



## Applied nutritional investigation

# Protective effects of dietary supplementation with natural $\omega$ -3 polyunsaturated fatty acids on the visual acuity of school-age children with lower IQ or attention-deficit hyperactivity disorder



Qiaoling Wu Ph.D.<sup>a</sup>, Tingting Zhou M.D.<sup>a</sup>, Liping Ma M.D.<sup>b</sup>, Dongjuan Yuan Ph.D.<sup>c</sup>, Yongmei Peng M.D., Ph.D.<sup>a,\*</sup>

<sup>a</sup> Department of Child Health Care, Children's Hospital, Fudan University, Shanghai, China

<sup>b</sup> Centre for Disease Control, Yuyao, China

<sup>c</sup> Department of Biochemistry and Molecular Biology, Guangdong Medical College, Zhanjiang, China

## ARTICLE INFO

## Article history:

Received 3 March 2014

Accepted 17 December 2014

## Keywords:

$\omega$ -3 polyunsaturated fatty acids

School-age children

Visual acuity

Dietary supplementation

Lower IQ

Attention-deficit hyperactivity disorder

## ABSTRACT

**Objective:** Little attention has been paid to the possible protective role of  $\omega$ -3 polyunsaturated fatty acids (PUFAs) on the visual acuity of school-age children with lower IQs or attention-deficit hyperactivity disorder (ADHD). The aim of this study was to evaluate the effect of dietary  $\omega$ -3 PUFAs on the visual acuity and red blood cell (RBC) fatty acid compositions of these children.

**Methods:** We randomly assigned 179 children with lower IQs or ADHD to receive ordinary eggs (control group,  $n = 90$ ) or eggs rich in C18:3  $\omega$ -3, eicosapentaenoic acid (EPA, 20:5  $\omega$ -3) and docosahexaenoic acid (DHA, 22:6  $\omega$ -3) for 3 mo (study group,  $n = 89$ ). Before and after the intervention, distance visual acuity was tested using an E chart and the RBC fatty acid composition was determined using capillary gas chromatography.

**Results:** Three months later, 171 children completed the follow-up with the exception of 8 children who were unavailable during follow-up. Both groups of children showed a significant improvement in visual acuity ( $P < 0.05$ ), however, visual acuity in the study group was significantly better than that of the control group ( $P = 0.013$ ). The C18:3  $\omega$ -3 ( $P = 0.009$ ), DHA ( $P = 0.009$ ) and  $\sum\omega$ -3 ( $P = 0.022$ ) levels of the intervention group were significantly higher than those of the control group, while the C20:4  $\omega$ -6 ( $P = 0.003$ ), C22:4  $\omega$ -6 ( $P = 0.000$ ),  $\sum\omega$ -6 ( $P = 0.001$ ),  $\sum\omega$ -6/ $\sum\omega$ -3 ( $P = 0.000$ ) and arachidonic acid/DHA ( $P = 0.000$ ) of the study group were significantly lower than those of the control group. No significant differences in the levels of C18:2  $\omega$ -6 ( $P = 0.723$ ), C20:2  $\omega$ -6 ( $P = 0.249$ ), C20:3  $\omega$ -6 ( $P = 0.258$ ), C20:5  $\omega$ -3 ( $P = 0.051$ ), or C22:5 ( $P = 0.200$ ) were found between the two groups.

**Conclusions:** Dietary supplementation with  $\omega$ -3 PUFAs improves both visual acuity and the RBC fatty acid profile in school-age children with lower IQs or ADHD.

© 2015 Elsevier Inc. All rights reserved.

This study was supported by the National Natural Science Foundation of China (grant no. 30972475). QLW was responsible for statistical analysis, drafting and editing of the manuscript. TTZ was involved in the participant recruitment and data collection. LPM was involved in the participant recruitment. DJY performed fatty acid analysis. YMP designed and supervised the study. All authors read and approved the final manuscript. The authors have no conflicts of interest to declare.

\* Corresponding author. Tel.: +86 021 64931229; fax: +86 021 64043900.

E-mail address: [ympeng@fudan.edu.cn](mailto:ympeng@fudan.edu.cn) (Y. Peng).

<http://dx.doi.org/10.1016/j.nut.2014.12.026>

0899-9007/© 2015 Elsevier Inc. All rights reserved.

## Introduction

The  $\omega$ -3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), mediate a wide variety of functions in the body. There have been numerous studies demonstrating that  $\omega$ -3 PUFAs play important roles in many aspects of health, such as cardiovascular diseases [1], inflammatory responses [2], neurodevelopmental conditions, [3] and visual function [4]. DHA is the principal  $\omega$ -3 fatty acid in the brain and retina, comprising 40% of the PUFAs in the brain and 60% of the PUFAs in the retina [5]. EPA, which plays a

more minor structural role but is essential for normal brain function, was found in trace amounts in the brain and the majority of other tissues [6]. Moreover,  $\omega$ -3 PUFAs and their derivatives help to regulate blood flow, neurotransmitter uptake, synaptic transmission, apoptosis, gene expression, ion channels, and immune functions during their biological processes [7]. However, the importance of dietary  $\omega$ -3 PUFAs in contributing to human vision development remains poorly understood, which is partly related to the complexity of fatty acid metabolism and the incomplete knowledge of the pathways of transfer and fatty acid uptake in the retina.

Many authors have reported that approximately 10% to 20% of school-age children in developing countries have various vision problems [8–12]. Vision problems adversely affect children's learning through both direct and indirect mechanisms, including compromising sensory perception, hampering cognition, and negatively affecting socioemotional development and connectiveness to school [13]. As children progress in school, their vision may deteriorate due to increasing class work and homework. They may become nearsighted, farsighted, or they may develop astigmatism, which may significantly affect visual acuity. The study and development of a feasible and efficient vision intervention is of great importance to promote children's visual acuity.

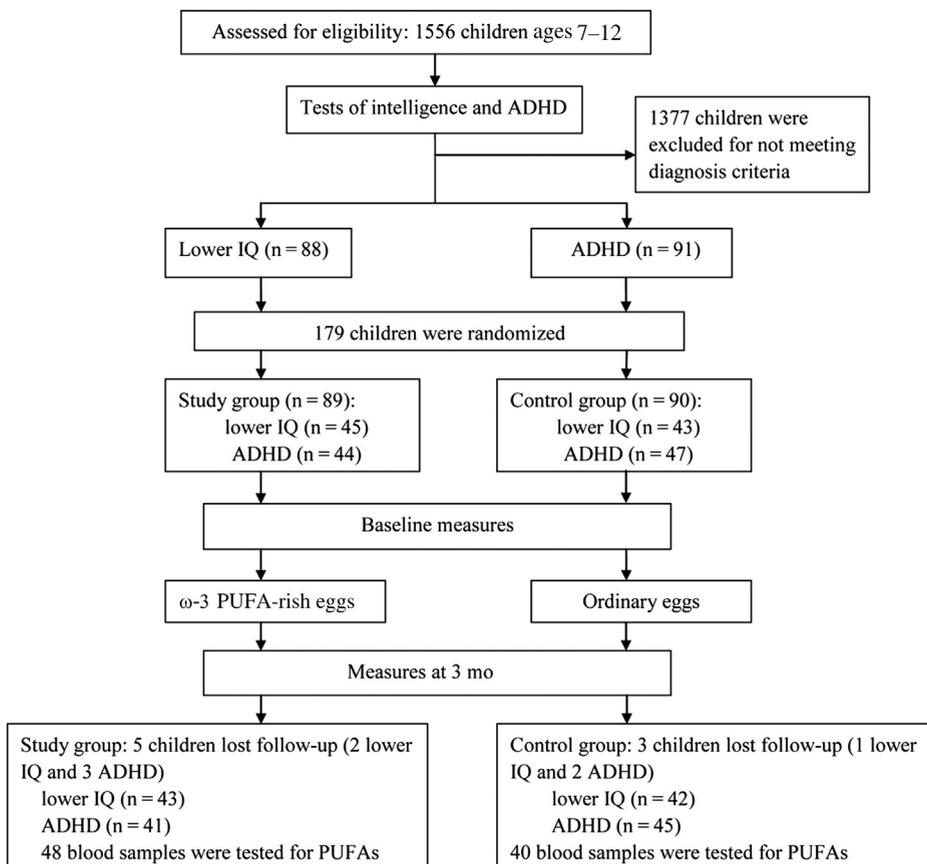
During the past decades, a growing body of research has revealed that  $\omega$ -3 PUFAs are closely related to the visual function development in infants [4,14–18]. However, reports on the studies of  $\omega$ -3 PUFAs and the visual acuity of school-age children are scarce. In the past several years, our research group studied the effects of  $\omega$ -3 PUFAs on lipid metabolism and function

improvement in children with lower IQs or attention-deficit hyperactivity disorder (ADHD). Because  $\omega$ -3 PUFAs were highly concentrated in both the retina and brain, when we studied the effects of  $\omega$ -3 PUFAs on children with ADHD or lower IQs, we also wanted to know whether  $\omega$ -3 PUFAs plays any role in improving the visual acuity of school-age children in these clinical groups. Moreover, it is of interest to explore the roles of  $\omega$ -3 PUFAs in the visual acuity of children, which is closely related to the integrity of the retina. Therefore, before and after supplementation with  $\omega$ -3 PUFAs, we tested the visual acuity of the school-age children with lower IQs or ADHD, and found that  $\omega$ -3 PUFAs seemed to play a valuable role in improving the visual function of such children. We then conducted a detailed analysis of visual acuity to examine the effects of  $\omega$ -3 PUFAs on visual acuity in children with lower IQs or ADHD. We also analyzed the fatty acid composition of red blood cell (RBC) membranes to evaluate any improvement in the fatty acid profiles of these children. The findings should provide valuable information about the use of natural dietary  $\omega$ -3 PUFAs to improve the visual acuity and fatty acid profiles of this age group. To avoid the side effects of drugs or chemically synthesized PUFAs tablets [19], a healthy and natural  $\omega$ -3 PUFA dietary supplement was used in this study.

#### Participants and methods

##### Study participants

The study population included 179 children ages 7 to 12 y, of whom 88 had lower IQs (<90) and 91 had ADHD. The children were screened from 1556 students in two township primary schools of Zhejiang Province, China. All the students were tested to obtain their IQ scores and to diagnose ADHD (Fig. 1).



**Fig. 1.** Participant and group characteristics. We screened 1556 school-age children; 88 lower IQ children and 91 ADHD children were enrolled; however, due to loss to follow-up, only 171 children finished the trial. ADHD, attention-deficit hyperactivity disorder; PIFA, polyunsaturated fatty acid.

Residents of the two townships, a relatively isolated area, have simple lives with similar diet habits. The IQ test was performed using the Combined Raven's Test [20], revised by Chinese psychologists according to the Standard Progressive Matrices and Colored Progressive Matrices of Raven's Progressive Matrices [21]. ADHD was diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition [22]. For the diagnosis of ADHD, the Chinese version of the Conners' Parent and Teacher Rating Scales-revised: Short forms (CPRS-R:S and CTRS-R:S, respectively) were used [23]. These rating scales about learning, attention, and behavior were completed by the teachers and either parent(s) or guardians.

The study was explained to the children and their parent(s) or guardians. Written informed consent was obtained. We recruited children ages 7 to 12 y who were free of diagnosed major eye diseases (congenital, traumatic or acute infective eye diseases). We excluded children who were taking any form of  $\omega$ -3 PUFAs supplements at the time of recruitment, or who consumed  $\omega$ -3 PUFAs supplements in the previous 3 mo. All of the enrolled children were diagnosed with ADHD for the first time in our study; therefore, none were taking any other medications for ADHD or had consumed any medications for ADHD. During the 3 mo, the use of drugs containing PUFAs or other medications for ADHD was forbidden. Baseline assessments, including age, sex composition, height, and weight were obtained from all of the recruited children.

### Design

This study was conducted according to the guidelines of the Declaration of Helsinki and all of the procedures were approved by the Ethics Committee of Children's Hospital, Fudan University, Shanghai, China (approval no:[2010] No. 50). The 179 children were assigned to either the study or the control group by the random number table method, based on the controlled and double-blind principle. The ADHD and lower IQ children screened from each town were assigned to the study group or control group, respectively; therefore, the number of children with ADHD and lower IQs in each group, and the number of children from each town included in each group were nearly equal in amount. The study group included 89 children: 45 with lower IQs and 44 with ADHD. There were 90 children in the control group: 43 with lower IQs and 47 with ADHD. Before group assignment, venous blood was collected for the detection of PUFAs.

For 3 mo, each child in the study group was given one  $\omega$ -3 PUFA-rich egg (egg 1, rich in EPA, DHA, and C18:3  $\omega$ -3) daily; each child in the control group was fed with an ordinary egg (egg 2) every day. The two types of eggs appeared similar. After completing the 3-mo intervention, venous blood of the children was extracted for fatty acid composition analysis. Distance visual acuity was tested before and after the intervention in the same children. All personnel joining the study were trained by the same trainer and the same instructions and study protocols were used throughout the experiment. With the exception of the eggs, no changes were made to the children's daily food intake. The eggs were all provided by Shanghai Zhanwang Corp of China. Table 1 presents the main  $\omega$ -3 PUFAs components of the two types of eggs, obtained using capillary gas chromatography.

### Sample collection and measurement

#### Fatty acid analysis

Fasting blood samples were obtained before and after the intervention. We extracted 2 mL venous blood and it was centrifuged at 3000g for 10 min. The RBCs were immediately separated for  $-70^{\circ}\text{C}$  cryopreservation until analysis. The determination process included the following steps: 100  $\mu\text{L}$  of RBCs were centrifuged to separate the red cell membrane, and the upper liquid was collected by n-hexane and 14% derivative of boron trifluoride-methanol solution. The supernatant was then dried with nitrogen and dissolved with 40  $\mu\text{L}$  of n-hexane. Capillary gas chromatography was used to examine the RBC fatty acid composition. The results are expressed in concentrations (g/100 g total fatty acid).

#### Visual acuity test

The E chart [24], a chart with the letter "E" in different directions (up, down, right, and left) and sizes, was placed in a transparent light box (the background luminance of the screen was 350  $\text{cd}/\text{m}^2$ , and the luminance of the letter E was

20  $\text{cd}/\text{m}^2$ ). The participants sat 5 m away from the light box, with their right eyes tested first, followed by the left. When one eye was being examined, the other eye was fully covered by a spoon. Participants were supposed to point out the directions of the letter E from the top row to the bottom row (letter E became smaller and smaller) until they identified the smallest letter E, and then the monocular visual acuity was recorded according to the given scores of every row. The test was conducted by local trained health workers or members of our study team. All test results were converted to the minimum angle of resolution logarithmic.

### Statistical analyses

All statistical tests were two-tailed, and the level of significance was set at  $\alpha = 0.05$ . SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The Shapiro-Wilk test was used to test the normal distribution of continuous date. Data of normal distributions were expressed as the mean  $\pm$  SD and tested by independent sample *t* tests, while data of skewed distributions were tested by the Mann-Whitney test or the Wilcoxon signed-ranks test and expressed as the mean  $\pm$  SD and the range. The Mann-Whitney test was applied for characteristics such as age and IQ; the  $\chi^2$  test was administered to test the sex composition and independent sample *t* tests were used to test the weight and height between the two groups. The Wilcoxon signed-ranks test was used to test the changes of visual acuity within the two groups before and after the intervention. The Mann-Whitney test was used to test the visual acuity across the two groups before and after the intervention. The Mann-Whitney test or independent sample *t* tests were used to test the fatty acid outcome measures across the two groups before and after the intervention.

## Results

### Baseline characteristics

Figure 1 presents the participant and group characteristics. Among the 179 recruited children, 171 completed the 3-mo assessment, including 97 boys and 74 girls. Eight children were lost to follow-up following randomization, with five from the study group (two with lower IQs and three with ADHD) and three (one with a lower IQ and two with ADHD) from the control group.

At the beginning, blood samples were collected from all 179 children. Owing to budgetary constraints, only 99 blood samples were randomly selected to test (59 from the study group and 40 from the control group); 3 mo later, because eight children were lost to follow-up and three of the blood samples lacked the volume needed for testing, only 88 blood samples (48 from the study group and 40 from the control group) were tested. In all, 171 children were assessed for distance visual acuity, with 84 from the study group and 87 from the control group; and the fatty acid compositions of 88 blood samples were analyzed before and after the intervention.

The average age of these children was  $9.57 \pm 1.30$  y. No significant differences in age ( $P = 0.920$ ), sex composition ( $P = 0.260$ ), IQ ( $P = 0.963$ ), height ( $P = 0.957$ ), or weight ( $P = 0.376$ ) were found between the study group ( $n = 84$ ; 43 with lower IQs and 41 with ADHD) and the control group ( $n = 87$ ; 42 with lower IQs and 45 with ADHD) (Table 2).

**Table 2**

Basic characteristics of the two groups

	Study group (n = 84)	Control group (n = 87)	P-value*
Age (mean $\pm$ SD) <sup>†</sup>	9.58 $\pm$ 1.20	9.56 $\pm$ 1.39	0.920
Sex composition (M/F) <sup>†</sup>	44/40	53/34	0.260
Height (cm) <sup>§</sup>	142.37 $\pm$ 8.75	142.48 $\pm$ 10.78	0.957
Weight (kg) <sup>§</sup>	36.91 $\pm$ 11.71	35 $\pm$ 8.52	0.376
IQ <sup>‡</sup>	96.89 $\pm$ 15.62	96.59 $\pm$ 18.01	0.963

\* Significant differences were not found.

<sup>†</sup> Values were analyzed by the Mann-Whitney test.

<sup>‡</sup> Values were analyzed by the  $\chi^2$  test.

<sup>§</sup> Values were analyzed by the independent samples *t* test.

**Table 1**

The Main  $\omega$ -3 PUFA Content of the Two Types of Eggs (mg/100 g)

	DHA	EPA	C18:3 $\omega$ -3	$\sum\omega$ -3*	$\sum\omega$ -6/ $\sum\omega$ -3
Egg 1	321.0	42.4	584.0	947.4	0.9
Egg 2	36.0	0	19.1	55.1	15.6

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid

\*  $\sum\omega$ -3 = DHA + EPA + 18:3  $\omega$ -3.

### Participant compliance

No difficulties or side effects were reported with the dietary supplementation.

The participants' lifestyles were unchanged throughout the study. All of the eggs were boiled and served without condiments. On weekdays, the teachers boiled and distributed the eggs and then monitored the students as they ate them; on weekends or holidays, the parent(s) or guardians took the eggs home, boiled them, and monitored the children as they ate the eggs. After the intervention, the C18:3  $\omega$ -3, DHA, and  $\sum\omega$ -3 levels in the study group were significantly higher than those of the control group. Furthermore, the count of consumed eggs showed good compliance, and the percentage of adherence to treatment in our study was 100%.

### PUFA levels

No significant differences in the levels of  $\omega$ -3 and  $\omega$ -6 PUFAs were found between the two groups before the intervention ( $P > 0.05$ ). After the intervention, the C18:3  $\omega$ -3 ( $P = 0.009$ ), DHA ( $P = 0.009$ ), and  $\sum\omega$ -3 ( $P = 0.022$ ) levels in the intervention group were significantly higher than those of the control group, whereas the C20:4  $\omega$ -6 ( $P = 0.003$ ), C22:4  $\omega$ -6 ( $P = 0.000$ ),  $\sum\omega$ -6 ( $P = 0.001$ ),  $\sum\omega$ -6/ $\sum\omega$ -3 ( $P = 0.000$ ), and arachidonic acid (AA)/DHA ( $P = 0.000$ ) levels in the study group were significantly lower than those of the control group. No significant differences in the levels of C18:2  $\omega$ -6 ( $P = 0.723$ ), C20:2  $\omega$ -6 ( $P = 0.249$ ), C20:3  $\omega$ -6 ( $P = 0.258$ ), C20:5  $\omega$ -3 ( $P = 0.051$ ), or C22:5 ( $P = 0.200$ ) were found between the two groups (Table 3).

### Visual acuity

No significant differences were found in visual acuity between the two groups before the intervention ( $P = 0.520$ ). After the intervention, the mean  $\pm$  SD of visual acuity in the study group changed from  $0.042 \pm 0.254$  to  $-0.011 \pm 0.232$ , and the improvement was significant ( $P = 0.000$ ). In the control group, the mean  $\pm$  SD of visual acuity changed from  $0.058 \pm 0.245$  to  $0.041 \pm 0.232$ , and the improvement was also significant ( $P = 0.038$ ; Table 4). However, the comparison between the two groups shows that the monocular visual acuity in the study group was significantly better than in the control group ( $P = 0.013$ ).

### Discussion

In this study, the  $\omega$ -3 PUFA-rich eggs were laid by hens that were fed castor beans; thus they were a natural source of  $\omega$ -3 PUFAs containing pure fatty acids instead of a chemically modified supplement. The eggs, with little or no side effects, were readily accepted by the children's parent(s) or guardians, who were critical to compliance throughout the trial process.

After the intervention, the study group showed significantly higher levels of C18:3  $\omega$ -3, DHA, and  $\sum\omega$ -3, and significantly lower levels of  $\sum\omega$ -6,  $\sum\omega$ -6/ $\sum\omega$ -3, and AA/DHA compared with those of the control group. The results implied that the eggs rich in  $\omega$ -3 PUFAs not only improve children's RBC  $\omega$ -3 PUFA profiles but also improve the total  $\omega$ -6 PUFAs levels. The high concentration of DHA in the membranes of retinal photoreceptors and the significantly higher levels of DHA in the study group could account for the better improvement in visual acuity in the study group. The value of  $\sum\omega$ -6/ $\sum\omega$ -3 significantly affects the ratio and rate of production of eicosanoids, a group of hormones intimately involved in the body's inflammatory and homeostatic processes. The shifts of this ratio can change the body's metabolic and inflammatory state, and a lower ratio of  $\sum\omega$ -6/ $\sum\omega$ -3 decreases inflammation [25,26]. Thus, diet supplementation with natural  $\omega$ -3 PUFAs can provide children with additional health benefits. Although a significant difference in the levels of EPA was not observed between the two groups, the EPA level of the study group was higher than that of the control group ( $0.62 \pm 0.33$  versus  $0.49 \pm 0.29$ , respectively;  $P = 0.051$ ). If the sample size was larger, the difference between the groups might have been significant. The observed differences of the  $\omega$ -3 and  $\omega$ -6 PUFA levels between the two groups after the intervention can be attributed to the different consumption of C18:3  $\omega$ -3, EPA, and DHA. It appears that, compared with ordinary eggs, eggs rich in  $\omega$ -3 PUFAs could better improve the PUFA composition of school-age children.

Possibly because the two types of eggs both contained a certain amount of DHA and C18:3  $\omega$ -3, children in both groups had significant improvements in visual acuity after the intervention. However, the improvement of visual acuity in the study group was significantly better than in the control group, which could be attributed to the different doses of  $\omega$ -3 PUFAs consumed during the 3 mo. In addition to being highly concentrated in retinal photoreceptor cells, DHA is also abundant in the visual cortex and other cortical areas [6,15,27]. It has been reported that

**Table 3**  
Comparison of the RBC  $\omega$ -3 and  $\omega$ -6 PUFA Profiles in the Two Groups Before and After the Intervention\* (g/100 g of total fatty acids)

	Before intervention			After intervention		
	Study group (n = 59)	Control group (n = 40)	P-value	Study group (n = 48)	Control group (n = 40)	P-value
C18:2 $\omega$ -6	10.78 $\pm$ 2.10	10.21 $\pm$ 2.94	0.273	10.96 $\pm$ 2.59	11.17 $\pm$ 2.84	0.723
C18:3 $\omega$ -3	0.12 $\pm$ 0.04	0.12 $\pm$ 0.05	0.702	0.15 $\pm$ 0.04	0.13 $\pm$ 0.05	0.009
C20:2 $\omega$ -6	0.31 $\pm$ 0.10	0.28 $\pm$ 0.12	0.300	0.42 $\pm$ 0.27 (0.04–1.48)	0.35 $\pm$ 0.17 (0.11–1.06)	0.249
C20:3 $\omega$ -6	1.78 $\pm$ 1.05 (0.74–5.88)	1.51 $\pm$ 0.52 (0.64–3.63)	0.333	1.87 $\pm$ 0.96 (0.73–6.87)	1.75 $\pm$ 0.68 (1.08–4.78)	0.258
AA (C20:4 $\omega$ -6)	17.77 $\pm$ 4.50 (10.97–32.16)	19.24 $\pm$ 7.51 (9.34–39.98)	0.840	21.81 $\pm$ 7.89 (9.23–43.15)	25.62 $\pm$ 8.07 (14.77–48.09)	0.003
EPA (C20:5 $\omega$ -3)	0.46 $\pm$ 0.21	0.49 $\pm$ 0.27	0.535	0.62 $\pm$ 0.33	0.49 $\pm$ 0.29	0.051
C22:4 $\omega$ -6	3.17 $\pm$ 0.86	3.10 $\pm$ 1.23	0.749	3.18 $\pm$ 0.91	4.08 $\pm$ 1.17	0.000
C22:5 $\omega$ -3	1.87 $\pm$ 0.45	1.98 $\pm$ 0.74	0.401	2.18 $\pm$ 0.70 (0.88–4.52)	2.30 $\pm$ 0.64 (1.22–3.81)	0.200
DHA (C22:6 $\omega$ -3)	6.30 $\pm$ 1.97	6.70 $\pm$ 3.10	0.471	10.25 $\pm$ 3.10 (4.55–19.55)	8.97 $\pm$ 3.45 (3.87–16.98)	0.009
$\sum\omega$ -6	37.73 $\pm$ 6.41	34.34 $\pm$ 11.41	0.095	35.99 $\pm$ 13.51 (24.70–65.74)	42.97 $\pm$ 11.35 (31.09–77.05)	0.001
$\sum\omega$ -3	8.75 $\pm$ 2.51	9.29 $\pm$ 3.99	0.452	13.21 $\pm$ 4.04 (5.80–26.02)	11.89 $\pm$ 4.25 (5.78–21.43)	0.022
$\sum\omega$ -6/ $\sum\omega$ -3 <sup>†</sup>	4.58 $\pm$ 1.27	4.06 $\pm$ 1.40	0.057	2.95 $\pm$ 0.42	3.81 $\pm$ 0.86	0.000
AA/DHA	2.97 $\pm$ 0.86	3.23 $\pm$ 1.47	0.318	2.13 $\pm$ 0.40	3.02 $\pm$ 0.82	0.000

AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

\* Data of normal distribution were tested by independent samples *t* test and expressed as the mean  $\pm$  SD, whereas data with skewed distributions were tested by the Mann-Whitney test and expressed as the mean  $\pm$  SD and range.

<sup>†</sup>  $\sum\omega$ -6/ $\sum\omega$ -3 = C18:3  $\omega$ -3 + EPA + C22:5  $\omega$ -3 + DHA/C18:2  $\omega$ -6 + C20:2  $\omega$ -6 + C20:3  $\omega$ -6 + AA + C22:4  $\omega$ -6.

**Table 4**Visual Acuity of Study and Control Groups Before and After Intervention (LogMAR, mean  $\pm$  SD\* and range)

	Study group (n = 168) <sup>†</sup>	Control group (n = 174) <sup>†</sup>	P-value <sup>‡</sup>
Before intervention	0.042 $\pm$ 0.254 (–0.30 to 0.90)	0.058 $\pm$ 0.245 (–0.30 to 0.90)	0.520
After intervention	–0.011 $\pm$ 0.232 (–0.30 to 1.00)	0.041 $\pm$ 0.232 (–0.30 to 0.60)	0.013
P-value <sup>§</sup>	0.000	0.038	

LogMAR, minimum angle of resolution logarithmic

\* Because some visual acuity data is negative and the SD is always positive, the SDs were greater than the means.

† Because monocular visual acuity was analyzed, these data are doubled: The number of tested monocular visual acuity = the number of tested children  $\times$  2; there are actually 84 and 87 children in the study and control group, respectively.

‡ Values were analyzed by the Mann-Whitney test.

§ Values were analyzed by the Wilcoxon signed-ranks test.

DHA is crucial for the regeneration of visual pigment rhodopsin, which plays a critical role in the visual transduction system that converts light hitting the retina to visual images in the brain [28]. Therefore, it is no wonder that the visual acuity of the children could be significantly improved by eggs high in DHA. DHA is mainly available from food (such as fish and algae oil), and can be synthesized in the human body from dietary C18:3  $\omega$ -3 with an efficiency of only a few percent [29]. Children who are picky eaters may have a diet lacking DHA, directly causing a deficiency in DHA levels in vivo. Possibly because DHA was found to be rapidly incorporated in the nervous tissue of the retina and the brain during the brain's growth spurt, which mainly takes place from the last trimester of pregnancy up to 2 y of age [30–33], the current reports about  $\omega$ -3 PUFA supplementation and visual function are almost entirely focused on infants [4,14–18]. Nevertheless, visual acuity was found to be fully mature between the ages of 5 and the mid teenage years, whereas in contrast, sensitivity was found to be fully mature between the ages of 8 and 19 y. Thus, the basic aspects of visual function probably mature later than previously thought [34], and a timely and effective dietary intervention during this period is of great importance.

Although investigations about  $\omega$ -3 PUFAs and the visual acuity of school-age children are scarce, similar findings have been reported in other age groups. One study found that a 90-d supplementation with DHA can improve both the plasma DHA levels and the right eye visual acuity of 74 healthy participants ages 45 to 77 y [35]. It has been reported that prenatal  $\omega$ -3 fatty acid intake had long-term positive effects on the visual function in school-age children [36]. The intensive studies in infancy and early childhood have demonstrated that  $\omega$ -3 PUFA supplementation in pregnant and lactating women or DHA-supplemented formulas could significantly improve the vision development of children [18,37–40]. However, some investigators have proposed alternative perspectives [41,42]. The huge variations between studies could be partly attributed to the differences in methodologies, such as the dose and main components of supplemented  $\omega$ -3 PUFAs, the study durations, and the sample sizes. Many previous studies in other age groups and the present work show evidence that  $\omega$ -3 PUFAs, especially DHA, play critical roles in the development of children's visual acuity.

To our knowledge, this is the first study to address the role of  $\omega$ -3 PUFAs in the visual acuity of children 7 to 12 y old. The compliance was excellent and was confirmed by the fatty acid composition in the RBC membrane. The approach of using a natural dietary supplement to improve children's visual acuity may be novel. By incorporating two different types of natural eggs into the intervention strategy, we designed a trial comparing two distinct dietary doses of  $\omega$ -3 PUFAs. Furthermore, this study involved students from a broad range of ages (7–12 y) in which different vision conditions may be present. Hence, our

findings could provide specific insights regarding the use of  $\omega$ -3 supplements to improve visual acuity of school-age children without lower IQs or ADHD. Moreover, our results suggested that ordinary eggs might not be sufficient for optimal development of children visual acuity.

There are limitations to the present study. Much of the data in our study did not meet the parametric test conditions, which possibly led to a decrease in the testing power. Additionally, our study did not analyze the dose–response relation between the amount of DHA supplementation and the improvement of visual acuity. In summary, our study leaves much to be desired. Further research should pay more attention to the above short-comings and the identification of the appropriate content of  $\omega$ -3 PUFAs in eggs to promote children's vision.

## Conclusions

Dietary supplementation with  $\omega$ -3 PUFA-rich eggs can improve visual acuity and the RBC fatty acid profiles of school-age children with lower IQs or ADHD. The DHA content of ordinary eggs may not be sufficient for the development of visual acuity in school-age children.

## Acknowledgments

The authors acknowledge the local teachers and health workers who provided much help throughout the study implementation. They acknowledge all of the supporters, student participants, and their parent(s) or guardians for making the study possible.

## References

- [1] Delgado-Lista J, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *Br J Nutr* 2012;107(Suppl 2):S201–13.
- [2] Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 2010;68:280–9.
- [3] Milte CM, Sinn N, Buckley JD, Coates AM, Young RM, Howe PR. Polyunsaturated fatty acids, cognition and literacy in children with ADHD with and without learning difficulties. *J Child Health Care* 2011;15:299–311.
- [4] Qawasmi A, Landeros-Weisenberger A, Bloch MH. Meta-analysis of LCPUFA supplementation of infant formula and visual acuity. *Pediatrics* 2013;131:E262–72.
- [5] Singh M. Essential fatty acids, DHA and human brain. *Indian J Pediatr* 2005;72:239–42.
- [6] Tassoni D, Kaur G, Weisinger RS, Sinclair AJ. The role of eicosanoids in the brain. *Asia Pac J Clin Nutr* 2008;17:220–8.
- [7] Richardson AJ. Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. *Int Rev Psychiatry* 2006;18:155–72.
- [8] Maul E, Barroso S, Munoz SR, Sperduto RD, Ellwein LB. Refractive error study in children: results from La Florida, Chile. *Am J Ophthalmol* 2000;129:445–54.

- [9] Murthy GV, Gupta SK, Ellwein LB, Munoz SR, Pokharel GP, Sanga L, et al. Refractive error in children in an urban population in New Delhi. *Invest Ophthalmol Vis Sci* 2002;43:623–31.
- [10] He M, Huang W, Zheng Y, Huang L, Ellwein LB. Refractive error and visual impairment in school children in rural southern China. *Ophthalmology* 2007;114:374–82.
- [11] Pi LH, Chen L, Liu Q, Ke N, Fang J, Zhang S, et al. Prevalence of eye diseases and causes of visual impairment in school-aged children in Western China. *J Epidemiol* 2012;22:37–44.
- [12] Vitale S, Cotch MF, Sperduto RD. Prevalence of visual impairment in the United States. *J Am Med Assoc* 2006;295:2158–63.
- [13] Basch CE. Healthier students are better learners: a missing link in school reforms to close the achievement gap. *J Sch Health* 2011;81:593–8.
- [14] Agostoni C, Harvie A, McCulloch DL, Demellweek C, Cockburn F, Giovannini M, et al. A randomized trial of long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria. *Dev Med Child Neurol* 2006;48:207–12.
- [15] Judge MP, Harel O, Lammi-Keefe CJ. A docosahexaenoic acid–functional food during pregnancy benefits infant visual acuity at four but not six months of age. *Lipids* 2007;42:117–22.
- [16] Smithers LG, Gibson RA, McPhee A, Makrides M. Higher dose of docosahexaenoic acid in the neonatal period improves visual acuity of preterm infants: results of a randomized controlled trial. *Am J Clin Nutr* 2008;88:1049–56.
- [17] Jacobson JL, Jacobson SW, Muckle G, Kaplan-Estrin M, Ayotte P, Dewailly E. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the Inuit of arctic Quebec. *J Pediatr* 2008;152:356–64.
- [18] Birch EE, Carlson SE, Hoffman DR, Fitzgerald-Gustafson KM, Fu VL, Drover JR, et al. The DIAMOND (DHA Intake And Measurement Of Neural Development) study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. *Am J Clin Nutr* 2010;91:848–59.
- [19] Lavie CJ, Milani RV, Mehra MR, Ventura HO. Omega-3 polyunsaturated fatty acids and cardiovascular diseases. *J Am Coll Cardiol* 2009;54:585–94.
- [20] Wang D, Qian M, Fang YY, Lai CC, Li D, Chen GP. Revision on the Combined Raven's Test for the Rural in China. *Psychol Sci*; 1989:23–7.
- [21] Raven J, Raven JC, Court JH. *Manual for Raven's Progressive Matrices and Vocabulary Scales*. San Antonio, TX: Harcourt Assessment; 2003 (updated 2004).
- [22] American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington, DC: American Psychiatric Association; 1994.
- [23] SS1 Gau, Shen HY, Soong WT, Gau CS. An open-label, randomized, active-controlled equivalent trial of osmotic release oral system methylphenidate in children with attention-deficit/hyperactivity disorder in Taiwan. *J Child Adolesc Psychopharmacol* 2006;16:441–55.
- [24] Basak SK. *Ophthalmology oral & practical* (3rd ed.). Kolkata, India: Current Book International; 2006.
- [25] Hooper L, Thompson RL, Harrison RA, Summerbell CD, Ness AR, Moore HJ, et al. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *BMJ* 2006;332:752–60.
- [26] Lands WE. Biochemistry and physiology of n-3 fatty acids. *FASEB J* 1992;6:2530–6.
- [27] Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991;54:438–63.
- [28] San Giovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 2005;24:87–138.
- [29] Anderson BM, Ma DW. Are all n-3 polyunsaturated fatty acids created equal? *Lipids Health Dis* 2009;8:33.
- [30] Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intra-uterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early Hum Dev* 1980;4:121–9.
- [31] Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Extra-uterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early Hum Dev* 1980;4:131–8.
- [32] Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr* 1992;120:S129–38.
- [33] Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. *J Neurochem* 1998;71:2528–33.
- [34] Leat SJ, Yadav NK, Irving EL. Development of visual acuity and contrast sensitivity in children. *J Optom* 2009;2:19–26.
- [35] Stough C, Downey L, Silber B, Lloyd J, Kure C, Wesnes K, et al. The effects of 90-day supplementation with the omega-3 essential fatty acid docosahexaenoic acid (DHA) on cognitive function and visual acuity in a healthy aging population. *Neurobiol Aging* 2012;33:824.e14.
- [36] Jacques C, Levy E, Muckle G, Jacobson SW, Bastien C, Dewailly E, et al. Long-term effects of prenatal omega-3 fatty acid intake on visual function in school-age children. *J Pediatr* 2011;158:83–90.
- [37] Birch EE, Castaneda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. *Am J Clin Nutr* 2005;81:871–9.
- [38] Birch EE, Garfield S, Castaneda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Hum Dev* 2007;83:279–84.
- [39] Innis SM, Friesen RW. Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. *Am J Clin Nutr* 2008;87:548–57.
- [40] Dunstan JA, Simmer K, Dixon G, Prescott SL. Cognitive assessment of children at age 2 1/2 years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* 2008;93:F45–50.
- [41] Jensen CL, Voigt RG, Llorente AM, Peters SU, Prager TC, Zou YLL, et al. Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. *J Pediatr* 2010;157:900–5.
- [42] Smithers LG, Gibson RA, Makrides M. Maternal supplementation with docosahexaenoic acid during pregnancy does not affect early visual development in the infant: a randomized controlled trial. *Am J Clin Nutr* 2011;93:1293–9.