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RESEARCH PAPER

The effect of phosphatidylserine administration on memory and symptoms of attention-deficit hyperactivity disorder: a randomised, double-blind, placebo-controlled clinical trial

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Abstract

Background: Attention-deficit hyperactivity disorder (ADHD) is the most commonly diagnosed behavioural disorder of childhood, affecting 3–5% of school-age children. The present study investigated whether the supplementation of soy-derived phosphatidylserine (PS), a naturally occurring phospholipid, improves ADHD symptoms in children.
Methods: Thirty six children, aged 4–14 years, who had not previously received any drug treatment related to ADHD, received placebo ($n = 17$) or 200 mg day^{-1} PS ($n = 19$) for 2 months in a randomised, double-blind manner. Main outcome measures included: (i) ADHD symptoms based on DSM-IV-TR; (ii) short-term auditory memory and working memory using the Digit Span Test of the Wechsler Intelligence Scale for Children; and (iii) mental performance to visual stimuli (GO/NO GO task).
Results: PS supplementation resulted in significant improvements in: (i) ADHD ($P < 0.01$), AD ($P < 0.01$) and HD ($P < 0.01$); (ii) short-term auditory memory ($P < 0.05$); and (iii) inattention (differentiation and reverse differentiation, $P < 0.05$) and inattention and impulsivity ($P < 0.05$). No significant differences were observed in other measurements and in the placebo group. PS was well-tolerated and showed no adverse effects.
Conclusions: PS significantly improved ADHD symptoms and short-term auditory memory in children. PS supplementation might be a safe and natural nutritional strategy for improving mental performance in young children suffering from ADHD.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common childhood disorders and can continue through adolescence and adulthood (Wilens *et al.*, 2002). ADHD symptoms include difficulties in paying attention and staying focused, controlling behaviour and hyperactivity. Symptoms often result in underperforming at school and often put a strain on family relationships. It is normal for all children to be inattentive, impulsive or hyperactive sometimes but, for children with ADHD, these symptoms are more severe and occur more often.

Attention-deficit hyperactivity disorder patients are classified into three subtypes: predominantly inattentive, predominately hyperactive-impulsive and combined hyperactive-impulsive and inattentive. A child must have symptoms for ≥ 6 months to a degree that is greater than other children of the same age to be diagnosed with the disorder. The exact cause of the disorder has yet to be specified; however, genetic influences, environmental toxins and nutritional deficiencies were proposed as risk factors (Pellow *et al.*, 2011). Conventional treatment for ADHD consists of behavioural interventions and stimulant and antidepressant medications. Stimulant drugs such as methylphenidate mimic the action of the neurotransmitter dopamine and norepinephrine and were shown to improve ADHD symptoms in most children. However, 20–30% of children do not respond to this class of drug or were unable to tolerate them as a result of adverse effects (Rapport *et al.*, 1994; Goldman *et al.*, 1998). Side effects may include anxiety, mood swings, loss of appetite, insomnia, increase in blood pressure and heart rate and, at higher doses, even paranoid psychoses (Brue & Oakland, 2002). Antidepressants are considered to increase neurotransmitter levels in the brain; however, numerous potential side effects are associated with these drugs (Pellow *et al.*, 2011). As a result of the potential negative side effects of common ADHD drugs, parents are looking for natural alternatives to manage the symptoms.

Phosphatidylserine (PS) is a naturally occurring phospholipid nutrient that is most concentrated in organs with high metabolic activity, such as the brain, lungs, heart, liver and skeletal muscle. PS is located mainly in the internal layer of the cell membrane and has a variety of unique regulatory and structural functions. It modulates the activity of receptors, enzymes, ion channels and signalling molecules and is involved in governing membrane fluidity (Pepeu *et al.*, 1996). PS is considered to be one of the most important brain nutrients beneficially influencing numerous neurotransmitter systems, such as acetylcholine, dopamine, serotonin and norepinephrine. PS has been shown to counteract the stress-induced activation of the hypothalamic-pituitary-adrenal axis when faced with a physical stressor (Starks *et al.*, 2008). In young healthy college students, PS supplementation resulted in an increase in mental performance during a calculus test (Parker *et al.*, 2011) and an increase in accuracy off the tee and a reduction of perceived stress in golf players (Jäger *et al.*, 2007).

A pilot study on 15 ADHD children, aged 6–12 years, indicated that the administration of 200 mg of soy-derived PS per day for 2 months significantly improved ADHD symptoms (Hirayama *et al.*, 2006). However, that study was designed as a pilot study without a control group. Recent studies showed that the supplementation of 300 mg of eicosapentaenoic and docosahexaenoic acid enriched PS in children with ADHD improved visual sustained attention performance (Vaisman *et al.*, 2008). This intervention also improved hyperactivity, especially in a subgroup of children with more pronounced hyperactive/impulsive behaviour, and reduced the emotional impact on parents (Manor *et al.*, 2012).

The present study aimed to confirm earlier findings (Hirayama *et al.*, 2006) showing that soy-derived PS supplementation improves ADHD symptoms in children in a placebo-controlled, randomised design.

Materials and methods

Subjects

The primary goal of the present study was to examine the effects of 2 months of PS supplementation on symptoms of attention deficit (AD) in children 4–14 years of age. Therefore, AD symptoms were the primary outcome measure in the present study and provided the basis for the sample sizes as determined by G*POWER analysis software (Faul *et al.*, 2007, 2009). Our rationale for sample size was based on a pilot study conducted by Hirayama *et al.* (2006). These investigators found that children similar in age to our population improved their AD score by 22%. Using the equation: effect size (ES) = (pre-ADHD scores – post-PS-ADHD scores)/the pooled SD of the ADHD scores, the study by Hirayama *et al.* (2006) had an ES of 0.45 = [(5.8–4.53)/2.82]. Based on the use of the analysis of variance (ANOVA) statistical model, an alpha level of 0.05, a power of 80 and an ES of 0.45, a total of 32 subjects (16 per experimental group) were needed to have sufficient power to detect the effects of PS on AD. However, allowing for a 20% dropout, forty children (4–14 years old) with ADHD, who had not received any drug treatment related to ADHD before, were recruited for the present study. The children were recruited from participants of the 'Kankyo Taiwa Camp' training program for ADHD children held by Department of Early Childhood Education and Care, Kurashiki City College. The interviews with the parents were performed by Professor S. Hirayama. The parents were blinded to the group allocation. The parents were informed that the purpose of the study was to investigate the effects of a dietary supplement that might have beneficial effects on ADHD symptoms and cognition in a placebo-controlled manner. The purpose of the study was not shared with the children. All children were diagnosed by their own psychiatrists prior to recruitment. The subjects were randomly assigned to the PS and control group.

The study was approved by the ethics committee of Kurashiki City College and written informed consent was obtained from the parents of each child.

Test methods

Inattention, hyperactivity and impulsivity were assessed using the ADHD diagnostic criteria of the American Psychiatric Association, DSM-IV-TR (Table 1). The assessments were performed as an interview with the parents of the subjects. Questions were answered with either 'yes' or 'no'. The answer 'yes' resulted in a score of one, whereas the answer 'no' resulted in a score of zero.

Short-term auditory memory and working memory were assessed using the Digit Span Test of the Wechsler Intelligence Scale for Children (WISC-III; Wechsler, 1991). Short-term auditory memory was evaluated by repeating a series of numbers, working memory by repeating the series of numbers in reverse order. Each series begins with two numbers and keeps increasing in length, with two sets of numbers at each length (Table 2).

Mental performance to visual stimuli was evaluated using a conditioned grabbing movement reflex with prior verbal instructions in a GO/NO-GO experiment (Luria, 1971; Terasawa *et al.*, 2000). The task consisted of three stages: familiarisation, differentiation and reverse differentiation.

In the familiarisation experiment subjects were instructed to grab a rubber ball in response to a red light being switched on. After a positive response, a conditioned reflex was formed. The stimulus was presented five times for 0.5–1.5 s with an interval between presentations of 3–6 s.

On completion of the familiarisation experiment, the subject moved immediately onto the differentiation phase in which red and yellow lights were used. The instruction was to grab the ball in response to the red light but not in response to the yellow light. Again, the stimulus was presented for 0.5–1.5 s at intervals of 3–6 s. The red and yellow lights were switched on 10 times each in a randomly determined order.

Immediately after the differentiation phase, the subject moved on to the reverse differentiation experiment. The instructions were reversed and the subject was told to grab the ball only in response to the yellow light. The presentation times, intervals and order were identical to those in the differentiation experiment. Children were assessed using the red and yellow lights, whereas the experiment involving junior high school students used lights of differing intensities (170 nit/30 nit). The stimulation light was installed at the subjects' eye level at a distance of 1 m.

The GO/NO-GO task resulted in a total of 12 measurements: 'forgot to grab the ball (inattention error)' and 'grabbed the ball at the wrong time (impulsivity error)', and 'total number of errors (inattention plus impulsivity error)' in the differentiation and reverse differentiation experiment, 'total number of inattention errors', 'total number of impulsivity errors', 'total number of inattention and impulsivity errors', as well as mean response time in all three phases.

Table 1 Attention-deficit hyperactivity disorder criteria (DSM-IV, American Psychiatric Association, 2000)

(1) Inattention
Often does not give close attention to details or makes careless mistakes
Often has trouble keeping attention on tasks or play activities
Often does not seem to listen when spoken to directly
Often does not follow instructions and fails to finish schoolwork or chores
Often has trouble organising activities
Often avoids, dislikes or doesn't want to do things that take a lot of mental effort for a long period of time (such as schoolwork or homework)
Often loses things needed for tasks and activities (e.g. toys, pencils, books, or tools)
Is often easily distracted
Is often forgetful in daily activities
(2) Hyperactivity
Often fidgets with hands or feet or squirms in seat when sitting still is expected
Often gets up from seat when remaining in seat is expected
Often excessively runs about or climbs when and where it is not appropriate
Often has trouble playing or doing leisure activities quietly
Is often 'on the go' or often acts as if 'driven by a motor'
Often talks excessively
(3) Impulsivity
Often blurts out answers before questions have been finished
Often has trouble waiting one's turn
Often interrupts or intrudes on others (e.g. intrudes into conversations or games)

Table 2 Wechsler Intelligence Scale for Children (WISC-III)

Series	Short-term auditory memory	Working memory
1	3-5	5-4
2	6-3	3-1
3	5-2-4	6-2-8
4	2-6-8	9-6-2
5	4-1-7-3	2-6-9-8
6	7-3-6-9	9-1-7-3
7	5-1-8-4-3	8-4-7-1-5
8	3-9-6-5-7	1-6-4-9-3
9	2-7-9-3-1-5	4-7-3-9-1-5
10	1-6-3-7-4-9	7-2-8-1-9-4
11	4-1-8-6-9-2-7	3-7-5-9-1-2-6
12	8-2-6-9-1-7-3	6-3-9-7-2-1-8

All the tests were conducted by the same investigator, who was a skilled expert in ADHD.

Supplementation

The previous pilot study using soft gel capsules resulted in a low compliance rate with children. For this study, cocoa-flavored chews were used, containing 100 mg of soy-derived PS per chewable. The PS and placebo chews were matched in taste and appearance and the children consumed two chews per day for 2 months. The parents supervised the daily intake of the study material in both groups and were asked not to change the current typical Japanese diet of their children during the duration of the study. Dietary PS can be found in meat, although it is most abundant in the brain and innardsm such as in liver and kidney. Only small amounts of PS can be found in dairy products or in vegetables, with the exception of white beans. The parents were advised on PS content in foods and were asked specifically not to change the current diet of the children with respect to foods naturally rich in PS.

Statistical analysis

The data are shown as the mean (SD). Statistical analysis was performed using STATCEL 2 (Oms-Publishing, Saitama, Japan). ANOVA and a paired *t*-test were employed for comparison of the data obtained within a group and between groups. The pre-data in both groups were compared by a nonpaired *t*-test. $P < 0.05$ was considered statistically significant.

Results

Nineteen of the 20 children in the PS group completed the study. One subject dropped out because of the subject's refusal to comply with the daily supplementation. Seventeen of the 20 children in the placebo group completed the study. Three children were withdrawn from the study by their parents without giving a specific reason. The drop-outs did not differ significantly from the completers in terms of age, severity of symptoms or symptom subgroups. Based on the power analysis, the present study is not underpowered and a completers analysis rather than intention to treat was performed. The PS and placebo chews were well-tolerated and no adverse effects were observed.

No significant differences between groups were observed with respect to presupplementation for age, sex (Table 3) and clinical presentation (ADHD symptoms, memory) (Table 4).

Over time (pre versus post), ADHD symptoms significantly improved in the PS group (ADHD: $P < 0.01$; AD: $P < 0.01$; HD: $P < 0.01$), whereas the placebo group showed no significant changes (ADHD: $P = 0.53$; AD: $P = 1.00$; HD: $P = 0.56$) (Table 4). Post-intervention, the PS group had a significantly better ADHD ($P < 0.01$) and AD ($P < 0.01$) score than the placebo group.

Table 3 Subject characteristics

	Phosphatidylserine ($n = 19$)	Placebo ($n = 17$)
Age (years), mean (SD)	9.1 (1.7)	8.7 (3.0)
Sex (male : female)	18 : 1	16 : 1

There was no significant pre- versus post-difference in the working memory in the WISC-III test; however, the short-term auditory memory was significantly improved in the PS (but not in the placebo group) over time ($P < 0.05$) (Table 4). One of the subjects in the PS group did not comply with the test method during the WISC-III assessment and the subject was excluded from the statistical analysis.

PS supplementation significantly improved ($P < 0.05$) inattention in the differentiation and the reverse differentiation test, the total number of errors in the reverse differentiation test (inattention and impulsivity), as well as total inattention errors and total errors over time in the GO/NO GO-task. PS had no effect on mean response times. Placebo supplementation did not improve any of the measurements over time (Table 4). Post-intervention, the PS group had significantly better inattention and total number of errors scores (inattention and impulsivity) in the reverse differentiation ($P = 0.03$ and $P = 0.01$, respectively) and differentiation and reverse differentiation ($P = 0.04$ and $P = 0.03$, respectively) tasks than the placebo group.

The magnitude of change observed in the present study is clinically important and is associated with a substantially improved functioning and quality of life in school and at home. Children in the PS group showed improved classroom behaviour and social skills, such as a reduction of walking around in the classroom or talking during class. In addition, children in the PS group showed improvements in putting things back in their proper places at home. Children in the placebo group did not show meaningful behavioural changes.

Discussion

The main finding of the present study is that supplementation of 200 mg of PS administered for 2 months significantly improved ADHD symptoms and short-term auditory memory in children.

PS supplementation significantly improved ADHD criteria defined by the American Psychiatric Association (DSM-IV-TR) over time and in comparison to placebo, post-supplementation. The number of ADHD symptoms

Table 4 Mean scores pre- and post-supplementation

	Phosphatidylserine (n = 19)			Placebo (n = 17)		
	Pre	Post	*P*-value	Pre	Post	*P*-value
DSM-IV-TR						
ADHD	11.4 (3.2)	7.2 (3.9)**	0.01	11.5 (3.4)	10.9 (4.6)	0.53
AD	6.7 (1.7)	4.4 (2.7)**	0.01	6.7 (1.8)	6.7 (2.4)	1.00
HD	4.8 (1.4)	2.7 (1.3)**	0.01	4.8 (1.6)	4.2 (2.0)	0.56
WISC-III (Digit span)						
Short-term auditory memory	6.6 (2.0)	7.7 (2.7)*	0.03	6.5 (3.1)	6.9 (2.4)	0.47
Working memory	4.6 (2.5)	4.9 (3.3)	0.55	3.9 (2.0)	3.2 (2.4)	0.08
GO/NO-GO task						
Differentiation						
Inattention	1.0 (1.8)	0.1 (0.3)*	0.04	0.6 (1.0)	0.3 (0.8)	0.29
Impulsivity	4.8 (3.2)	4.1 (2.3)	0.36	4.2 (2.3)	3.8 (2.3)	0.62
Total number of errors	5.6 (3.4)	4.2 (2.4)	0.09	4.8 (1.0)	4.1 (2.6)	0.32
Reverse differentiation						
Inattention	1.8 (3.3)	0.1 (0.3)*	0.03	0.3 (1.0)	0.8 (1.3)	0.20
Impulsivity	3.4 (2.7)	2.9 (2.8)	0.40	3.2 (1.4)	3.2 (2.6)	1.00
Total number of errors	5.3 (4.6)	3.2 (2.7)*	0.03	3.5 (1.8)	3.9 (3.1)	0.51
Differentiation and reverse differentiation						
Total inattention errors	2.8 (4.7)	0.2 (0.6)*	0.02	0.9 (1.4)	1.0 (1.9)	0.81
Total impulsivity errors	8.2 (5.6)	7.0 (4.6)	0.29	7.4 (3.1)	7.0 (4.6)	0.77
Total number of errors	10.8 (7.3)	7.2 (4.6)*	0.02	8.3 (3.0)	8.0 (5.4)	0.83

Data are the mean (SD).

Measurement showing a statistically significant difference within a group over time (pre–post) (*$P < 0.05$, **$P < 0.01$). One subject was excluded in the phosphatidylserine (PS) group during the WISC-III test for noncompliance with the test method. The data for this subject were excluded (n = 18).

ADHD, attention-deficit hyperactivity disorder.

decreased by an average of 4.2 [pre: 11.4 (3.2), 7.2 (3.9); −37%]. In addition, PS significantly improved each category, inattention and hyperactivity-impulsivity.

Working memory arises from networks of the prefrontal cortex (PFC) pyramidal cells that are assumed to maintain task relevant information even when the stimuli are no longer present. The cognitive functions of the PFC are the most advanced and the most vulnerable to disruption. Attentional focus and inhibition of inappropriate motor responses are based on optimal PFC functioning. PFC cognitive functions erode when we are exposed to stress or when we are fatigued. Working memory functions can be impaired when facing even a mild stressor (Arnsten & Robbins, 2002). Many neurotransmitters and neuromodulators (dopamine, serotonin, acetylcholine, glutamate, norepinephrine) contribute to PFC cognitive functioning. PS supplementation has been shown to benefit dopamine release (Mazzari & Battistella, 1980) and glutamatergic neurotransmission (Cohen & Müller, 1992). PS benefits acetylcholine release by maintaining adequate acetylcholine supply (Casamenti *et al.*, 1979) and increases the availability of endogenous choline for *de novo* synthesis and release, whereas similar treatments with phosphatidylcholine had no effect (Pedata *et al.*, 1985).

Working memory has often been assessed in neuropsychological research with the retention and oral repetition of digit spans in reverse order. Both children and adults with ADHD have shown decreased performance with backwards repetition of digit spans (Barkley, 1997). PS supplementation in the present study resulted in an improvement of retention and oral repetition of digit spans in reverse order [working memory: +7%; pre: 4.6 (2.5), post: 4.9 (3.3)]; however, the improvement failed to reach statistical significance. Auditory memory is the ability to process, analyse and recall orally presented information. It is one of the most important learning skills and children with weak auditory memory can show delayed learning of language and might take longer to learn how to read. PS supplementation significantly ($P < 0.05$) improved short-term auditory memory [+17%; pre: 6.6 (2.0), post: 7.7 (2.7)], whereas the placebo group showed no significant improvement.

PS significantly improved inattention errors in the GO/NO GO-task under differentiation and reverse differentiation conditions. Both differentiation and reverse differentiation conditions require attention control. The differentiation experiment is conducted subsequent to the formation experiment and because the task is the same in both tests (grab a ball in response to a red light), habitua-

tion occurred in the children and the task was more easily completed in the pre- and post-treatment phases. The visual stimulation to induce and to restrain a response under the reverse differentiation conditions requires a higher level of attention as a result of the switch of the target. PS supplementation appeared to be more effective in the more challenging test (i.e. the reverse differentiation task) because the total amount of inattention and impulsivity errors significantly improved in the PS group compared to placebo. PS appears to have a beneficial influence on the frontal lobes that are linked to attention (Shallice, 1988).

PS supplementation had no significant effect on impulsivity in the GO/NO GO task, whereas it improved hyperactivity-impulsivity in the DSM-IV-TR assessment over time. The impulsivity evaluation of the GO/NO-GO task is based on errors made to a simple visual stimulation (colour of light), whereas the DSM-IV-TR test considers multiple assessments of daily life. Total impulsivity errors improved by 15% in the GO/NO GO task [pre: 8.2 (5.6), post: 7.0 (4.6)] in the PS group over time; however, the improvements failed to reach statistical significance. Changing the supplementation conditions such as the duration and dose might improve impulsivity in the GO/NO GO-task in a future study.

Attention-deficit hyperactivity disorder presents ongoing challenges for children resulting in stress. PS supplementation results in an increase in stress resistance and improvements in memory relevant signalling processes. PS has been shown to blunt stress induced increases in cortisol (Starks *et al.*, 2008) and to significantly decrease β-1 power in the right hemispheric frontal brain regions before and after stress (Baumeister *et al.*, 2008). These beneficial effects result in an increase in performance during a calculus test (Parker *et al.*, 2011) and an increase in motor skills in golf players (Jäger *et al.*, 2007). Protein kinase C (PKC) plays an important role in controlling memory relevant signalling process. Vital neural structures in the brain that are involved in cognition and mood regulation contain the highest concentrations of PKC (Saito *et al.*, 1998). PS supplementation plays a significant role in the maintenance of neuronal excitability and message transfer within brain cells. PS has been shown to activate tyrosine hydroxylase (Raese *et al.*, 1976), the sodium-potassium activated ATPase (Tsakiris & Deliconstantinos, 1984) and PKC (Kaibuchi *et al.*, 1981).

The development of ADHD likely involves a broad range of genetic, social, developmental, prenatal, environmental and nutritional factors. Nutritional deficiencies, including deficiencies in zinc, selenium, methionine and omega-3 fatty acids, were linked to defects in neuronal plasticity and impact behaviour in children with ADHD (Dufault *et al.*, 2009). Phospholipid deficiencies are linked to impairments in neuronal structure and function, especially during early development (Pellow *et al.*, 2011). Dietary deficiency in essential fatty acids and phospholipids during childhood may increase the risk of developing ADHD-type symptoms (Sadiq, 2007). Correcting underlying imbalances through PS supplementation may be an important treatment strategy in cases where deficiency exists. Additional nutritional strategies might include fish oil supplementation, or eating foods rich in essential fatty acids, as well as supplementation with vitamin B_6 and magnesium (Pellow *et al.*, 2011) or L-theanine to improve some aspects of sleep quality (Lyon *et al.*, 2011).

The PS used in the study by Hirayama *et al.* (2006) was administered in the form of a capsule that reduced the compliance rate. Children complained that the capsule looked like a drug and that the product was difficult to ingest. In the present study, we provided PS as a chewable flavoured with cocoa. The more food-like dosage form with a classic food flavour scored excellent on taste and resulted in a high compliance rate in this target group. Soy-derived PS administered in the present study (200 mg for 2 months) might even be more effective than the omega-3-enriched PS used in recent studies (300 mg of PS for 3 months; Vaisman *et al.*, 2008; Manor *et al.*, 2012) on ADHD symptoms. Studies on the cortisol response to a physical stressor showed similar results for bovine-cortex PS (800 mg for 10 days; Monteleone *et al.*, 1992) and soy-derived PS (600 mg for 10 days; Starks *et al.*, 2008). This indicates that the fatty acid composition of soy-derived PS does not negatively influence its efficacy compared to bovine cortex or omega-3-enriched forms of PS in this population.

In conclusion, PS has been shown to significantly improve ADHD symptoms and short-term memory. PS supplementation appears to be a safe and natural nutritional strategy for improving mental performance in young children suffering from ADHD. Further research is needed to determine the optimal dose and duration of PS supplementation in children with ADHD.

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Conflicts of interest, sources of funding and authorship

RR, TI and TH work for companies that distribute phosphatidylserine. All of the other authors declare that they have not conflicts of interest.

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SH created the study protocol, and collected and analysed all the data. KT handled the statistical analysis. RJ, SH and MP contributed to the writing of the paper. TI, YT, RR, RJ and TH concieved the idea for the study and contributed to the study design. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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