

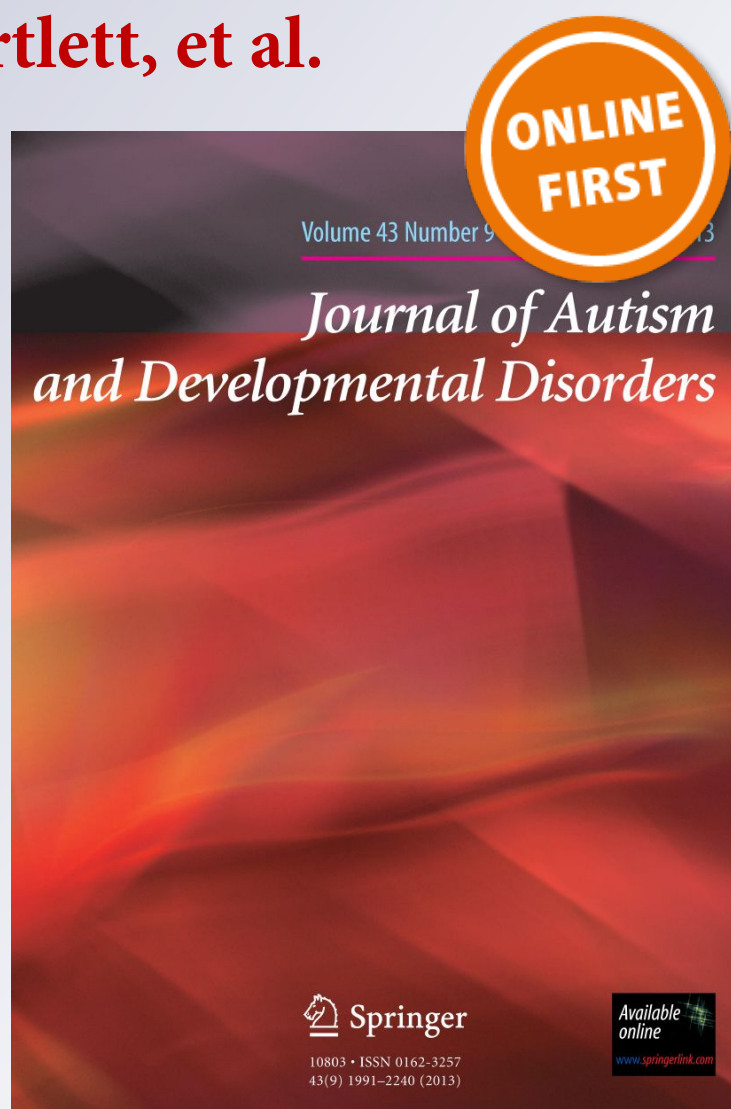
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Effect of Omega-3 and -6 Supplementation on Language in Preterm Toddlers Exhibiting Autism Spectrum Disorder Symptoms

Kelly W. Sheppard^{1,6} · Kelly M. Boone¹ · Barbara Gracious^{2,7} · Mark A. Klebanoff^{3,6} · Lynette K. Rogers^{3,6} · Joseph Rausch¹ · Christopher Bartlett^{4,6} · Daniel L. Coury^{1,5} · Sarah A. Keim^{1,6}

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Abstract Delayed language development may be an early indicator of autism spectrum disorder (ASD). Early intervention is critical for children with ASD, and the present study presents pilot data on a clinical trial of omega-3 and -6 fatty acid supplementation and language development, a secondary trial outcome, in children at risk for ASD. We randomized 31 children to receive an omega-3 and -6 supplement or a placebo for 3 months, and measured their language abilities at baseline and after supplementation. Gesture use, but not word production, increased for children in the treatment group more than children in the placebo group. These results suggest possible effectiveness of omega-3 and -6 supplementation for language development in children at risk for ASD.

Keywords Children born preterm · Autism spectrum disorder · Language development · MacArthur Bates communicative development inventory · Omega-3 fatty acids · Omega-6 fatty acids

Introduction

Delays in language development are a hallmark of autism spectrum disorder (ASD, for review: Groen et al. 2008), and understanding the language development of children at risk for ASD is crucial to early interventions. Gesture use is an early marker of language development (Cattani et al. 2010; Gordon and Watson 2015; LeBarton et al. 2015; LeBarton and Iverson 2016; Parlade and Iverson 2015; Sansavini et al. 2011), and studying gesture use can provide an earlier window into language difficulties than word comprehension or use. Children with ASD or those who are considered high risk for ASD use fewer gestures than typically developing children (Gordon and Watson 2015;

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✉ Kelly W. Sheppard
kelly.sheppard@nationwidechildrens.org

- ¹ Center for Biobehavioral Health, The Research Institute at Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205, USA
- ² Center for Innovation in Pediatric Practice, The Research Institute at Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205, USA
- ³ Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205, USA
- ⁴ Battelle Center for Mathematical Medicine, The Research Institute at Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205, USA

- ⁵ Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205, USA
- ⁶ Department of Pediatrics, College of Medicine, The Ohio State University, 700 Children's Dr, Columbus, OH 43205, USA
- ⁷ Department of Psychiatry and Behavioral Health, College of Medicine, The Ohio State University, 1670 Upham Drive, Suite 130, Columbus, OH 43210, USA

LeBarton and Iverson 2016), and gesture use in children with ASD has been linked to later language abilities (Gordon and Watson 2015; Mundy et al. 1987). Children who were later diagnosed with ASD also displayed less coordinated communication from 12 to 18 months, defined as using gestures with vocalizations, than peers who were later diagnosed with a language delay (Parlade and Iverson 2015). Gesture use, therefore, represents an important early indicator of ASD risk that may also help distinguish among related diagnoses.

Infants born preterm represent a population at risk for both ASD (Johnson et al. 2010; Kuzniewicz et al. 2014; Pritchard et al. 2016; Verhaeghe et al. 2016) and early childhood language delays (Cattani et al. 2010; Foster-Cohen et al. 2007; Sansavini et al. 2011; Spittle et al. 2017). In both children with ASD and children born preterm, later language difficulties have been linked to worse scores on neonatal assessments such as the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNSNS, Spittle et al. 2017), altered brain activity such as delayed N400 when processing spoken language (Coderre et al. 2017), and early gross and fine motor abilities (Mody et al. 2017), indicating an early neurological basis for later language delays. Investigating gesture use in children born preterm may help parents and clinicians identify those at risk for ASD or language delay diagnoses and intervene earlier.

Interventions designed to prevent language deficits should address both altered neurological development (such as that set in motion by premature birth) and provide behavioral adaptations that will help children develop similarly to their typically developing peers. With a focus on prevention, nutritional support of the brain has been shown to be key to preventing later deficits in children born preterm, and a Cochrane review concluded that supplementation during infancy with omega-3 fatty acids was beneficial to children born preterm (Simmer et al. 2008). Omega-3 fatty acids are one family of polyunsaturated fatty acids (PUFA) that accrete rapidly in the brain during gestation, particularly in the third trimester (Martinez 1992; Martinez and Mougan 1998). Infants born preterm possibly benefit from supplementation with omega-3 fatty acids because they miss this period of considerable omega-3 fatty acid accretion in utero.

Deficiencies in omega-3 fatty acids have been shown to affect neuronal structure and function by reducing neurite elongation (Ikemoto et al. 1997), reducing neuron size in the hippocampus (Ahmad et al. 2002a, b), and reducing basal acetylcholine levels and muscarinic receptor binding in the hippocampus (Aid et al. 2003), and all three changes are associated with worse working memory performance in animals (Catalan et al. 2002; Moriguchi et al. 2000). Additionally, deficiencies in omega-3 fatty acids have been linked to increased serotonin receptor density (Delion et al.

1994) and increased serotonin receptor binding (du Bois et al. 2006) along with decreased dopaminergic receptor density, decreased dopamine levels, and decreased dopamine presynaptic terminals (Delion et al. 1994; Zimmer et al. 2000), and both omega-3 fatty acid deficiency and changes in dopamine and serotonin have been linked to depression (McNamara 2010; McNamara et al. 2007, 2010; McNamara and Liu 2011; Weiser et al. 2015), attention-deficit/hyperactivity disorder (Jucaite et al. 2005; Spencer et al. 2007), and bipolar disorder (Patrick and Ames 2015; Schneider et al. 2012).

There is some limited evidence that supplementation with omega-3 fatty acids reduces language deficits (Johnson et al. 2016; Meldrum et al. 2012; Richardson and Montgomery 2005) and ASD symptoms (Amminger et al. 2007; Parletta et al. 2016; Keim et al. under review). Specifically, supplementation with omega-3 and -6 fatty acids was related to increased gesture use at 12 and 18 months (Meldrum et al. 2012), phonological decoding and visual analysis, two measures considered indicative of neural processing of written material (Johnson et al. 2016), and children who received omega-3 fatty acid supplementation were no longer behind their peers in reading level (Richardson and Montgomery 2005). However, other studies have found no effect on any included measures of language ability in typically-developing children (Auestad et al. 2001; Lauritzen et al. 2005; Scott et al. 1998). For ASD symptoms, studies have shown reductions in hyperactivity (Amminger et al. 2007) and stereotypy (Amminger et al. 2007; Bent et al. 2014), and there has been early evidence of reductions in total ASD symptoms from one cross-sectional study (Parletta et al. 2016) and the report of primary outcomes of the supplementation trial being reported here (Keim et al. under review). However, other studies have reported no effects on hyperactivity or stereotypy (Bent et al. 2014; Mankad et al. 2015) or total ASD symptoms (Voigt et al. 2014), leading to a lack of definitive recommendations as to whether and how to supplement children at risk for poor outcomes.

Omega-3 and -6 fatty acids have complementary roles in neuronal structure and function, with omega-6 fatty acids linked to protein kinase activity (Schaechter and Benowitz 1993), ion currents (Fang et al. 2011), and the induction of long-term potentiation (LTP), a process associated with consolidation of memory and learning (Bliss and Collingridge 1993; Kato et al. 1991; Lynch and Voss 1994). Omega-3 and -6 fatty acids are metabolized using the same metabolic resources (Lee et al. 2016; Nakamura and Nara 2004), and there is evidence of competition for metabolic resources (Bourre et al. 1993, 1989; Gibson et al. 2013; Harnack et al. 2009). Omega-3 and -6 fatty acids at the top of the metabolic chain (Supplementary Fig. 1) are processed through several desaturase and elongase steps to

produce long chain fatty acids further down the pathway, such as eicosapentaenoic acid (EPA, omega-3), docosahexaenoic acid (DHA, omega-3), and arachidonic acid (ARA, omega-6), which are fatty acids that have been strongly linked to human health (for review: Bowen et al. 2016; Dyall and Michael-Titus 2008; Harris 2006; Innis 2014; Kris-Etherton et al. 2010; Lapillonne and Moltu 2016). There is some evidence that supplementation with a combination of omega-3 and omega-6 fatty acids is more beneficial to cognitive function than supplementation with omega-3 fatty acids alone (Koletzko et al. 2008; Willatts and Forsyth 2000).

The present study was a pilot randomized clinical trial (RCT) that aimed to determine the feasibility of a full-scale trial of the effect of omega-3 and -6 fatty acid supplementation on ASD symptoms in children born preterm who were at increased risk for a diagnosis of ASD. A secondary goal of the trial, and the focus of the analyses reported here, was to explore treatment effects on language abilities in these children at higher risk for ASD.

Methods

Setting and Participants

The Preemie Tots trial was a pilot, single-site, double-blinded, RCT (NCT01683565) examining the effect of an omega-3 and -6 supplement on development in preterm infants considered at risk for ASD based on pre-specified screening criteria. The study, conducted from November 2012 to March 2015, was reviewed and approved by the Institutional Review Board at Nationwide Children's Hospital (NCH), Columbus, OH, and each child's primary caregiver provided written informed consent.

Children 18–38 months old who (1) were born at fewer than 30 completed gestational weeks, and either (2) admitted to the NCH neonatal intensive care unit (NICU) after birth or (3) had a Neonatology Clinic follow-up visit at NCH were recruited for the study. Primary caregivers of children who were potentially eligible completed a questionnaire that included the Pervasive Developmental Disorders Screening Test – II, Stage 2 (PDDST-II), the Brief Infant Toddler Social and Emotional Assessment (BITSEA), and one joint attention item from the Ages and Stages Questionnaire, “Looks in the direction you are pointing, when you are pointing at something”. Children who scored >5 on the PDDST-II, below the 15th percentile on the BITSEA competence scale, did not respond to their name, or did not respond to joint attention bids were further assessed for trial eligibility (n = 201).

Toddlers who met the above criteria had to weigh between the 5th and 95th percentiles of the World Health

Organization (WHO) growth standards for age and sex, and the primary caregiver had to be proficient in English. Children were excluded if they consumed high-DHA foods, such as omega-3 fatty acid supplements, fatty fish, or beverages with added DHA, more than twice per week, were known to be unable to tolerate venipuncture, or had been diagnosed with any of the following conditions: fragile X, Rett syndrome, Angelman syndrome, tuberous sclerosis complex, deafness, blindness, quadriplegic cerebral palsy, fish or canola oil allergy, type 1 diabetes, or a bleeding disorder. Children with a score of <70 on the Bayley Scales of Infant and Toddler Development (Bayley) clinical assessment (within the past year) or who had had a recent non-febrile seizure without a clear and resolved etiology were also excluded. In all, 106 children met the inclusion criteria. The enrollment goal was 40 children, and due to funding limitations, 31 children were ultimately enrolled in the trial.

Procedures

Children were randomized to one of four color-coded groups (two colors for treatment, two colors for placebo) using a varying block size of multiples of 4 with 1:1 allocation to treatment and placebo. All investigators and staff remained blinded to participant allocation throughout the trial, and a statistician at a separate institution prepared the randomization scheme. Twins were allocated to the same group to avoid accidental treatment crossover. The treatment group received 3 months of an oral omega-3, -6, and -9 fatty acid supplement that included, as a single liquid daily dose of 2.5 ml, 338 mg EPA, 225 mg DHA, 280 mg total omega-6 fatty acids (including 83 mg GLA), and 306 mg total omega-9 fatty acids (Omega-3-6-9 Junior™, Nordic Naturals, Inc., Watsonville, CA). The placebo group received 3 months of oral canola oil, also as a single liquid daily dose of 2.5 ml, which contained 129 mg palmitic acid, 39 mg stearic acid, 513 mg linoleic acid (LA, omega-6), 225 mg alpha-linolenic acid (ALA, omega-3), and 1346 mg oleic acid. Both oils were lemon-flavored (lemon oil flavor added to the placebo by NCH Investigational Drug Services (IDS)), and both products were packaged identically and distributed to the families through the hospital pharmacy.

Baseline Study Visit (Day 0)

The primary caregiver completed questionnaires on the following: demographics, developmental delays, diagnoses, and prescriptions and supplements (if any), the child's diet focused on number of servings of fatty fish, moderately fatty fish, and white fish/shellfish each month, and child behavior which included the Toddler Behavior Assessment

Questionnaire – Short Form (TBAQ-SF), the Child Behavior Checklist (CBCL), the MacArthur-Bates Communicative Development Inventory (CDI; to assess language), the Vineland Adaptive Behavior Scales (VABS; to assess socialization), and the Infant/Toddler Sensory Profile (ITSP; to assess sensory processing). Children's weight, height, and head circumference were measured, and a blood sample for fatty acid quantification was obtained by a trained phlebotomist. Primary caregivers were given an adherence diary to record whether the child received the provided oil each day over a 2-week period. The families received all adherence diaries at the beginning of the study, and they returned the diaries at the second and final study visits.

Second Study Visit (45 Days Post-Randomization)

Children randomized into the RCT returned for a second study visit that occurred 45 \pm 9 days post-randomization. Data collection at the second study visit provided a time to review the adherence diary and answer any questions. The primary caregiver completed the Willett Food Frequency Questionnaire (FFQ, Willett et al. 1985) about the child's typical diet, and the child's weight, height, and head circumference were measured. The child also provided another blood sample for fatty acid quantification.

Final Study Visit (3 Months Post-Randomization)

Children randomized into the RCT returned for a final study visit that took place 90 \pm 28 days post-randomization. The ASD screening questionnaires (e.g., BITSEA and PDDST) and baseline study visit questionnaires were repeated. The child's weight, height, and head circumference were again measured, and the child provided a final blood sample for fatty acid quantification.

Data Reduction and Statistical Analyses

The data used for the current analyses were the CDI language assessments at the baseline study visit and final study visit and treatment allocation. The CDI is a widely used measure of language development (Alexander Pan et al. 2004; Arriaga et al. 2008; Bruckner et al. 2007; Dale 1991; Fenson et al. 2000; Rescorla 1991; Ring and Fenson 2000) that includes a Words and Gestures form for ages 8–18 months, a Words and Sentences form for ages 16–30 months, and a CDI-III for use with children 30–37 months old. Given the delays in language development commonly seen in children born preterm, a combination of the short form of the CDI Words and Gestures and Words and Sentences forms was created for use in the present study. By collecting data on both gesture and word use, we ensured

that we collected as comprehensive a view of each child's language development as possible. Due to the use of a modified set of questions from both the Words and Gestures and Words and Sentences forms, the data were scored following the standard CDI scoring system with slight modifications. A gesture score was calculated by summing the number of Yes responses to the gesture use questions (Supplementary Table 1, 12 gestures total), a word score was calculated by summing the number of words the primary caregiver indicated the child used (Supplementary Table 1, 100 words total), and a words and gestures score was calculated by summing both gestures and words used.

An important element of gesture use is the functional utility of the gesture. Deictic gestures are considered those that are bound to context and usually include a specific referent in the environment (e.g., pointing to obtain a specific object), whereas representational gestures are not bound to context and usually represent a referent that may or may not be present (e.g., shaking head for 'no', Garton 1985). Representational gestures, therefore, tend to be used for more general social communication. To explore potential differences in deictic and representational gesture use in the present sample, the 12 gestures from the CDI forms were divided into deictic gestures (5) and representational gestures (7) as shown in Supplementary Table 1, and the sums of each type of gesture were included in analyses. Deictic gestures were very commonly endorsed, especially at the final visit with only two children in the placebo group having only four deictic gestures and the rest of the children having all five deictic gestures. Therefore, a logistic regression was run predicting deictic gestures with completed weeks' gestation, BITSEA ASD scale scores, and PDDST scores to obtain the probability of having all five deictic gestures. These probabilities were also included as outcome variables in the model. Additionally, the children's scores were compared to normative data available through Wordbank (Frank et al. 2016).

All analyses were conducted with SAS software (v9.4, SAS Institute, Cary, NC). All analyses were conducted according to intent-to-treat (all randomized children were included), and no interim analyses were conducted. Due to our small sample size and the inclusion of twins, each set of twins was analyzed as a single observation by calculating the mean of the pair for all variables. We used mixed effects regression according to the method described by Winkens et al. (2007) to compare changes in language abilities (continuous variables for total gestures, total words, total words and gestures, deictic gestures, representational gestures, and the probability of having all deictic gestures) in the treatment and placebo groups controlling for baseline scores. Treatment-by-time interaction terms were included as fixed effects to serve as estimates of the treatment effect. No covariates were included in the models. Data were first

assessed for violations of the general linear model, and only the distributional issues with deictic gestures mentioned above were found. Continuous variables are reported as mean \pm standard deviation (SD), and categorical variables are reported as percentages.

Results

Participant Characteristics

The treatment and placebo groups did not differ on any demographic or anthropometric measures at the baseline study visit, including gestational weeks at birth, height, weight, sex, maternal education, or monthly

family income (Table 1). The toddlers in both groups grew across the 3-month intervention, and both height and weight increased similarly in the treatment and placebo groups. As reported in the primary outcomes paper (Keim et al. under review), the toddlers in the treatment and placebo groups had similar risk for ASD at baseline, and supplementation with omega-3 and -6 fatty acids significantly improved BITSEA ASD scale scores across the intervention. Toddlers in the treatment group also had increased DHA, EPA, and total omega-3 blood levels and decreased ARA levels and omega-6 to omega-3 fatty acid ratios at the final study visit compared to the toddlers in the placebo group whose fatty acid levels did not change significantly. Overall, the randomization worked to create two similar samples at baseline, and the supplementation

Table 1 Descriptive statistics for the Premie Tots sample

	Baseline		Final visit	
	Treatment (n = 14)	Placebo (n = 13)	Treatment (n = 12)	Placebo (n = 12)
Gestational weeks	26.5 (1.7)	26.9 (1.7)		
Corrected age (months)	30.8 (5.9)	29.6 (6.2)	33.9 (6.5)	32.4 (6.1)
Weight (lbs)	12.9 (2.5) ^a	12.0 (2.3) ^a	13.4 (2.8) ^b	12.4 (2.2) ^b
Height (cm)	88.3 (7.0) ^a	85.2 (7.0) ^a	91.1 (7.8) ^b	88.9 (7.5) ^b
Family monthly income				
<\$1667	38.5% (5)	10.0% (1)		
\$1667–\$2917	23.1% (3)	20.0% (2)		
\$2917–\$3378	7.7% (1)	20.0% (2)		
>\$3378	30.8% (4)	50.0% (5)		
Maternal education				
<A.S. degree	57.1% (8)	69.2% (9)		
A.S. degree or beyond	42.9% (6)	30.8% (4)		
Female	38.5% (5)	10.0% (1)		
Ever breastfed	92.3% (12)	90.0% (9)		
Participant diagnosed with ASD	7.7% (1)	30.0% (3)		
Sibling diagnosed with ASD	15.4% (2)	0	23.1% (3) [^]	
BITSEA ASD score	7.1 (3.7) ^c	6.5 (4.1) ^c	5.3 (2.6) ^d	7.5 (3.3) ^c
PDDST score	6.79 (2.2)	5.2 (2.5)	6.0 (2.6)	5.4 (2.7)
VABS play	17.7 (8.8)	19.8 (11.9)	22.8 (8.3)	25.0 (6.7)
VABS relate	28.7 (10.7) ^a	28.9 (10.0) ^a	40.1 (9.4) ^b	23.8 (11.7) ^a
DHA (% mmol)	4.9 (1.1) ^c	5.1 (1.2) ^c	6.6 (1.1) ^d	5.6 (1.0) ^c
EPA (% mmol)	0.7 (0.4) ^c	0.9 (0.5) ^c	1.6 (0.4) ^d	0.9 (0.5) ^c
ARA (% mmol)	16.8 (1.4) ^c	16.1 (1.6) ^c	15.7 (1.7) ^d	16.4 (1.0) ^c
Omega-3 (% mmol)	7.3 (1.0) ^c	8.0 (1.3) ^c	9.6 (1.1) ^c	8.2 (0.8) ^c
Omega-6 (% mmol)	42.8 (5.9)	51.5 (22.7)	42.7 (5.8)	42.4 (4.8)
Omega-6/Omega-3 Ratio ^{^*}	6.1 (1.4) ^a	6.6 (3.0) ^a	4.5 (0.9) ^b	5.1 (0.8) ^b

Different letters in a row signify significant differences ($p < 0.05$)

ASD autism spectrum disorder, BITSEA brief infant toddler social emotional assessment, PDDST pervasive developmental disorders screening test-II, VABS Vineland Adaptive Behavior Scales, DHA docosahexaenoic acid, EPA eicosapentaenoic acid, ARA arachidonic acid

^{a,b}Significant difference from the baseline to final study visits

^{c,d}Significant difference between treatment groups

[^]One child had a sibling diagnosed with ASD between the baseline and final study visits

with omega-3 and -6 fatty acids predictably altered fatty acid blood levels.

The present sample was quite different from typically developing children in their gesture and word use. Based on normative data from WordBank, the 50th percentile of 30-month-olds who completed the English CDI Words and Sentences form produced 541 words (79.6% of the 680 words queried). In the present sample, the toddlers produced a mean of 36 words ($SD=33.2$, range 2.5–100, 36% of words queried) at the baseline visit and a mean of 42.9 words ($SD=31.4$, range 9.5–100, 42.9% of the words queried) at the final study visit. Based on the percentages of words endorsed, on extrapolation, the current sample would have endorsed around 244 words on the full Words and Sentences form at baseline and around 291 words at the final visit. These production scores place them between the 10th and 20th percentile for 28-month-olds at baseline and the final visit. The children in the present sample were performing closer to typically-developing 18-month-olds, yet their average age was 30 months.

As has been reported by others, the emergence of word use in the Preemie Tots sample followed a similar pattern to word use among typically developing children, simply at a delay (Cattani et al. 2010; Sansavini et al. 2011). The top ten most-endorsed words in the Preemie Tots sample were very similar to highly endorsed words in typically-developing 28-month-olds, such as 'bye', 'hi', 'no', and 'ball'. However, the caregivers still endorsed these words

at lower rates. The caregivers of 97–99% of typically developing 28-month-olds endorsed all of the top ten words, whereas only 79% of caregivers endorsed 'ball' and only 78% endorsed 'dog' in our sample.

Effect of Omega-3 and -6 Supplementation on Language in Preterm Infants

Children in the treatment and placebo groups did not differ on any language measures at baseline (Table 2). Children who received an omega-3 and -6 supplement for 3 months increased their combined gesture and word use from baseline significantly more than children who received the placebo oil ($F(1,26)=5.37$, $p<0.05$, Table 2). The difference between the treatment and placebo groups in combined gesture and word use was driven by significant differences in gesture use as the treatment group produced significantly more gestures than the placebo group after supplementation ($F(1, 26)=5.47$, $p<0.05$, Fig. 1a and Table 2), but there were no significant differences between the treatment and placebo group in word use alone over the course of the intervention ($F(1,26)=0.01$, $p>0.05$, Fig. 1b and Table 2). Instead, all children increased somewhat in their word use over the 3 months ($F(1,26)=7.05$, $p<0.05$, Fig. 1b and Table 2). For specific types of gestures, children in the treatment group increased their representational gesture use compared to children who received the placebo ($F(1,22)=4.72$, $p<0.05$, Fig. 1d and Table 2). There

Table 2 Comparison of Preemie Tots CDI scores in treatment and placebo groups across the study

	Baseline		Final visit		Change from baseline to final visit	
	Treatment (n = 14)	Placebo (n = 13)	Treatment (n = 12)	Placebo (n = 12)	Treatment	Placebo
CDI words and gestures	12.5 (2.4)	12.1 (1.4)	12.9 (1.5)* [^]	11.0 (2.3) [^]	0.4 [†]	−1.1 [†]
CDI gestures	9.6 (2.3)	9.1 (1.4)	10.9 (1.4)*	9.0 (2.3)	1.3	0.1
CDI words	40.7 (37.7)	31.0 (28.2)	45.8 (33.4) [^]	39.7 (30.4) [^]	5.1 [†]	8.7 [†]
Deictic gestures	4.8 (0.4)	4.5 (0.7)	5.0 (0)	4.8 (0.5)	0.2	0.3
Representational gestures	5.3 (1.4)	4.7 (1.8)	6.2 (1.1)*	4.3 (2.3)	0.9	−0.4
Probability of having all deictic gestures ^z	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	0	0

All values represent the count of words or gestures or both except the probability of having all deictic gestures

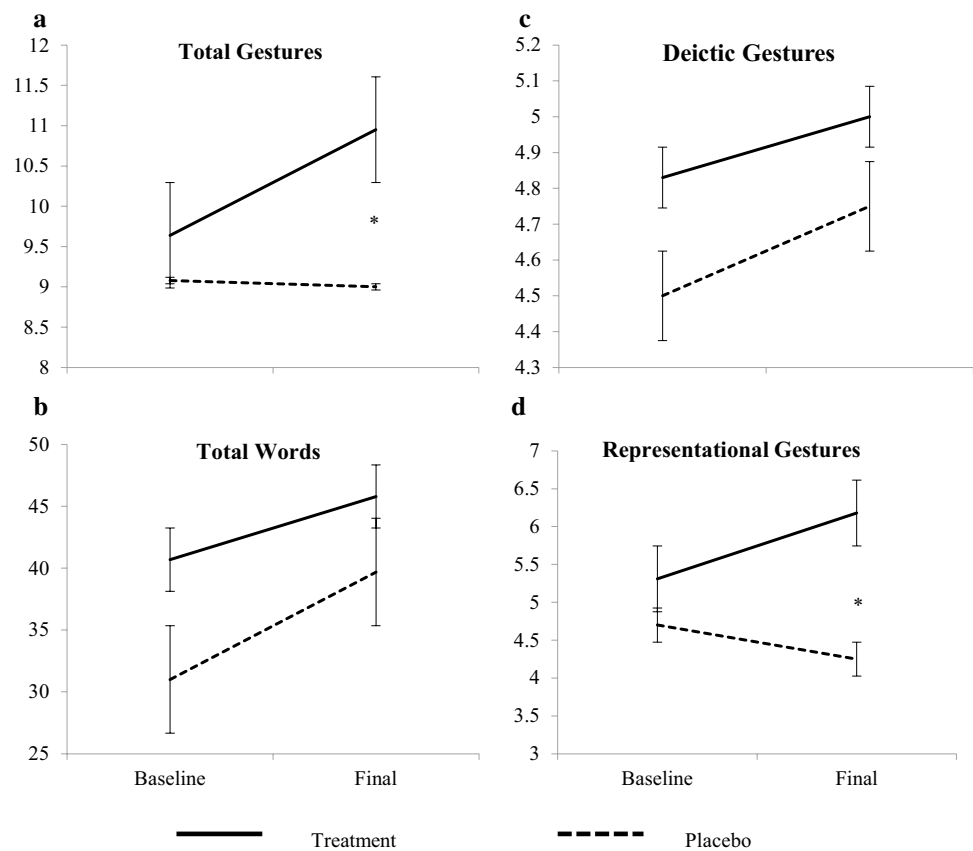
CDI MacArthur-Bates communicative development inventory

*Significantly different from the placebo group at the same time point ($p<0.05$). [^]Significantly different from baseline in the same group ($p<0.05$)

[†]Significantly different change from baseline to final visit

^zAt baseline, one toddler in the placebo group had only three deictic gestures, and three toddlers in the placebo group and two toddlers in the treatment group had only four deictic gestures. The rest had all five. At the final visit, all children in the treatment group had all five deictic gestures, and two children in the placebo group only had four deictic gestures. Therefore, the model was run after first creating a binary variable where 0=did not have all deictic gestures and 1=had all deictic gestures. The likelihood of having all deictic gestures was then predicted by weeks of completed gestation, BITSEA ASD scores, and PDDST scores. The likelihoods were output as a separate variable and predicted by time and treatment group with the original mixed model. The values in the table represent the likelihood that a child in each group at each data collection time had all deictic gestures

Fig. 1 CDI gesture and word scores across time in the treatment and placebo groups. **a** Total gestures, **b** total words, **c** deictic gestures, and **d** representational gestures. Dotted lines indicate the placebo group, and solid lines indicate the treatment group. Asterisk denotes statistically a significant difference ($p < 0.05$). Bars indicate standard error



were no differences in deictic gesture use between the two groups neither when comparing the sums of deictic gestures ($F(1,22)=2.08$, $p > 0.05$, Fig. 1c and Table 2) nor when comparing the probabilities of having all five deictic gestures ($F(1,22)=0.5$, $p > 0.05$, Table 2). These results indicate that supplementation with omega-3 and -6 fatty acids increased broader social communicative gesture use among children born preterm.

Discussion

The present study demonstrated that supplementation with omega-3 and -6 fatty acids increased representational gesture use in children 18–36 months old who were born preterm and had a high risk for ASD. Word use was not altered by supplementation. These results indicate that very early language development might, on confirmation, improve in children born preterm at high risk for ASD using omega-3 and -6 fatty acids. This finding is especially important because gesture use is considered an early indicator of language development that is linked to later language abilities (Cattani et al. 2010; Gordon and Watson 2015; LeBarton et al. 2015; LeBarton and Iverson 2016; Parlade and Iverson 2015; Sansavini et al. 2011) and can serve as an early

indicator of possible language delays (Gordon and Watson 2015; LeBarton and Iverson 2016; Parlade and Iverson 2015). This study provides support for a larger trial to confirm the usefulness of omega-3 and -6 fatty acid supplementation to promote language development in children born preterm.

The present study adds to the body of literature indicating that omega-3 and -6 fatty acid supplementation is effective for improving cognitive functions in children born preterm (Clandinin et al. 2005; Fewtrell et al. 2004; Henriksen et al. 2008; Simmer et al. 2008; Willatts and Forsyth 2000). There is evidence that omega-6 and omega-3 fatty acids are maximally processed when administered in combination, and such combinations may be important in improving developmental outcomes (Gibson et al. 2013; Harnack et al. 2009). Over-supplementation with omega-3 fatty acids leads to detrimental consequences (Wainwright et al. 1999, 1997), and the addition of omega-6 fatty acids provided in the supplement used in this study may afford a more optimal metabolic environment. Supplementation with omega-6 fatty acids has been controversial. Previous work predominantly used linoleic acid (LA, omega-6) in supplements, and demonstrated that adding omega-6 fatty acids reduced the amount of longer chain omega-3 PUFAs in the blood and may have contributed to worse cardiovascular

outcomes in several adult samples (Barden et al. 2016; Farvid et al. 2014; Serhan and Savill 2005). However, the treatment group in the present study received omega-6 fatty acids in the form of gamma-linoleic acid (GLA, omega-6), and actually had significantly reduced ARA blood levels in addition to the increased DHA levels. The use of GLA in the supplement may have reduced the need to use delta-6 desaturase to process LA to GLA. In doing so, the delta-6 desaturase would be available to process omega-3 fatty acids instead, further enhancing omega-3 availability.

The present study adds important information about how omega-3 and -6 fatty acid supplementation may affect gesture use in children born preterm. As gesture use is an important early indicator of language development, the use of omega-3 and -6 supplementation to help children at risk for language delays is promising. Omega-3 and -6 fatty acid supplements are readily available and easy for families to administer. In this study, specifically representational gesture use was improved with omega-3 and -6 fatty acid supplementation. The use of gestures requires coordination of communication goals and the motor system to produce the appropriate gesture. Omega-3 and -6 fatty acids have been shown to affect executive functions in older, typically developing children (Sheppard and Cheatham 2017), which are higher order cognitive functions that similarly require coordination among brain areas. Both deictic and representational gesture use require the former type of coordination (motor and communication goals), but representational gesture use may require more complex cognitive process coordination to represent the most abstract goal. Due to their complementary roles in neuronal function, it may be that we found effects on representational gesture use in this smaller sample because of the greater coordination of brain activity required. This greater coordination was in turn more highly affected by supplementation with omega-3 and -6 fatty acids. Supplementation provided to children born preterm may be particularly effective at aiding the development of skills that require coordination among brain areas.

The effect of the omega-3 and -6 fatty acid supplementation also may not directly target language abilities but social communication more broadly. The fact that representational gestures were more affected by supplementation than deictic gestures may reflect the fact that supplementation with omega-3 and -6 fatty acids affects overall social communication. This interpretation is supported by the correlations between representational gesture use and VABS Relate scale scores ($r_s=0.7$ and 0.8 , $p_s<0.05$ for baseline and final visit, respectively) and the lack of correlations between VABS Relate scale scores and deictic gesture use ($r_s=0.1$ and 0.4 , $p_s>0.05$ for baseline and final visit, respectively). VABS Relate scale scores also increased significantly in the treatment group across time (Table 1), supporting a potential effect of omega-3 and -6

supplementation on overall social communication. However, deictic gesture use, and not representational gesture use, has been previously linked to later language abilities in children with ASD (Ozcaliskan et al. 2016). The present study did not employ a measure designed to provide a comprehensive look at specific types of gestures, and therefore effects on deictic gesture use could simply be masked by the use of a less sensitive tool. Another possible explanation of the results could be that omega-3 and -6 fatty acid supplementation aided brain development more generally, and helped children born preterm progress faster in social communication more broadly. Omega-3 and -6 fatty acids are incorporated into different phospholipid pools in cell membranes (Chalon et al. 1998; Clandinin et al. 1991; Jumpsen et al. 1997a, b), and those phospholipid pools are not distributed uniformly in the brain (Breckenridge et al. 1973). By supplementing with omega-3 and -6 fatty acids, more diffuse effects may have occurred than would have been seen with solely omega-3 fatty acid supplementation.

The participants in this study were also at risk for an ASD diagnosis by virtue of their preterm birth. The improvement seen in more social communicative gestures may have occurred because omega-3 and -6 fatty acids have been linked to ASD symptoms (Amminger et al. 2007; Bent et al. 2014; Parletta et al. 2016; Keim et al. under review). The improvement seen in this study may have been related to the participants demonstrating improvements in ASD symptoms and not specific to language. However, the correlations between BITSEA ASD scale scores and all CDI scores were generally weak (ranging from -0.02 to -0.22 at baseline and 0.01 to -0.33 at the final visit), indicating that the changes reported in the BITSEA ASD scale scores and the changes in CDI scores reported here could represent two different effects. An interesting question for future prospective research is how to select children at risk for ASD for intervention. The children in the present study were screened using two standardized screening tools (BITSEA and PDDST) in addition to two key components of ASD (response to joint attention and response to name). Other studies have recruited children at risk due to having a sibling with ASD (e.g., Parlade and Iverson 2015). Both methods of identifying children at risk have their advantages, but they may also represent two different ASD etiologies that may or may not benefit from different treatment. Only three participants in the present study had a sibling diagnosed with ASD, and this sample may therefore be different from other samples commonly studied as at-risk for ASD. Future studies should examine the relevance of these differences on potential outcomes as efforts continue to be made to identify indicators of developmental challenges and intervene earlier.

The present study has limitations to address in future work. The sample size for this pilot was quite small, and

the results need to be replicated in a larger sample. The children in this study were not screened specifically for meeting criteria for ASD but to capture children at risk through preterm birth and broader symptom scores on commonly used ASD screeners. The modification of the CDI is a weakness in that the data are not as directly comparable to studies that employed the standard instruments. However, the modifications proved to be a strength in that a large portion of the sample would have received scores in the bottom 5th percentile on the age-appropriate Words & Sentences measure, and it allowed for study of language development across developmental periods that bridged two versions of the measure. Merging the two questionnaires and including all gestures allowed us to analyze the different types of gestures and allowed for variability in scores for this sample of children known to have delayed language development.

In summary, this pilot study offers initial, suggestive evidence of the efficacy of omega-3 and -6 fatty acid supplementation in improving aspects of early language development in children at risk for ASD, specifically gesture use. The use of a combined omega-3 and -6 fatty acid supplement may be important for providing preterm children with an appropriate balance of omega-3 and -6 fatty acids that allows for the most efficient use of metabolic resources and the greatest production of fatty acids known to be important for brain development, especially DHA and EPA. The effects were seen in the use of representational gestures, which may also indicate general improvements in social communication rather than language, per se. Future research with larger samples over a longer period of time will help clarify the findings reported here. Studies that stratify children by other important factors, such as baseline ASD symptomatology and reason for at-risk status (e.g., sibling with a diagnosis, genetic predisposition, premature birth) will help clarify the role of omega-3 and -6 fatty acids in language abilities in children at risk for ASD.

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Author Contributions KWS ran the statistical analyses, interpreted the results, and drafted and revised the manuscript; KMB participated in study design, oversaw data collection, participated in analyses, and revised the manuscript; JR participated in study conception and design, oversaw statistical analyses, and revised the manuscript; SAK, BG, MAK, LKR, CB, and DLC conceived of and designed the study, had overall oversight of the project, participated in statistical analyses and interpretation of data, and revised the manuscript.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical Approval All procedures performed in this study were in accordance with institutional ethical standards and with the 1964 Helsinki declaration and its later amendments.

Informed Consent Written informed consent (parental permission for the children) was obtained from all individual participants included in this study.

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