Meta-analysis of LCPUFA Supplementation of Infant Formula and Visual Acuity

abstract

BACKGROUND AND OBJECTIVE: Long-chain polyunsaturated fatty acids (LCPUFAs) are hypothesized to affect visual acuity development in infants. Randomized controlled trials (RCTs) have been conducted to assess whether supplementation of LCPUFAs of infant formulas affects infant visual acuity. This meta-analysis was conducted to evaluate whether LCPUFA supplementation of infant formulas improves infants' visual acuity.

METHODS: PubMed and PsycInfo were searched for RCTs assessing the efficacy of LCPUFA supplementation of infant formulas on infant visual acuity. RCTs assessing the effects of LCPUFA supplementation on visual acuity (by using either visual evoked potential or behavioral methods) in the first year of life were included in this meta-analysis. Our primary outcome was the mean difference in visual resolution acuity (measured in logarithm of minimum angle of resolution [logMAR]) between supplemented and unsupplemented infants. We also conducted secondary subgroup analyses and meta-regression examining the effects of LCPUFA dose and timing, preterm versus term birth status, and trial methodologic quality.

RESULTS: Nineteen studies involving 1949 infants were included. We demonstrated a significant benefit of LCPUFA supplementation on infants' visual acuity at 2, 4, and 12 months of age when visual acuity was assessed by using visual evoked potential and at 2 months of age by using behavioral methods. There was significant heterogeneity between trials but no evidence of publication bias. Secondary analysis failed to show any moderating effects on the association between LCPUFA supplementation and visual acuity.

CONCLUSIONS: Current evidence suggests that LCPUFA supplementation of infant formulas improves infants' visual acuity up to 12 months of age. *Pediatrics* 2013;131:e262–e272

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KEY WORDS

infant formula, unsaturated fatty acids, visual acuity, long-chain polyunsaturated fatty acids, meta-analysis

ABBREVIATIONS

AA—arachidonic acid
BM—behavioral method
CI—confidence interval
df—degrees of freedom
DHA—docosahexaenoic acid
EPA—eicosapentaenoic acid
LCPUFA—long-chain polyunsaturated fatty acid
logMAR—logarithm of the minimum angle of resolution
PL—preferential looking
RCT—randomized controlled trial
VEP—visual evoked potential
WMD—weighted mean difference

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Infant formula is the sole source of energy and nutritional requirements for many infants during the first 12 months of life. The US Food and Drug Administration estimates that by 1 year of age, 75% of infants are formula fed.¹ Given the widespread dependence on infant formula, formula is designed to mimic breast milk's composition to optimize growth and development of infants. Differences in long-chain polyunsaturated fatty acids (LCPUFAs) between unsupplemented infant formula and breast milk have been hypothesized to affect infant growth and development.^{2,3} Docosahexaenoic acid (DHA) and arachidonic acid (AA) are the 2 main LCPUFAs that constitute an integral structural part of membranes of the cells of the central nervous system and retina.⁴ Although human fetuses can synthesize DHA and AA *de novo* from their essential fatty acid precursors after 26 weeks of gestation, the synthesis varies widely between infants, being very minimal in some.^{4–6} Postnatally, breast milk is the main source of LCPUFAs and their metabolites; however, the amount varies among mothers.^{7,8} Infants with diets deficient in LCPUFAs have been demonstrated to have low levels of DHA and AA in plasma and red blood cell membranes.⁹ A clear dose-response relationship between DHA and AA supplemented in infant formula and levels of DHA and AA in red blood cell membranes has been demonstrated in several studies.^{10–15}

Although many LCPUFAs play an essential structural and functional role in cell membranes of retinal tissue, DHA is considered the major LCPUFA that has a vital role in retinal cells. DHA constitutes a fundamental structural part of the retinal photoreceptor membrane, where it comprises >50% of the phospholipid content of the retinal membrane bilayer; therefore, retinal cells have the highest concentration of

DHA in the human body.^{16,17} DHA concentration affects the enzyme activity of membranes of retinal photoreceptors and thus their function.¹⁸ DHA is necessary for photoreceptor differentiation and the activity of rhodopsin, an essential pigment needed for photo-transduction in retinal photoreceptors.¹⁹ The hypothesis that abnormal levels of LCPUFAs, specifically DHA, would have an impact on visual function and visual acuity of animals has previously been studied. DHA levels and electroretinogram amplitudes were found to be reduced in rats fed diets deprived of n-3 fatty acids.²⁰ DHA has also been shown to affect neuronal functionality by incorporating in neuronal cell membranes of gray and white matter, both of which are part of the primary visual cortex and higher processing centers.²¹ DHA is therefore not only necessary for optimal retinal function but also is necessary for the proper function of the visual processing centers, optic tract, and optic nerve.

Given the importance of LCPUFAs in the composition and function of retinal tissue and higher visual processing areas, several randomized controlled trials (RCTs) have studied whether supplementation of infant formula with LCPUFAs improves visual acuity as assessed by visual evoked potential (VEP) or behavioral methods (BMs). Several of these trials showed improved visual acuity with supplementation,^{14,22–27} whereas other trials have not.^{28–36} We conducted a meta-analysis to determine whether supplementing infants with formula enriched with LCPUFAs would improve visual acuity outcomes at and before 1 year of age compared with infants fed nonenriched formula. We also used meta-regression to examine the effect of doses of LCPUFAs in supplements, starting time and duration of supplementation, and study quality on visual acuity outcomes.

METHODS

Search Strategy

Two reviewers (AQ, ALW) used the search terms “(Infant Nutrition [Mesh] OR Infant Formula [Mesh]) AND Fatty acids, Unsaturated [Mesh]” in PubMed, “Omega-3 and Infant Formula” in Scopus, and “Fatty Acids and Infant Formula” in PsychInfo to search for relevant citations. The search in PubMed (1965 to 2011) was further limited by using RCT and meta-analysis filters. References from relevant articles and related systematic reviews were also searched for additional studies. Authors of some articles were contacted for missing information when necessary. There were no limitations on the basis of the language of publication.

Inclusion Criteria

The inclusion criteria of our meta-analysis were (1) RCTs assessing infant formulas with LCPUFAs to unsupplemented formula, (2) trials assessing infant visual acuity as an outcome by using VEP or BMs, including preferential looking (PL) or acuity card procedures, (3) supplementation starting within 1 month after birth, (4) evaluation of visual acuity occurring at the ages of 2, 4, and/or 12 months, and (5) articles published in peer-reviewed journals. RCTs were considered as such if the investigator defined them as an RCT in the methods section of the article.

Meta-analytic Procedure and Information Extraction

Visual resolution acuity is a measure of the finest spatial detail an observer can discriminate on a visual image and can be measured reliably early in life. Visual acuity is assessed in infants by 2 methods: BMs and VEP. Both methods use a grating of either checkerboard patterns or a series of black and white stripes to assess acuity. VEPs represent the cortical electrical activity of the

brain that occurs when observing the grating; they can be subdivided into transient VEPs or steady state VEPs. Steady-state VEP includes sweep or swept VEP as a subcategory. These subdivisions are based on the speed of reversing of the grating or checkerboard and the subsequent cortical response. BMs, on the other hand, are the PL method and acuity card method. Both methods are based on the fact that humans and other species have a visual preference toward patterned compared with uniform backgrounds, namely black and white stripes as opposed to gray backgrounds. In the PL method that test binocular grating acuity by using gray with black and white grating cards, the tester, through a peephole, observes the behavior of the infant and to which card he prefers looking. The other method is the acuity cards, which is a more rapid version of PL, used mainly in research settings.³⁷

We divided visual acuity measurements into these 2 main categories on the basis of the assessment method used. Data from trials were entered into 2 separate Microsoft Excel spreadsheets based on the method used to assess visual acuity (BM or VEP). Visual acuity results were reported by researchers by using 2 different metrics: logMAR and cycle/degree. We converted all results to logMAR for use in this meta-analysis. For conversion of the mean from cycles/degree into logMAR, we used the following equation: mean in logMAR = $\log [30/(\text{mean in cycle/degree})]$.²⁹ The SD was converted from octaves into logMAR by using the following: SD in logMAR = $0.3 \times \text{SD in octaves}$. Mean visual acuity and SD values were extracted from figures if not reported in text or tables. For preterm infants, all values represent those extracted at corrected age rather than chronologic age. If the number of subjects was not reported in a study at a specific time in the visual acuity assessment series, the authors were

contacted for additional information. Whenever we did not receive information, the number of subjects that participated in the following visual acuity assessment was used. If visual acuity was reported for each eye separately, the average visual acuity of both eyes was calculated and used. Data on additional potentially moderating variables such as number of subjects, start date and duration of LCPUFA supplementation, and dose of eicosapentaenoic acid (EPA), DHA, and AA in the supplemented formula were collected from each trial. Individual study quality was evaluated by using Jadad scale.³⁸ Using the Web site ULRICHWEB,³⁹ we were able to identify which articles were published in peer-reviewed journals. All trials included started supplementation within 1 month of birth.

Our primary analysis was done by stratifying the methods of visual acuity assessment into subgroups, each at 3 different times: 2, 4, and 12 months. We included trials as contributing to the 2-, 4-, and 12-month time points if visual acuity assessment occurred within 1 month of the scheduled time period (ie, 3 to 5 months postpartum for 4-month outcome and 11 to 13 months for 12-month outcome). We also intended to include a >18-month time point for assessment of visual acuity, but because only 3 studies^{40–42} measured visual acuity by using VEP or BM beyond this time, articles assessing visual acuity beyond this time point were excluded from the meta-analysis.

A random effects model in RevMan 5.1⁴³ was used to conduct the meta-analysis to assess the efficacy of LCPUFA supplementation for each of the 2 methods to assess visual acuity (BM and VEP) at each of the 3 time points. Weighted mean difference (WMD) of mean and SD of logMAR for each trial was the summary measure for each analysis.

Publication bias was evaluated by using the funnel plot generated for each

subgroup. Heterogeneity of effect of supplementation trials was assessed visually by using forest plots and from the I^2 statistic generated by RevMan for each subgroup. We conducted a sensitivity analysis to examine whether to use random-effects or fixed-effects models for our meta-analysis.

For secondary analysis, we conducted a stratified subgroup analysis and several meta-regression analyses at each time point for each of the measures of visual acuity. We performed a stratified subgroup analysis to determine if preterm versus term birth status influenced the efficacy of LCPUFA supplementation on visual acuity. We used the test for subgroup differences in RevMan to determine whether subgroups reduced overall heterogeneity.⁴⁴ Meta-regression was performed in SPSS 19.0 (SPSS Inc, Chicago, IL) by using linear regression. Trials were weighted by using the generic inverse variance method. Effect size (WMD of logMAR difference) of trials was entered as the dependent variable, with the variables of interest being the independent variable. We used meta-regression techniques to examine the association between the effect of LCPUFA supplementation on visual acuity and naturally continuous variables such as (1) start of supplementation, (2) study sample size, (3) study quality (as measured by using the Jadad scale), and (4) doses of fatty acids in supplementation preparations. We examined doses of EPA, DHA, and AA in LCPUFA preparations, as well as the DHA:AA ratio in supplementation. For all outcomes at 12 months, we additionally conducted a meta-regression to determine the effects of duration of supplementation. For our primary analyses examining the efficacy of LCPUFA supplementation on visual acuity at 2, 4, and 12 months, we used a significance threshold of $P < .01$. For all subgroup analyses and meta-regression, we also used a threshold of $P < .01$ for statistical significance to

decrease the likelihood of a false-positive error. Any significant findings in secondary analyses should be regarded as exploratory because we did not adjust for inflation of a false-positive error from our 50 secondary analyses.

RESULTS

Our search resulted in a total number of 286 trials, 16 of which met our inclusion criteria and were included. Figure 1 is a flow diagram of the studies identified in our search. The characteristics of the included studies are shown in Table 1. The number of participants included in these studies was 1949. Ten trials showed a favorable effect of LCPUFA supplementation to infant formula on visual acuity,^{14,22,24–27,36,40,42,45} whereas 9 others did not demonstrate a significant effect.^{28–35,11} Contributions of each of the trials to the assessment method used are shown in Table 2.

Efficacy of LCPUFA Supplementation on Infant Visual Acuity as Assessed by VEP

A total number of 10 trials evaluating visual resolution acuity of 852 infants by

using the VEP method were included in this meta-analysis. The analysis showed a significant positive effect of LCPUFA supplementation to infant formula on infant visual resolution acuity at all 3 time points (WMD = -0.08 [95% confidence interval (CI): -0.14 to -0.03], $z = 2.82$, $P = .005$ for 2 months; WMD = -0.07 [95% CI: -0.13 to -0.02], $z = 2.77$, $P = .006$ for 4 months; and WMD = -0.11 [95% CI: -0.20 to -0.03], $z = 2.61$, $P = .009$ for 12 months). There was significant heterogeneity between studies at all times tested ($\chi^2 = 12.58$, degrees of freedom [df] = 3 [$P = .006$] and $I^2 = 76\%$ for studies at 2 months; $\chi^2 = 40.21$, $df = 9$ [$P < .00001$] and $I^2 = 78\%$ for studies at 4 months; and $\chi^2 = 38.48$, $df = 3$ [$P < .00001$] and $I^2 = 92\%$ for studies at 12 months). Figure 2 A–C is forest plots that show the effects of LCPUFAs on visual acuity as measured by VEP at 2, 4, and 12 months. Funnel plots demonstrated no evidence of publication bias. Meta-regression showed no significant association between sample size and measured study effect ($\beta = 5.9E-5$ [95% CI = 0.000 to 0.001], $t = 0.282$, $P = .78$ for 2 months; $\beta = 1.902E-5$ [95% CI = -0.001 to

0.001], $t = 0.052$, $P = .96$ for 4 months; and $\beta = 0.0$ [95% CI = -0.002 to 0.002], $t = -0.430$, $P = .71$ for 12 months). Results of our meta-analysis did not differ appreciably when the fixed-effect model was used as opposed to the random-effect model.

Efficacy of LCPUFA Supplementation on Infant Visual Acuity as Assessed by Behavioral Methods

Twelve trials including 1095 infants evaluated visual acuity by using BM. Meta-analysis demonstrated a significant benefit of LCPUFA supplementation on visual acuity (as assessed by using BMs) at the age of 2 months (WMD: -0.08 [95% CI: -0.14 to -0.02], $z = 2.68$, $P = .007$) but not at 4 months (WMD: -0.01 [95% CI: -0.04 to 0.02], $z = 0.77$, $P = .44$) or 12 months (WMD: 0.01 [95% CI: -0.02 to 0.03], $z = 0.53$, $P = .60$). Figure 3 A–C is forest plots depicting the association between LCPUFA supplementation and visual acuity. Significant heterogeneity between studies was observed only at the age of 2 months ($\chi^2 = 26.01$, $df = 8$ [$P = .001$]; $I^2 = 69\%$). There was no evidence of publication bias on funnel plots at the 3 developmental time points. There was no significant association between study effect and sample size at the times assessed. We also found no significant association between trial methodologic quality and reported effect of LCPUFA supplementation on visual acuity at any time point. Analysis by using fixed-effect models showed similar results as those reported in random-effect models.

Effect of Supplementation on Preterm Versus Term Infants

Stratified subgroup analysis examining the effect of preterm birth status on the association between LCPUFA supplementation and visual acuity failed to show any significant difference between preterm and term infants.

* The number of articles was 336, duplicated articles were omitted

FIGURE 1

Selection of eligible trials and reasons for exclusion. Each citation is counted only once for clarity.

TABLE 1 Characteristics of Included Trials

Author	Year	N	Preterm Versus Term ^a	Start (d After Birth) ^b	Duration (m)	DHA (22:6), %		EPA (20:5), %	AA (20:4), %
Birch et al (14)	2010	343	Term	1–9	12	0.32		NR	0.64
						0.64		NR	0.64
						0.96		NR	0.64
Birch et al (25)	2005	103	Term	0–5	12	0.36		0	0.72
Fang et al (31)	2005	28	Preterm	0–14	6	0.05		NR	0.10
van Wezel-Meijler et al (29)	2002	42	Preterm	14–21	8	0.34		NR	0.70
Innis et al (30)	2002	194	Preterm	0–14	<1–2	DHA	0.34	NR	0
						DHA + AA	0.33	NR	0.60
						Both	0.34	NR	0.30
Auestad et al (33)	2001	239	Term	0–9	12	Egg	0.14	0	0.45
						Fish/fungal	0.13	0.04	0.46
						Both	0.14	0.02	0.46
O'Connor et al (36)	2001	427	Preterm	0–3	14	Fish/fungal	0.27	0.08	0.43
						Egg/fish	0.24	0	0.41
						Both ^c	0.26	0.04	0.42
Makrides et al (28)	2000	68	Term	0–7	12	DHA	0.35	0.10	0
						DHA + AA	0.34	0	0.34
						Both	0.35	0.05	0.17
Hørby Jørgensen et al (34)	1998	37	Term	0–25	14 d (median)	DHA	0.32	0.39	0.06
						DHAGF	0.32	0.37	0.06
						Both	0.32	0.38	0.06
Birch et al (26)	1998	79	Term	0–2	4	DHA	0.35	0	0.02
						DHA + AA	0.36	0	0.72
						Both	0.36	0.00	0.37
Auestad et al (11)	1997	134	Term	0–7	12	DHA	0.23	0.07	0
						DHA + AA	0.12	0	0.43
						Both	0.18	0.04	0.22
Carlson et al (32)	1996	59	Preterm	2–5	5	0.20		0.06	NR
Carlson et al (35)	1996	39	Term	NR	12	0.10		0.00	0.43
Makrides et al (22)	1995	89	Term	NR	7.5	0.36		0.58	0.01
Carlson et al (27)	1993	79	Preterm	0–21	11	0.20		0.30	NR
Birch et al (24)	1992	73	Preterm	0–10	3	0.40		0.6	0.10

Doses are defined in g/100 g of fatty acids. DHAGF, docosahexaenoic acid and γ -linolenic acid formula; NR, not reported.

^a Timing for preterm infants was calculated after delivery.

^b Start of supplementation.

^c Both, indicates the average DHA content in both preparations in a trial.

TABLE 2 Timing and Contribution of Each Study and the Assessment Method Used

Author	Year	VEP (m)			BM (m)		
		2	4	12	2	4	12
Birch et al (14)	2010	Y	Y	Y	N	N	N
Birch et al (25)	2005	Y	Y	Y	N	N	N
Fang et al (31)	2005	N	Y	N	N	Y	N
van Wezel-Meijler et al (29)	2002	N	N	N	N	Y	Y
Innis et al (30)	2002	N	N	N	Y	Y	N
Auestad et al (33)	2001	N	N	N	Y	Y	Y
O'Connor et al (36)	2001	N	Y	N	Y	Y	N
Makrides et al (28)	2000	N	Y	N ^a	N	N	N
Hørby Jørgensen et al (34)	1998	N	Y	N	N	N	N
Birch et al (26)	1998	Y	Y	Y	Y	Y	Y
Auestad et al (11)	1997	Y	Y	Y	Y	Y	Y
Carlson et al (32)	1996	N	N	N	Y	Y	Y
Carlson et al (35)	1996	N	N	N	Y	Y	Y
Makrides et al (22)	1995	N	Y	N	N	N	N
Carlson et al (27)	1993	N	N	N	Y	Y	Y
Birch et al (24)	1992	N	Y	N	N	Y	N

N, no; Y, yes.

^a Assessment done at the age of 8.5 months and was not included in any category.

Subgroup analysis was only performed at 4 months for trials using VEP because of the lack of trials in preterm infants examined at 2 and 12 months. We observed no significant difference in the effect of LCPUFA supplementation on VEP-measured visual acuity in preterm compared with term infants (test for subgroup differences: $\chi^2 = 0.39$, $df = 1$ [$P = .53$], $I^2 = 0\%$). LCPUFA supplementation demonstrated a significant benefit on visual acuity in term infants (WMD: -0.08 [95% CI: -0.10 to -0.05], $z = 6.79$, $P < .00001$) but failed to reach statistical significance in preterm infants, although the point estimate of the benefit was larger (WMD: -0.12 [95% CI: -0.29 to 0.05], $z = 1.39$, $P = .16$). Figure 2B depicts the effect of

FIGURE 2
A, Difference in visual acuity as assessed by VEP at the age of 2 months between infants fed formula supplemented with LCPUFAs and nonsupplemented formula. B, Difference in visual acuity as assessed by VEP at the age of 4 months between infants fed formula supplemented with LCPUFAs and nonsupplemented formula. C, Difference in visual acuity as assessed by VEP at the age of 12 months between infants fed formula supplemented with LCPUFAs and nonsupplemented formula.

LCPUFA in preterm and term infants separately.

Subgroup analysis on trials using BMs as a means of detecting visual acuity improvement after LCPUFA supple-

mentation showed some variation. At the age of 2 months, there was a significant difference between preterm and term groups (test for subgroup differences: $\chi^2 = 10.72$, $df = 1$ [$P = .001$],

$I^2 = 90.7\%$). At this age, it was shown that LCPUFA had no significant effect on visual acuity of the preterm infant group (WMD: -0.03 [95% CI: -0.07 to 0.00], $z = 1.78$, $P = .07$). In contrast, analysis on term infants demonstrated a positive significant benefit from LCPUFAs (WMD: -0.12 [95% CI: -0.16 to -0.08], $z = 5.74$, $P < .00001$). At 4 months of age, there was no significant difference between the preterm and term groups (test for subgroup differences: $\chi^2 = 6.48$, $df = 1$ [$P = .01$], $I^2 = 84.6\%$), although subgroup analysis showed a significant effect of LCPUFAs on the preterm infant group (WMD: -0.03 [95% CI: -0.06 to -0.01], $z = 2.52$, $P = .01$) compared with term infants (WMD: 0.03 [95% CI: -0.01 to 0.06], $z = 1.34$, $P = .18$). Stratified subgroup analysis at the age of 12 months failed to show a significant difference between both groups (test for subgroup differences: $\chi^2 = 0.43$, $df = 1$ [$P = .51$], $I^2 = 0\%$). Neither the preterm group (WMD: 0.02 [95% CI: -0.03 to 0.07], $z = 0.84$, $P = .40$) nor the term group (WMD: 0.00 [95% CI: -0.03 to 0.03], $z = 0.09$, $P = .93$) demonstrated any significant effect of LCPUFA supplementation on infant visual acuity. Figure 3 A–C is forest plots demonstrating the differences between the preterm and term groups.

Dose of LCPUFA in Supplemented Infant Formula

Meta-regression on the efficacy of LCPUFA was not significantly associated with the dose of DHA and AA supplementation. This lack of association was observed when either of the assessment methods were used to detect improved visual acuity. Furthermore, the DHA:AA ratio failed to show any significant association with the efficacy of LCPUFA. Meta-regression on the EPA dose, however, demonstrated better visual acuity with increased doses of EPA at 4 months of age ($\beta = -0.194$ [95% CI: -0.333 to -0.056], $t = -3.03$, $P < .01$). This improved visual acuity

FIGURE 3

A, Difference in visual acuity as assessed by BMs at the age of 2 months between infants fed formula supplemented with LCPUFAs and nonsupplemented formula. B, Difference in visual acuity as assessed by BMs at the age of 4 months between infants fed formula supplemented with LCPUFAs and nonsupplemented formula. C, Difference in visual acuity as assessed by BMs at the age of 12 months between infants fed formula supplemented with LCPUFAs and nonsupplemented formula.

FIGURE 3
Continued

was only detected when BMs were used.

Effect of Supplementation Timing on Visual Acuity

Our meta-regression failed to show any significant association between start of supplementation and duration of supplementation on the efficacy of LCPUFA on visual acuity, regardless of the assessment method used.

DISCUSSION

In this meta-analysis, we demonstrate a significant benefit of LCPUFA supplementation of infant formulas on infant visual acuity at several stages of development within the first year of life. LCPUFA supplementation of infant formulas showed a significant benefit on visual acuity assessed by VEP at 2, 4, and 12 months of age. When BMs were used to assess visual acuity, a significant benefit was observed only at 2 months of age. The results of our meta-analysis confirm the results seen in

some previous meta-analyses in term and preterm infants that were conducted before the completion of several of our included trials.^{2,46} However, our results stand in contrast to 2 recently published Cochrane systematic reviews that failed to show a benefit of LCPUFA supplementation on visual acuity.^{47,48} These meta-analyses failed to combine trials that (1) measured visual acuity in logMAR and cycles/degree and (2) assessed preterm and term infants separately. These decisions substantially reduced the power of these meta-analyses to detect potential benefits of LCPUFA supplementation.

The results of our meta-analysis provide more evidence to support sensitivity differences between the main visual acuity assessment methods, BMs and VEP, in detecting visual acuity in infants. Infant visual acuity progresses rapidly through the first year of life.^{17,49} There exists a 5-octave difference between the visual acuity of a newborn and an adult. This difference corre-

sponds, by using a Snellen chart, to a visual acuity of 20/640 compared with 20/20 in adults. Visual acuity progresses dramatically through the first year of life as assessed by VEP, with acuities of 20/100, 20/60, and 20/30 for the ages of 2, 4, and 12 months, respectively. When assessed by using BMs, however, the acuities are 20/300, 20/150, and 20/60 for the ages of 2, 4, and 12 months, respectively.^{17,50} We suspect therefore that the VEP method was able to detect more subtle benefits of DHA, AA, and other LCPUFA supplementation that were not detected by BMs even at periods when visual acuity development plateaued (11 to 12 months). It is worth noting, however, that there are differences in the areas evaluated by these 2 methods.^{51,52} BMs are subjective, requiring the infant to move his or her head and orient toward the area of preference; in other words, BMs involve both the visual cortex and areas of higher processing.⁵² In contrast, the VEP method assesses the visual cortex as stimulated by retinal

inputs. This difference in the areas evaluated by these methods could be accountable for the observed variation in LCPUFA benefit as assessed by these 2 methods.

Trials included in our meta-analysis varied in terms of the dose of the supplemented LCPUFA. In general, trials that used doses of DHA and AA similar to that found in human milk in a ratio of 1:1 or higher, especially those assessing visual acuity by using VEP, tended to show a positive effect of supplementation.^{14,15,25,26,36} Conversely, trials that used doses of <0.32% of DHA were likely to show a nonsignificant effect on visual acuity.^{11,31,32,35} Meta-regression of dose of LCPUFAs, however, failed to show any significant effect of DHA or AA dose supplemented on the efficacy of LCPUFAs. This result is in support of larger dose-response trials.¹⁴ Although there was a significant effect of supplemented formulas on visual acuity compared with nonsupplemented formulas, higher doses failed to show any significant benefit compared with the current practice dose of 0.32%. We therefore hypothesize that a dose of 0.32% of DHA with a similar or higher dose of AA, while maintaining a ratio of DHA and AA of at least 1:1, is sufficient for optimal visual acuity maturation. Higher doses of DHA or AA did not show any significant effect on visual acuity improvement in infants fed LCPUFA-supplemented formulas.

Our meta-analysis demonstrated a significant moderating effect of preterm status on the association between LCPUFA supplementation and visual acuity as assessed by BMs at 2 and 4 months. However, the moderating effect of preterm status was inconsistent between time points. LCPUFA supplementation showed a greater effect in improving visual acuity as assessed by BMs at 4 months but showed a lesser effect at 2 months in preterm

compared with term infants. Research has shown that visual cortical sensitivity to retinal stimuli is significantly lower in preterm compared with term infants and that a greater degree of prematurity is associated with worse visual cortical sensitivity.⁵³ It is well established that there is a difference in visual development between preterm and term infants, but it is unclear how or whether these visual acuity deficits in preterm infants moderate the effects of LCPUFA supplementation. The lack of a consistent directional nature of the moderating effects of birth status and the absence of significant moderating effects on VEP and later behavioral measures of visual acuity leave open a strong possibility that our findings on the moderating effects of birth status are caused by a false-positive error.

Our meta-analysis provides strong evidence to support LCPUFA supplementation of infant formula to enhance infant visual development. However, there are several limitations to our results. We demonstrated significant heterogeneity between trials in this meta-analysis. We conducted multiple meta-regression and stratified subgroup analyses to identify sources of heterogeneity. These additional analyses failed to provide a consistent source for this heterogeneity and also introduced a strong possibility of a false-positive error in secondary analyses. A potential explanation that could account for the heterogeneity seen is the amount of DHA and AA supplied, as well as the ratio of different LCPUFAs. Some studies,^{22,24,27,32,35} especially those carried out before clear recommendations were provided regarding the amount of DHA, AA, and EPA, and the ratio of DHA and AA, had different amounts, as well as different ratios of LCPUFAs. These differences may have caused the significant heterogeneity seen. Our meta-analysis could have been insensitive to

dose effects of supplementation if they were nonlinear or reached a threshold over the dose ranges studied. Another possible explanation could be the source of fatty acids supplied. The trials included were not uniform in terms of LCPUFA source; algae, tuna, fungi, eggs, borage oil, or a mixture of these sources were used in different trials. Each provided a different mixture of LCPUFAs, as well as other n-6, n-3 fatty acids, different triglycerides, and phospholipids.⁶ Last, maternal diet during pregnancy is another potential cause of heterogeneity. It is well known that fetuses are able to synthesize only minor amounts of LCPUFAs from their precursors α -linoleic acid and linoleic acid and that they depend mainly on placental transfer of these fatty acids for optimal growth and development.^{4,5} It has been shown in previous studies^{54,55} that maternal intake of LCPUFAs increases maternal blood and cord levels, as well as fetal levels, of these LCPUFAs. Differences in maternal intake of LCPUFAs therefore will cause a variation in fetal and neonatal levels of these fatty acids, and subsequent differences in baseline levels of LCPUFAs when supplementation is started. This difference in LCPUFAs may be another explanation of heterogeneity seen between studies. We also were unable to examine the association between LCPUFA supplementation and visual acuity at later, meaningful developmental time points because of the scarcity of data.^{40,42,45}

Overall, our meta-analysis demonstrates a significant benefit of LCPUFA supplementation to infant formula on infant visual acuity during the first year of life. The results of our meta-analysis, however, cannot be generalized beyond the age of 12 months. Further trials are required to assess the efficacy of LCPUFA supplementation on infant's visual acuity for children older than 1 year of age.

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