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Plasma polyunsaturated fatty acids are directly associated with cognition in overweight children but not in normal weight children

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Short title: Polyunsaturated fatty acids and cognition

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List of abbreviations:

AA: arachidonic acid

ALA: α -linolenic acid

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

PUFA: polyunsaturated fatty acids

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ABSTRACT

Aim: Polyunsaturated fatty acids are essential nutrients for the normal development of the brain. We investigated the associations between plasma polyunsaturated fatty acids and cognition in normal weight and overweight children.

Methods: The study recruited 386 normal weight children and 58 overweight children aged six to eight years and blood samples were drawn after a 12-hour fast. We assessed plasma polyunsaturated fatty acids using gas chromatography, cognition using Raven's Coloured Progressive Matrices, and overweight and obesity using the age-specific and sex-specific cut-offs from the International Obesity Task Force. The data were analysed by linear regression analyses adjusted for age and sex.

Results: Higher proportions of eicosapentaenoic acid in plasma triacylglycerols ($\beta=0.311$, $p=0.020$, $p=0.029$ for interaction) and docosahexaenoic acid in plasma triacylglycerols ($\beta=0.281$, $p=0.038$, $p=0.049$ for interaction) were both associated with higher Raven's scores in overweight children but not in normal weight children. Higher eicosapentaenoic acid to arachidonic acid ratios in triacylglycerols ($\beta=0.317$, $p=0.019$) and phospholipids ($\beta=0.273$, $p=0.046$) were directly associated with the Raven's score in overweight children but not in normal weight children.

Conclusions: These findings suggest that increasing the consumption of fish and other sources of eicosapentaenoic acid and docosahexaenoic acid may improve cognition among overweight children.

Key words: cognition, fish consumption, overweight, plasma, polyunsaturated fatty acids

KEY NOTES

- Polyunsaturated fatty acids are essential for normal brain development, but few studies have investigated their association with childhood cognition.
- Our study of 444 normal and overweight children aged 6-8 years showed that higher proportions of polyunsaturated fatty acids in plasma were related to better cognition in overweight, but not normal weight, children.
- The findings suggest that increasing the consumption of polyunsaturated fatty acids would help improve cognition among overweight children.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are essential nutrients for normal development of the brain and cognition (1,2) and should be obtained from diet because the body has a limited ability to synthesise them (2,3). A typical Western diet is low in sources of PUFAs, and less than half of the children in Western countries meet the recommended intake of PUFAs and particularly those of n-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (4,5).

Some studies suggest that a higher dietary intake of PUFAs has been related to better short-term memory in children (6,7). Furthermore, studies have suggested that a higher intake of n-3 PUFA supplements, especially DHA, may improve cognitive functions during the perinatal period and infancy but not in childhood or adulthood (7–10). Few studies have investigated the associations of the plasma biomarkers of PUFA status with cognition in children and these studies have provided inconsistent results (7,11). Nevertheless, one study found that increased whole-blood DHA and EPA concentrations after a three month school meal intervention were related to greater improvements in reading performance in nine to 11-year-old children (12). Furthermore, paediatric overweight, which has emerged as an important global public health concern

(13), has also been linked to impaired development of the brain and cognition (14).

There have not been any studies on the associations between plasma PUFAs and cognition in normal weight and overweight or obese children. We therefore investigated these associations in a population-based sample of children and examined whether being overweight or obese modified these associations.

METHODS

Study population

The present analyses were based on the baseline data from the Physical Activity and Nutrition in Children (PANIC) study, which is an on-going physical activity and diet intervention study in a population sample of primary school children from the city of Kuopio, Finland (4). A total of 736 children aged 6–8 years were invited to participate in the baseline examinations between 2007 and 2009 and 512 (70%) participated. The participants did not differ from other children of the same age, whose data were obtained from school health examinations (data not shown), when it came to sex distribution, age or their body mass index standard deviation score (BMI-SDS). After excluding children with developmental disabilities, attention deficit hyperactivity disorder or incomplete data from the analyses, the final study sample comprised 444 children: 219 girls and 225 boys. The PANIC Study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Finland, and the children and their parents gave their written informed consent.

Assessment of body size and composition

The body fat percentage was measured by the Lunar Prodigy Advance dual-energy x-ray absorptiometry device (GE Medical Systems, Madison, Wisconsin, USA) (15). Body height and weight were measured and BMI-SDS was calculated using Finnish references (16). The prevalence of overweight was defined using the age-specific and sex-specific cut-offs from the International Obesity Task Force (17).

Assessment of fatty acids

Blood samples were drawn after 12-hour fasting. Plasma PUFAs were analysed as described earlier (18) and were expressed in mol%. In short, the plasma samples were extracted with chloroform-methanol (2:1) and the lipid fractions were separated by solid phase extraction with an aminopropyl column. The fatty acids in plasma cholesteryl esters, phospholipids and triacylglycerols were transmethyated with 14% boron trifluoride in methanol and were analyzed by the 7890A gas chromatograph (Agilent Technologies Inc., Wilmington, Delaware, USA) equipped with a 25-m FFAP column. Cholesteryl nonadecanoate (Nu Chek Prep Inc., Elysian, Minnesota, USA), trionadecanoin and phosphatidylcholine dinonadecanoyl (Larodan Fine Chemicals, Malmö, Sweden) served as internal standards. In the analyses, we used the proportions of α -linolenic acid (ALA), EPA, DHA and docosapentaenoic acid (DPA) and the ratio of EPA or DHA to arachidonic acid (AA) (19).

Assessment of cognition

We used Raven's Coloured Progressive Matrices (Raven's CPM) to assess non-verbal reasoning (20). One trained researcher administered these assessments. Raven's CPM includes 36 large figures with a part missing. The children were asked to select the correct part that completed the figure from six alternatives presented beneath the large figure. Raven's CPM requires the ability to find similarities, differences, and discrete patterns, does not depend on acquired knowledge or language skills (20), and has been suggested to represent all-core components of executive functions (21). The Raven's CPM score was the number of correct answers, ranging from zero to 36.

Other assessments

Total physical activity and total sedentary behaviour were assessed using the PANIC Physical Activity Questionnaire, which was filled out by the parents (22). The parents were also asked to report their highest completed or on-going educational levels - vocational school or less, polytechnic, university - and the annual house-hold income in Euros, from the ranges $\leq 30,000$, $30,001-60,000$, and $\geq 60,001$. The level of the more educated parent was used in the analyses. We assessed food consumption and nutrient intake by food records administered by the parents on four predefined consecutive days. Of the food diaries, 99.5% included two weekdays and two weekend days and 0.5% included three weekdays and one weekend day. We assessed the number of meals per day based on data from the food records, classifying breakfast, lunch, and dinner as main meals and all eating and drinking between main meals as snacks. We categorised the children into those who had eaten all main meals daily and those who had skipped any of their main meals. The food records were analysed and we calculated total energy intake using the Micro Nutrica dietary analysis

software, Version 2.5 (The Social Insurance Institution of Finland), that uses Finnish and international data on the nutrient composition of foods (23). Parents were asked to report any medically diagnosed Attention-deficit hyperactivity disorder or other developmental disorder. The research physician assessed pubertal status using the five-stage criteria described by Tanner (24). The boys were defined as having entered clinical puberty, if their testicular volume assessed by an orchidometer was stage two or more, which is more than 3ml. The girls were defined as having entered puberty, if their breast development had started, which is also stage two or more. Genomic DNA was isolated from the blood mononuclear cells using the QIAamp DNA Blood kit (Qiagen, Hilden, Germany). Common fatty acid desaturase polymorphisms (rs174547 and rs174616) were extracted from the Human Core Exome Chip (Illumina, San Diego, California, USA). The genotypes were determined by using GenomeStudio (Illumina, San Diego, California, USA). Quality control was carried out using the free, open-source whole genome association analysis toolset PLINK Version 1.07.

Statistical methods

We performed all data analyses using SPSS Statistics software, Version 19.0 (IBM Corp., Armonk, New York, USA). The Student's t-test was used to investigate the differences in basic characteristics between normal weight and overweight or obese children. The associations of plasma PUFA proportions with the Raven's CPM score were analysed by linear regression adjusted for age and sex. Data were additionally adjusted for body fat percentage, clinical puberty, eating main meals, the dietary intakes of carbohydrates and sucrose, physical activity, sedentary behaviour, parental education, annual household income, and common fatty acid desaturase gene polymorphisms (rs174547 and rs174616).

which were entered into the model one by one with age and sex. We used general linear models adjusted for age and sex to study whether overweight and obesity modified the relationships between plasma PUFAs in cholesteryl esters, phospholipids, and triacylglycerols and the Raven's CPM score. Because of small number of pubertal children in the present study sample, we also performed the analyses without children who had entered clinical puberty.

RESULTS

The characteristics of normal weight and overweight or obese children are presented in Table 1. Higher proportions of EPA ($p=0.029$ for interaction) and DHA ($p=0.049$ for interaction) in plasma triacylglycerols and a higher EPA to AA ratio in plasma triacylglycerols and phospholipids were associated with a higher Raven's CPM score in overweight or obese children but not in normal weight children (Table 2). Additional adjustment for annual household income slightly attenuated the associations of the proportions of DHA ($\beta=0.233$, $p=0.086$) and EPA ($\beta=0.261$, $p=0.055$) in plasma triacylglycerols as well as EPA to AA ratio in plasma triacylglycerols ($\beta=0.284$, $p=0.037$) and phospholipids ($\beta=0.223$, $p=0.105$) with the Raven's CPM score in overweight or obese children. Other adjustments had no effects on these associations. The results remained virtually the same after excluding the children who had entered clinical puberty.

DISCUSSION

We found that higher proportions of DHA and EPA in plasma triacylglycerols and a higher EPA to AA ratio in plasma triacylglycerols and phospholipids were associated with a higher Raven's CPM score among overweight or obese

children. However, none of the plasma PUFAs were related to the Raven's CPM score among normal weight children.

There are few studies on the associations between the proportions of blood or plasma PUFAs and cognition in school-aged children (7,11). Montgomery et al (11) found no association between the proportion of DHA in blood and working memory but observed a direct relationship between the proportion of combined DHA and EPA in blood and working memory in children (11). Boucher et al (7) found no associations between the proportions of DHA, EPA, or other n-3 fatty acids in blood phospholipids and working memory in children. However, these studies did not take the possible modifying effect of adiposity into account. Our results suggest that the relationship between EPA, DHA, and the EPA to AA ratio and cognition was stronger in overweight or obese children than in normal weight children.

One explanation for the direct associations of plasma EPA, DHA and the EPA to AA ratio with cognition in the present study, may be that a higher dietary intake of EPA and DHA may reduce low-grade inflammation and improve brain structures and functions, which have been observed to be impaired in overweight children compared to normal weight children (2). Accordingly, some evidence has suggested inverse associations of whole blood PUFA concentration and the EPA to AA ratio with inflammatory markers in children and that body adiposity may modify these associations (25). Therefore, an adequate intake of EPA and DHA may be more important in the normal development of the brain and cognition among overweight and obese children than in normal weight children.

We found direct associations between EPA and DHA in plasma triacylglycerols, which have been suggested to reflect the fatty acid intake of the last few days, with the Raven's CPM score in overweight or obese children. However, we observed no associations between these fatty acids in plasma

cholesteryl esters or phospholipids, which reflect the dietary fat composition of preceding months (26), with cognition in children. The blood sampling and the cognitive testing were not performed on the same day and that makes it difficult to conclude that eating foods high in EPA and DHA before the cognitive test had beneficial effects on cognitive performance in overweight children. Nevertheless, direct associations of the EPA to AA ratio in plasma triacylglycerols and phospholipids with cognition in overweight or obese children suggest that a higher intake of EPA and DHA over months and years could have positive effects on the brain (2), particularly in overweight and obese children.

Household income partly explained the associations between plasma EPA and DHA with the Raven's CPM score. Low household income and poverty have been inversely associated with brain development and cognitive functions in children (27,28). We previously found that children from families with a lower household income were less likely to eat fish, which is the main source of EPA and DHA in Finland, than other children (4). Together with these observations, our current results suggest that poorer cognition in overweight or obese children from families with a lower household income may be partly modified by a lack of sources of EPA and DHA. However, further longitudinal studies are needed to explore the modifying effects of diet on the development of brain and cognition in children from a lower socioeconomic background.

The strengths of the present study included a relatively large population sample of children and the rigorous methods used to assess the plasma proportions of PUFAs. We also had an opportunity to control the data for a number of possible confounding factors in the analyses. The limitations of our study are the cross-sectional design which does not allow us to make conclusions about the causality of the relationships and the use of only one

measure of cognition instead of more comprehensive cognitive testing. There was limited statistical power in the analyses due to the relatively small number of overweight or obese children.

CONCLUSION

Our results suggest that the proportions of EPA and DHA in plasma triacylglycerols and the EPA to AA ratio in plasma triacylglycerols and phospholipids were directly associated with cognition in overweight or obese children but not in normal weight children. These findings provide some evidence that increasing the consumption of fish and other sources of EPA and DHA may improve cognition, particularly among overweight or obese children.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare

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Table 1. The characteristics of 386 normal weight children and 58 overweight or obese children

	Normal weight children (n=386)	Overweight children (n=58)	<i>P</i> -value
Age (years)	7.6 (0.4)	7.7 (0.4)	0.059
Body height (cm)	128.3 (5.4)	131.9 (5.2)	<0.001
Body weight (kg)	25.6 (3.4)	35.3 (4.3)	<0.001
BMI-SDS	-0.45 (0.86)	1.59 (0.44)	<0.001
Body fat percentage (%)	17.6 (5.9)	34.3 (5.5)	<0.001
Raven Coloured Progressive Matrices score	24.2 (5.1)	23.0 (5.3)	0.109
18:3n-3 α -linolenic acid in plasma TG (mol %)	1.74 (0.57)	1.71 (0.58)	0.697
20:5n-3 EPA in plasma TG (mol %)	0.66 (0.24)	0.62 (0.21)	0.207
22:5n-3 DPA in plasma TG (mol %)	0.63 (0.47)	0.57 (0.45)	0.371
22:6n-3 DHA in plasma TG (mol %)	1.46 (0.77)	1.45 (0.71)	0.962
18:3n-3 α -linolenic acid in plasma CE (mol %)	1.04 (0.22)	1.07 (0.23)	0.386
20:5n-3 EPA in plasma CE (mol %)	1.25 (0.55)	1.32 (0.43)	0.357
22:6n-3 DHA in plasma CE (mol %)	0.80 (0.21)	0.83 (0.26)	0.260
18:3n-3 α -linolenic acid in plasma PL (mol %)	0.40 (0.11)	0.40 (0.14)	0.708
20:5n-3 EPA in plasma PL (mol %)	1.27 (0.52)	1.32 (0.45)	0.488
22:5n-3 DPA in plasma PL (mol %)	1.28 (0.20)	1.29 (0.19)	0.849
22:6n-3 DHA in plasma PL (mol %)	4.66 (1.07)	4.75 (0.95)	0.538

Values are means (standard deviations) from the Students t-test. BMI-SDS = body mass index standard deviation score, based on Finnish reference values (Saari et.al.); CE= Cholesteryl esters; DHA= Docosahexaenoid acid; DPA= Docosapentaenoic acid; EPA=Eicosapentaenoic acid; PL= phospholipids; TG=triacylglycerols

Table 2. The associations of polyunsaturated fatty acids in plasma triacylglycerols, cholesteryl esters, and phospholipids with Raven's Coloured Progressive Matrices score in normal weight and overweight or obese children.

		Normal weight children (n=386)	Overweight or obese children (n= 58)
Fatty acid	Common name of the fatty acid		
18:3n-3	α -linolenic acid in plasma TG*	0.015	0.165
20:5n-3	EPA in plasma TG*	0.027	0.311*
22:5n-3	DPA in plasma TG*	-0.040	0.224
22:6n-3	DHA in plasma TG*	0.035	0.281*
	<i>EPA to AA</i> ratio in plasma TG	0.012	0.317 *
	<i>DHA to AA</i> ratio in plasma TG	0.021	0.164
18:3n-3	α -linolenic acid in plasma CE *	0.020	0.058
20:5n-3	EPA in plasma CE *	-0.005	0.152
22:6n-3	DHA in plasma CE*	-0.002	-0.097

	<i>EPA to AA in plasma CE</i>	-0.022	0.251	
	<i>DHA to AA ratio in plasma CE*</i>	0.004	0.023	
18:3n-3	α -linolenic acid in plasma PL	-0.038	0.039	
20:5n-3	EPA in plasma PL	-0.009	0.251	D
				a
22:5n.3	DPA in plasma PL*	0.009	0.074	t
22:6n-3	DHA in plasma PL	0.045	0.091	a
	<i>EPA to AA ratio in plasma PL*</i>	0.021	0.273*	a
	<i>DHA to AA ratio in plasma PL*</i>	0.038	0.171	e

tandardized regression coefficients (β) from linear

regression models adjusted for age and sex.

*Logarithmic transformation was carried out, because of skewed distributions.

CE= Cholesteryl esters; AA=Arachinoid acid;

DHA= Docosahexaenoid acid; DPA=

Docosapentaenoic acid; EPA=Eicosapentaenoic

acid;PL= phospholipids;

TG=triacylglycerols.* $P<0.05$.