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Abnormal fatty acids in Canadian children with autism

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ABSTRACT

Objective: Fatty acids are critical for pediatric neurodevelopment and are abnormal in autism, although prior studies have demonstrated conflicting results and methodological differences. To our knowledge, there are no published data on fatty acid in Canadian children with autism. The aim of this study was to investigate red blood cell and serum fatty acid status to identify whether abnormalities exist in Canadian children with autism, and to enhance future cross-study comparison.

Methods: Eleven Canadian children with autism (3 girls, 8 boys; age 3.05 ± 0.79 y) and 15 controls (9 girls, 6 boys; age 3.87 ± 1.06 y) met inclusion criteria, which included prior Diagnostic and Statistical Manual diagnosis of autism spectrum disorder, no recent medication or supplements, no specialty diets, and no recent illness.

Results: The children with autism demonstrated lower red blood cell docosahexaenoic acid ($P < 0.0003$), eicosapentaenoic acid ($P < 0.03$), arachidonic acid ($P < 0.002$), and ω-3/ω-6 ratios ($P < 0.001$). They also demonstrated lower serum docosahexaenoic acid ($P < 0.02$), arachidonic acid ($P < 0.05$), and linoleic acid ($P < 0.02$) levels.

Conclusions: Fatty acids in both serum and red blood cells were abnormal in this small group of Canadian children with autism than in controls, underlining a need for larger age- and sex-matched investigations in this community. A potential role for fatty acid abnormalities within the complex epigenetic etiology of autism is proposed in relation to emerging understanding of relationships between cobalamin metabolism, gut microbiota, and propionic acid production.

Introduction

The role of essential fatty acids (EFAs) in childhood development and neurodevelopmental abnormalities is an evolving area of science. It is known that essential polyunsaturated fatty acids (PUFAs) are critical for normal brain development in children [1]. Infants of mothers supplemented with EFAs, and docosahexaenoic acid (DHA) in particular, demonstrate improved cognitive and psychomotor development [2]. There is a growing body of evidence for the potential utility of fatty acid supplementation in attention deficit disorder and attention-deficit hyperactivity disorder [3–5].

In children with autism, controlled supplementation trials demonstrate therapeutic promise but lack statistical power [6–9]. Cross-sectional studies measuring the fatty acid profiles of children with autism in different countries have demonstrated conflicting results. Overall, the majority of studies show lower levels of the EFA metabolites DHA and arachidonic acid (AA) [7–11]. However, differences in methodology render cross-study comparisons difficult.

To our knowledge, there are currently no published data for fatty acid levels in the serum or red blood cells (RBCs) of children with autism in Canada. The present study sought to measure RBC and serum fatty acid levels in a subgroup of Canadian children with autism compared with age-matched controls. The

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concomitant determination of RBC and serum fatty acid levels is intended to enhance future cross-study comparison with pediatric autism data sets in other countries.

Participants and methods

Study participants

Twenty children (age 3.90 ± 1.68 y; 4 girls, 16 boys) with a recent diagnosis of autism spectrum disorder (ASD) were recruited through the regional Community Care Access Centre, the Rotary Children's Centre, and local physicians and pediatricians. ASD diagnosis was established by regional developmental pediatricians and psychologists in accordance with Diagnostic and Statistical Manual criteria before study participation. Study eligibility required verbal parental confirmation of diagnosis; hard copy confirmation of diagnosis was not collected. After prior allocation of blood samples to the previously published assessment of trace element status [12] among this study group, sufficient blood for serum and RBC lipid analysis was available for 10 and 11 of the children with autism, respectively.

Of the 20 healthy control children recruited through Guelph University and city-run day cares, only 15 (age 3.87 ± 1.06 y; 9 girls, 6 boys) met full study eligibility criteria. Eligibility criteria for both children with autism and controls included no history of specialty diets (eg, gluten-free/casein-free), no recent history of nutritional supplements or pharmaceutical medications, and no recent illness in the 2 wk before blood collection. After prior allocation of blood samples to the previously published assessment of trace element status [12], sufficient blood was available for RBC lipid analysis in all 15 control children but for serum lipid analysis in 14.

Because of recruitment challenges, the study children were successfully matched for age but not sex, which may represent a limitation to interpretation of the results.

Study design

This study was designed as a one-time cross-sectional comparison of EFAs in RBCs and serum to assess potential differences between children with autism and healthy controls of the same age. A 5-d food diary was requested for all participants. However, compliance with diet documentation for the children with autism was too poor to provide reliable comparison data. This absence of intake data may limit interpretation of the phospholipid findings, but is consistent with other observational and supplementation phospholipid studies in children with autism.

Human ethics approval was carried out by Institutional Review Board Services, Aurora, Ontario, Canada.

Sample collection and treatment

Morning fasting blood samples were collected in lithium-heparin trace element–free evacuated tubes. For serum fatty acids, the samples were centrifuged at 2.7 *g* for 15 min at 25°C. The recovered serum was portioned and stored at −80°C until analyzed. The fatty acid compositions of total serum phospholipid were measured after extraction and derivatization of the fatty acid methyl esters, followed by capillary gas-liquid chromatography (GLC) [13].

The fatty acid compositions of the RBC preparations were determined after lipid extraction by a modification of a previously described method [14]. Aliquots of the lipid extract (in chloroform) were taken to dryness under nitrogen and subjected to transmethylation [15] followed by GLC analyses of the resulting fatty acid methyl esters based on previous studies [16,17]. GLC analyses were conducted following injection into a Varian GLC (Varian, Palo Alto, CA, USA) with a 60-m DB-23 capillary column (0.32 mm internal diameter).

When insufficient blood volume was collected from the young participants, red cell phospholipid assessment was prioritized over serum assessment.

Statistical analysis

Comparison of serum and RBC phospholipid levels between autistic and control children was carried out using the Student's *t* test with significance set at 0.05. Important trends were identified where statistical *P* values fell between 0.05 and 0.1. No corrections were made for multiple comparisons.

Results

RBC levels of fatty acids for children with and without autism are summarized in Table 1. The children with autism demonstrated significantly lower RBC DHA and eicosapentaenoic acid (EPA) status. RBC AA levels and the ratio of total ω-3 to ω-6 fatty acids were significantly lower in the children with autism. There was a trend toward lower linoleic acid (LA) levels in children with autism than in controls ($P < 0.07$).

Table 1
Red blood cell fatty acids

	Autistic children (n = 11) (3 girls, 8 boys) Mean (SD)	Control children (n = 15) (9 girls, 6 boys) Mean (SD)	*P* value
Age (y)	3.05 (0.79)	3.87 (1.06)	
DHA (%)	2.23 (1.42)	4.5 (1.43)	0.0003
EPA (%)	0.4 (0.52)	0.64 (0.27)	0.03
ALA (%)	0.09 (0.07)	0.08 (0.08)	NS
LA (%)	10.18 (3.65)	12.15 (1.39)	0.07
AA (%)	9.72 (5.43)	15.8 (1.3)	0.002
ω-3: ω-6	0.16 (0.05)	0.24 (0.06)	0.001
AA:DHA	4.59 (1.63)	3.69 (1.15)	NS
AA:EPA	57.86 (94.79)	28.70 (15.38)	NS

AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; NS, nonsignificant

The differences in serum fatty acids of children with and without autism are summarized in Table 2. Serum DHA ($P < 0.02$), AA ($P < 0.05$), and LA ($P < 0.02$) levels were lower for children with autism than for controls. The autistic children also demonstrated a trend toward higher ratios of AA to DHA ($P < 0.06$) and lower ratios of total ω-3 to ω-6 ($P < 0.07$).

Discussion

In the present study, AA levels were lower in both RBCs and plasma in the children with autism. These findings are consistent with lower plasma AA levels among children with autism in Japan, Saudi Arabia, and France [11,12,18] and lower red cell AA among children with autism in Scotland and the United States [19,20].

In the present study, the children with autism demonstrated lower serum LA and a trend toward lower RBC LA ($P < 0.07$). However, results from comparative studies are highly variable; children with autism demonstrated higher plasma LA in Saudi Arabia [12], higher RBC LA in Scotland [19], and lower blood spot LA in Egypt [8], whereas other studies found no difference in plasma [11] or RBC [21] LA.

LA is a precursor to AA. The literature has shown that AA levels are consistently lower than levels of LA in children with autism [11,12,18,19,20]. Given the relative inconsistency of LA levels in the literature, AA status in pediatric autism may be more

Table 2
Serum fatty acids

	Autistic children (n = 10) (2 girls, 8 boys) Mean (SD)	Control children (n = 14) (8 girls, 6 boys) Mean (SD)	*P* value
Age (y)	2.95 (0.76)	3.79 (1.05)	
DHA (%)	2.39 (0.57)	3.4 (1.5)	0.02
EPA (%)	0.64 (0.28)	0.83 (1.49)	NS (0.1)
ALA (%)	0.21 (0.10)	0.22 (0.09)	NS
LA (%)	19.63 (3.75)	22.72 (2.51)	0.02
AA (%)	9.7 (1.32)	10.66 (1.4)	0.05
ω-3: ω-6	0.12 (0.02)	0.15 (0.06)	0.07
AA:DHA	4.19 (0.71)	3.56 (1.17)	0.06
AA:EPA	17.96 (8.04)	16.64 (7.82)	NS

AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; NS, nonsignificant