use of SAS (SAS Institute, Cary, NC) computer programs. Fisher's exact test was used to evaluate categorical responses to health-related questions (20). Continuous variables were analyzed by using Student's two-tailed t test. Some of the continuous data did not meet normality assumptions necessary to perform t tests so the Kruskal-Wallis nonparametric test was applied. A P value of 0.05 was considered statistically significant. Continuous data are expressed as arithmetic mean ± SD. A total of 112 tests were conducted, each with a type I error of 0.05. Six significant relations would be expected by chance; 35 were established.

RESULTS

Descriptive, behavioral, and health-related data on the subjects in the study are summarized in Table 1. Initially, the control group, the unmedicated subjects with ADHD, and the medicated subjects with ADHD were compared. However, with the exception of "problems getting to sleep," no significant differences between the medicated and unmedicated subjects with ADHD were found. "Problems getting to sleep" is not surprising because a common side effect of stimulant medication is insomnia (21). These data, therefore, were analyzed by comparing the control subjects with the subjects with ADHD (unmedicated and medicated combined) and are presented below. Control subjects and subjects with ADHD were not found to differ significantly with regard to age, height, weight, body mass index, or socioeconomic status. Eighty-one percent (35 of 43) of the control subjects had been breast-fed compared with 45% (24 of 53) of the subjects with ADHD ($P < 0.0003$). The duration of breast-feeding was significantly longer in the control subjects than in subjects with ADHD ($P < 0.0001$). The incidence of asthma was significantly higher in the subjects with ADHD ($P < 0.01$), but the frequencies of other allergies were not. Subjects with ADHD complained of significantly more stomachaches ($P < 0.008$) than did the control subjects. There was no difference in the two groups regarding the number of colds in the last year, but subjects with ADHD complained of more ear infections ($P < 0.02$) and used more antibiotics ($P < 0.02$) since birth than did the control subjects. A greater percentage of the subjects with ADHD had had tubes placed in their ears because of repeated ear infections compared with the control subjects ($P < 0.009$). Previous studies of children with ADHD have also reported higher frequencies of allergies (22, 23), somatic complaints (15, 24) and ear infections (15, 25).

Parents' ratings regarding the frequency of symptoms indicative of EFA deficiency are summarized in **Table 2.** Again, initially, the control group, the unmedicated subjects with

TABLE 2
Signs of possible essential fatty acid (EFA) deficiencies in control subjects and subjects with attention-deficit hyperactivity disorder (ADHD)[1]

	Control subjects (n = 43)	ADHD (n = 53)
Thirst (%)[2]	16.3	45.3[4]
Frequent urination (%)[2]	7.0	34.0[4]
Dry hair (%)[2]	0	13.2[5]
Dandruff (%)[2]	0	7.6
Dry skin (%)[2]	4.7	11.3
Follicular keratoses (%)[2]	11.6	5.7
Brittle nails (%)[2]	2.3	1.9
Total EFA deficiency score (%)[6]	9.3	39.6[7]
Fluid intake (mL)[8]	1691 ± 522	1764 ± 664

[1] Categorical variables expressed as percentages were analyzed by Fisher's exact two-tailed test. Fluid intake was analyzed by Student's two-tailed t test.

[2] Percent of control subjects or subjects with ADHD whose symptoms were rated as one of the two most severe categories. Each subject's symptoms were rated on a 4-point scale: 0 = not at all, 1 = just a little, 2 = pretty much, and 3 = very much.

[6] Percent of subjects with a total EFA deficiency score > 3. The total EFA deficiency score is the sum of the scores (on a 4-point scale) for each of the seven individual items.

[8] $\bar{x}$ ± SD.

[4,5,7] Significantly different from control subjects: [4] $P < 0.004$, [5] $P < 0.02$, [7] $P < 0.0009$.

ADHD, and the medicated subjects with ADHD were compared, but with the exception of dandruff ($P < 0.05$), no significant differences were found. (The seemingly positive influence of medication on dandruff is perhaps an anomalous finding.) Therefore, the data were analyzed by comparing the control subjects with the subjects with ADHD (unmedicated and medicated), and these results are presented. The percentage of subjects described as having "pretty much" or "very much" (the two most severe degrees) thirst ($P < 0.004$), frequent urination ($P < 0.002$), and dry hair ($P < 0.02$) were significantly higher (based on Fisher's exact two-tail test) in the subjects with ADHD. The same test showed that the total score of the seven deficiency symptoms combined was significantly higher in the subjects with ADHD than in the control subjects ($P < 0.0009$). However, t tests showed that mean fluid intake, calculated from the 3-d diet records, was not significantly different.

The results of the analysis of plasma FAMEs expressed as area percentages are given in **Table 3.** When mean plasma concentrations were compared between the unmedicated and medicated subjects with ADHD, t tests revealed that the only significant difference was for 18:3n−3 ($P < 0.02$). The medicated group ($n = 24$) had mean concentrations of 0.01 ± 0.04 and the unmedicated group ($n = 29$) had mean concentrations of 0.06 ± 0.10. Because no other significant differences were found between the unmedicated and the medicated subjects with ADHD, the data in Table 3 were analyzed by comparing the control subjects with the subjects with ADHD (unmedicated and medicated combined). The subjects with ADHD had mean concentrations of 20:4n−6 ($P < 0.02$), 20:5n−3 ($P < 0.02$), and 22:6n−3 ($P < 0.03$) significantly lower than those of the control subjects. Also, the mean content of 18:1 was significantly higher ($P < 0.05$) and 24:1 was significantly lower ($P < 0.05$) in subjects with ADHD. The mean content of total n−3 fatty acids was lower ($P < 0.001$) in the ADHD group. However, there was no difference between the two groups in total mean concentrations of n−6 fatty acids. The mean ratio of total n−6 to n−3 fatty acids in the subjects with ADHD compared with the control subjects was significantly increased ($P < 0.002$).

TABLE 3
Fatty acid composition of polar lipids isolated from plasma of control subjects and subjects with attention-deficit hyperactivity disorder (ADHD)[1]

Plasma fatty acids	Control subjects ($n = 43$)	ADHD ($n = 53$)
	area %	
Saturated		
13:0	ND[2]	ND
14:0	ND[2]	ND
16:0	24.02 ± 2.71	23.50 ± 1.43
17:0	0.47 ± 0.07	0.46 ± 0.08
18:0	14.79 ± 1.01	14.57 ± 0.98
20:0	0.07 ± 0.11	0.04 ± 0.09
22:0	0.65 ± 0.26	0.66 ± 0.38
24:0	ND[2]	ND
Monounsaturated		
16:1	0.41 ± 0.22	0.39 ± 0.17
18:1	11.12 ± 1.43	11.76 ± 1.64[3]
24:1	0.60 ± 0.29	0.45 ± 0.35[3]
n−6		
18:2n−6 *cis*	22.47 ± 2.44	23.09 ± 2.77
18:2n−6 *trans*	0.23 ± 0.18	0.25 ± 0.17
18:3n−6	0.07 ± 0.13	0.10 ± 0.40
20:3n−6	3.01 ± 0.57	3.14 ± 0.69
20:4n−6	11.00 ± 1.40	10.33 ± 1.42[4]
22:4n−6	0.65 ± 0.17	0.60 ± 0.22
22:5n−6	0.57 ± 0.19	0.54 ± 0.21
n−3		
18:3n−3	0.06 ± 0.09	0.04 ± 0.08
20:5n−3	0.24 ± 0.21	0.15 ± 0.21[4]
22:5n−3	1.35 ± 0.32	1.29 ± 0.39
22:6n−3	2.04 ± 0.58	1.78 ± 0.45[5]
Totals and ratios		
Σ n−6 fatty acids	37.88 ± 1.82	37.62 ± 2.57
Σ n−3 fatty acids	3.65 ± 0.66	3.22 ± 0.60[6]
Σ n−6: Σ n−3	10.68 ± 1.79	12.04 ± 2.30[7]

[1] $\bar{x}$ ± SD. Variables were analyzed by using Student's two-tailed t test. Because some of the variables did not meet normality assumptions, t tests were supplemented by a nonparametric method, the Kruskal-Wallis test.
[2] None detected.
[3-7] Significantly different from control subjects: [3] $P < 0.05$, [4] $P < 0.02$, [5] $P < 0.03$, [6] $P < 0.001$, [7] $P < 0.002$.

The results of analysis of total RBC FAMEs expressed as area percentages are given in **Table 4.** Similar to the plasma fatty acid results, the mean concentrations of 20:4n−6 ($P < 0.02$), 22:4n−6 ($P < 0.03$), and 22:6n−3 ($P < 0.06$) in RBCs were reduced in the ADHD group compared with the control subjects. However, 20:5n−3 was not detected in the RBCs. The mean concentration of one saturated fatty acid–16:0–was significantly lower in RBCs in subjects with ADHD whereas the mean concentrations of 22:0 and 22:5n−6 were significantly higher ($P < 0.05$). Whereas the mean concentration of 18:1 was significantly elevated in plasma in the group with ADHD, it was significantly reduced in subjects with ADHD in the RBCs ($P < 0.05$). No difference in 24:1 was observed. None of the other fatty acid indexes was different.

The total EFA-deficiency score, comprising the sum of the seven possible EFA-deficiency-symptom scores, was used to identify a subgroup of subjects with ADHD who might be more likely to have EFA deficiencies. A subject with ADHD was considered to have a low score if his total deficiency score was ≤ 3, and a high score if his EFA-deficiency score was 4–12. A subgroup of 21 children with ADHD, who showed high EFA-deficiency scores, was compared with the remaining boys with ADHD ($n = 32$), who showed low EFA-deficiency scores. The mean area percentages of FAMEs were compared for all plasma and RBC fatty acids, and those that differed significantly between control subjects and subjects with ADHD are presented in **Table 5.** All the other fatty acids were checked and none was found to be significantly different between the two groups. Plasma concentrations of 20:4n−6 ($P < 0.001$) and 22:6n−3 ($P < 0.003$) were significantly lower in those with high EFA-deficiency scores. Values for these fatty acids for the group with low EFA-deficiency scores were not significantly different from those of the control subjects. Total n−3 fatty acids were significantly reduced in the subjects with high EFA-deficiency scores ($P < 0.007$).

RBC fatty acid concentrations were not significantly different between the two subgroups and were found to be either equally different from control subjects (as for 20:4n−6) or somewhat more different from the subgroup with low scores than from the subgroup with high scores for 18:1, 24:1, and

TABLE 4
Fatty acid composition of total lipids isolated from red blood cells (RBCs) of control subjects and subjects with attention-deficit hyperactivity disorder (ADHD)[1]

RBC fatty acids	Control subjects (n = 35)	ADHD (n = 46)
	area %	
Saturated		
13:0	3.89 ± 6.03	4.88 ± 5.85
14:0	0.08 ± 0.18	0.05 ± 0.16
16:0	16.44 ± 1.73	15.45 ± 1.78[2]
18:0	14.54 ± 1.64	14.32 ± 1.62
22:0	0.87 ± 0.89	1.23 ± 0.90[3]
24:0	3.12 ± 2.46	3.48 ± 2.25
Monounsaturated		
18:1	12.37 ± 2.01	11.26 ± 2.33[1]
20:1	ND[4]	ND
24:1	2.04 ± 1.60	2.61 ± 1.74
n−6		
18:2n−6	9.70 ± 1.54	9.28 ± 2.24
20:3n−6	1.73 ± 0.63	1.72 ± 0.82
20:4n−6	15.12 ± 2.39	13.74 ± 2.75[2]
22:4n−6	5.21 ± 0.90	4.72 ± 1.07[5]
22:5n−6	0.27 ± 0.68	0.73 ± 1.18[3]
n−3		
18:n−3	ND[4]	ND
20:5n−3	ND[4]	ND
22:5n−3	1.54 ± 1.35	1.58 ± 1.51
22:6n−3	2.18 ± 1.45	1.61 ± 1.31[6]
Totals and ratios		
Σ n−6 fatty acids	32.05 ± 4.46	30.26 ± 5.38
Σ n−3 fatty acids	3.72 ± 2.77	2.95 ± 2.59
Σ n−6: Σ n−3	8.01 ± 3.47	10.63 ± 6.36

[1] $\bar{x}$ ± SD. Variables were analyzed by using Student's two-tailed t test. Because some of the variables did not meet normality assumptions, t tests were supplemented by a nonparametric method, the Kruskal-Wallis test. Eight samples from the control group and seven samples from the ADHD group were lost as a result of experimental error.

[2,3,5,6] Significantly different from control subjects: [2] $P < 0.02$, [3] $P < 0.05$, [5] $P < 0.03$, [6] $P < 0.06$.

[4] None detected.

TABLE 5
Fatty acid composition of plasma polar lipids and red blood cell (RBC) total lipids from subjects with attention-deficit hyperactivity disorder (ADHD) with low compared with high essential fatty acid (EFA)-deficiency symptom scores[1]

Fatty acids	Low EFA-deficiency score[2] (n = 32)	High EFA-deficiency score[3] (n = 21)
	area %	
Plasma polar lipids		
Monounsaturated		
18:1	11.58 ± 1.65	12.03 ± 1.63
24:1	0.46 ± 0.31	0.45 ± 0.43
n−6		
20:4n−6	10.82 ± 1.39	9.59 ± 1.13[4]
n−3		
20:5n−3	0.17 ± 0.24	0.12 ± 0.16
22:6n−3	1.92 ± 0.50	1.58 ± 0.28[5]
Totals		
Σ n−3 fatty acids	3.38 ± 0.67	2.98 ± 0.36[6]
RBC total lipids[7]		
Monounsaturated		
18:1	11.16 ± 2.38	11.63 ± 2.29
24:1	2.83 ± 2.65	2.27 ± 1.50
n−6 fatty acids		
20:4n−6	13.77 ± 2.65	13.71 ± 2.96
22:4n−6	4.74 ± 1.19	4.70 ± 0.92
22:5n−6	0.71 ± 1.13	0.71 ± 1.25
n−3 fatty acids		
20:5n−3	ND[8]	ND
22:6n−3	1.45 ± 1.18	1.85 ± 1.47
Fluid intake (mL)	1574 ± 573	2064 ± 701[9]

[1] $\bar{x}$ ± SD. Based on total EFA deficiency score, which equals the sum of the scores (on a 4-point scale) for each of seven individual items: thirst, frequent urination, dry hair, dandruff, dry skin, follicular keratoses, and brittle nails. Variables were analyzed by using Student's two-tailed t test. Because some of the variables did not meet normality assumptions, t tests were supplemented by a nonparametric method, the Kruskal-Wallis test.

[2] Total EFA-deficiency score ≤ 3.

[3] Total EFA-deficiency score > 3.

[7] Low total EFA-deficiency score (n = 27) compared with high total EFA-deficiency scores (n = 19).

[8] None detected.

[4–6,9] Significantly different from subjects with a low EFA-deficiency score: [4] $P < 0.001$, [5] $P < 0.003$, [6] $P < 0.007$, [9] $P < 0.01$.

22:6n−3. Fluid intake was increased significantly in the group with high EFA-deficiency scores ($P < 0.01$). This group also showed a significantly greater incidence of allergic rhinitis ($P < 0.006$), temper tantrums ($P < 0.01$), and problems getting to sleep ($P < 0.05$) than did subjects with ADHD with low EFA-deficiency scores. Medication use was not significantly different between the group with low EFA-deficiency scores (15 of 32) and the group with high scores (9 of 21).

The results of the food-composition analysis of the 3-d diet records of control subjects and subjects with ADHD are summarized in **Table 6.** There were no significant differences between the control subjects and subjects with ADHD in energy, protein, carbohydrate, vitamin, and mineral intakes. However, for the recorded 3 d, the ADHD group consumed on average more fat ($P < 0.02$) and more polyunsaturated fatty acids (PUFAs) ($P < 0.05$). When subjects with ADHD with low EFA-deficiency scores were compared with those with high EFA-deficiency scores, there were no significant differences in dietary intake, except fluid consumption. Note that the food-composition data banks are not always complete with respect to individual nutrients.

Correlation statistics for 20:4n−6, 22:6n−3, the Conners' Parent and Teacher Rating Scales, the EFA-deficiency score, and duration of breast-feeding are shown in **Table 7.** Concentrations of 20:4n−6 were negatively correlated with the total deficiency score, whereas 22:6n−3 was negatively correlated with the Conners' Parent Rating Scale (but not the Teachers' Rating Scale) and with the EFA-deficiency score. The EFA-deficiency score was positively correlated with the Parent Rating Scale. The duration of breast-feeding was negatively correlated with the Conners' Parent and Teacher Rating Scales.

DISCUSSION

ADHD, the most common childhood behavioral disorder, is the label given to children who show a chronic pattern of

TABLE 6
Food-composition data from 3-d diet records in control subjects and in subjects with attention-deficit hyperactivity disorder (ADHD)[1]

Nutrient	Control subjects (n = 42)	ADHD (n = 50)
Energy (kJ)	8799 ± 1885	9585 ± 2412
Protein (g)	74 ± 18	76 ± 22
Fat (g)	75 ± 18	87 ± 28[2]
Polyunsaturated fatty acids (g)[3]	9.0 ± 4.8	11.7 ± 7.2[4]
Monounsaturated fatty acids (g)[3]	20.1 ± 4.8	23.3 ± 10.2
Saturated fatty acids (g)[3]	27.7 ± 7.9	30.7 ± 10.1
Cholesterol (mg)	236 ± 112	262 ± 114
Energy composition		
Protein (% of energy)	13.9 ± 2.3	13.3 ± 2.4
Carbohydrates (% of energy)	54.7 ± 5.7	53.5 ± 6.3
Fat (% of energy)	31.5 ± 4.6	33.3 ± 4.9
Carbohydrates (g)	293 ± 76	310 ± 84
Dietary fiber (g)	2.8 ± 2.0	2.3 ± 1.6
Calcium (mg)	1088 ± 398	1047 ± 417
Iron (mg)	14.1 ± 6.7	14.9 ± 7.1
Magnesium (mg)	218 ± 82	197 ± 58
Zinc (mg)	8.8 ± 3.8	8.9 ± 4.0
Ascorbic acid (mg)	136 ± 111	133 ± 90
Vitamin A (RE)	1046 ± 646	1041 ± 546
Vitamin B-6 (mg)	1.6 ± 0.8	1.7 ± 0.8
Vitamin B-12 (μg)	4.9 ± 3.0	4.8 ± 2.5
Thiamin (mg)	1.7 ± 0.6	1.8 ± 0.6
Riboflavin (mg)	2.2 ± 0.9	2.5 ± 0.9
Niacin (mg)	19.9 ± 6.9	21.9 ± 8.8
Folate (μg)	239 ± 146	259 ± 171
Pantothenic acid (mg)	4.1 ± 2.3	4.3 ± 3.3

[1] $\bar{x}$ ± SD. Variables were analyzed by using Student's two-tailed t test. Because some of the variables did not meet normality assumptions, t tests were supplemented by a nonparametric method, the Kruskal-Wallis test.
[2,4] Significantly different from control subjects: [2] $P < 0.02$, [4] $P < 0.05$.
[3] Missing values for some foods in the database.

inattention, impulsivity, and hyperactivity. Many studies have suggested a variety of etiologies for this disorder, thus ADHD appears to be multifactorial. A few studies have focused on EFA status in subjects with ADHD. The results of the study presented here show that this sample of subjects with ADHD had significantly lower amounts of specific polar lipid fatty acids in plasma (20:4n−6, 20:5n−3, and 22:6n−3), and also lower concentrations of total fatty acids in RBCs (20:4n−6, 22:4n−6, and 22:6n−3). Mitchell et al (15) also found significantly lower plasma concentrations of 20:4n−6 and 22:6n−3 in subjects with hyperactivity. These researchers also reported significantly lower concentrations of 20:3n−6, but the current study found no significant differences in area percentages for this PUFA.

Because previous work indicated that some subjects with ADHD showed signs of a possible EFA deficiency (11, 15), parents were asked to evaluate symptoms that have been reported in the literature to be associated with EFA deficiency in animals and humans: thirst (12), frequent urination (15), dry skin (26), dry hair (12), follicular keratoses (27), dandruff (12), and brittle nails (28). In this study, boys with ADHD were reported to have greater thirst, more frequent urination, and a greater incidence of dry skin than control subjects.

To test whether subgroups within the ADHD group might differ in fatty acid content of plasma polar lipids and RBC lipids, the subjects with ADHD were divided into two groups according to low or high EFA-deficiency scores. Fluid intake was significantly greater in the group with high EFA-deficiency scores compared with the group with low EFA-deficiency scores. Subjects with high EFA-deficiency scores had significantly lower plasma polar lipid concentrations of 20:4n−6 and 22:6n−3 than did those subjects with ADHD having low EFA-deficiency scores. Subjects with low EFA-deficiency scores had plasma polar lipid fatty acid concentrations similar to those of control subjects. These same trends were not seen with the RBC samples, because no differences were observed in the RBC fatty acid concentrations between these two groups. The reason for the discrepancy in results between the two sites of measurement is not clear, but may be due to the presence of different pools of fatty acids in the RBCs: a slow-turnover "structural" pool and a second pool that is in equilibrium with the plasma pool (29, 30). Future studies will address this issue.

Decreased cellular concentrations or pool sizes of 20:4n−6 and 22:6n−3 could adversely affect behavior (31, 32). First, lower concentrations of substrate might decrease the production of eicosanoids, which act as mediators or modulators of nerve transmission in the central nervous system (32). Second, because 22:6n−3 is the predominant PUFA in the polar lipids of the cerebral cortex and retina, especially in cell membranes that are the most fluid and metabolically active (32), decreased concentrations of this fatty acid might negatively affect the function of the retina and cerebral cortex in several ways. A decrease in the concentration of 22:6n−3 could affect fluidity and transport processes (33–35). The PUFA composition of membranes affects not only their biological and physical properties but also that of the membrane-bound proteins (33–35).

Several observations in rats and/or monkeys have supported a relation between fatty acid depletion and behavioral or neurological dysfunction. Enslen et al (36) showed that rats fed a diet low in n−3 fatty acids had decreased exploratory behavior in a new environment. However, Wainwright (37) noted a lack of conclusive evidence for lower fatty acid concentrations affecting learning independently of performance by influencing sensation, motivation, or other factors that affect performance. Connor et al (38) and Reisbick et al (39, 40) fed monkeys a diet deficient in n−3 fatty acids and reported decreased visual acuity and polydipsia. In a study of home cage behavior, Reisbick et al (41) reared monkeys on a diet deficient in n−3 fatty acids and reported significantly more bouts of total locomotion in deficient monkeys than in control monkeys on a soybean-oil diet or a standard reference group. Holman et al (42) reported the case of a young child maintained on total parenteral nutrition rich in 18:2n−6 but low in 18:3n−3. They found that neurological symptoms developed but were totally corrected by supplementation with 18:3n−3. In other case studies (43) symptoms of n−3 deficiency included impaired visual acuity, neurological dysfunction, and dermatitis. These symptoms disappeared when feedings were supplemented with n−3 fatty acids.

The reason for the differences in 20:4n−6 and 22:6n−3 contents of plasma polar lipids and RBC total lipids between the control subjects and boys with ADHD in this study is not clear. Primary deficiency was the first factor considered, but a lower intake of n−6 fatty acids does not appear to be a causative factor because the plasma 18:2n−6 content was not different between the groups; there was no indication of the

TABLE 7
Estimated Pearson correlation coefficients for all subjects[1]

	Conners' Parent Rating Scale	Conners' Teacher Rating Scale	EFA-deficiency score[2]	20:4n−6	22:6n−3	Duration of breast-feeding
Conners' Parent Rating Scale	1.00	0.67[3]	0.33[3]	−0.14	−0.27[3]	−0.38[3]
Conners' Teacher Rating Scale		1.00	0.23[3]	−0.07	−0.17	−0.27[3]
EFA-deficiency score			1.00	−0.37[3]	−0.33[3]	−0.14
20:4n−6				1.00	0.49[3]	0.25[3]
22:6n−3					1.00	0.20[3]
Duration of breast-feeding						1.00

[1] SAS analysis (PROC CORR) of correlation coefficients (20). n = 82–96 subjects.
[2] Total essential fatty acid (EFA)-deficiency score equals the sum of the scores (on a 4-point scale) for each of seven individual items: thirst, frequent urination, dry hair, dandruff, dry skin, follicular keratoses, and brittle nails.
[3] $P < 0.05$.

presence of Mead acid (20:3n−9), which is elevated in EFA deficiency (44); and the results of the dietary analysis suggest that the subjects with ADHD consumed more PUFAs than did the control subjects. On the other hand, the possibility of a low n−3 intake cannot be ruled out. n−3 Fatty acid deficiency is characterized by lower concentrations of 20:5n−3, 22:5n−3, and 22:6n−3 in plasma but higher concentrations of 22:4n−6 and 22:5n−6 (43). We did find that subjects with ADHD had lower concentrations of 20:5n−3 and 22:6n−3 in the plasma whereas 22:5n−6 was significantly higher only in the RBCs. Additionally, dietary intake data did not provide an accurate estimate of n−3 intake because there was incomplete nutritional information for many foods.

Another possible reason to explain the lower concentrations of 20:5n−3, 22:6n−3, and 20:4n−6 is a poorer ability to convert 18-carbon fatty acids to longer, more highly unsaturated fatty acids. The data suggest that this may be the case for n−6 fatty acids, because the 20:4n−6 content of serum polar lipids can be used as a general measure of 18:2n−6 metabolism (44). However, not all of the results are consistent with this reasoning, because some long-chain PUFAs were not different between the groups: 22:5n−6 in RBCs was actually higher in the ADHD group.

Additional potential physiologic causes for the lower concentrations of 20:4n−6 and 22:6n−3 in plasma and RBC polar lipids could include increased metabolism of specific fatty acids like 20:4n−6 and 22:6n−3 to eicosanoids or an impaired systemic or cellular transport system. Thus, a multiplicity of physiologic and environmental causes might be expressed on an individual basis and may explain why two previous studies in which supplementation with primrose oil (a concentrated source of 18:3n−6) failed to improve behavior significantly (45, 46). Supplementation of oils to ameliorate the observed abnormalities in fatty acid concentrations of subjects with ADHD might be successful if treatment were based on individual fatty acid profiles. Additionally, the link between lower concentrations of EFA metabolites in plasma phospholipids and the expression of behavioral abnormalities in some subjects with ADHD needs to be established before supplementation with specific oils should be considered, except in an experimental context.

We thank Carol Karahadian, Thomas Kuczek, and Leo Galland for their technical advice and encouragement.

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