Efficacy of Adding Large Doses of Arachidonic Acid to Docosahexaenoic Acid against Restricted Repetitive Behaviors in Individuals with Autism Spectrum Disorders: A Placebo-Controlled Trial

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Abstract

Objectives: It has been documented that behavioral addictions resemble substance addiction in many domains, including the repetitive patterns of interests and behavior displayed by addicts and their failure to resist impulses to perform an act that are harmful to themselves or others. Restricted and repetitive patterns of interests and behavior resembling the characteristics of behavioral addictions are also a core feature of autism spectrum disorders (ASD). Impaired delivery of afferent signals has been proposed to be a factor in pathophysiology of ASD. The polyunsaturated fatty acids, arachidonic acid (ARA) and docosahexaenoic acid (DHA) play key roles in the maturation of the brain network. Supplementation of larger doses of ARA added to DHA may therefore improve repetitive and addictive behavior.

Methods: To estimate the efficacy of this supplementation in individuals with ASD, we conducted a 16-week double-blind, randomized placebo-controlled trial in 13 individuals with ASD. The outcomes were measured using the Aberrant Behavior Checklist (ABC), and the repetitive behavior subdomains of the Autism Diagnostic Interview-Revised (ADI-R). To study the mechanisms underlying the effects of this supplementation regimen, we examined the plasma levels of main polyunsaturated fatty acids (i.e., eicosapentaenoic acid, DHA and ARA).

Results: Our supplementation regimen significantly improved ABC-measured social withdrawal and ADI-R C3 subdomain (stereotyped and repetitive motor manereiments). Their scores of the ADI-R C3 subdomain were significantly correlated with ABC-measured inappropriate speech (r=0.397, P<0.01). The changes in the plasma ARA levels at the end of the placebo-controlled trial was significantly increased in the treatment group.

Conclusion: The observed improvement in repetitive and addictive behavior might have been related to reduction in the scores of the ABC-measured social withdrawal and ABC-measured inappropriate speech. These findings suggest that improvements in impaired social interactions may contribute to improvements in repetitive and addictive behavior.

Keywords: Repetitive behavior; Behavioral addictions autism spectrum disorders; Arachidonic acid; Clinical trial; Signal transduction.

Introduction

Autism spectrum disorders (ASD) refers to a group of developmental disorders (autistic disorder, Asperger's syndrome, and pervasive developmental disorder not otherwise specified) characterized by reduced social interactions; impairments in language function; and restricted repetitive and stereotyped patterns of behavior, interests and activities [3]. These behaviors include stereotypical movements, repetitive play, inflexible routines, and a ritualistic onset of attention on consistency [20], which are often the case with internet addiction [8]. When such behaviors are interrupted, a child with ASD may protest or exhibit anxiety or aggression [20]. Interestingly, it has been documented that several behaviors, besides psychoactive substance ingestion produce short-term reward that may engender persistent behavior despite knowledge of aversive consequences (i.e., diminished control over the behavior). Proposed revision for the DSM include for the first time “behavioral addiction” [17]. In this regard, restricted and repeated patterns of behavior observed in ASD may be included in behavioral addictions.

Many of the drugs such as risperidone [34], and selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, fluvoxamine [47], citalopram or sertraline [40] are useful in treating disruptive repetitive behaviors. SSRIs are of limited efficacy in children and adolescents, but antipsychotics such as risperidone and aripiprazole have been shown to reduce the severity if these symptoms [48]. However, use of these SSRI and antipsychotics is limited by their adverse effects, particularly weight gain, fatigue, agitation, irritability [34,47] and tardive dyskinesia [35]. The neuropeptide oxytocin has been found to decrease compulsive and repetitive behaviors; however, this effect was only lasted 240 minutes after its infusion [18], and thus the long-term effects of oxytocin on repetitive behaviors are unclear. Thus treatments for the core compulsive addictive patterns of behaviors observed in ASD have few adverse effects.

Imagining studies have suggest that social impairment in ASD

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is be associated with reductions in long-distance connectivity between frontal and temporal higher cortices and other structures that promote detail-oriented processing strategies [9]. Such reduced connectivity might be due to inadequate numbers and timing of long-distance afferents, which would affect the delivery of afferent signals to higher-order cortical regions [9]. It is therefore possible that restricted and repetitive patterns of behavior in ASD may be related to impaired signaling based on abnormal connectivity in the central nervous system (CNS). The CNS is rich in polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA) and arachidonic acid (ARA). DHA and ARA are essential for brain development [22], and ARA has a key role insignaling [5,42]. Also, altered fatty acid metabolic pathways may contribute to pathophysiology of ASD [6]. Infant formula with 0.36% DHA and 0.72% ARA promotes mental development [7]. ARA may therefore mitigate impaired social interaction and compulsive behavioral addictions in individuals with ASD. Previous double-blind randomized placebo-controlled studies found that DHA or eicosapentaenoic acid (EPA) added to small doses of ARA supplementation reduced behavioral problems in children with Attention Deficient/Hyperactive Disorder [36], learning difficulties (LD) [41], or ASD [4]. However, there are no double-blind randomized placebo-controlled studies of the effect of large doses of ARA, added to DHA, on social impairment, and restricted and repeated patterns of behavior observed in ASD. In this preliminary study we opted for a double-blind, randomized placebo-controlled trial to measure the treatment effects on social interaction and restricted and repeated patterns of behavior in individuals with ASD of larger doses of ARA added to DHA supplementation. To determine the role of ARA in our supplementation regimen, we examined plasma levels of main PUFAs (EPA, DHA and ARA).

Methods

Behavioral characteristics of ASD

Restricted and repetitive patterns of behavior are a core feature of autism spectrum disorders [23]. Several behaviors, besides psychoactive substance ingestion, that has become the major focus of a person’s life to the exclusion of other activities, or that has harm the individual or others physically, mentally, or socially is considered an behavioral addiction [14,17]. The essential feature of behavioral addictions is the failure to resist an impulse, drive, or temptation to perform an act that is harmful to the person or to others [14]. Each behavioral addiction is characterized by a recurrent pattern of behavior that has this essential feature within a specific domain [14]. The repetitive engagement in these behaviors ultimately interferes with functioning in other domains. Drawing these strands together, restricted and repetitive patterns of behavior in ASD may be considered in behavioral addictions.

Subjects

Our study includes 13 individuals (12 males and 1 female) in the age range 6-28 years (mean age±SD, 14.6±6.0 years), who had been diagnosed with autistic disorder, showing delays in the development of spoken language (n=1) or Asperger’s disorder (n=12). For a diagnosis of ASD, semi-structured clinical interviews based on the Autism Diagnostic Interview Revised (ADI-R) were used [38] as were DSM-IV checklists [3] and available information from schools and child psychiatric services concerning the development in childhood. Each diagnosis was established at the screening by the agreement of two independent experienced psychiatrists. The subjects were randomly assigned to receive either the experimental medication or a matched placebo as described below.

At screening and at the end of the trial, the subjects underwent a physical examination (sitting blood pressure, heart rate, weight, and height), and clinical laboratory measurements (clinical chemistry, platelet activation, and hematology). The inclusion criteria were as fellows: (1) absence of medical or comorbid psychiatric disorders; (2) weight of at least 16 kg; (3) verbal or performance intelligence quotient (IQ) above 80 at the baseline as measured by the Wechsler Intelligence Scale for children and adolescents aged 6-16 years (WISC-III) [45] or for adults (WAIS-R) [46], as the total IQ is at least 80 in subjects with Asperger disorders [33]; and (4) a score of greater than 10 on the social withdrawal subscale of the Aberrant Behavior Checklist (ABC) [2] at the baseline. The fourth criterion was employed because the mean score on the social withdrawal subscale of the ABC in subjects aged 5-30 years was found to be less than 10 in 531 subjects with neuropsychiatric diseases (e.g., deafness and psychoses) [1]. The five exclusion criteria were as follows: (1) hospitalization for their behavioral symptoms within the previous 3 months; (2) the presence of clinically significant abnormal laboratory data; (3) presence of neurological disorders, sensory or chromosomal abnormalities; (4) treatment of ADHD symptoms with stimulants was allowed during the study provided that the patient’s dosage had remained stable for at least 3 months before and during the study. Finally,(5) individuals who received program teaching appropriate social skills within the previous 3 months.

The study was conducted at the Department of Child and Adolescent Psychiatry, Sawa Hospital, which is a psychiatric institute attached to the Ashiya University, in Osaka. The study protocol was approved by the appropriate ethics committee of the Sawa Hospital. The committee was given a full description of the study, after which written informed consent was obtained from each participant’s parents, or the participant, or both.

Study drugs

Commercially available Aravita (SUNTGA40S; Suntoy Ltd, Osaka, Japan) contains 40 mg/capsule of DHA, 40 mg/capsule of ARA, and 0.16 mg/capsule of astaxanthin (carotenoid antioxidant). We purchased the Aravita and identical capsules containing olive oil from the Suntory Ltd.

Study design

This study was conducted in the Research Institute of Pervasive Developmental Disorders of Ashiya University between October 2008 and January 2009 through a local advertisement. After a 14-day screening period, during which we examined whether the participants met the above mentioned described inclusion and exclusion criteria for ASD, the 13 eligible participants were randomly assigned either to a supplementation with larger doses of ARA added to DHA (n=7; aged range, 6 to 20 years; mean age, 13.9±5.3 years), or matching placebo (n=6; mean age, 15.5±7.4 years; five were aged 7 to 18 years and one was 28 years-old). The assignment of the subjects was based on demographic data, medical and psychiatric history, routine laboratory evaluation, and baseline scores for the two outcome measures, i.e., the ABC [2] and ADI-R repetitive behavior domain [26]. The subject assignment was performed by two pharmacological scientists who were not directly involved in the study. In a double-blind randomized placebo-controlled trial conducted for 16 weeks, the daily experimental medication was 6 capsules of ARA-enriched triglyceride (SUNTGA20S). The placebo was an identical capsule.
containing olive oil. Four participants, who ranged in age from 6 to 10 years, received a daily supplementation dose of 120 mg.

Assessment of efficacy

Measures of restricted and repetitive patterns of behavior were obtained using an restricted repetitive behavior domain (C-domain) on the Autism Diagnostic Interview-Revised (ADI-R) [26] and the ABC at the baseline, and at 4, 8, 12, and 16 weeks during the placebo-controlled trial. The ADI-R is a semi-structured interview administered to parents or caregivers, and includes three domains related to early development, social behavior, communication skill, and repetitive behavior as a measure of clinical features of ASD [11, 21,39]. It's algorithms for the social, communication and repetitive stereotyped behavior domains were validated (Lord et al., 2000) [26]. The ADI-R domains (communication, social behavior and repetitive behavior) were used to adapt several times over the period [37]. For example, the ADI-R has been used to measure longitudinal changes over two years on ADI-R scores of pre-school children with ASD [39] and developmental changes of ASD (Fetteau et al., 2003) [11].

The repetitive stereotyped behaviors included algorithm items C1 (encompassing preoccupation or circumscribed pattern of interest), C2 (apparently compulsive adherence to non-functional routines or rituals), C3 (stereotyped and repetitive motor mannerisms) and C4 (preoccupations with parts of objects or non-functional elements of materials) [26,28]. The ABC was intended primarily to evaluate treatment in ASD psychopharmacological and behavioral intervention trials [2] involving children and adolescents with normal IQ levels [18]. It consists of 58 items, each scored on a 4-point scale (0: not a problem, through 3: problem in severe in degree). The items fall into five subscales: (1) irritability, (2) social withdrawal, (3) stereotypy, (4) hyperactivity and (5) inappropriate speech in conjunction with a clearly established and validated factorial structure [2].

Safety assessment

The safety of the supplementation regimen was assessed by monitoring and recording adverse events throughout the 16-week placebo-controlled trial. Adverse events were either reported spontaneously by the participants or the participants’ guardians, or were noted by the investigator.

Assays of plasma levels of PUFAs

Plasma samples were obtained from blood anticoagulated with ethylene-diaminetetra-acetic acid and frozen at -80°C until analysis. Plasma levels of PUFAs were measured at the baseline, after the intervention at week 8, 16 during the placebo-controlled trial, and at week 24 and 32 during the open-label treatment. The measurements were conducted by specialists at the SRL, Inc, Tokyo.

Plasma levels of PUFAs

The fatty acid composition of the total phospholipid fraction of the patients’ plasma was determined as fellows [15]. Total lipids were extracted from the plasma according to the method of Bligh and Dyer [7]. After transmethylation with HCl-methanol, the fatty acid composition was analyzed by gas chromatography (GC2010 Shimadzu Co, Japan). A total of 24 long-chain fatty acids were identified. The sensitivity of detection of our method for measuring the plasma levels of DHA and ARA was 0.2μg/mL. The intra- and inter-assay coefficients of ARA were 110.14 μg/mL and 100.63 μg/mL, respectively, and those of DHA were 73.87 μg/mL and 68.07 μg/mL, respectively. The plasma level of each PUFA is expressed as the percentage weigh of total fatty acids, as mean±standard deviation values (Table 3).

Data analysis

Repeated-measures analysis of variance (ANOVA) was used to compare ASD and control groups, with baseline scores as the covariate, treatment as the between-subject-factor, and test session as a repeated factor, so as to adjust for pretreatment differences. Multiple comparison procedures are commonly used in ANOVA after obtaining a significant omnibus test results [32]. We used the Bonferroni test as a multiple comparison [32]. The data were not normally distributed, so that the group comparisons at the baseline and at 16 weeks were carried out using the non-parametric Mann-Whitney U-test. As the scores for the ADI-R C3 sub domain (stereotyped and repetitive motor mannerisms) were significantly different between the two groups during the 16-week placebo-controlled trial, the relationships between this subdomain and the five subscales scores of the ABC were compared among the two groups using Pearson’s correlation. The X² test was used for categorical variables. All tests of hypotheses were performed using a 2-sided significance levels of 0.05. To analyze the data, we used SPSS version 18.

Results

Sample characteristics

Of the 13 participants, four were aged 6-10 years, 8 were aged 13-20, and one was 28-years-old. All individuals displayed the above-mentioned characteristics of behavioral addictions. The following restricted and repetitive behaviors in the 13 participants: playing video game (n=2), reading circulars about electric devices (n=1), petting a small dog (n=1), preoccupied with a particular briefcase (n=1), being preoccupied with red ink ballpoint pens (n=1), being preoccupied with particular model of shoes (n=1), being preoccupied with handkerchiefs (n=2), and being preoccupied with stuffed toys (n=2), and displaying restricted and stereotyped interests in particular TV personalities (n=1) or TV programs (n=1). There were no significant differences in age (U=20.0, P=0.95), or in baseline scores of the ABC

| Variable | Group | Comparison
<table>
<thead>
<tr>
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<tr>
<td>Age</td>
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<td>3.7 (0.8)</td>
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<td>1.8 (1.8)</td>
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<td>0.7 (1.6)</td>
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<td>3.1 (1.0)</td>
<td>3.2 (0.8)</td>
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<td>ABC</td>
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<td>Irritability</td>
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<td>12.7 (7.6)</td>
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<td>26.7 (10.7)</td>
<td>30.0 (7.9)</td>
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<td>Stereotypy</td>
<td>7.6 (7.8)</td>
<td>8.0 (6.8)</td>
</tr>
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<td>Hyperactivity</td>
<td>18.3 (9.9)</td>
<td>20.3 (15.5)</td>
</tr>
<tr>
<td>Inappropriate speech</td>
<td>7.3 (6.8)</td>
<td>4.5 (4.9)</td>
</tr>
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</table>

Table 1: Behavioral scores at the baseline.
and ADI-R repetitive stereotyped behavior domain scores between the two groups (all P values exceeded 0.05) (Table 1). Total ABC and the ADI-R repetitive behavior domain were 74.5±30.4, and 12.3±3.0, respectively. In children and adolescents with ASD, the total ABC score was 85.6±27.3 [27] and 44.7±24.6 [19], respectively, and in another study of the ASD the total score for the repetitive stereotyped behavior domain of the ADI-R was 4.7±1.3 [29]. Our patients were thus considered to have severe for the entire group.

Efficacy results

In the treatment group the ABC social withdrawal scores (F=3.08, P<0.01) and ADI-R C3 subdomain (stereotyped and repetitive motor manernisers) score (F=3.38, P<0.05) were significantly reduced relative to the placebo group during the 16-week placebo-controlled trial, (Table 2). The ADI-R C3 subdomain score was significantly correlated with ABC-measured inappropriate speech (r=0.397, P<0.01). The number of individuals that achieved 30% improvements in total scores for the ADI-R C3 subdomain from the baseline was significantly greater in the treatment group compared to the placebo groups (X²=26.0, P<0.01).

Adverse events

No adverse events were reported in either group in placebo-controlled trial during the placebo-controlled trial.

Plasma levels of EPA, DHA and ARA

Plasma levels of EPA, DHA and ARA were not significantly different between the groups. At the end of the placebo-controlled trial, the difference in the plasma ARA levels from the baseline was significantly higher (U=7.0, P<0.05) in the treatment group than the placebo group (Table 3).

Discussion

Growing evidence suggests that behavioral addictions resemble substance addictions in many domains, including their natural history, phenomenology, tolerance,comorbidity, overlapping genetic contribution, neurobiological mechanisms, and response to treatment [14]. It has been documented that video game addiction included long-lasting, overcome use,and the individuals with this addiction lower social competence, and increased impulsivity. In these regards, video game addiction is similar to addictive behaviors [13]. As described above, behavioral addictions have become the major focus of a person’s life to the exclusion of other activities, and/or that have begun to harm the individual or others physically, mentally, or socially [10,14,17]. DSM-IV criteria for substance abuse [3] are as follows: (1) recurrent substance use resulting in a failure to fulfill major roles obligations at work, school, orhome; (2) recurrent substance use in situations in which it is physically hazardous; (3) recurrent substance-related legal problems; (4) continuous substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance. In the current study, we observed the following restricted and repetitive behaviors in the 13 individuals with ASD: the recurrent and repetitive use of one of their

<table>
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<th>Variables: mean (SD)</th>
<th>4 week</th>
<th>8 week</th>
<th>12 week</th>
<th>16 week</th>
<th>4 week</th>
<th>8 week</th>
<th>12 week</th>
<th>16 week</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
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<td>ADI-R repetitive behavior domains</td>
<td></td>
<td></td>
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<tr>
<td>Encompassingpreoccupatuion</td>
<td>3.1 (1.5)</td>
<td>3.4 (0.8)</td>
<td>3.0 (1.5)</td>
<td>2.6 (1.5)</td>
<td>2.8 (1.6)</td>
<td>2.0 (1.8)</td>
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<td>1.46</td>
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<td>Compulsive adherenc</td>
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<td>0.7 (0.8)</td>
<td>2.9 (1.6)</td>
<td>0.0 (0.0)</td>
<td>0.5 (1.2)</td>
<td>0.3 (0.8)</td>
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<td>0.62</td>
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<td>0.0 (0.0)</td>
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<td>0.3 (0.8)</td>
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<td>3.18</td>
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<td>2.3 (1.1)</td>
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<td>1.7 (1.4)</td>
<td>2.0 (1.3)</td>
<td>1.8 (1.2)</td>
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<td>14.4 (14.3)</td>
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<td>Inappropriate speech</td>
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<td>3.8 (2.1)</td>
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<td>5.3 (4.1)</td>
<td>0.79</td>
<td>0.51</td>
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</table>

Treatment group received supplementation with arachidonic acid (ARA) and docosahexaenoic acid (DHA) for 16 weeks. ADI-R, Autism Diagnostic Interview-Revised; ABC, Aberrant Behavior Checklist.

1) Repeated measures analysis of variance (ANOVA) was used when baseline scores as a covariate with treatment as the between-subject-factor and test session repeated factor. * P<0.05.
2) ADI-R algorithm item C 1: Encompassing preoccupation or circumscribed pattern of interest.
3) ADI-R algorithm item C 2: Apparently compulsive adherence to nonfunctional routines or rituals.
4) ADI-R algorithm item C 3: Stereotyped and repetitive motor manernisers.
5) ADI-R algorithm item C 4: Preoccupation with parts of objects or nonfunctional elements of materials.

Table 2: Mean scores on the Aberrant Behavior Checklist (ABC) and the Social Responsiveness Scale (SRS) for a 16-week randomized double-blind placebo-controlled trial.
findings suggest that ARA-related upregulation of signal transduction is an important structural component of neural membranes [22]. These changes preferentially modulate signal transduction [22,44], whereas DHA is contributing to the pathogenesis of ASD. It is well known that ARA fluidity [43] and give rise to altered lipid signal transduction [42], composition or omega-6/omega-3 fatty acids may affect membrane fluidity [43] and repetitive motor mannerisms in the placebo-controlled trial. There were no significant differences in the plasma levels of EPA, DHA and ARA; however, at the end of clinical trial the plasma ARA levels were significantly higher in the treatment group compared to the control group. Previous studies on supplementation with DHA, EPA and ARA yielded significant improvements in ADI-R scores [3, 33]. However, in the present study plasma ARA levels were significantly higher in the treatment group compared to the control group. The ARA/DHA ratios in the present study was 0.088 [37] or 0.083 [41]. The ARA/DHA ratio in the present study may be important. The ARA/DHA ratios in the present study are small compared to previous studies [4,36,41], however, we find significant improvement in scores of the ABC-measured social withdrawal and ADI-R C3 subdomain (repetitive addictive behavior). There was no significant difference in the subjects’ scores of the ABC-measured stereotypy. Considering that the stereotypy subscale of the ABC is primarily limited to questions related to repetitive motor movements rather than the full range of repetitive behaviors [12], further studies are needed on this matter.

In this study, the subjects’ scores of the ADI-R “stereotyped and repetitive motor mannerisms” subdomain were significantly correlated with ABC-measured inappropriate speech ($r=0.397, P<0.01$). A previous study reported that the scores of ABC-measured irritability, social withdrawal, and hyperactivity were significantly correlated with Repetitive Behavior Scale-Revised [12]. The present findings suggest that the scores for ABC-measured social withdrawal as well as inappropriate speech might have contribute to the improvements in the subjects’ scores for ADI-R C subdomain. Further studies on these relationships are necessary.

Considering the underlying mechanisms

Since altered fatty acid metabolic pathways may be involved in pathophysiology of ASD [6,43], the dose ratio of ARA to DHA in the supplementation trial may be important. The ARA/DHA ratios in the two previous clinical studies was 0.088 [37] or 0.083 [41]. The ARA/DHA ratio in the present study was 1.0, suggesting an important contribution of ARA to the reduction in social impairment. A significant increase in plasma ARA levels at the end of the 16-week placebo-controlled trial in the treatment groups may reflect contribution of ARA to the present findings. Alterations in fatty acid composition or omega-6/omega-3 fatty acids may affect membrane fluidity [43] and give rise to altered lipid signal transduction [42], contributing to the pathogenesis of ASD. It is well known that ARA preferentially modulates signal transduction [22,44], whereas DHA is an important structural component of neural membranes [22]. These findings suggest that ARA-related upregulation of signal transduction in relation to neuronal network function might have been involved in the mitigation of social impairment.

The present study has several limitations. First, number of participants was small. Second, the study included participants with a large age range. Although the control group included one 28-aged adult, there was no large discrepancy in age profiles of the two groups. In addition, 12 of the 13 participants had accepted programs that taught appropriate social skills, and one male adult worked in a big car factory, enabling the development of social peer-based support. Moreover, the 13 participants avoided behavioral intervention and courses that taught appropriate social skills for 3 months before and during the study period. Our results are therefore unlikely to have been affected by the age range or any behavioral interventions. Third, we found statistically significant changes in only one of the five subscales of the ADI-R. Finally, the underlying mechanism of the observed improvement should be examined in more detail.

Adverse effects

In previous studies, high doses of arachidonate-enriched triglycerides (SUNTAGA40S) as a source of ARA had no adverse effects on health or growth in rats [25,31], indicating the safety of SUNTAGA40S. In the present study, no adverse effects were detected in either group, although the result is not decisive because of the small sample size.

Conclusion

The present findings suggest that observed improvements in impaired social interaction and repetitive stereotyped behavior were induced by our supplementation regimen possibly via the ARA-related up regulation of signal transduction.

Financial Disclosure

Dr. Koshiba and Professor Yui received a Grant-in-Aid for Scientific Research on Innovative Areas (Grant No 21200017) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, but report no conflict of interests related to the current manuscript.

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