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Linoleic and docosahexaenoic acids in human milk have opposite relationships with cognitive test performance in a sample of 28 countries ☆

W.D. Lassek [a,*], S.J.C. Gaulin [b]

[a] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, 130 Desoto Street, Pittsburgh, PA 15213, United States
[b] Department of Anthropology, University of California at Santa Barbara, Santa Barbara, CA 93106-3210, United States

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ABSTRACT

Polyunsaturated fatty acids play critical roles in brain development and function, and their levels in human breast milk closely reflect the long-term diet. The fatty acid contents of human milk samples from 28 countries were used to predict averaged 2009 and 2012 test scores in mathematics, reading, and science from the Program for International Student Assessment. All test scores were positively related to milk docosahexaenoic acid (r=0.48 to 0.55), and negatively related to linoleic acid (r= −0.28 to −0.56). Together, these two human milk fatty acids explained 46% to 48% of the variance in scores, with no improvement in predictive power when socioeconomic variables were added to the regression. The (log) ratio of linoleic to arachidonic acid was negatively related to scores (r= −0.45 to −0.48). Statistical effects were similar for the two sexes. In a separate US sample, estimated dietary linoleic was negatively related to the levels of all long-chain n-3 and n-6 plasma fatty acids. High levels of dietary linoleic may impair cognition by decreasing both docosahexaenoic and arachidonic acids in the brain.

1. Introduction

Docosahexaenoic acid (DHA, 22:6n-3) plays critical roles in neurogenesis, neurite growth, impulse transmission, and synaptic function [1–3]; but while many studies show beneficial effects of dietary DHA on cognition, those involving supplemental dietary DHA have mixed results [2,3]. While conflicting results have been attributed to methodological differences, another possibility is that higher dietary levels of n-6 fatty acids (FA) may oppose the effects of dietary DHA in enriching neural membranes and enhancing cognition because of competitive interactions between the two types of polyunsaturated FA [4]. Most studies have failed to control for the effects of n-6 [5]. By far the most abundant form of dietary n-6 is linoleic acid (LA, 18:2n-6) which is found in high concentrations in many vegetable oils, and a number of studies have suggested possible adverse effects of LA on animal and human cognition [6–18].

Potential adverse effects of LA are of practical significance because of dietary shifts in many countries towards increased consumption of LA in vegetable oils and products from grain-fed animals [19–21]. In the US, for example, LA increased from 2.3% to 7.2% of total food energy from 1909 to 1999 [19], and levels of LA have also been increasing in European countries [22]. Because the FA composition of brain phospholipids is critical to neurodevelopment and physiology, these dietary shifts may have unintended consequences for brain function and cognition.

Investigating the cognitive effects of n-3 and n-6 FA in humans involves several methodological difficulties. Studies involving supplemental n-3 may involve time periods which are too short to affect amounts in the brain because of slow turnover in the brain and adipose [23,24]. Studies which measure FA intake using dietary histories are subject to inaccurate reporting [25]. Blood levels of FA may better reflect long-term intake, but identifying significant effects may be constrained by a lack of variability within local or regional diets. Despite the significant limitations of ecological studies [26], the use of population-level data offers another avenue of investigation and may generate useful hypotheses.

Because polyunsaturated fatty acids in human milk are largely determined by the long-term diet of lactating mothers [27,28], the fatty acid composition of human breast milk in samples from different countries varies with the composition of the national diet [29,30], making such milk samples suitable for ecological investigations [31,32]. Because of the influence of diet on fatty acids in the brain, such national dietary differences may influence the cognitive performance of children and may be reflected in mean national scores on international standardized tests such as those

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* Corresponding author. Tel.: +1 412 681 3867; fax: +1 412 624 3737.
E-mail address: will.lassek@gmail.com (W.D. Lassek).

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given through the Program for International Student Assessment (PISA) of the Organization for Economic Co-operation and Development (OECD).

An earlier study [32] found that mean levels of DHA in maternal breast milk in 28 countries accounted for more than 20% of the variance in scores on the 2009 PISA math test when economic measures were controlled. Here, we extend the focus of that earlier study by simultaneously considering breast milk n-6 and n-3 FA, and by expanding the measures of cognitive performance to include three subject tests over two cycles of administration.

2. Methods

Measures of cognitive performance were averages of the 2009 and 2012 national mean scores for the mathematics, reading, and science tests administered by the PISA test program [33,34]. A minimum of 4500 students from each country were required for the PISA sample. PISA test scores are strongly correlated with independent estimates of national IQ [35].

The earlier study [32] used only math test data from a single cycle (2009). Averaging scores over two test cycles and using results from three different tests may provide a more valid and generalizable index of national cognitive performance. Because participation in the PISA program has been increasing with each cycle, data from the two most recent test cycles (2009 and 2012) were used to maximize the number of countries with data suitable for analysis. PISA scores for both years were available for 58 countries.

National averages of human maternal milk FA content from published reports were used as a measure of FA availability to children. To acquire human milk data, we searched MEDLINE combining MESH search terms “milk” and “fatty acids” and thereby identified 128 studies providing human milk FA values as percentages of total FA in 50 countries. The mean sample size for the 128 studies was 52, and the number of samples varied from 1 to 23 per country. Specifics on the studies used for each country are available at http://onlinelibrary.wiley.com/doi/10.1111/mcn.12060/suppinfo.

Although different methods may have been used for extracting and analyzing milk FA in different studies, FA percentages may be less sensitive to such differences than concentrations. Specific FA measured varied among studies; the number of countries with data for specific FA is shown in Table 2. The 28 countries with both milk and cognitive test data are shown in Fig. 1. Since PISA test scores are strongly correlated with GDP *per capita* [35], we used this value (GDPPC) and also educational dollars per pupil (DPP) as covariates, paralleling previous research [32]. Recent data for GDPPC were obtained from United Nations sources [36]; DPP data were obtained from the OECD [37].

To provide insight into how dietary n-3 and n-6 FA may affect the composition of the blood lipids used to produce breast milk, the relationship between dietary and plasma FA was explored in a representative American sample of 1741 adults aged 20+ from the National Health and Nutrition Examination Survey (NHANES) for 2003–2004 [38]. The intake of individual dietary FA included in the data set was estimated from foods consumed in two detailed dietary histories per participant, each based on 24-h recall obtained by trained NHANES personnel [39] The nutrients in each food item were then determined by NHANES staff using the National Nutrient Database for Standard Reference [40]. Plasma levels of 24 FA were independently measured [41].

2.1. Analysis

The ratios of milk DHA to LA (DHA/LA), AA to DHA (AA/DHA), and LA to AA (LA/AA) were calculated. To minimize statistical problems of skew and non-normality associated with ratios [42,43], log-transformed FA ratios were also used in inferential analyses. The joint explanatory power of n-3 and n-6 FA was also demonstrated without ratio variables by including both FA variables in multiple regression analyses. Possible sex differences in the association between predictor variables and test scores were also evaluated.

Table 1
n-3 and n-6 fatty acid synthetic pathways with enzymes shown in italics, in the formula for fatty acid the first numeral is the number of carbons and the second is the number of double bonds.

step	n-6 FA		n-3 FA
	18:2n-6 LA linoleic		18:3n-3 ALA α-linolenic
1		*Δ6 desaturase*	
	18:3n-6 GLN γ-linoleic		18:4n-3 SA stearidonic
2		*elongase*	
	20:3n-6 DGLA dihomo-γ-linolenic		20:4n-3 ETA eicosatetraenoic
3		*Δ5 desaturase*	
	20:4n-6 AA arachidonic		20:5n-3 EPA eicosapentaenoic
4		*elongase*	
	22:4n-6 DTA docosatetraenoic		22:5n-3 DPA3 docosapentaenoic
5		*elongase*	
	24:4n-6 TTA tetracosatetraenoic		24:5n-3 TPA3 tetracosapentaenoic
6		*Δ6 desaturase*	
	24:5n-6 TPA6 tetracosapentaenoic		24:6n-3 THA tetracosahexaenoic
7		*beta oxidation*	
	22:5n-6 DPA6 docosapentaenoic		22:6n-3 DHA docosahexaenoic

Table 2
Mean, standard deviation, variability, and sample size for variables used in analysis.

	Mean	Coefficient of variation	Number of countries
Milk fatty acids, %total			
Saturated	43.32 ± 9.56	0.221	33
Monounsaturated	35.69 ± 6.45	0.181	34
Total polyunsaturated	20.80 ± 8.85	0.425	33
LA	14.53 ± 3.82	0.263	43
ALA	0.99 ± 0.54	0.545	44
AA	0.53 ± 0.23	0.434	44
Total long-chain n-3[a]	0.73 ± 0.38	0.521	30
DHA	0.38 ± 0.23	0.605	50
DPA	0.19 ± 0.09	0.474	33
EPA	0.13 ± 0.09	0.692	36
Untransformed ratios			
DHA/LA	0.027 ± 0.177		42
DHA/AA	0.735 ± 0.406		44
LA/AA	31.5 ± 16.8		44
Socioeconomic *variables*			
GDPPC[b,c]	24407 ± 15047		28
DPP[c,d]	5922 ± 4219		28
PISA test scores			
Math[e]	473.2 ± 55.7		58
Reading[e]	473.7 ± 46.5		58
Science[e]	479.2 ± 50.5		58
3-test average[e]	475.4 ± 50.5		58

[a] Sum is affected by different *n*'s.
[b] Per capita gross domestic product.
[c] Data only for countries included in the multivariate analyses.
[d] Dollars per student expended on education.
[e] Mean of 2009 and 2012 scores.

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Various applications of the general linear model (GLM: bivariate Pearson correlation coefficients and multiple linear regression) were used to test for cross-national relationships between the individual milk FA variables, the economic variables (GDPPC, DPP) and the three PISA test scores. Dependent variables included test scores by gender and combined in math, science, reading, and the average of the 3 tests over two cycles of administration. Independent variables included milk DHA, LA, AA, EPA and DPA, ratios of DHA to LA and LA to AA and their logs, GDPPC, and DPP. Fisher's *r*-to-*z* transformation was used to evaluate sex differences in the correlates of PISA scores.

In a separate analysis using US individuals as the units of analysis, GLM techniques were again used to examine the relationship between dietary and plasma levels of individual FA. SPSS-20 was used throughout.

3. Results

3.1. Descriptive statistics

Descriptive statistics are given in Table 2. About one fifth of the FA in typical human maternal milk were PUFA, with LA predominating. Although there was 40% more AA than DHA on average, when all long-chain n-3 were combined they jointly predominated over AA by nearly 40%. The ratio of DHA to LA was very small and highly variable across the sample of 42 countries. Coefficients of variation were higher for n-3 than for n-6 FA.

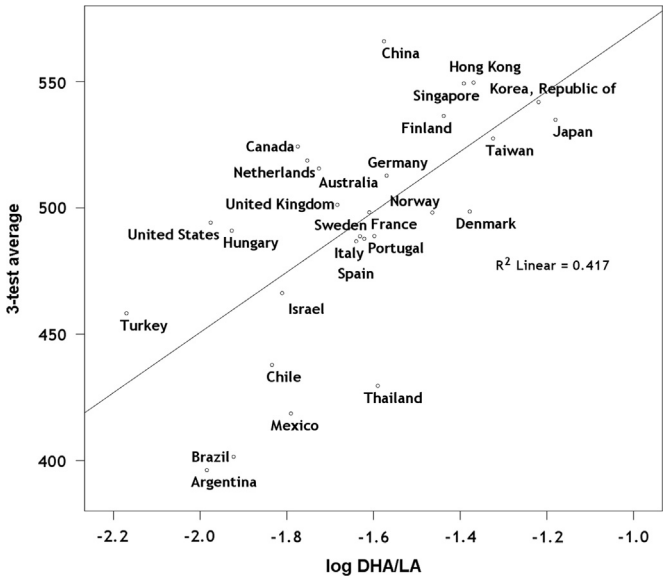

Fig. 1. Relationship between log DHA/LA in maternal milk and average PISA test score for 2009/2012.

3.2. Significant correlations among human milk FA

Although LA can be converted to AA, their percentages were not significantly related in maternal milk ($r=0.098$, $p=0.526$, $n=44$ countries). AA was positively correlated with two n-3 FA, DHA ($r=0.326$, $p=0.021$, $n=43$) and DPA ($r=0.447$, $p=0.010$, $n=32$). By contrast, the log ratio of LA/AA was negatively related to both DHA ($r=-0.312$, $p=0.042$, $n=43$) and DPA ($r=-0.462$, $p=0.008$, $n=33$). DHA was correlated with the two other long-chain n-3 FA, EPA ($r=0.546$, $p=0.001$, $n=43$) and DPA ($r=0.840$, $p<0.0001$, $n=33$). There were no significant correlations between ALA or LA and n-3 LCPUFA.

3.3. Correlations of human milk FA and socioeconomic measures with PISA test scores

The socioeconomic variables GDPPC and DPP were strongly correlated with test scores (Table 3) and accounted for 19% to 26% and 14% to 29% of the variance, respectively. Relevant to covariance issues, milk DHA was unrelated to these SES variables ($r<0.06$), whereas LA had a significant negative relationship with DPP ($r=-0.421$, $p=0.013$) and a marginal negative correlation with GDPPC ($r=-0.265$, $p=0.082$).

Milk DHA, and the DHA/LA ratio and its log were significant positive correlates for all three cognitive scores, with a somewhat stronger statistical effect on math and science, while milk LA was a significant negative correlate for reading and science scores but not math (Table 3). Log DHA/LA accounted for 38% to 45% of the variance in scores; this ratio's relationship with the average PISA test score is illustrated in Figs. 1 and 2. Scores were 1.9 standard deviations (85 points) higher in the highest vs. the lowest quartile of the DHA/LA ratio distribution.

The log ratio of milk LA/AA was strongly *negatively* related to test scores, accounting for 20% to 23% of the variance in scores. By itself, milk AA (not shown) had a weak positive relationship with scores, with non-significant correlations from +0.065 to +0.136, $p>0.49$). All correlations with test scores and socioeconomic variables were similar for males and females and did not differ statistically.

Though not shown in Table 3, n-3 DPA ($n=24$) and the sum of DHA, EPA, and DPA ($n=22$) were also strongly positively correlated with PISA test scores with coefficients similar to those for DHA, but DHA was used in subsequent analysis because of the larger sample size and its well documented role in cognition. There were no significant correlations of test scores with ALA or EPA.

Table 3
Bivariate Pearson correlations between averaged 2009/2012 PISA test scores in 28 countries, GDPPC (gross domestic product per capita), DPP (dollars per pupil), and milk DHA, LA, log DHA/LA, and log LA/AA.

	Math		Reading		Science		3-test average	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
GDPPC	0.431	0.020	0.510	0.005	0.503	0.005	0.513	0.0001
DPP	0.370	0.048	0.439	0.017	0.425	0.022	0.540	0.0001
DHA	0.552	0.006	0.477	0.010	0.487	0.009	0.492	0.007
LA	−0.275	0.157	−0.558	0.003	−0.530	0.004	−0.356	0.063
DHA/LA	0.597	0.001	0.531	0.004	0.561	0.002	0.609	0.001
log DHA/LA	0.674	0.0001	0.619	0.0004	0.618	0.0005	0.646	0.0002
LA/AA	−0.503	0.006	−0.504	0.006	−0.513	0.005	−0.520	0.005
log LA/AA	−0.465	0.013	−0.449	0.017	−0.478	0.010	−0.469	0.012

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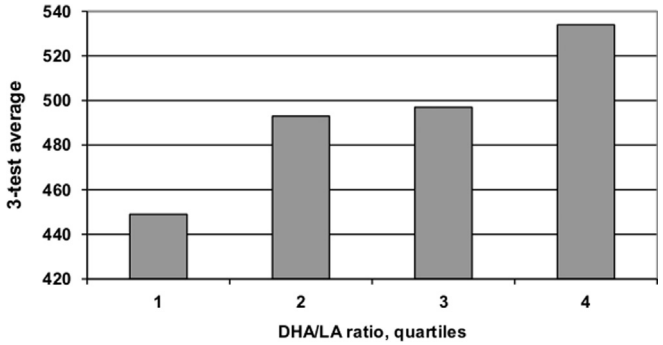

Fig. 2. DHA/LA ratio in maternal milk by quartile and average PISA test score for 2009/2012, (ANOVA $F=6.70$, $n=28$, $p=0.002$).

Table 4
Multiple regression using milk DHA and LA to predict averaged 2009/2012 PISA test scores in 28 countries, with β coefficients and r^2 for the model.

Subject	DHA		LA		Model	
	β	p	β	p	r^2	p
Math	0.697	0.0001	−0.483	0.003	0.479	<0.001
Reading	0.640	0.0002	−0.551	0.001	0.464	<0.001
Science	0.604	0.0003	−0.616	0.0003	0.484	<0.001
3-test average	0.656	0.0001	−0.551	0.0008	0.480	<0.001

3.4. Multiple regression models predicting test scores

In multiple-regression models using milk DHA and LA to jointly predict test scores (Table 4), the statistical effect of both variables on scores was considerably strengthened and together they consistently explained more than 46% of the variance. If ALA was added to the regressions, it was positively but not significantly related to scores (β 0.095 to 0.133, $p>0.35$). If AA was added, its statistical effect was negative but also not significant (β −0.131 to −0.165, $p>0.30$). Coefficients for scores of males and females treated separately (not shown) were very similar and did not differ statistically.

Strikingly, despite their strong bivariate relationships with test scores (Table 3), neither GDPPC nor DPP were significant nor did they improve r^2 when added to the models with DHA and LA; apparently these two milk FA better account for the variance in test scores attributable to these socioeconomic variables.

Based on the regression coefficients (121.7 for DHA and −8.0 for LA), an increase of one standard deviation in the milk DHA percentage was associated with an increase in the average PISA test score by 0.56 standard deviations (28.1 points), while a similar increase in the milk LA percentage was associated with a decrease in the average score by 0.60 standard deviations (30.1 points). All results were similar when 2009 and 2012 test scores for the 28 countries are analyzed separately.

3.5. Dietary LA and plasma FA

In the NHANES 2003–2004 sample with individual plasma FA values and estimates of dietary FA from diet histories, dietary LA was significantly *negatively* correlated with plasma levels of all of the long-chain n-6 and n-3 FA, including n-6 DGLA (−0.113, $p<0.001$) and AA (−0.086, $p<0.001$), and n-3 EPA (−0.056, $p=0.019$), DPA (−0.083, $p<0.001$), and DHA (−0.114, $p<0.001$). Using multiple regression, plasma DHA was positively related to dietary DHA ($\beta=0.248$, $p<0.001$) and negatively related to dietary LA ($\beta=-0.143$, $p<0.001$), with $r^2=0.073$ ($n=1743$).

4. Discussion

A previous study explored whether national differences in PISA math test performance were related to apparent national differences in the supply of DHA, as reflected in the percentage of this FA in maternal breast milk [32]. The current study expands on that research in two important ways. First, it extends the measure of cognitive performance to include tests in three different subject areas conducted in two different test cycles. Second, in addition to replicating the previously reported strong positive relationship between breast milk DHA and test scores, the current study documents a strong, independent, and negative relationship between breast milk LA and test scores as well as a negative relationship between the LA/AA ratio and test scores.

DHA and other long-chain n-3 were strongly positively related to PISA test scores for math, reading, and science, with similar statistical effects in 15-year-old males and females. PISA test scores have been shown to reflect national cognitive ability [35]. This extends the result of the previous study of DHA and math scores [32] with three test scores over two test cycles. Milk AA levels had only very small and nonsignificant (though positive) correlations with test scores, as found previously for math scores [32].

Milk LA was not only a significant negative predictor for two of the three test scores, but adding LA to a regression with milk DHA substantially strengthened the effects of both variables, which together accounted for more than 46% of the variance in each of the three test scores. The log ratio of DHA to LA accounted for a similar amount of variance (42%). This is substantially greater than the 21% accounted for by DHA alone for the 2009 PISA math score [32]. When DHA was held constant, a one standard deviation increase in the percentage of LA in milk FA was associated with a decrease of 0.6 standard deviations in the average test score (30 points).

Neither of the two socioeconomic variables, which separately accounted for 14% to 29% of the variance in test scores, was significant when added to the regressions with milk DHA and LA. This may be due to their shared variance with milk LA; when DHA is the only FA in the regression, the socioeconomic variables are significant predictors of math scores [32].

The log LA/AA ratio was also a strong negative predictor of test scores suggesting that higher levels of dietary LA in relation to AA may be detrimental to cognitive functioning. The difference between these two n-6 FA is also implied by the fact that milk DHA and DPA were positively correlated with milk AA but negatively correlated with milk LA/AA. This is consistent with a study which found that the ratio of AA to LA in infant red blood cells at 24 months was strongly positively related to developmental scores [17].

The results of the current study are also consistent with other studies showing a negative effect of LA on cognition. Higher LA and LA/ALA ratios in the diet have been linked to adverse cognitive effects in many animal studies [8–9,18], with similar negative effects in human studies. A higher concentration of LA in maternal blood or milk has been negatively linked to neurodevelopment in premature infants in three studies [10–12]. In full-term children, a higher ratio of n-6 to n-3 in the maternal diet during pregnancy was negatively related to neurodevelopment at age 2–3 [13]. A higher ratio in the current diet was also negatively related to performance in cognitive tasks at age 7–9 [14]. In older children, cheek cell LA was negatively related to performance on two cognitive tests [15], and higher dietary LA has also been linked to cognitive impairment in older men [16]. In our analysis of the relationship between dietary and plasma FA in an American sample, dietary LA was negatively related to the amount of all long-chain FA in the plasma, especially DHA, suggesting suppression of desaturase enzymes and possible competition with DHA

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for incorporation into plasma phospholipids, as found in other studies (see below).

The opposing statistical effects of dietary LA (negative) and DHA (positive) on plasma DHA were the same as the opposing statistical effects of milk LA and DHA on test scores. Since blood lipids are the immediate source of FA in breast milk, this suggests a direct connection between diet and FA available for lactation.

4.1. How can dietary LA reduce DHA in the brain?

The potential adverse cognitive effects of LA may be related to decreased availability of DHA in the brain. The opposite relationships of DHA and LA with cognitive performance found in this and other studies suggest competition between them, and it may be useful to enumerate possible metabolic pathways for such effects. There are at least four possible ways in which higher levels of dietary LA can decrease brain DHA, including 1) competitive interference with conversion of n-3 FA into DHA, 2) inhibition of desaturase enzymes necessary for the synthesis of all long-chain FA, 3) competition with DHA for incorporation into plasma phospholipids, and 4) competition for inclusion in brain phospholipids between DHA and LA's elongation product, n-6 docosapentaenoic acid (DPA6).

The main source for DHA in the brain is DHA in the blood, with most carried in plasma phospholipids [44,45]. This DHA may come directly from the diet or from hepatic conversion of dietary α-linolenic acid (ALA) or other shorter-chain n-3 FA (see Table 1), although conversion of ALA to DHA is very limited [46,47]. This conversion is further restricted with higher levels of dietary LA because the same enzymes are needed to produce longer-chain n-6 or n-3 FA from shorter-chain forms (Table 1). DHA synthesis by hepatic cells was much lower with a LA/ALA ratio of 4:1 than 1:1 [48], and a ratio of 7:1 blocked conversion [4]. Reducing LA from 7–10% to 3–4% of food energy substantially increased conversion of ALA to longer chain n-3 FA [49,50].

Higher levels of LA in the diet and blood may also down-regulate production of the desaturase enzymes required to convert shorter-chain forms of both n-3 and n-6 to long-chain derivatives [48,51–54]. Decreased availability of desaturase enzymes limits production of *all* longer-chain FA—both n3 and n6—including DHA and arachidonic acid (AA), an n-6 FA which also plays a critical role in brain function [55,56].

In addition to reducing synthesis of long-chain FA, higher levels of dietary LA may result in competition between LA and DHA for limited phospholipid acylation sites in the liver with LA occupying sites that would otherwise go to DHA [4,57]. This may explain why higher levels of dietary LA increase LA and decrease DHA in blood phospholipids [4,58,59]. In young children receiving DHA from fish oil supplements, the amount of n-3 incorporated into plasma phospholipids was unrelated to n-3 in the background diet but *negatively* related to the amount of dietary n-6 [60]. Children with higher levels of dietary n-6 had lower levels of n-3 in phospholipids despite receiving supplemental n-3. Conversely, two recent studies show that reducing dietary LA while leaving n-3 intake unchanged results in significant increases in DHA in plasma phospholipids [45,61].

Finally, through its long-chain n-6 product in the brain, the n-6 form of DPA (DPA6 22:5n-6), LA may interfere with incorporation of DHA into neural membranes. Since milk DHA is the end result of any prior competition between LA and ALA for incorporation into blood lipids, local competition between DHA and n-6 FA for incorporation into neural phospholipids could help to account for the negative association between milk LA and cognitive performance observed in the present study.

A number of animal studies have found that increasing the amount of dietary LA increases DPA6 in brain phospholipids while AA remains largely unchanged [62–66], and this pattern has also been seen in the blood and brain of human infants [67–70]. Conversely, reducing dietary LA decreases DPA6 in plasma phospholipids, but not AA [45]. DPA6 appears to be incorporated into brain phospholipids more readily than DHA so that when equal amounts were made available to cortical neurons, three times more DPA6 than DHA appeared in neuron phospholipids [71]. Thus, higher levels of DPA6 resulting from increasing dietary LA can displace DHA in brain phospholipids [71–73]. Supporting this, animal studies show that when DPA6 increases in animals fed increased amounts of LA, there is a corresponding decrease in DHA in brain phospholipids [66,67–73].

Studies in humans give similar results. Infants fed formulas with 12–15% LA, had more DPA6 and less DHA in the brain than nursing infants [67,68]. A similar pattern was observed in red cell and plasma phospholipids [69] and in the brains [70] of infants fed formula with a high vs. low LA/ALA ratio. In studies where dietary ALA is held constant and LA increased, there is again little change in LA or AA in the brain, while DPA6 increases and DHA decreases [6,62]. Similarly, a study comparing fatty acids in the tissues of Tanzanian infants with low intakes of LA compared with western populations found higher levels of DHA and lower levels of DPA6 in the brain [74].

DPA6 does not provide the same support as DHA for secondary neurite branching and growth [62,75,76], and a higher ratio of DPA6 to DHA in fetal brain phospholipids also leads to markedly impaired neurogenesis [77]. Monkeys fed a lifetime diet high in LA and low in n-3 have decreased functional connectivity in the cortex and impaired distributed cortical networks [7]. Even if dietary DHA is subsequently increased, replacement of DPA6 by DHA and recovery of DHA in neural membranes is quite slow [78–80].

Adverse effects of higher levels of brain DPA6 on cognition have been found for rodent learning, [18,65,81] exploratory behavior [82], and primate vision [66]. In children, DPA6 in cheek cells was negatively related to attention scores [15], and higher levels of DPA6 in red blood cells in the mother were negatively related to verbal IQ in offspring at age 8 [83] with similar adverse cognitive effects in an elderly sample [84].

These multiple mechanisms for competition between dietary LA and DHA may explain why some studies have shown weaker effects of dietary or supplementary DHA on cognition in children: Children with higher dietary LA may be less responsive to the beneficial effects of dietary DHA (see [60]).

4.2. Limitations

Because they are based on ecological analysis, the associations found in this study between breast milk FA and PISA test scores should be considered as preliminary and as a basis for planning and implementing further research. In addition to limitations common to ecological studies, this study is limited by the data sources used. Although the results are statistically significant, the sample size of 28 countries is small, and a larger sample could produce different results. Breast milk FA data came from 128 separate studies, and samples may not represent countries as a whole. Means from different samples were averaged for individual countries, with variable numbers of samples for different countries and variable sample sizes. The use of percentages of fatty acids in milk lipids may also influence comparisons of different fatty acids. While human maternal milk is likely to reflect the national diet, the proportion of children receiving breast milk and the duration of nursing probably varies by country. The composition of commercial or home-made formulas used in lieu of nursing or as supplements may also vary and is likely to be less reflective of the

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national diet. Children in countries where nursing is more common and prolonged may have cognitive advantages.

The amount of LA in maternal milk samples could be correlated with other dietary variables that are also related to cognitive performance. For example, in the U.S.A. more than half of dietary LA comes from commercial baked goods (breads, pizza, cookies, and cakes), potato and corn chips, fries, and snacks; and meat dishes made with vegetable oils [85]. A heavier reliance on such foods could be correlated with economic or educational opportunity, and other ingredients could also have cognitive effects.

Although standardized international PISA test scores were used as the outcome variable, test questions must be translated into the language of each country, and student samples may not be equally representative of national populations. The use of the average of the 2009 and 2012 test scores may help to reduce this source of variability. While two socioeconomic control variables were not significant in the multiple regressions, other factors could be correlated with both test scores and breast milk FA.

The relationship between dietary and plasma FA is based on data from only one country and is affected by the limitations of dietary estimates based on 24-h recall, the database used to determine nutrient values, and the degree to which they accurately reflect the long-term diet.

Most of these uncertainties are likely to constitute random rather than systematic error and thus should not elevate the chance of mistakenly rejecting the null hypothesis. The robusticity of the results—across all three tests—and the congruence of the observed statistical effects with known neurodevelopmental influences and physiological pathways, and with other studies, may warrant some attention.

4.3. Conclusions

This study supports the hypothesis that high LA intake could contribute to suboptimal cognitive performance and is consistent with other studies showing adverse metabolic and cognitive effects of LA. Such findings suggest that to maximize cognitive function in children it may be desirable not only to increase dietary n-3 FA, but to also decrease the amount of LA in the diet. They indicate the need for larger-scale studies which examine the relationships and interactions between both n-3 and n-6 in the diet, corresponding amounts in blood and tissues, and measures of cognitive functioning.

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