Title: Plasma and red blood cell n3 fatty acids correlate positively with the WISC-R verbal and full-scale intelligence quotients and inversely with Conner's parent-rated ADHD index t-scores in children with high functioning autism and Asperger's syndrome.

Beata Joanna Kozielec - Oracka[1], Yoeju Min[2], Amritpal S Bhullar[3], Barbara Stasiak[4], Kebreab Ghebremeskel[2]

[1]Psychiatric Clinic for Children and Youth, The Children's Memorial Health Institute, Warsaw, Poland

[2] Lipidomics and Nutrition Research Centre, School of Human Sciences, London Metropolitan University, London, UK

[3] Division of Human Nutrition, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada

[4]Mental Health Clinic for Children and Youth, Masovian Neuropsychiatry Center, Warsaw, Poland

Corresponding author:

Name: Professor Kebreab Ghebremeskel

Address: Lipidomics and Nutrition Research Centre, School of Human Sciences, London Metropolitan University
166-220 Holloway Road, London N7 8DB, UK

Tel: +44 20 7133 2440

Fax: +44 20 7133 2453

Email: k.ghebremeskel@londonmet.ac.uk

Short title: Fatty acids, cognitive ability and behaviour of children with high functioning autism and Asperger's syndrome

Keywords: N3 and n6 polyunsaturated fatty acids, behaviour, cognition, high functioning autism, Asperger's syndrome.

Declaration of conflicts of Interest: The authors declare no conflicts of Interest

ABSTRACT

Findings of the fatty acid status of people with autism spectrum disorders have been incongruent perhaps because of the diversity of the condition. A ***cross-sectional*** design study was used to investigated fatty acid levels and relationships between fatty acids, and cognition and behaviour in a homogenous group of children with autism spectrum disorder. Children with Asperger's syndrome (AS) /high functioning autism (n=44) and healthy siblings (n=17) were recruited from the Diagnostic and Therapeutic Centre for Children with Autism, Warsaw, Poland. In the AS group, plasma phosphatidylcholine 22:5n3 correlated positively with verbal (r=0.357, p=0.019) and full scale (r=0.402, p=0.008) IQs, red blood cell phosphatidylcholine 22:5n3 with verbal (r=0.308, p=0.044), performance (r=0.304, p=0.047) and full scale (r=0.388, p=0.011) IQs and red blood cell phosphatidylethanolamine 22:5n3 with verbal (r=0.390, p=0.010) and full scale (r=0.370, p=0.016) IQs. Whilst, plasma phosphatidycholine 20:5n3 (r=-0.395, p=0.009), 22:6n3 (r=-0.402, p=0.007) and total n3 fatty acids (r=-425, p=0.005), red blood cell phosphatidlycholine 20:5n3 (r=-0.321, p=0.036) and red blood cell phosphatidylethanolamine 20:5n3 (r=-0.317, p=0.038), 22:6n3 (r=-0.297, p=0.05) and total n3 fatty acids (r=-0.306, p=0.046) correlated inversly with ADHD index. Similarly, inattention was negatively related with plasma phosphatidylcholine 22:6n3 (r=-0.335, p=0.028), and total n3 fatty acids (r=-0.340, p=0.026), oppositional with plasma phosphatidylcholine 18:3n3 (r=-0.333, p=0.029), 20:5n3 (r=-0.365, p=0.016), total n3 fatty acids (r=-0.293, p<0.05), red blood cell phosphatidylcholine 18:3n3 (r=-0.337, p=0.027) and red blood cell ethanolamine 18:3n3 (r=- 0.333, p=0.029), 20:5n3 (r=-0.328, p=0.032), 22:6n3 (r=0.362, p=0.017) and total n-3 fatty acids (r=-0.298, p<0.05) and hyperactivity with plasma phosphatidylcholine 22:6n3 (r=-0.320, p=0.039). In contrast, there were inverse correlations between red blood cell phosphatidylcholine 18:2n6 and performance (r= -0.358, p=0.019) and full scale (r= -0.320, p=0.039) IQs, and direct correlations between red blood cell phosphatidylcholine 22:4n6 (r=0.339, p=0.026) and 22:5n6 (r=0.298, p<0.05) and ADHD index, between red blood cell phosphatidylcholine 22:4n6 (r=0.308, p=0.044) and inattention, between plasma phosphatidylcholine 22:4n6 (r=0.341, p=0.025), red blood cell phosphatidylcholine 20:4n6 (r=0.314, p=0.041) and total n6 fatty acids (r=0.336, p=0.028) and oppositional and plasma phosphatidylcholine 20:3n6 (r=0.362, p=0.018) and red blood cell phosphatidylcholine 20:3n6 (r=0.401, p=0.009) and hyperactivity. The findings of the ethnically homogenous children with Asperger's syndrome/high functioning autism study revealed positive associations between 22:5n3 and cognition, and negative relationships between 20:5n3 and 22:6n3 and behavioural problem. In contrast, cognitive ability and behavioural problems were negatively and positively associated with n6 fatty acids. Further investigation is required to establish whether there is a cause and effect relationship. Regardless, it would be prudent to ensure that children with the condition have optimum n3 PUFA intake.

Introduction

High functioning autism and Asperger's syndrome are developmental disorders classified within related neurological conditions known as autistic spectrum disorders (ASD). Individuals with the conditions have seriously impaired social and communication skills despite normal cognitive development and well-developed speaking ability. These difficulties often lead to social isolation. The common symptoms include obsessive adherence to routines, excessive preoccupation with a single and narrow subject of interest, rhythm and intonation problems in speech, delayed motor skills and impaired social communication, interaction and imagination skills [1-2]. Besides, they suffer regularly from sleep disturbances [3], food selectivity, and picky eating [4-5]. Although certain drugs are given to treat anxiety, depression and ADHD symptoms co-existing with the disorder [6-7], there is no known curative drug for the core symptoms [8-10]. Therefore, the mainstream management remains social skills training and behaviour, occupational, speech therapies, and support and management training for parents [11-13].

The long-chain polyunsaturated fatty acids, docosahexaenoic acid (DHA, 22:6n3) and arachidonic acid (AA, 20:4n6) are vital structural components of brain cell membranes. Moreover, eicosapentaenoic (EPA, 20:5n3) and DHA have been shown to improve memory, learning and cognition [14], protect against cognitive decline and dementia [15] and attenuate social communication and social interaction deficits associated with psychological/psychiatric disorders [16]. These effects are most likely due to the modulation of neuronal function by the aforementioned fatty acids and their metabolites. Indeed, there is evidence that long-chain n3 polyunsaturated fatty acids play a critical role in receptor function, neurotransmitter release, and signalling [17-18]. Also, they are known to promote neurite outgrowth of sensory and hippocampal neurons [19-21].

Studies have investigated the fatty acid status of people with Alzheimer's disease [22], Parkinson's disease [23-24], schizophrenia [25], depressive and anxiety disorders [26-27], attention deficit hyperactivity disorder [28-30], autistic spectrum disorder [31-36]) of various population groups. There is a paucity of comprehensive and well-thought-out investigations on the fatty acid composition of plasma and red blood cell polar and non-polar lipids and neurological and behavioural disorders from Poland and elsewhere.

Aim – The aim of this cross-sectional design study was to investigate blood fatty acid status of polish children with high functioning autism/Asperger’s syndrome and assess relationships between fatty acids and behaviour and cognitive ability. The study was a cross-sectional design.

Sample size, participants and methods

Sample size – Docosahexaenoic acid (DHA) is the central nervous system's most limiting and vital structural and functional fatty acid. We wanted to find out if the level of this nutrient is compromised in individuals with high functioning autistic (HFA) and Asperger's syndrome (AS). In our previous pilot study of healthy Polish Children, the level DHA in plasma choline phosphoglycerates was 2.1±0.85%. We wanted to detect a 20% difference in DHA levels between high functioning autistic (HFA) and Asperger's syndrome (AS) and their healthy control siblings (HC) with an 85% power at the 5% significant level. We expected a 20% loss of sample during laboratory processing. Therefore, the required sample size was 88 (44 HFA/AS & 44 HC). Gow and colleagues [37] detected a relationship between n3 fatty acids and emotion processing in 31 boys with attention deficit hyperactivity disorder. Therefore, we anticipated sample size of 44 would be sufficient to reveal correlations between n3 fatty acids and the behaviour in the HFA/AS.

Participants - Seventy-two children – 55 HFA/AS and 17 HC siblings of the HFA/AS - were enrolled from the Therapeutic Centre for Children with Autism, Warsaw, Poland. Of these, forty-four (n=44; female 5, Male 39) HFA/AS and seventeen (n=17; female 10, male 7) HC siblings were recruited into the study. The seventeen healthy children were siblings of the seventeen HFA/AS participants. All of the 44 HFA/AS had siblings; however, only seventeen HFA/AS children's parents consented for their healthy offspring to participate in the study. Eleven HFA/AS children did not meet the inclusion criteria and were excluded from participation. The inclusion criteria were age greater than five and under twenty years and diagnosis with HFA or AS. Children with an IQ score below 80, comorbid severe conditions and illness, recent drug-prescription history and those on special diets or nutritional supplements were excluded from recruitment. Ethical approval from the Mother and Child Care Institute Bioethics Committee (Warsaw, Kasprzaka, Poland) and informed consent from the parents or guardians of the children were obtained. Blood specimen, about 5 ml, was collected before breakfast from those who consented to participate in the study.

Anthropometry and blood pressure - Weight in kilogram and height in centimetre were assessed with a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham B5 5QB, UK) and a measuring board (Schorr, Weight and Measure, LLC, Olney, Maryland, USA), respectively. Blood pressure was measured with the use of a validated digital sphygmomanometer.

Diagnosis and assessment of intelligence quotient and behaviour - High functioning autism and Asperger's syndrome were diagnosed using the International Classification of Diseases (ICD-10, version 2010, [38]. Wechsler Intelligence Scale for Children (WISC-R) and Conners' Parent Rating Scale-Revised (S, Polish version) were used for the assessments of intelligence quotient (full scale,

verbal. Performance IQ and subsets) and behavioural profile, respectively. To maintain consistency, medical and psychological assessments were conducted by BJKO and BS, respectively.

Fatty acid analysis - Plasma and total erythrocyte lipids were extracted by the modified method of Folch et al. [39]) by homogenising 1 ml of sample in 90 ml chloroform: methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) under oxygen-free nitrogen. Phospholipids were separated by thin-layer chromatography (TLC) on silica gel plates using the developing solvents, chloroform, methanol: and methylamine (65:35:15 v/v/v). Petrol ether, diethyl ether, formic acid and methanol (85:15:2.5:1 v/v/v/v) were used to separate the neutral lipids. The phospholipid and neutral lipid bands were visualised by spraying the developed plates with a methanolic solution of 2, 7-dichlorofluorescein (0.01% w/v) and identified using authentic standards. Fatty acid methyl esters (FAMEs) were prepared under nitrogen by heating the phospholipid bands in 4 ml of 15% methanolic acetyl chloride in a sealed vial at 70C° for 3 hours. The resulting FAMEs were extracted with petroleum spirit, dried, dissolved in heptane and subsequently separated by a gas chromatograph (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a BPX 20 capillary column (60 m x 0.32 mm ID, 0.25: film). The injector, oven and detector temperatures were 250, 230 and 280°C, respectively, and hydrogen was used as a carrier gas.

Quality certified fatty acid methyl ester standard mixture (Supelco® 37 Component FAME Mix. U47885-U, Sigma-Aldrich, Dorset, UK) and GC-MS authenticated fatty acid methyl esters prepared from lipid extract of (1) vegetable seed oils containing alpha-linolenic, gamma-linolenic and stearidonic acids, and (2) bovine brain L-A-phosphatidylethanolamine Type 1 (Sigma-Aldrich, Dorset, UK), were used for identification. Peak areas were computed using the EZChrom chromatography data system (Scientific Software, Inc., San Ramon, CA).

Data analyses

The data are expressed as mean ± standard deviation. Cronbach's Alpha was used to measure internal consistencies of mental abilities (IQ) and Conner's Parent Rating Scale items that measure the same construct. Statistical differences between fatty acid levels of the children with and without autism spectrum disorder and linear relationships between fatty acid levels and IQ scores and Conner's parent rating scale t-scores were investigated with the use of an independent test, and Stepwise regression and Pearson's correlation coefficient (r), respectively. P-values of less than 0.05 are considered statistically significant. An independent t-test rather than a paired t-test was used to compare the fatty acid status of the case (HFA/AS) and reference (HC siblings) groups. This is because most of the HFA/AS children did not have matching sibling (natural pairing) participants. The data were tested for normal distribution and, when deemed necessary, normalised before statistical analyses. Analyses were

performed using IBM SPSS, version 25 (International Business Machines Corporation, New York, USA).

Results

Demography and blood pressure - Age, height, weight, body mass index and systolic and diastolic blood pressure values of the autistic children and healthy controls are shown in table 1. There was no difference in any of the variables mentioned above between the two groups ($p>0.05$).

Cognitive abilities of HFA/AS - *C*ognitive abilities of the children with Asperger's syndrome are presented in table 1. The internal consistency value (Cronbach alpha coefficient) of the mental ability (Wechsler Intelligence) sub-scales was 0.84. The scaled full-scale intelligence quotient (IQ) scores of the 44 affected children ranged between 83 and 152. One (2.3%) had a score of 83 (low average), 15 (34.1%) 90 to 109 (average), 8 (18.2%) 110 to 119 (high average), 10 (22.7%) 120 to 129 (superior) and 10 (22.7%) 130 or higher (very superior).

Connor's Parental Rating Scale T-Score of HFA/AS – The Cronbach alpha coefficient (internal consistency value) of the Connor's Parent Rating Scale items was 0.78. An appreciable number of the forty-four children had clinically significant behavioural problems (T-score higher than 65) – attention deficit hyperactivity disorder 21 (47.7%), inattention 23 (52.3%), oppositional 19 (43.2%) and hyperactivity 18 (40.9%).

Plasma triacylglycerol (PT) - Fatty acid composition of plasma triacylglycerol is presented in table 2. The children with autistic spectrum disorder compared with their healthy counterparts had higher levels of C14:0, C16:0, C16:1n7 and total saturated fatty acids ($p<0.0001$) and lower C18:0 ($p=0.014$), C18:1n9 and total monounsaturated fatty acids ($p<0.0001$), 20:3n6 ($p=0.002$), 22:4n6 and 22:5n6 ($p=0.007$), 20:5n3 ($p=0.033$), 22:5n3 ($p<0.005$) and total n-3 fatty acids (($p=0.025$).

Plasma cholesterylesters (PCE) - C14:0 ($p=0.004$), C16:0 ($p<0.0001$), C16:1n7 ($p=0.008$), total saturated fatty acids ($p<0.0001$) and total monounsaturated fatty acids ($p=0.041$) were higher and 18:2n6 ($p<0.0001$), 20:3n6 ($p=0.036$), 22:5n6 ($p=0.0017$), total n6 fatty acids ($p<0.0001$), 20:5n3 ($p=0.045$), 22:5n3 ($p=0.003$), 22:6n3 ($p=0.011$) and total n3 fatty acids ($p=0.035$) lower in the children with autistic spectrum disorder than in the healthy controls (table 3).

Plasma phosphatidylcholine (PPC) – The children with Asperger's syndrome compared with their healthy siblings had higher levels of 14:0, 16:0 ($p<0.0001$), total saturates ($p=0.002$), 16:1n7 (($p<0001$), and lower 20:1n9 ($p=0.004$), 24:1n9 ($p=0.039$),18:2n6 ($p=0.033$), 20:2n6 ($p<0.001$),

20:3n6 ($p<0.01$), 22:4n6 ($p=0.004$), 22:5n6 ($p=0.019$), total n6 fatty acids ($p=0.015$) and 22:5n3 ($p=0.047$) (table 4).

Red blood cell phosphatidylcholine (RBC PC) – Table 5 shows fatty acid composition of the children without (healthy siblings) and with Asperger's syndrome. The levels of 16:0 ($p<0.001$), total saturated fatty acids ($p=0.002$), 18:1n9 ($p<0.01$) and total monounsaturated fatty acids ($p=0.004$) were higher and 18:2n6 ($p=0.032$), 20:2n6 ($p=0.039$), 20:3n6 ($p=0.015$), 22:4n6 ($p=0.03$), total n3 fatty acids ($p<0.001$) and 20:5n3, 22:5n3, 22:6n3 ($p<0.05$) and 18:0 dimethylacetal lower in the children with Asperger's syndrome.

Red blood cell phosphatidylethanolamine (RBC PE) – Compared with their healthy siblings, the children with Asperger's syndrome had reduced levels of 18:2n6 ($p=0.003$), 20:3n6 ($p=0.009$), 22:4n6 ($p<0.05$), total n6 fatty acids ($p=0.003$) and elevated 20:2n9 ($p=0.009$), 18:3n6 ($p=0.007$) and 16:0 dimethylacetal ($p=0.012$) (Table 6).

Cognitive abilities and fatty acids of HFA/AS children - Data of the relationships between plasma and red blood cell fatty acids and cognitive abilities are presented in table 7. There were positive relationships between plasma phosphatidylcholine 22:5n3 and verbal ($r=0.357$, $p=0.019$) and full scale ($r=0.402$, $p=0.008$) IQs, between red blood cell phosphatidylcholine 22:5n3 and verbal ($r=0.308$, $p=0.044$), performance ($r=0.304$, $p=0.047$) and full scale ($r=0.388$, $p=0.011$) IQs and between red blood cell phosphatidylethanolamine 22:5n3 and verbal ($r=0.390$, $p=0.010$) and full scale ($r=0.370$, $p=0.016$) IQs. In contrast, red blood cell phosphatidylcholine 18:2n6 correlated inversely with performance ($r= -0.358$, $p=0.019$) and full scale ($r= -0.320$, $p=0.039$) IQs.

Behavioral problems and fatty acids of *HFA/As children* – Table 8 illustrates relationships between behavioral problems (ADHD index, inattention, oppositional and hyperactivity). Plasma phosphatidycholine 20:5n3 ($r=-0.395$, $p=0.009$), 22:6n3 ($r=-0.402$, $p=0.007$) and total n3 fatty acids ($r=-425$,$p=0.005$), red blood cell phosphatidlycholine 20:5n3 ($r=-0.321$, $p=0.036$) and red blood cell phosphatidylethanolamine 20:5n3 ($r=-0.317$, $p=0.038$), 22:6n3 ($r=-0.297$, $p=0.05$) and total n3 fatty acids ($r=-0.306$, $p=0.046$) corrleated negativly with ADHD index. Conversly, there were postive correlations between ADHD index and red blood cell phosphatidylcholine 22:4n6 ($r=0.339$, $p=0.026$) and 22:5n6 ($r=0.298$, $p<0.05$).

Inattention was negatively related with plasma phosphatidylcholine 22:6n3 ($r=-0.335$, $p=0.028$), and total n3 fatty acids ($r=-0.340$, $p=0.026$) and positively with red blood cell phosphatidylcholine 22:4n6 ($r=0.308$, $p=0.044$) (table 8).

There were inverse relationships between oppositional and plasma phosphatidylcholine 18:3n3 (r=-0.333, p=0.029), 20:5n3 (r=-0.365, p=0.016), total n3 fatty acids (r=-0.293, p<0.05), red blood cell phosphatidylcholine 18:3n3 (r=-0.337, p=0.027) and red blood cell ethanolamine 18:3n3 (r=-

0.333, p=0.029), 20:5n3 (r=-0.328, p=0.032), 22:6n3 (r=0.362, p=0.017) and total n-3 fatty acids (r=-0.298, p<0.05). Conversely, plasma phosphatidylcholine 22:4n6 (r=0.341, p=0.025), red blood cell phosphatidylcholine 20:4n6 (r=0.314, p=0.041) and total n6 fatty acids (r=0.336, p=0.028) were directly associated with oppositional (table 8).

Hyperactivity (table 8) correlated inversely with plasma phosphatidylcholine 22:6n3 (r=-0.320, p=0.039) and directly with plasma phosphatidylcholine 20:3n6 (r=0.362, p=0.018) and red blood cell phosphatidylcholine 20:3n6 (r=0.401, p=0.009).

Discussion

The findings of the ethnically homogenous children with Asperger's syndrome/high functioning autism study revealed positive associations between 22:5n3 and cognition, and negative relationships between 20:5n3 and 22:6n3 and behavioural problems. In contrast, cognitive ability and behavioural problems were negatively and positively associated with n6 fatty acids.

Findings of the fatty acid status of people with autistic spectrum disorder have not been consistent. Some have reported reduced levels of long-chain n3, n6 or n3 & n6 in whole blood [31, 40], serum [32,41]), plasma [34, 42-43], red blood cells [30, 33] and plasma and red blood cells [35] of patients with autism spectrum disorder compared with their non-autistic counterparts. In contrast, Bu et al. [44] and Bell et al. [45] did not find any difference between the two groups, and Yui et al. [36] reported higher levels of EPA, DHA, DHA/AA and EPA/AA and lower AA in plasma of those with autism. The reasons for the contradictory findings are unclear. It is plausible that differences in the blood lipids analysed (total lipid, phospholipids, phospholipid fractions), background diet and ethnic and/or disorder heterogeneity of the participants may have played a role.

The participants who participated in the current study were homogenous with regard to ethnicity (Polish Caucasians) and disorder (Asperger's syndrome without severe comorbidities and IQ higher than 80). Besides, unlike in most of the previous investigations, plasma triacylglycerol (PT), cholesterylesters (PCE) and phosphatidylcholine (PPC) and Red blood cell phosphatidylcholine (RBC PC) and phosphatidylethanolamine (RBC PE) were assayed. The findings revealed that Asperger's syndrome/high-functioning autism is associated with abnormal blood fatty acid levels. The abnormality was manifested in the plasma, and red blood cell lipid groups (PT, PCE, PPC, RBC PC & RBC PE) analysed. But, the anomaly was less remarkable in the RBC PE.

Previous studies have focused on the reductions of some of the n3, n6 or n3 and n6 fatty acids in individuals with autistic spectrum disorders. However, the current investigation shows that saturated and monounsaturated fatty acid levels are also anomalous in patients with the disorder. Levels of total saturated fatty acids were significantly higher in PT, PCE, PPC, RBC PC in the children with HFA/AS. Similarly, their total monounsaturated fatty acid levels were higher in PCE & RBC PC and lower in PT. These abnormalities are likely to have physiological implications because saturated (16:0, 18:0, etc.) and monounsaturated (16:1n7, 18:1n7, 18:1n9, etc.) are integral components of phospholipid molecular species containing DHA, AA or other n3 and n6 fatty acids.

The primary cause of blood fatty acid perturbations in autism is not elucidated. However, several postulates have been put forward – mitochondrial dysfunction [46-47], lipid peroxidation and compromised antioxidant protections [48-49], aberration of polyunsaturated fatty acid metabolism [33, 50], FADS1, FADS2 and ELOVL2 gene polymorphism [51] and dietary intake [32]. The current investigation of children with Asperger's syndrome and their siblings, which revealed abnormality of saturated, monounsaturated, n3 and n6 fatty acid profile, suggests that the problem is most likely due to mitochondrial and peroxisomal dysfunctions.

The present study showed positive correlations between docosapentaenoic (22:5n3) and cognitive function (verbal, performance and full-scale IQs) and negative between DHA, EPA, total n3, and behavioural deviations. These associations are unlikely to be spurious because they are evident in plasma (PPC) and red blood cell (RBC PC & RBC PE) phosphoglycerides. The observed correlations do not demonstrate cause and effect relationships; nevertheless, it has been shown that n3 fatty acid supplementation improves concentration and sociability and reduces irritability, aggression and hyperactivity [52-55]. Conversely, other investigators did not report clinical benefits from n3 fatty acid supplementation [56-58]. The inconsistent findings could be due to the heterogeneity of the participants' disorder, genetic variations in fatty acid metabolism, and bassline fatty acid status.

Cognitive function correlated significantly with docosapentaenoic (22:5n3, n3 DPA) but not with docosahexaenoic acid (DHA). This finding was unexpected since previous studies have reported positive relationships between DHA (dietary intake or blood level) and verbal memory [59], working memory [60-61], composite memory performance score [62], declarative memory [63] and immediate memory [64]. Besides, DHA is an integral structural component of neuronal membrane lipids [65-66] and plays a critical role in developmental and functional processes [67-68]. Although n3 DPA is the second abundant structural n3 LCPUFA in the brain [69]), there is a paucity of rigorous research-based information on n3 DPA and cognitive and behavioural functions. However, n3 DPA and its lipid mediator metabolites (protectins, resolvins, maresins, isoprostanes) have been shown to reduce the symptoms of depression [70-71].

This cross-sectional study has several strengths and limitations. The main strengths are (1) The homogeneity of the HFA/AS and healthy control groups (they are all indigenous Polish) and of the autism spectrum disorders (HFA/AS). The two conditions have overlapping symptoms; indeed, in DSM-5, HFA and AS are classified as the same group. (2) The seventeen healthy control subjects (reference group) are siblings of the seventeen HFA/AS who participated in the study. This would have helped reduce biases associated with family-linked genes and dietary intake differences. (3) Fatty acid compositions of plasma (circulating) and red blood cell (structural) lipid fractions and their relationships with cognition and behaviour functions are evaluated. Structural fatty acid composition data are of paramount importance for assessing the impact of fatty acid status on functional outcomes. Circulating (plasma) fatty acid compositions do not reflect function if there is a dysfunction in fatty acid incorporation into cellular membrane phospholipids.

The study is a cross-sectional design, and therefore it incorporates the inherent biases associated with the design. The other limitations are (1) An imbalance of sample size in the two arms of the study (44 HFA/AS, Case Group and 17 HC, Reference Group) and a lack of functional data of the reference group. Twenty-seven parents of the HFA/AS group were reluctant to allow their healthy offspring to participate in the study, and we were only able to recruit seventeen healthy siblings. Indeed, even the seventeen parents who gave their consent did not approve functional (cognitive and behaviour) evaluation. (2) We do not have dietary or genetic information. An effort was made to collect nutritional data; however, most of the parents of the children were either unwilling or unable to complete and return the dietary questionnaires. Besides, the few dietary questionnaires that were returned were incomplete and unusable. Because of financial constraints, we could not employ a nutritionist to investigate the dietary aspect and do pertinent genetic analyses, such as FADS and ELOVL polymorphisms. (3) Percent fatty acid compositions of plasma and red blood cell lipid fractions were analysed. In the absence of dietary intake information, fatty acid concentrations data would have enhanced our understanding of the observed fatty acid status abnormality in the HFA/AS and helped facilitate the interpretation.

Conclusion

The findings of the ethnically homogenous children with Asperger's syndrome/high functioning autism study revealed a positive correlation between n3 DPA and cognition, a negative relationship between EPA and DHA and behavioural problems. In contrast, cognitive ability and behavioural problems were negatively and positively associated with n6 fatty acids. Further investigation is required to establish

whether there is a cause and effect relationship. Regardless, it would be prudent to ensure that children with the condition have optimum n3 PUFA intake.

ACKNOWLEDGEMENTS

Very sincere thanks are due to the children and their parents, support staff of the Mental Health Clinic for Children and Youth, Masovian Neuropsychiatry Center, Warsaw, Poland, London Metropolitan University (provided laboratory facility) and Vifor Pharma Ltd, Switzerland (for financial support).

AUTHORS' CONTRIBUTIONS

BJKO conceived the idea, designed and initiated the study, BS helped with recruitment and demographic data collection and psychological assessment, ASB analysed the blood samples and collated the data, KG and YM analysed the data, BJKO and KG wrote the manuscript.

References

1. Cederlund M, Hagberg B, Billstedt E, Gillberg IC, and Gillberg C (2008) Asperger Syndrome and Autism: A Comparative Longitudinal Follow-Up Study More than 5 Years after Original Diagnosis. J. Autism Dev. Disord 38 (1): 72–85.
2. Faridi F and Khosrowabadi R (2017) Behavioral, Cognitive and Neural Markers of Asperger Syndrome," Basic Clin. Neurosci. J. 8(5): 349–360.
3. Baker EK and Richdale AL (2017) Examining the Behavioural Sleep-Wake Rhythm in Adults with Autism Spectrum Disorder and No Comorbid Intellectual Disability. J. Autism Dev. Disord. 47(4): 1207–1222.
4. Aponte CA and Romanczyk RG (2016) Assessment of feeding problems in children with an autism spectrum disorder. Res. Autism Spectr. Disord. 21: 61–72.
5. Kral TVE, Eriksen WT, Souders MC and Pinto-Martin JA (2013) Eating Behaviors, Diet Quality, and Gastrointestinal Symptoms in Children With Autism Spectrum Disorders: A Brief Review. J. Pediatr. Nurs. 28(6): 548–556.
6. Gadow KD, Guttmann-Steinmetz S, Rieffe C and DeVincent CJ (2012), "Depression Symptoms in Boys with Autism Spectrum Disorder and Comparison Samples. J. Autism Dev. Disord. 42(7): 353–1363.
7. Stevens T, Peng L and Barnard-Brak L (2016) The comorbidity of ADHD in children diagnosed with autism spectrum disorder. Res. Autism Spectr. Disord. 31: 11–18.
8. Buck Buck TR, Viskochil J, Farley M, Coon H, McMahon WM, Morgan J and Bilder DA (2014) Psychiatric Comorbidity and Medication Use in Adults with Autism Spectrum Disorder. J. Autism Dev. Disord. 44(12): 3063–3071.
9. Hsia Y, Wong AYS, Murphy DGM, Simonoff E, Buitelaar JK, and Wong ICK (2014) Psychopharmacological prescriptions for people with autism spectrum disorder (ASD): a multinational study. Psychopharmacology (Berl.) 231(6): 999–1009. .
10. Madden JM, Lakoma MD, Lynch FL, Rusinak D, Owen-Smith AA, Coleman KJ, Quinn VP, Yau VM, Qian YX and Croen LA (2017) Psychotropic Medication Use among Insured Children with Autism Spectrum Disorder. J. Autism Dev. Disord. 47(1): 144–154.
11. Barnhill GP (2016) Supporting Students With Asperger Syndrome on College Campuses: Current Practices. Focus Autism Dev. Disabil. 31(1): 3–15.
12. Peters R and Brooks R (2016) Parental perspectives on the transition to secondary school for students with Asperger syndrome and high-functioning autism: a pilot survey study: Parental perspectives on the transition to secondary school for students with Asperger syndrome and high-functioning autism: a pilot survey study. Br. J. Spec. Educ. 43(1): 75–91.

13. Tse J, Strulovitch J, Tagalakis V, Meng L, and Fombonne E (2007) Social Skills Training for Adolescents with Asperger Syndrome and High-Functioning Autism. J. Autism Dev. Disord. 37(10):1960–1968.

14. Muldon MF, Ryan CM, Sheu L, Yao JK, Conklin SM and Manuck SB (2010) Serum phospholipid docosahexaenoic acid is associated with cognitive functioning during middle adulthood. Journal of Nutrition 140: 848-853.

15. Jicha GA and Markesbery WR (2010) Omega-3 fatty acids: potential role in the management of early Alzheimer's disease. Clinical interventions in aging 5: 45-61.

16. Murakami K, Miyake Y, Sasaki S, Tanaka K, Arakawa M (2010) Fish and n-3 polyunsaturated fatty acid intake and depressive symptoms: Ryukyus Child Health Study. Paediatrics 126(3): e623-630.

17. Innis SM and de la Presa O (2001) Dietary fatty acid composition in pregnancy alter neurite membrane fatty acids and dopamine in newborn rat brain. J. Nutr. 131(1): 118-122.

18. Mitchell DC, Niu SL and Litaman BJ (2003) Enhancement of G protein-coupled signalling by DHA phospholipids. Lipids 38(4):437-443.

19. Calderon F and Kim Hee-Yong (2004) Docosahexaenoic acid promotes neurite growth in hippocampal neurons. J. Neurochem. 90(4): 979-88.

20. Dagai L, Peri-Naor R and Birk RZ (2009) Docosahexaenoic acid significantly stimulates immediate early response genes and neurite outgrowth. Neurochem. Res. 34: 867-875.

21. Robson LG, Dyall S, Sidloff D and Michael-Titus AT (2010) Omega 3 polyunsaturated fatty acids increase the neurite outgrowth of rat sensory neurons throughout development and in aged animals. Neurobiology of Aging 3: 678-687.

22. Astarita G, Jung K-M,Vasilevko V, Dipatrizio NV, Martin SK, Cribbs DH, Head E, Cotman CW, Daniele Piomelli D (2011) Elevated stearoyl-CoA desaturase in brains of patients with Alzheimer's disease. PLoS One 6(10): e24777.

23. Fabelo N, Martin V, Santpere G, Marin R, Torrent L, Ferrer I and Díaz M (2011) Severe alterations in lipid composition of frontal cortex lipid rafts from Parkinson's disease and incidental Parkinson's disease. Mol. Med. 17: 1107–1118.

24. Schulte EC, Altmaier E, Berger HS, Do KT, Kastenmüller G, Wahl S, Adamski J, Peters A, Krumsiek J, Suhre K, Haslinger B, Ceballos-Baumann BA, Gieger C and Winkelmann J (2016) Alterations in Lipid and Inositol Metabolisms in Two Dopaminergic Disorders. PLoS One 11:e0147129.

25. Hoen WP, Lijmer JG, Duran M, Waanders RJA, Van Beveren NJM and De Haan L (2013) Red blood cell polyunsaturated fatty acids measured in red blood cells and schizophrenia: a meta-analysis. Psychiatry Res . 207(1-2): 1-12.

26. Lin P-Y, Huang S-Y and Su K-P (2010) A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. Biol. Psychiatry 68:140–147.

27. Thesing CS, Bot M, Milaneschi Y, Giltay EJ and Penninx BWJH (2018) Omega-3 and omega-6 fatty acid levels in depressive and anxiety disorders. Psychoneuroendocrinology 87:53–62.

28. Colter AL, Cutler C, Meckling KA (2008) Fatty acid status and behavioural symptoms of attention deficit hyperactivity disorder in adolescents: a case-control study. Nutrition Journal Nutrition Journal 7:8 doi:10.1186/1475-2891-7-8

29. Hawkey E and Nigg JT (2014) Omega-3 fatty acid and ADHD: Blood level analysis and meta-analytic extension of supplementation trials. Clin. Psychol Rev. 34(6):496–505.

30. Parletta N, Niyonsenga T and Duff J (2016) Omega-3 and Omega-6 Polyunsaturated Fatty Acid Levels and Correlations with Symptoms in Children with Attention Deficit Hyperactivity Disorder, Autistic Spectrum Disorder and Typically Developing Controls. PLOS ONE 11(5): e0156432.

31. Vancassel S, Durand G, Barthélémy C, Lejeune B, Martineau J, Guilloteau D, Andrès C and Chalon S (2001) Plasma fatty acid levels in autistic children. Prostaglandins Leukot. Essent. Fat. Acids 65(1): 1–7.

32. Al-Farsi YM, Waly MI, Deth RC, Al-Sharbati MM, Al-Shafaee M, Al-Farsi O, Maha M. Al-Khaduri MM, Al-Adawi S, Hodgson NW, Gupta I and Ouhtit A (2013) Impact of nutrition on serum levels of docosahexaenoic acid among Omani children with autism. Nutrition 29: 142-146

33. Brigandi S, Shao H, Qian S, Shen Y, Wu B.-L and Kang J (2015) Autistic Children Exhibit Decreased Levels of Essential Fatty Acids in Red Blood Cells. Int. J. Mol. Sci. 16(5): 10061-10076.

34. Mostafa GA and Al-Ayadhi LY (2015) Reduced levels of plasma polyunsaturated fatty acids and serum carnitine in autistic children: Relation to gastrointestinal manifestations. Behav. Brain Funct. 11:4, doi 10.1186/s12993-014-0048-2

35. Jory J (2016) Abnormal fatty acids in Canadian children with autism. Nutrition 32(4): 474–477.

36. Yui K, Imataka G, Kawasaki Y and Yamada H (2016) Down-regulation of a signaling mediator in association with lowered plasma arachidonic acid levels in individuals with autism spectrum disorders. Neurosci. Lett. 610: 223–228.

37. Gow RV, Sumich A, Vallee-Tourangeau F, Crawford MA, Ghebremeskel K, Allain A. Bueno AA, Hibbeln JR, Taylor E, Wilson DA, Rubia K (2013) Omega-3 fatty acids are related to abnormal emotion processing in adolescent boys with attention deficit hyperactivity disorder. Prostaglandins, Leukotrienes and Essential Fatty Acids 88: 419–429

38. ICD-10 International. Statistical. Classification of Diseases and. Related Health. Problems. 10th Revision. Volume 2. Instruction manual. 2010 Edition.

39. Folch J, Lees M, Slone-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues, Journal of Biological Chemistry 226: 497-409.

40. Meguid NA, Atta HM, Gouda AS, and Khalil RO (2008) Role of polyunsaturated fatty acids in the management of Egyptian children with autism. Clin. Biochem. 41(13): 1044–1048.

41. Esparham AE, Smith T, Belmont JM, Haden M, Wagner LE, Evans RG, Drisko JA (2015) Nutritional and Metabolic Biomarkers in Autism Spectrum Disorders: An Exploratory Study. Integrative Medicine 14 (2): 40-53.

42. Weist MM, German JB, Harvey DJ, Watkins SM and Hertz-Picciotto I (2009) Plasma fatty acid profiles in autism: A case-control study. Prostaglandins Leukot. Essent. Fatty Acids 80: 221–227.

43. El-Ansary AK, Bacha AG and Al-Ayahdi LY (2011) Impaired plasma phospholipids and relative amounts of essential polyunsaturated fatty acids in autistic patients from Saudi Arabia. Lipids Health Dis 2011;10:63.

44. Bu B, Ashwood P, Harvey D, King IB, Van de Water J and Jin L-W (2006) Fatty acid compositions of red blood cell phospholipids in children with autism. Prostaglandins Leukot Essent Fatty Acids 74(4):215–21.

45. Bell JD, Miller D, MacDonald DJ, MacKinlay EE, Dick JR, Cheseldine S, Boyle RM, Graham C and O'Hare AE (2010) The fatty acid compositions of erythrocyte and plasma polar lipids in children with autism, developmental delay or typically developing controls and the effect of fish oil intake. British Journal of Nutrition 103: 1160–1167.

46. Pastural E, Ritchie S, Lu Y, Jin W, Kavianpour A, Su-Myat K, Heath D, Wood PL, Fisk M and Goodenowe DB (2009) Novel plasma phospholipid biomarkers of autism: Mitochondrial dysfunction as a putative causative mechanism. Prostaglandins Leukot. Essent. Fatty Acids 81:253–264.

47. Cheng N, Rho JM and Masino S (2017) Metabolic Dysfunction Underlying Autism Spectrum Disorder and Potential Treatment Approaches. Front Mol. Neurosci. 10:34. doi: 10.3389/fnmol.2017.00034.

48. Chauhan A, Chauhan V, Brown WT and Cohen I (2004) Oxidative stress in autism: Increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin-the antioxidant proteins. Life Sci. 75(21): 2539–2549.

49. Meguid NA, Dardir AA, Abdel-Raouf ER and Hashish A (2011) Evaluation of Oxidative Stress in Autism: Defective Antioxidant Enzymes and Increased Lipid Peroxidation. Biol Trace Elem. Res. 143:58-65.

50. Tamiji J and Crawford DA (2010) The neurobiology of lipid metabolism in autism spectrum disorders. Neurosignals 18: 98–112.

51. Sun C, Zou M, Wang X, Xia W, Ma Y, Liang S, Hao Y, Wu L and Fu S (2018) FADS1-FADS2 and ELOVL2 gene polymorphisms in susceptibility to autism spectrum disorders in Chinese children. BMC Psychiatry 18:283 https://doi.org/10.1186/s12888-018-1868-7

52. Bell JG, MacKinlay EE, Dick JR, MacDonald DJ, Boyle RM and Glen AC (2004) Essential fatty acids and phospholipase A(2) in autistic spectrum disorders. Prostaglandins Leukot Essent Fatty Acids 71(4) 201-4

53. Amminger GP, Gregor E Berger GE, Schäfer MR, Klier C, Friedrich MH and Feucht M (2007) Omega-3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study. Biol. Psychiatry 61(4):551-3.

54. Meiri G, Bichovsky Y and Belmaker RH (2009) Omega 3 fatty acid treatment in autism. J. Child Adolesc. Psychopharmacol. 19: 449–451.

55. Mazahery H, Stonehouse W, Delshad M, Kruger MC, Conlon CA, Beck KL and von Hurst PR (2017) Relationship between Long Chain n-3 Polyunsaturated Fatty Acids and Autism Spectrum Disorder: Systematic Review and Meta-Analysis of Case-Control and Randomised Controlled Trials. Nutrients 9 (2): 155, doi:10.3390/nu9020155.

56. Williams K, Williams K and Marraffa C (2012) No evidence yet to support omega-3 fatty acids as a treatment for autism. Journal of Paediatrics and Child Health 48(6): 534-536

57. Mankad D, Dupuis A, Smile S, Roberts W, Brian J, Lui T, Genore L, Zaghloul D , Alana Iaboni A, et A Marcon PMA and Anagnostou E (2015) A randomised, placebo controlled trial of omega-3 fatty acids in the treatment of young children with autism. Mol. Autism 6(1):18, DOI 10.1186/s13229-015-0010-7

58. De Crescenzo F, D'Alò GL, Morgano GP, Minozzi S, Mitrova Z, Saulle R, Cruciani F, Fulceri F, Davoli M, Scattoni ML, Nardocci F, Schünemann HJ and Amato L (2020) Impact of polyunsaturated fatty acids on patient-important outcomes in children and adolescents with autism spectrum disorder: a systematic review. Health Qual. Life Outcomes 18: 28, doi: 10.1186/s12955-020-01284-5.

59. Tan ZS, Harris WS, Beiser AS, Au R, Himali JJ, Debette S, Pikula A, Decarli C, Wolf PA, Vasan RS, Robins SJ and Seshadri S (2012) Red blood cell -3 fatty acid levels and markers of accelerated brain aging. Neurology 78: 658–664, doi: 10.1212/WNL. 0b013e318249f6a9 PMID: 22371413

60. Muldoon MF, Ryan CM, Sheu L, Yao JK, Conklin SM and Manuck SB (2010) Serum phospholipid docosahexae nonic acid is associated with cognitive functioning during middle adulthood. J. Nutr. 140:848–853.

61. Samieri C, Feart C, Proust-Lima C, Peuchant E, Dartigues JF, Amieva H, Barberger-Gateau P (2011) Omega-3 fatty acids and cognitive decline: Modulation by ApoE(epsilon)4 allele and depression. Neurobio. Aging 32(12):2317.e13-2317.e22

62. Phillips MA, Childs CE, Calder PC, Rogers PJ (2012) Lower omega-3 fatty acid intake and status are associated with poorer cognitive function in older age: A comparison of individuals with and without cognitive impairment and Alzheimer's disease. Nutr. Neurosci. 15: 271–277.

63. Titova OE, Sjogren P, Brooks SJ, Kullberg J, Ax E, Kilander L, Riserus U, Cederholm T, Larsson E-M, Johansson L, Ahlström H, Lind L, B Schiöth HB and Benedict C (2013) Dietary intake of eicosapentaenoic and docosahexaenoic acids is linked to gray matter volume and cognitive function in elderly. Age (Dordr) 35(4):1495–505.

64. Whalley LJ, Fox HC, Wahle KW, Starr JM and Deary IJ (2004) Cognitive aging, childhood intelligence, and the use of food supplements: possible involvement of n-3 fatty acids. Amer. J. Clin. Nutr. 80: 1650–1657.

65. Kim H-Y, Edsall L, Garcia M, Zhang (1999) The Release of Polyunsaturated Fatty Acids and Their Lipoxygenation in the Brain. Adv. Exp. Med. Biol. 447:75-85.

66. Carver JD, Benford VJ, Han B and Cantor AB (20091) The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. Brain Research Bulletin 56 (2): 79–85.

67. Cao D, Kevala K, Kim J, Moon H-S, Jun SB, Lovinger D, Lovinger D and Kim H-Y (2009) Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. J. Neurochem. 111(2): 510–521.

68. Gharami K, Das M and Das S (2015) Essential role of docosahexaenoic acid towards development of a smarter brain. Neurochemistry International 89: 51-62.

69. Drouin G, Rioux V and Legrand P (2019) The n-3 docosapentaenoic acid (DPA): A new player in the n-3 long chain polyunsaturated fatty acid family. Biochimie 159:36-48.

70. Laino CH, Garcia P, Podestá MF, Höcht C, Slobodianik N and Reinés A (2014) Fluoxetine potentiation of omega-3 fatty acid antidepressant effect: evaluating pharmacokinetic and brain fatty acid-related aspects in rodents, J. Pharm. Sci. 103(10): 3316-3325.

71. Matsuoka YJ, Sawada N, Mimura M, Shikimoto R, Nozaki S, Hamazaki K, Hamazaki K, Uchitomi Y and Tsugane S (2017) Dietary fish, n-3 polyunsaturated fatty acid consumption, and depression risk in Japan: a population-based prospective cohort study, Transl. Psychiatry 7(9):e1242, doi: 10.1038/tp.2017.206.https://doi.org/10.1038/ tp.2017.206.

Table 1: Mean (±SD) Demographic Data, Connor's Parental Rating Scale T-Score, and WISC-R Performance and Verbal index (sub-scale and scale) and Full Scale IQ Scores of Polish Children and Teenagers with (HFA/AS, n=44) and without (HC n=17) Autism

Demographic variables	**HFA/AS**	**HC Siblings**
Age (years)	10.4±2.9	11.6±3.3
Weight (kg)	43.1±20.2	45.3±17.5
Height (cm)	146.2±18.9	148.0±18.0
Body mass index	19.2±4.6	19.8±3.9
Systolic blood pressure (mm Hg)	104.8±12.6	
Diastolic blood pressure (mm Hg)	67.6±9.8	
Connor's Parental Rating Scale T- Score		
ADHD Index	67.4±8.9 (49-88)	
Inattention	64.9±9.7 (41-86)	
Oppositional	66.7±11.5 (42-88)	
Hyperactivity	63.5±12.6 (45-88)	
WISC-R variables		
Information	14.1±3.1 (8-19)	
Similarities	13.2±3.2 (3-19)	
Arithmetic	12.2±3.7 (5-19)	
Vocabulary	12.7±2.8 (7-19)	
Comprehension	12.1±3.3 (6-19)	
Scaled Verbal IQ	118.2±15.3 (87-158)	
Picture completion	11.5±3.3 (3-19)	
Picture arrangement	12.9±2.7 (7-17)	
Block design	11.9±3.7 (1-19)	
Object assembly	11.4 ±3.3 (5-18)	
Coding	8.9 ± 3.4 (3-18)	
Scaled Performance (nonverbal) IQ	109.7±17.1 (61-137)	
Scaled Full Scale IQ	116.0±15.4 (83-152)	

Table 2: Mean (±sd) Percent Plasma Triacylglycerol Fatty Acids of Polish Children and Teenagers with (HFA/AS, n=44) and without (HC, n=17) Autism

Fatty acids	**HFA/AS**	**HC Siblings**	**P-values**
14:0	1.48±0.9	0.49±0.5	<0.0001
16:0	25.3±4.7	18.5±5.1	<0.0001
18:0	3.73±1.3	4.70±1.6	= 0.014
Σ Saturates	30.5±6.1	23.7±5.1	<0.0001
16:1n7	3.29±1.3	1.89±1.0	<0.0001
18:1n7	2.36±0.5	2.62±0.4	= 0.32
18:1n9	42.4±4.1	48.0±3.8	<0.0001
20:1n9	0.36±0.2	0.48±0.2	= 0.035
24:1n9	0.03±0.02	0.04±0.01	>0.05
Σ Monoenes	48.5±3.8	53.0±3.3	<0.0001
18:2n6	14.9±3.5	15.7±2.2	>0.05
18:3n6	0.37±0.1	0.36±0.1	>0.05
20:2n6	0.25±0.1	0.28±0.2	>0.05
20:3n6	0.27±0.06	0.41±0.2	= 0.002
20:4n6	1.25±0.4	1.36±0.6	>0.05
22:4n6	0.16±0.06	0.25±0.1	= 0.007
22:5n6	0.14±0.06	0.22±0.1	= 0.007
ΣN6	17.4±3.7	18.6±2.6	>0.05
18:3n3	1.37±0.7	1.58±0.8	>0.05
20:5n3	0.29±0.2	0.40±0.2	= 0.033
22:5n3	0.36±0.1	0.52±0.2	<0.005
22:6n3	0.70±0.4	1.1±0.8	>0.05
ΣN3	2.77±1.1	3.71±1.5	= 0.025
N6/N3 ratio	6.84±1.2	5.81±1.9	p=0.05

Table 3: Mean (±sd) Percent Plasma Cholesteryl Ester Fatty Acids of Polish Children and Teenager's with (HFA/AS, n=44) and without (HC, n=17) Asperger's Syndrome

Fatty Acids	**HFA/AS**	**HC Siblings**	**P-value**
14:0	0.55±0.2	0.39±0.2	= 0.004
16:0	11.6±0.7	9.15±1.4	<0.0001
18:0	0.76±0.1	0.80±0.2	>0.05
Σ Saturates	12.9±0.9	10.3±1.4	<0.0001
16:1n7	2.60±1.0	2.07±0.5	= 0.008
18:1n7	1.03±0.2	1.02±0.2	>0.05
18:1n9	20.6±1.7	19.7±2.7	>0.05
20:1n9	0.04±0.01	0.05±0.04	>0.05
24:1n9	0.02±0.01	0.06±0.06	= 0.006
Σ Monoenes	24.3±2.3	22.9±2.9	= 0.041
18:2n6	50.6±3.2	54.4±4.0	<0.0001
18:3n6	0.83±0.4	0.82±0.5	>0.05
20:2n6	0.04±0.04	0.05±0.05	>0.05
20:3n6	0.58±0.1	0.69±0.2	= 0.036
20:4n6	6.86±1.3	7.09±1.5	>0.05
22:4n6	0.02±0.01	0.04±0.03	>0.05
22:5n6	0.04±0.01	0.06±0.04	= 0.017
ΣN6	59.0±2.8	63.1±3.7	<0.0001
18:3n3	0.67±0.3	0.73±0.3	<0.05
20:5n3	0.78±0.4	1.05±0.7	= 0.045
22:5n3	0.03±0.01	0.06±0.04	= 0.003
22:6n3	0.49±0.1	0.61±0.03	= 0.011
ΣN3	2.05±0.7	2.50±0.9	= 0.035
N6/N3 ratio	31.3±8.7	29.0±9.3	>0.05

Table 4: Mean (±sd) Percent Plasma Phosphatidylcholine Fatty Acids of Polish Children & Teenagers with (HFA/AS, n=44) and without (HC, n=17) Autism

Fatty acids	**HFA/AS**	**HC Siblings**	**P-value**
14:0	0.33±0.1	016±0.2	<0.0001
16:0	26.5±1.9	22.6±4.1	<0.0001
18:0	13.8±1.0	14.7±1.4	= 0.004
Σ Saturates	40.6±2.1	37.5±3.4	= 0.002
16:1n7	0.75±0.3	0.44±0.2	<0.0001
18:1n7	1.41±0.3	1.39±0.2	>0.05
18:1n9	13.7±2.4	13.1±2.4	>0.05
20:1n9	0.14±0.04	0.17±0.04`	= 0.004
24:1n9	0.05±0.01	0.06±0.03	= 0.039
Σ Monoenes	16.03±2.6	15.1±2.5	>0.05
18:2n6	22.6±2.8	24.3±2.9	=0.033
18:3n6	0.10±0.04	0.12±0.05	>0.05
20:2n6	0.26±0.05	0.31±0.07	<0.001
20:3n6	2.93±0.8	3.69±1.0	<0.01
20:4n6	10.1±1.6	10.4±1.8	>0.05
22:4n6	0.31±0.07	0.39±0.1	= 0.004
22:5n6	0.26±0.09	0.32±0.1	= 0.019
ΣN6	36.6±2.4	39.6±4.4	= 0.015
18:3n3	0.38±0.2	0.38±0.1	>0.05
20:5n3	0.85±0.4	1.08±0.6	>0.05
22:5n3	0.92±0.2	1.05±0.3	= 0.047
22:6n3	3.60±1.0	4.10±1.6	>0.05
ΣN3	5.80±1.4	6.65±2.2	>0.05
16:0DMA	0.37±0.09	0.38±0.1	>0.05
18:0DMA	0.08±0.02	0.10±0.03	= 0.016
N6/N3 ratio	6.64±1.7	6.61±1.8	>0.05

Table 5: Mean (±sd) Percent Red Blood Cell Phosphatidylcholine Fatty Acids of Polish Children & Teenagers with (HFA/AS, n=44) and without (HC, n=17) Autism

Fatty Acids	**HFA/AS**	**HC Siblings**	**P-value**
14:0	0.26±0.08	0.27±0.08	>0.05
16:0	34.8±1.3	33.4±1.6	<0.001
18:0	11.5±0.8	11.7±0.9	>0.05
20:0	0.18±0.05	0.13±0.1	>0.05
Σ Saturates	47.9±1.1	46.8±1.4	= 0.002
16:1n7	0.34±1	0.34±01	>0.05
18:1n7	1.44±0.2	1.37±0.2	>0.05
18:1n9	17.3±1.0	16.5±1.0	<0.01
20:1n9	0.27±0.06	0.27±0.08	>0.05
24:1n9	-	-	-
Σ Monoenes	19.3±1.1	18.5±1.0	= 0.004
18:2n6	18.9±1.6	20.0±1.8	= 0.032
18:3n6	0.16±0.05	0.13±0.05	>0.05
20:2n6	0.25±0.05	0.27±0.03	= 0.039
20:3n6	1.72±0.4	2.0±0.3	= 0.015
20:4n6	6.81±1.1	6.81±1.1	>0.05
22:4n6	0.43±0.1	0.50±0.08	= 0.03
22:5n6	0.27±0.1	0.31±0.08	>0.05
ΣN6	28.6±1.3	30.0±1.5	<0.001
18:3n3	0.28±0.09	0.28±0.06	>0.05
20:5n3	0.55±0.2	0.64±0.3	<0.05
22:5n3	0.54±0.1	0.60±0.08	<0.05
22:6n3	1.71±0.5	2.01±0.9	<0.05
ΣN3	3.15±0.7	3.61±1.3	>0.05
16:0DMA	0.48±0.08	0.49±0.1	>0.05
18:0DMA	0.26±0.06	0.34±0.1	= 0.013
N6/N3 ratio	9.56±2.3	9.16±2.2	>0.05

Table 6: Mean (±sd) Percent Red Blood Cell Phosphatidylethanolamine Fatty Acids of Polish Children & Teenagers with (HFA/AS, n=44) and without (HC, n=17) Autism

Fatty Acids	HFA/AS	**HC Siblings**	**P-value**
14:0	0.10±0.04	0.09±0.03	>0.05
16:0	11.0±1.0	10.7±1.4	>0.05
18:0	5.61±0.4	5.71±0.5	>0.05
20:0	0.11±0.03	0.11±0.05	>0.05
Σ Saturates	17.4±1.0	17.2±1.5	>0.05
16:1n7	0.13±0.05	0.13±0.06	>0.05
18:1n7	0.78±0.1	0.77±0.1	>0.05
18:1n9	14.8±1.1	14.9±1.1	>0.05
20:1n9	0.29±0.06	0.37±0.1	= 0.009
24:1n9	-	-	-
Σ Monoenes	15.96±1.3	16.1±1.2	>0.05
18:2ω6	4.32±0.7	5.03±1.1	= 0.003
18:3n6	0.32±0.06	0.26±0.08	= 0.007
20:2n6	0.15±0.05	0.18±0.04	>0.05
20:3n6	0.88±0.2	1.05±0.3	= 0.009
20:4n6	24.3±1.7	24.4±1.4	>0.05
22:4n6	5.36±1.2	6.01±1.1	<0.05
22:5n6	0.61±0.2	0.66±0.1	>0.05
Σ N6	35.9±2.4	37.6±1.7	= 0.003
18:3n3	0.15±0.04	0.18±0.05	>0.05
20:5n3	1.25±0.7	1.09±0.5	>0.05
22:5n3	4.30±0.7	4.20±0.4	>0.05
22:6n3	6.55±1.4	6.46±1.2	>0.05
Σ N3	12.3±2.3	11.9±1.7	>0.05
16:0 DMA	4.68±0.5	4.31±0.4	= 0.012
18:0 DMA	9.47±1.2	9.50±1.3	>0.05
18:1 DMA	1.57±0.3	1.53±0.3	>0.05
N6/N3 ratio	3.03±0.7	3.2±0.5	>0.05

Table 7: Correlation (Pearson, r) between Plasma and Red Blood N3 and N6 Fatty Acids, and Scaled Verbal, Performance and Full-Scale IQ of Polish Children & Teenagers with Autism (HFA/AS, n=44)

Plasma phosphatidylcholine	**Verbal IQ**	**Performance IQ**	**Full-Scale IQ**
18:2n6	- 0.244 (p>0.05)	- 0.163 (p>0.05)	- 0.228 (p>0.05)
20:3n6	0.029 (p>0.05)	0.110 (p>0.05)	0.153 (p>0.05)
20:4n6	0.168 (p>0.05)	0.125 (p>0.05)	0.157 (p>0.05)
22:4n6	0.01 (p>0.05)	0.05 (p>0.05)	0.134 (p>0.05)
22:5n6	- 0.013 (p>0.05)	- 0.089 (p>0.05)	- 0.007(p>0.05)
ΣN6	- 0.161 (p>0.05)	- 0.071 (p>0.05)	- 0.098 (p>0.05)
18:3n3	0.041 (p>0.05)	0.042 (p>0.05)	0.144 (p>0.05)
20:5n3	0.174 (p>0.05)	0.157 (p>0.05)	0.143 (p>0.05)
22:5n3	**0.357 (p=0.019)**	0.281 (p>0.05)	**0.402 (p=0.008)**
22:6n3	0.124 (p>0.05)	0.033 (p>0.05)	0.057 (p>0.05)
ΣN3	0.0189 (p>0.05)	0.102 (p>0.05)	0.125 (p>0.05)
RBC phosphatidylcholine			
18:2n6	- 0.194 (p>0.05)	- **0.358 (p=0.019)**	- **0.320 (p=0.039)**
20:3n6	0.073 (p>0.05)	0.095 (p>0.05)	0.177 (p>0.05)
20:4n6	0.073 (p>0.05)	0.123 (p>0.05)	0.180 (p>0.05)
22:4n6	- 0.159 (p>0.05)	0.133 (p>0.05)	0.048 (p>0.05)
22:5n6	- 0.220 (p>0.05)	- 0.101 (p>0.05)	- 0.153 (p>0.05)
ΣN6	- 0.165 (p>0.05)	**- 0.304 (p=0.047)**	0.215 (p>0.05)
18:3n3	0.093 (p>0.05)	0.148 (p>0.05)	0.027 (p>0.05)
20:5n3	0.105 (p>0.05)	0.173 (p>0.05)	0.156 (p>0.05)
22:5n3	**0.308 (p=0.044)**	**0.304 (p=0.047)**	**0.388 (p=0.011)**
22:6n3	0.014 (p>0.05)	0.004 (p>0.05)	0.029 (p>0.05)
Σ N3	0.125 (p>0.05)	0.119 (p>0.05)	0.091(p>0.05)
RBC phosphatidylethanolamine			
18:2n6	- 0.116 (p>0.05)	- 0.158 (p>0.05)	- 0.175 (p>0.05)
20:3n6	- 0.170 (p>0.05)	- 0.111 (p>0.05)	0.039 (p>0.05)
20:4n6	0.037 (p>0.05)	0.039 (p>0.05)	0.096 (p>0.05)
22:4n6	- 0.237 (p>0.05)	- 0.077 (p>0.05)	- 0.134 (p>0.05)
22:5n6	- 0.244 (p>0.05)	- 0.210 (p>0.05)	- 0.232 (p>0.05)
ΣN6	- 0.149 (p>0.05)	- 0.073 (p>0.05)	- 0.073 (p>0.05)
18:3n3	0.088 (p>0.05)	0.009 (p>0.05)	0.070 (p>0.05)
20:5n3	0.217 (p>0.05)	0.116 (p>0.05)	0.149 (p>0.05)
22:5n3	**0.390 (p=0.010)**	0.182 (p>0.05)	**0.370 (p=0.016)**
22:6n3	0.051 (p>0.05)	0.087 (p>0.05)	0.066 (p>0.05)
ΣN3	0.216 (p>0.05)	0.036 (p>0.05)	0.115 (p>0.05)

Table 8: Correlation (Pearson, r) between Plasma and Red Blood Cell N3 and N6 Fatty Acids, and Conner's Parent Rating Scale T-scores of Polish Children and Teenagers with Autism (HFA/AS, n=44)

Plasma phosphatidylcholine	**ADHD Index**	**Inattention**	**Oppositional**	**Hyperactivity**
18:2n6	0.024 (p>0.05)	0.063 (p>0.05)	0.009 (p>0.05)	- 0.266 (p>0.05)
20:3n6	0.121 (p>0.05)	0.051 (p>0.05)	0.236 (p>0.05)	**0.362 (p=0.018)**
20:4n6	0.083 (p>0.05)	0.181 (p>0.05)	0.190 (p>0.05)	0.041 (p>0.05)
22:4n6	0.164 (p>0.05)	0.020 (p>0.05)	**0.341(p=0.025)**	0.283 (p>0.05)
22:5n6	0.048 (p>0.05)	0.150 (p>0.05)	0.234 (p>0.05)	0.198 (p>0.05)
ΣN6	0.038 (p>0.05)	0.038 (p>0.05)	0.234 (p>0.05)	0.139 (p>0.05)
18:3n3	- 0.141 (p>0.05)	- 0.043 (p>0.05)	**- 0.333 (p=0.029)**	-0.116 (p>0.05
20:5n3	**- 0.395 (p=0.009)**	- 0.270 (p>0.05)	**- 0.365 (p=0.016)**	- 0.098 (p>0.05)
22:5n3	- 0.117 (p>0.05)	- 0.168 (p>0.05)	0.085 (p>0.05)	- 0.017 (p>0.05)
22:6n3	**- 0.402 (p=0.007)**	**- 0.335 (p=0.028)**	- 0.280 (p>0.05)	**- 0.320 (p=0.039)**
ΣN3	**- 0.425 (p=0.005)**	**- 0.340 (p=0.026)**	**- 0.319 (p=0.035)**	- 0.269 (p>0.05)
N6/N3 LCPUFA	**0.312 (p=0.041)**	0.280 (p>0.05)	0.234 (p>0.05)	0.217 (p>0.05)
RBC phosphatidylcholine				
18:2n6	0.015 (p>0.05)	0.100 (p>0.05)	0.047 (p>0.05)	0.157 (p>0.05)
20:3n6	0.166 (p>0.05)	0.156 (p>0.05)	0.286 (p>0.05)	**0.401(p=0.009)**
20:4n6	0.179 (p>0.05)	0.070 (p>0.05)	**0.314 (p=0.041)**	0.189 (p>0.05)
22:4n6	**0.339 (p=0.026)**	**0.308 (p=0.044)**	0.256 (p>0.05)	0.189 (p>0.05)
22:5n6	**0.298 (p<0.05)**	0.275 (p>0.05)	0.170 (p>0.05)	0.164 (p>0.05)
ΣN6	0.224 (p>0.05)	0.252 (p>0.05)	**0.336 (p=0.028)**	0.114 (p>0.05)
18:3n3	- 0.206 (p>0.05)	- 0.122 (p>0.05)	**- 0.337 (p=0.027)**	- 207 (p>0.05)
20:5n3	**- 0.321 (p=0.036)**	- 0.181 (p>0.05)	- 0.254 (p>0.05)	- 0.219 (p>0.05)
22:5n3	0.171 (p>0.05)	0.139 (p>0.05)	0.284 (p>0.05)	0.131 (p>0.05)
22:6n3	- 0.089 (p>0.05)	- 0.040 (p>0.05)	- 0.130 (p>0.05)	- 0.156 (p>0.05)
Σ N3	- 0.138 (p>0.05)	- 0.063 (p>0.05)	- 0.151 (p>0.05)	- 0.157 (p>0.05)
N6/N3 LCPUFA	0.062 (p>0.05)	0.059 (p>0.05)	0.101 (p>0.05)	0.187 (p>0.05)
RBC Phosphatidylethanolamine				
18:2n6	0.125 (p>0.05)	0.252 (p>0.05)	0.135 (p>0.05)	0.134 (p>0.05)
20:3n6	0.151 (p>0.05)	0.222 (p>0.05)	0.143 (p>0.05)	0.206 (p>0.05)
20:4n6	0.023 (p>0.05)	0.110 (p>0.05)	0.701 (p>0.05)	0.180 (p>0.05)
22:4n6	0.236 (p>0.05)	0.056 (p>0.05)	0.099 (p>0.05)	0.052 (p>0.05)
22:5n6	0.130 (p>0.05)	0.021 (p>0.05)	0.022 (p>0.05)	0.216 (p>0.05)
ΣN6	0.171 (p>0.05)	0.035 (p>0.05)	0.212 (p>0.05)	0.140 (p>0.05)
18:3n3	- 0.076 (p>0.05)	- 0.088 (p>0.05)	**- 0.333 (p=0.029)**	- 0.164 (p>0.05)
20:5n3	**- 0.317 (p=0.038)**	- 0.128 (p>0.05)	**- 0.328 (p=0.032)**	- 0.160 (p>0.05)
22:5n3	- 0.087 (p>0.05)	0.059 (p>0.05)	0.086 (p>0.05)	0.047(p>0.05)

22:6n3	**- 0.297 (p<0.05)**	- 0.154 (p>0.05)	**- 0.362 (p=0.017)**	- 0.246 (p>0.05)
ΣN3	**- 0.306 (p=0.046)**	- 0.150 (p>0.05)	**- 0.298 (p=0.049)**	- 0.188 (p>0.05)
N6/N3 LCPUFA	**0.316 (p=0.039)**	0.175 (p>0.05)	0.278 (p>0.05)	0.252 (p>0.05)