Polyunsaturated fatty acids: Do they have a role in the pathophysiology of autism?

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ORIGINAL ARTICLE

Abstract

OBJECTIVES: There is now some evidence that alterations in fatty acids may play a role in the pathophysiology of autism. The aim of the present study was to examine whether autism is accompanied by abnormalities in the composition of the polyunsaturated fatty acids (PUFAs) in plasma phospholipids.

METHODS: The plasma phospholipid omega-3 (ω3) and omega-6 (ω6) PUFA fractions and the ω3/ω6 ratio were measured in 16 high-functioning male youngsters with autism (age 12–18) and 22 healthy volunteers. Group mean differences were assessed by means of analysis of variance (ANOVA).

RESULTS: In autism there was a significant increase in the fraction of C22:6ω-3 (docosahexaenoic acid, DHA) and an increase in the total ω3/ω6 ratio.

DISCUSSION: The results of this study suggest that an increase of the plasma phospholipid ω3 PUFAs, in particular DHA, and of the total ω3/ω6 ratio may take part in the pathophysiology of autism. One hypothesis is that an increase of ω3 PUFAs may cause alterations in the serotonergic turnover and the immune response system, both known to be associated with autism. Caution must be exercised against highly concentrated ω3 PUFAs supplementation.

Introduction

Polyunsaturated fatty acids (PUFAs), which comprise 25–30 % of the fatty acids in the human brain, are necessary for normal brain development and function. They contribute to the composition of neuronal membranous phospholipids and play an important role in brain membrane fluidity and function.

PUFA's can be divided into two series: ω6-series and ω3-series. These are essential fatty acids because they cannot be synthesized de novo. They are either directly provided by diet or derived from essential dietary precursors, linoleic acid (18:2ω-6) and α-linoleic acid (18:3ω-3), respectively.

There is evidence that omega-3 fatty acids are involved in brain function of humans [33]. Studies have demonstrated that PUFAs are involved in the regulation of many biochemical events in the brain such as neurotransmitter release and uptake [27], receptor function in the CNS [49] and enzymatic processes [10]. A correlational relationship has been described between PUFAs in plasma and metabolism of serotonin in the central nervous system [30].

PUFAs also have immunomodulatory properties. The ω6 PUFA arachidonic acid (AA) gives rise to the eicosanoid family of pro-inflammatory mediators (prostaglandins, leukotrienes and related metabolites) and through these regulates the activities of inflammatory cells, the production of cytokines and the various balances of the immune system [12]. Omega-3 PUFAs on the other hand have anti-inflammatory properties [54]. Therefore alterations of the PUFA composition of brain cell membranes may influence the inflammatory state of the brain and may cause an activation of the immune response system (IRS) [41,42].

There is growing evidence that abnormalities in PUFA metabolism may play a role in the development of neuronal dysfunction and in the etiopathophysiology of a range of psychiatric disorders such as depression [63] and schizophrenia [8].

Furthermore, some studies have shown a potential therapeutic benefit of PUFA dietary supplementations in these disorders [50,58,37].

An impairment of PUFA metabolism has also been postulated to occur in a range of neurodevelopmental disorders including ADHD [53], dyspraxia [59], dyslexia [60], and autism [52]. Autism is defined by the early onset of a constellation of difficulties in reciprocal social interactions and communication and restricted, repetitive behaviors or interests [DSM-4, 4]. The neurobiological basis of autism has become generally accepted.

Bell et al. reported a single case study of a patient with autism spectrum disorder that had reduced percentages of HUFAs in the RBC membranes. They also found evidence of instability of HUFA compositions [7]. A similar instability has been recorded in schizophrenic patients [51]. Vancassel et al. examined the phospholipid fatty acids in the plasma of a population of autistic subjects compared to mentally retarded controls [62]. The results showed a marked reduction in the plasma levels of DHA (C22:6ω-3) in autism, reduced levels of ω3 fatty acids without significant reduction of ω6 fatty acids and consequently a significant increase in the ω6/ω3 ratio. Finally, there is some evidence that PUFA dietary supplementations may also be of potential benefit in autism. There has been a case report of an autistic patient in which addition of omega-3 fatty acid supplement to an existing pharmacological regimen dramatically alleviated agitation and anxiety [32].

Biological studies on children and adolescents with autism are often hampered by a lack of homogeneity of study groups. Using mentally retarded subjects as control group is not ideal considering there is an overlap between autism and mental retardation as far as symptomatology and pathophysiology are concerned [28].

Based on previous research, we hypothesized to find a decrease in the plasma ω3 PUFA levels and / or an increase in the ω6 PUFAs, resulting in a decrease in the ω3/ω6 ratio in a large homogenous group of high functioning (IQ 70 or higher) post-pubertal subjects with autism compared to a group of matched normal controls.

Subjects and methods

Subjects.

In the present study forty male subjects (n=18 with autism and n=22 normal controls) aged between 12 and 20 years old and with an I.Q.>55 participated. One subject in the pathology group had a mild mental retardation (I.Q. between 55–60), all other subjects showed a borderline intellectual functioning (I.Q. between 71–84) or normal intellectual functioning (I.Q. between 85–120). All subjects passed the onset of puberty (Tanner-stage III–IV) and were of the Caucasian race.

Normal volunteers and autistic patients had a normal hematological screening. All subjects were free of any infections, inflammatory or allergic reactions for at least 2 weeks prior to blood samplings. Exclusion criteria for autistic patients and healthy volunteers were: subjects suffering from a neurological, inflammatory, endocrine or clinically significant chronic disease; immunocompromized subjects; subjects with an active seizure disorder; subjects with tuberous sclerosis, FRAXA or other chromosomal disorders; and subjects receiving drugs with known or potential interaction with immune and endocrine functions. All healthy youngsters had a negative past, present or family history for psychiatric disorders such as autistic-, bipolar-, schizophrenic-, paranoid-, organic mental- and eating disorders and psychoactive substance use. None was a regular drinker and none had ever been taking psychotropic drugs. All were free of any medications and substance abuse for at least one month. This was checked by a drug screening in the urine.

Clinical Evaluation

The autistic youngsters were recruited from the outpatient clinic of child- and adolescent psychiatry in Antwerp, Belgium; the mental health agencies of the same city; and from a Residential Treatment Center for Autistic Youngsters in Booischot, Belgium. We employed the DSM-IV [19]]criteria to make the diagnosis of autism. The diagnosis was made on the basis of a consensus between, at least three clinicians (psychiatrists and psychologists), working with the autistic subjects in residential, semi-residential or day-care centers. The Autism Diagnostic Interview-Revised (ADI-R) [40], a semi-structured interview, was performed with the parents of youngsters with autism by a trained Master's level clinician (who was blind to clinical status before the interview) or by the primary author. Consensus meetings were held after the structured interview with the clinician.

In order to evaluate the associated behaviors frequently seen in autism and eventual comorbidity and the absence of psychopathology in the control-group, all subjects completed the Youth Self Report (YSR) scale [1] during the initial screening. Parents of subjects completed the Child Behavior Checklist (CBCL) [2]and the Aberrant Behavior Checklist (ABC) [3].

Methods

All subjects received a VMA-free diet during 24 hours preceding the onset of blood-and urine collections. Subjects were kept at rest during the blood collections. Blood was drawn at 7:45 a.m. (±15 min) after an overnight fast.

Lipids were extracted from plasma with methanol/chloroform according to a modified Folch method. In short, 1 ml of methanol and 2 ml of chloroform were added to 1 ml of plasma. The mixture was centrifuged at 4 °C. The upper layer was removed by aspiration and the interface by filtration. The filtrate was evaporated to dryness under a N2 flow and the residue was dissolved in chloroform. After every step, the samples were flushed with N2 to avoid fatty acid oxidation. Plasma and RBC phospholipids were isolated by thin layer chromatography [13]. The phospholipid band was scraped off and the fatty acids were converted into methyl esters by transesterification using 2 ml of a mixture of methanol/HCl as methylating agent for 4 h at 95 °C. After cooling and addition of distilled water, the methyl esters were extracted with petroleum ether (boiling point 40–60 °C) and evaporated to dryness under a N2 flow not exceeding 40 °C.

The fatty acids were analyzed by temperature-programmed capillary gas chromatography (Varian model 3900 gas chromatograph, Walnut Creek, CA, USA) on a 25-m × 250 :m × 0.2 :m film thickness 10% cyanopropylphenyl – 90% biscyanopropyl polysiloxane column (Rtx®-2330, Restek, U.S.A.). Working conditions are published in detail somewhere else [25]. Peak identification was done based on the retention times using authentic standards [14]. Any plasmalogens present yield dimethyl acetals (DMA) which appear together with the methyl esters in the same chromatogram. They are included in the fatty acid compositions.

The results for individual fatty acids and DMA were expressed as weight % (wt %) of the total fatty acids/ vinyl ethers in the phospholipid fraction. The following sums were calculated: S DMA; saturated fatty acids (S SAFA); monounsaturated fatty acids (S MUFA); polyunsaturated fatty acids (S PUFA); highly unsaturated fatty acids (S HUFA); highly unsaturated fatty acids from the ω3 series (S ω3 HUFA); highly unsaturated fatty acids from the ω6 series (S ω6 HUFA); ω3 fatty acids (S ω3) and ω6 fatty acids (S ω6). The mean melting point (MMP) of the fatty acids, which is believed to be inversely related to membrane fluidity, was calculated as the sum of the mole fraction multiplied by the melting point of each fatty acid. It takes into consideration the chain length, branching and unsaturation of every fatty acid present and therefore, the transition from rigidity to fluidity [34]. The double bond index was calculated as the sum of the mole fraction of each fatty multiplied by the number of olefinic bonds [6].

In order to minimize the analytical variability, all blood specimens for the assays of the above parameters in autistic patients and healthy volunteers were assayed in a single run with a single lot number of reagents and consumables employed by a single operator.

Statistics

Normality of distribution was ascertained with the Kolmogorov-Smirnov test. The independence of classification systems has been checked by means of analysis of contingence (χ2-test). Relationships between variables were assessed by means of analysis of Pearson's product moment or through multiple regression analysis. Group mean differences were assessed by means of analysis of variance (ANOVA) and linear discriminant analysis (LDA). The statistical analyses in Table 1 were examined after p-correction (at p=0.005, two tailed).

Results

Table 1 shows that in autism there was a significant increase of the PL C22:6ω-3 (docosahexaenoic acid, DHA) fraction (mean score 3.40 %, SD 0.8 in autism and 2.83 %, SD 0.6 in normal controls; F= 5.47; p= 0.02). Total ω3 was significantly higher in autistic subjects than in normal controls (mean score 6.18 %, SD 1.0 in

Table 1. Selected fatty acids (weight%) and combinations in serum phospholipids in normal controls and subjects with autism.

Variables	Autism	Controls	F*	df	p
PL C20:5ω-3	0.69	0.67	0.08	1/35	0.76
PL C20:4ω-6	9.17	9.09	0.03	1/35	0.84
PL C22:6ω-3	3.40	2.83	5.47	1/35	0.02
Total ω6	36.0	35.8	0.07	1/35	0.77
Total ω3	6.18	5.49	5.35	1/35	0.02
ω/ω6	0.17	0.15	3.74	1/35	0.05

All results shown as mean(SD)
F* All results of ANOVA

autism and 5.49 %, SD 0.7 in normal controls; F= 5.35; p= 0.02).

There was no significant differences in the PL C20:4ω-6 PUFA fraction between autistic subjects than in normal controls(mean score 9.17 %, SD 0.2 in autism and 9.09 %, SD 1.1 in normal controls; F= 0.03; p= 0.84).

There was an increase in the total ω3/ω6 ratio(mean score 0.17, SD 0.03 in autism and 0.15, SD 0.02 in normal controls; F= 3.74; p= 0.05).

Discussion

The major finding of this present study is that plasma DHA (C22:6ω-3) levels are increased in subjects with autism, compared to healthy controls. These results are statistically significant. With respect to plasma EPA (C20:5ω-3) levels, there is no significant difference between autistic youngsters and normal controls.

These findings suggest there is an increase of the plasma phospholipid ω3 PUFAs and of the total ω3/ω6 ratio in autism. These results are in contradiction with previous findings and our starting hypothesis.

As far as previous findings are concerned, we refer to the study on PUFAs and autism by Vancassel et al. [62]. In comparison with the present study, there was a lack of homogeneity of their study group. Their study population consisted of 15 children, 4 girls and 11 boys, aged between 3 and 17 years. The autistic subjects in the present study were controlled for the intervening effects of variables such as age (all subjects were post-pubertal, between the age of 12 and 18 years old), sex (all males), race (all Caucasian) and IQ (no mental retardation).

Furthermore, Vancassel et al. compared autistic subjects to mentally retarded controls, which is not a suitable control group [28].

With respect to our starting hypothesis, we suggested that PUFAs may play a role in the pathophysiology of autism in two ways.

There is ample evidence that autism is accompanied by disturbances of the serotonergic system [20] and may be associated with an activation of the IRS [21]. As outlined above PUFAs are also known to play a role in these processes.

Firstly, PUFAs are known to be involved in the regulation of neurotransmitters in the central nervous system. Changes in PUFA levels may induce changes of the brain cell membrane fluidity, which in turn has a strong influence on the expression and function of the serotonin (5-HT) reuptake sites and serotonin receptors [9,48,24,23]. Previous research indicated that serotonergic dysfunctions could be caused by w3 PUFA depletion. Serotonin also plays an important role in developmental disorders because of its role as a morphogenetic agent and a differentiation factor in the developing brain [5]. There is some evidence for a role of 5-HT in the pathophysiology of autism. Selective 5-HT reuptake inhibitors (SSRI's) have beneficial effects in the treatment of patients with autism [46]. Tryptophan depletion techniques result in a significant increase in autistic behaviors [45]. PET-scan studies reveal decreased 5-HT synthesis in frontal cortex and thalamus, but elevated 5-HT synthesis in the contralateral dentate nucleus [15]. There is some preliminary evidence of linkage and association between the 5-HT transporter gene and autistic behavior [18,38]. Croonenberghs et al. reported an increase in the platelet H – paroxetine Kd values in autism, suggesting a decreased affinity for 5-HT in the platelet 5-Ht transporter system [21].

A second way of linking PUFAs with autism is through the activation of the immune response system (IRS).

Findings of previous research on autism include: increased urinary concentrations of neopterin [47], elevated plasma interferon-alpha concentrations [47], increased levels of the T8 antigen and IL-2 [55], and increased concentrations of IL-12 and Interferon-gamma [56]. It is interesting to note that activation of the IRS may induce some of the behavioral symptoms of autism through an increased production of proinflammatory cytokines. For example, IFNg, IL-1 and IL-6 may induce symptoms, such as sleep disorders, social withdrawal, suppression of social, locomotor and exploratory behavior and anhedonia [22,39].

As mentioned before PUFAs are involved in the production of cytokines such as Interferon-gamma, TNF, IL-1 and IL-6 [43]. Omega-6 PUFAs function as precursors of pro-inflammatory eicosanoids [35], omega-3 PUFAs on the other hand show anti-inflammatory and immunosuppressive actions [11]. An imbalance of ω3 to ω6 PUFAs, more specifically a decrease of the ω3/ω6 ratio may therefore cause an overproduction in pro-inflammatory cytokines [26] and a chronic activation of the Immune Response System (IRS), which is also seen in autism. Present study however shows an increase in the ω3/ω6 ratio in subjects with autism.

Different hypotheses can be proposed to explain these findings.

Fatty acid metabolism is very complex and can be influenced by many factors, both constitutional and environmental.

In trying to explain underlying mechanisms in psychiatric and developmental disorders, some studies suggest an abnormality in phospholipase enzyme activity (PLA2) and/or concentration. PLA2 releases fatty acids from membrane phospholipids and is important for general membrane phospholipid turnover.

Bell et al. implies an elevation of PLA2 in a single case study of an individual with ASD (autistic spectrum disorder) [7]. He also studied two individuals with Asperger's Syndrome (a milder form of ASD) and found that red cell membrane HUFA concentrations in these subjects showed a remarkable stability, relative to control samples, consistent with earlier speculations that phospholipase activity may be reduced in this condition [36]. In this case we would expect a reduced turnover of PUFAs, a reduced breakdown and incorporation of PUFAs into cell membranes and consequently an elevation of plasma

PUFA levels. This is in consistency with the results of our study.

Other studies [19] show that an abnormality in apo-lipoprotein-mediated PUFA (DHA) transportation and concentration into the brain may play a role in the patho-physiology of aggressive and impulsive behaviors, which are also observed in autistic children. Impulsive violence and aggressive behaviors have been repeatedly linked to low concentrations of cerebrospinal fluid of 5-hydroxy indoleacetic acid (CSF 5-HIAA) [44], a metabolite that reflects serotonin turnover predominantly in the frontal cortex [57]. Low concentrations of CSF 5-HIAA have also been reported in autistic children [16,17]. J. Hibbeln reports that the serotonin turnover rate in the CNS may be modulated by DHA [29]. He found that plasma levels of DHA were inversely correlated with CSF 5-HIAA among people with violent, impulsive behaviors, sug-gesting that they may have a defect in the mechanisms that regulate the transportation and selective concentra-tion of DHA into the brain. Such a defect would result in the accretion of DHA in plasma and not reflect brain concentrations [31]. One could postulate that autistic children share the same defect, explaining the findings in present study, that plasma DHA levels are raised in autism. Considering a possible negative correlation be-tween plasma DHA and CSF 5-HIAA in autism, similar as in people with impulsive behaviors, this may suggest that elevating plasma DHA concentrations could directly reduce central serotonin concentrations, and perhaps worsen symptoms associated with autism.

In other words, an increase in plasma ω3 PUFA levels and ω3/ω6 ratio, as seen in the autistic subgroup of this study may reflect a depletion of ω3 levels (DHA) and a decrease of ω3/ω6 ratio in the CNS. This would offer an explanation for the central serotonergic hypofunction and the pro-inflammatory state of the brain, both hy-pothesized to play a possible role in the pathophysiology of autism.

Limitations of the present study include
1) the fact that we examined the phospholipid ω3 and ω6 fractions in plasma. Although reported abnor-mal in many studies, these may be confounded by di-etary intake and reflect short-term turnover. It's un-clear if plasma PUFA levels reflect the PUFA status in the brain. In trying to understand the role of abnor-mal PUFA metabolism in the pathophysiology of au-tism, more research is needed to examine the correla-tion between plasma PUFA levels and red blood cell membrane PUFA concentrations on the one hand and abnormalities in the serotonergic turnover and the im-mune response system on the other hand. Measuring fatty acids in red blood cell membranes would be more reliable as it more closely reflects long term PUFA turnover and the fatty acid composition of the neuro-nal cell membranes [22];
2) the small number of autistic subjects and controls (n=26) and the lack of inclusion of autistic patients

with specific psychopathological dimensions, e.g. ag-gressive and obsessive behaviors.

In conclusion the present study is the first to suggest that an increase in plasma ω3 levels, in particular DHA levels and of the total ω3/ω6 ratio may play a role in the pathophysiology of autism. Although underlying mechanisms in these processes are still not clear, one hypothesis is that an increase in ω3 PUFAs (DHA) may cause alterations in the serotonergic turnover and im-mune response system, both known to be associated with autism. Caution must then be exercised against highly concentrated ω3 PUFAs supplementation.

Acknowledgments

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