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Follow-up Formula Consumption in 3- to 4-Year-Olds and Respiratory Infections: An RCT

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KEY WORDS

DHA, prebiotics, yeast β-glucan, acute respiratory infection, children

ABBREVIATIONS

ARI—acute respiratory infection
DHA—docosahexaenoic acid
FUF—follow-up formula
GOS—galacto-oligosaccharides
IL—interleukin
PDX—polydextrose
TGF—tumor growth factor
WBC—white blood cell

Dr Li conceptualized and designed the study, supervised data collection, interpreted the data, and reviewed and revised the manuscript; Dr Jin conceptualized and designed the study, supervised data collection, and reviewed the manuscript; Dr Liu conceptualized and designed the study, coordinated the data collection and analysis, interpreted the data, and reviewed and revised the manuscript; Ms Zhuang analyzed and interpreted the data and reviewed and revised the manuscript; Dr Scalabrin conceptualized and designed the study, analyzed and interpreted the data, and reviewed and revised the manuscript; and all authors approved the final manuscript.

This trial has been registered at www.clinicaltrials.gov (NCT01488435).

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WHAT'S KNOWN ON THIS SUBJECT: Inadequate nutrient intake can compromise a child's nutritional status, which may affect immune function. Improving dietary intake via a follow-up formula may support appropriate immune responses and improve a child's ability to resist infection.

WHAT THIS STUDY ADDS: Children who consumed an experimental follow-up formula had fewer episodes and shorter duration of acute respiratory infections, as well as less antibiotic treatment, and fewer days missed of day care due to illness.

abstract

OBJECTIVE: Children are vulnerable to diet inadequacies, which may affect immune function. Our objective was to determine if a follow-up formula (FUF) containing DHA, the prebiotics PDX and GOS, and yeast β-glucan affects incidence of respiratory infections and diarrheal disease in healthy children.

METHODS: In a double-blind, randomized, controlled, prospective trial, 3-4 year old children were fed 3 servings per day of either a FUF with 25 mg DHA, 1.2 g PDX/GOS, and 8.7 mg yeast β-glucan per serving or an unfortified, cow's milk-based beverage (control) for 28 weeks. Fecal and blood samples were collected to assess immune markers and iron/zinc status. Incidence of acute respiratory infections (ARI), diarrheal disease, and antibiotic treatment were obtained from medical records.

RESULTS: The FUF group had fewer episodes and shorter duration of ARI (mean days [SE]; control = 4.3 [0.2]; FUF = 3.5 [0.2]; $P = .007$), less antibiotic use (n [%]; control = 21 [14%]; FUF = 8 [5%]; $P = .01$), and fewer missed days of day care due to illness. No diarrheal disease was diagnosed in either group. The FUF group had higher interleukin-10 and white blood cell count at the end of the study. There were no differences in hemoglobin, serum ferritin and zinc, or fecal secretory immunoglobulin A.

CONCLUSIONS: Daily consumption of a FUF was associated with fewer episodes and shorter duration of ARI, as well as less antibiotic use. The children who consumed the FUF had increased interleukin-10 and white blood cells, suggesting an antiinflammatory mechanism and/or an increase of effector immune cells. *Pediatrics* 2014;133:e1533–e1540

Children experience rapid growth and are vulnerable to diet inadequacy, which may affect immune function.[1,2] The transition from a diet of human milk and/or infant formula to a diet consisting of cow's milk, nonmilk beverages, and solid foods may compromise a child's nutritional status.[3] Much of the current understanding of nutritional impact on immune outcomes derives from animal studies, which are easily controlled for specific dietary components or from populations whose nutrient deficiencies are endemic.[4] A systematic review of studies on diets of well-nourished children <5 years of age from developed countries identified a prevalence of incomplete adherence to dietary guidelines, indicating that there is room for improvement.[5]

Docosahexaenoic acid (DHA) has been associated with improved immune outcomes and fewer respiratory infections in infants and children.[6–9] Previous studies of children <6 years of age in the United States have reported inadequate intake of DHA and corresponding low DHA status.[7,10] In one study, children who consumed a cow's milk–based formula supplemented with DHA had improved DHA status and fewer respiratory illnesses, compared with children consuming unsupplemented formula.[7] Dietary components, such as prebiotic oligosaccharides, can also influence host immune responses.[11,12] Prebiotics may promote an increase in beneficial gut bacteria such as bifidobacteria[13,14] and support respiratory and intestinal health.[15–17] Another nutrient with immunomodulatory properties is yeast β-glucan, a polysaccharide isolated from the cell wall of *Saccharomyces cerevisiae*.[18] Consumption of yeast β-glucan has been associated with fewer symptoms of acute respiratory infection (ARI) in healthy adults.[19–21] A β-glucan from a different fungal source was reported to promote an increase of blood NK lymphocytes and fewer ARIs in children with recurrent ARIs.[22] However, no data on immune benefits of dietary intake of yeast β-glucan in children is currently available.

Respiratory infections, followed by diarrhea, are the leading cause of morbidity and mortality among children <5 years of age in China and worldwide.[23] Furthermore, diarrhea can compromise the nutritional status and be a risk factor for respiratory infections.[24] Our objective was to evaluate whether a follow-up formula (FUF) enriched with DHA, a prebiotic blend of polydextrose (PDX) and galacto-oligosaccharides (GOS), and yeast β-glucan has an effect on the incidence of ARI and/or diarrheal disease in healthy children attending day care in China.

METHODS

Population

Children (aged 3–4 years) who had been attending day care for up to 3 months and were consuming cow's milk or a cow's milk–based beverage before randomization were eligible. Exclusion criteria were (1) consumption of prebiotics or probiotics in the 15 days before randomization; (2) diarrhea or ARI during the 48 hours before randomization; (3) a z score of weight-for-height < -3; or (4) serious concurrent illness. The study sponsor (Mead Johnson Nutrition, Evansville, IN) provided a computer-generated randomization schedule and sealed consecutively numbered envelopes to the study site. Study formulas, designated by unique codes known only to the sponsor, were assigned by the study site to eligible children by opening the next sequential envelope. Product labels and envelopes were constructed to prevent unblinding. The study products were identical in odor, color, and flavor (vanilla). The study was conducted at a day care in Jinhua, Zhejiang Province, China from November 2011 to May 2012. The Shanghai Nutrition Society institutional review board approved the protocol, and a parent/legal guardian provided signed informed consent before enrollment.

Design

In this double-blind, randomized, controlled, parallel-designed, prospective trial, children were fed an experimental FUF, according to the CODEX Alimentarius definition,[25] enriched with 25 mg DHA, 1.2 g blend of PDX/GOS (1:1 ratio), and 8.7 mg yeast β-glucan (Wellmune WGP, Biothera, Eagan, MN) per serving, or a nonenriched, cow's milk–based beverage (control). Previous studies have demonstrated health benefits of similar levels of DHA[7] and PDX/GOS[26] in children. Because of the lack of published studies in children, the level of β-glucan was extrapolated from the efficacious range of daily intake in adults.[20,21] Study products were given 3 times per day for 28 weeks. Each serving consisted of 40 g of powder mixed with 200 mL water. See Table 1 for study product nutrient composition.

Outcomes

The primary outcome was incidence of ARI and/or diarrheal disease. ARI was defined as upper respiratory infections, including common cold, pharyngitis, tonsillitis, otitis media, infectious sinusitis and rhinitis, and lower respiratory infections, including pneumonia, bronchiolitis, and bronchitis. Diarrheal disease was defined as ≥3 liquid/semiliquid stools in 24 hours with fever and/or vomiting and/or dehydration and compromised general status. Secondary outcomes included duration of ARI and diarrheal disease, systemic antibiotic treatment, allergic manifestations, days missed at day care due to illness, stool pattern, and growth. All children were referred to a single designated study clinic.

TABLE 1 Nutrient Composition of Study Formulas

Per 40 g Serving of Powder	Control	FUF
Energy, kcal	180	180
Protein, g	7.3	7.3
Fat, g	6.6	6.6
DHA, mg	—	25
Carbohydrate, g	23	23
Dietary fiber, g (1:1 ratio polydextrose/galacto-oligosaccharides)	—	1.2
Yeast β-1,3/1,6-glucans, mg	—	8.7
Vitamin A, IU	380	630
Vitamin D, IU	31	119
Vitamin E, IU	0.33	2.6
Vitamin K_1, mcg	0.41	9.5
Thiamine, mcg	57	210
Riboflavin, mcg	520	490
Vitamin B_6, mcg	42	183
Vitamin B_{12}, mcg	0.72	0.72
Niacin, mcg	144	2200
Folic acid, mcg	7.8	31
Pantothenic acid, mcg	770	1160
Biotin, mcg	5.4	4.7
Vitamin C, mg	2.4	29
Choline, mg	28	44
Calcium, mg	280	290
Phosphorus, mg	200	187
Magnesium, mg	25	26
Sodium, mg	97	96
Potassium, mg	400	420
Chloride, mg	330	320
Iodine, mcg	13.4	15.2
Iron, mg	0.05	3.0
Zinc, mg	0.72	2.3
Manganese, mcg	5	19.2
Copper, mcg	4.8	82

—, indicates that formula did not contain nutrient.

Stool and blood samples were collected at baseline and at the end of study to assess stool parasites by direct microscopy, peripheral blood cell count, serum ferritin and zinc, and immune markers by enzyme-linked immunosorbent assay (fecal secretory immunoglobulin A and serum interleukin [IL]-10, tumor growth factor [TGF]-β1, TGF-β2, IL-4, and IFN-ϒ). Laboratory analyses were conducted by R&D Systems (Shanghai, China). Weight and height measurements were obtained at randomization and every 4 weeks thereafter and converted into z scores based on World Health Organization references.[27]

Sample Size and Statistics

A sample size of 125 per group was needed to achieve 90% power, assuming a control group proportion of 0.5 and a test group proportion of 0.3 at an α level of .05. The frequencies of ARI, diarrheal disease, or allergic manifestations, as well as number of missed days of day care due to illness, were compared with the Cochran-Mantel-Haenszel test. The average duration of ARI was analyzed by analysis of variance and antibiotic treatment and incidence of fecal parasites by Fisher's exact test. Fecal and serum immune markers, serum ferritin and zinc, and peripheral blood count values were compared by using Kruskal-Wallis test, whereas changes from baseline to end of study were analyzed by using analysis of covariance, with baseline values as covariates. Stool frequency and consistency and weight and height z scores were analyzed by using repeated measures analysis of variance.

RESULTS

Study Population and Clinical Outcomes

The study initially enrolled 310 children, and 264 completed the study. Reasons for discontinuation were participants' move to another city (control = 13; FUF = 19) or parental decision (control = 10; FUF = 4). Demographic and baseline characteristics (race, age, gender distribution, and anthropometric measures) were similar between groups, except weight-for-age z-scores of females (mean z score [SE]; control = 0.3 [0.1] vs FUF = 0.1 [0.1]; P = .03). During the study, height-for-age z-scores of females were higher in the control group compared to FUF at study week 28. Both genders in the two groups had an increase in weight-for-age and height-for-age z scores from baseline to end of study ($P < .001$). Weight-for-height z scores also increased (girls: 0.2 and 0.1–0.4 and 0.5; boys: 0.9 and 0.5–1.1 and 0.8, in controls and FUF, respectively; $P < .001$). Intake of study products was similar between groups. Children consuming the FUF had fewer episodes and shorter average duration of ARI compared with control, and fewer children in the FUF group were treated with systemic antibiotics (Table 2 and Fig 1). The mean duration of each antibiotic treatment was 3 days, and none of the children received more than a single course of antibiotics.

The FUF group missed fewer days of day care due to illness (Table 3). The median number of days absent was 0 for both groups (60%–70% missed no days due to illness), and the average number of days missed due to illness was 0.8 and 0.5 for control and FUF groups, respectively. The percentage of children who missed at least 1 day of day care due to illness was lower in the FUF group compared with control (29% vs 37%; absolute risk reduction = 0.08; number needed to treat = 13).

No lower respiratory infections were diagnosed, and no children were hospitalized during the study. Only 1 case of allergic manifestation (food allergy) was reported. No diarrheal disease was reported; however, diarrhea was to be confirmed using strict preestablished criteria, potentially excluding mild cases of diarrhea that could be of infectious origin. No differences in stool consistency or frequency between groups were observed.

Fecal/Blood Outcomes

Children consuming FUF had higher levels of serum IL-10 at 28 weeks compared with control. No differences were observed in fecal secretory immunoglobulin A, TGF-β1, TGF-β2, IL-4, and IFN-ϒ (Table 4). There were no differences

TABLE 2 Frequency of ARI During the 28-Week Study Period

	Number of ARI Episodes				P
	None	1	2	3	
Control, n (%)	73 (47)	68 (44)	11 (7)	2 (1)	.04
FUF, n (%)	90 (58)	58 (37)	8 (5)	0	

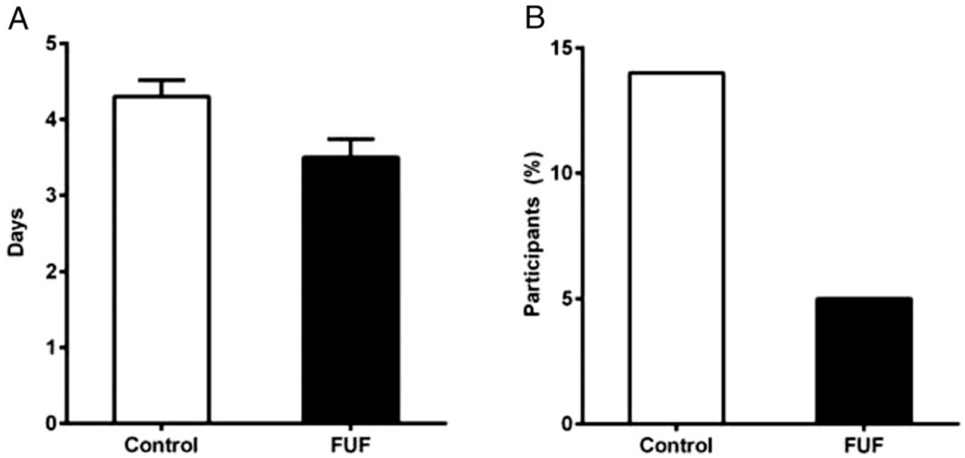

FIGURE 1
A, Average duration in days of ARI for those who had ARIs (Mean [SE]; control = 4.3 [0.2] days; FUF = 3.5 [0.2] days; $P = .007$). B, Participants (%) receiving systemic antibiotic treatment (*n* [%]; control = 21 [14%]; FUF = 8 [5%]; $P = .01$).

TABLE 3 Frequency of Day-Care Days Missed Due to Illness During the 28-Week Study Period

	Number of Days Missed							*P*
	None	1	2	3	4	5	6	
Control, *n* (%)	97 (63)	20 (13)	18 (12)	12 (8)	3 (2)	2 (1)	2 (1)	.01
FUF, *n* (%)	111 (71)	25 (16)	11 (7)	7 (4)	2 (1)	0	0	

in serum ferritin and zinc, hemoglobin, hematocrit, or red blood cells (Table 5). Based on World Health Organization criteria for anemia (hemoglobin <11.0 g/dL) and iron deficiency (ferritin < 12.0 ng/mL),[28] the overall study population had a prevalence of anemia of 65% ($n = 202$) and 58% ($n = 153$) and iron deficiency of 41% ($n = 108$) and 42% ($n = 104$) at onset and end of study, respectively. There were no differences in the number of participants who remained anemic or iron deficient at the end of the study. Children consuming FUF had higher white blood cell (WBC) count at 28 weeks; a difference in the change in WBC from baseline between groups was also observed (Table 6). The only fecal parasites detected were *Blastocystis hominis* (27%, both control and FUF groups, at baseline; 18%, control, and 24%, FUF, at end of study) and *Ascaris lumbricoides* (only 1 participant in control group, at baseline). No antiparasite treatment was given during the study, and there were no differences in fecal parasites.

DISCUSSION

We report that children who consumed an FUF had fewer episodes and shorter duration of ARI, fewer systemic antibiotic treatments, and missed fewer days at day care due to illness compared with children who consumed an unfortified, cow's-milk based beverage. They also had higher serum levels of IL-10, as well as higher blood leukocytes. No occurrence of diarrheal disease was reported during the study.

ARI was identified as causing symptoms 22% to 40% of the time during a 2-year observation period in an epidemiologic study in 0-5 year old children from 10 countries, including two in Asia.[29] The number of episodes of ARI observed in the current study (Table 2) was proportionally lower than the number of episodes previously reported in 3- to 4-year-old children in the United States (average 4.7 episodes per year).[30] We are not aware of data on frequency of ARI in 3- to 4-year-old children in China to which our findings could be compared. ARI is commonly diagnosed in both children who stay at home and those who attend day care; however, it is reported to be more frequent in the latter, especially among those who attend day care during the first year of life.[31,32] The impact of late day care exposure (>2 years of age) on ARI at 3- to 4-years of age was reported in 2 distinct populations in the United States and the Netherlands, with divergent outcomes of either decreasing[32] or increasing[33] ARI frequency. Regardless of whether ARI frequency was higher or lower than expected in our control group, it was significantly lower in the FUF compared with the control group, with potential repercussions in overall health and development.

In the United States, rhinovirus has been identified as the most common cause of ARI in children requiring physician consultation.[30] Likewise, recent data from China indicate that the most common ARI agents in children were respiratory syncytial virus, parainfluenza virus, and rhinovirus.[34] All ARIs diagnosed in our study were upper respiratory infections, which are usually less severe and have shorter duration than lower respiratory infections. The mean duration of ARI reported in the control group (4.3 days) was

TABLE 4 Comparison of Immune Markers Between Study Groups[a]

	Control Median (IQR[b])	FUF Median (IQR[b])	*P*
Fecal Secretory IgA, mg/dL			
Baseline	151 (59–324)	199 (70–144)	.19
Week 28	449 (227–760)	511 (234–788)	.30
Baseline to wk 28	237 (84–432)	238 (103–450)	.38
IL-10, pg/mL			
Baseline	3.9 (≤3.2–7.5)	4.4 (≤3.4–7.2)	.62
Week 28	5.8 (4.1–8.0)	6.5 (4.8–9.4)	.04
Baseline to wk 28	0.5 (–1.0 to 3.0)	1.3 (–1.0 to 4.0)	.24
TGF–β1, pg/mL			
Baseline	24042 (20179–29814)	24040 (19807–27696)	.38
Week 28	22319 (18021–29588)	22967 (17743–30973)	.52
Baseline to wk 28	−2467 (–8184 to 5878)	1340 (–5119 to 7769)	.13
TGF-β2, pg/mL			
Baseline	338.3 (<243.4–521.5)	323.8 (<243.4–420.1)	.40
Week 28	305.3 (<243.4–443.6)	362.9 (<243.4–502.8)	.26
Baseline to week 28	−31.2 (–66.0 to 0.0)	−8.9 (–45.0–0.0)	.39
IL-4, pg/mL[c]			
Baseline	<1.6 (<1.6-<1.6)	<1.6 (<1.6–<1.6)	1.00
Week 28	<1.6 (<1.6–<1.6)	<1.6 (<1.6–<1.6)	.15
IFN-γ, pg/mL[c]			
Baseline	<15.6 (<15.6–<15.6)	<15.6 (<15.6–<15.6)	.99
Week 28	<15.6 (<15.6–<15.6)	<15.6 (<15.6–<15.6)	.30

Ig, immunoglobulin; IQR, interquartile range.
[a] All markers except fecal secretory IgA were measured in serum.
[b] IQR = 25%–75% interquartile range.
[c] Changes from baseline to Week 28 not analyzed because most of the samples were under detection limit.

TABLE 5 Comparison of Zinc and Iron Status Between Study Groups

	Control Median (IQR[a])	FUF Median (IQR[a])	*P*
Serum zinc, μmol/L			
Baseline	22.1 (18.1–25.6)	22.2 (18.0–25.7)	.88
Week 28	23.7 (21.3–28.5)	24.4 (21.6–28.0)	.45
Baseline to wk 28	3.4 (–2.0 to 11.0)	3.5 (–2.0 to 8.0)	.85
Serum ferritin, ng/mL			
Baseline	13.3 (<10–24.2)	15.4 (<10–25.0)	.27
Week 28	13.8 (<10–21.9)	16.8 (<10–26.7)	.10
Baseline to wk 28	0.0 (–4.0 to 3.0)	0.0 (–4.0 to 9.0)	.31
Hemoglobin, g/dL			
Baseline	10.6 (10.2–11.2)	10.7 (10.1–11.1)	.52
Week 28	10.9 (10.4–11.3)	10.8 (10.4–11.3)	.96
Baseline to wk 28[b]	0.1 (0.06)	0.2 (0.06)	.32
Hematocrit, %			
Baseline	32.3 (30.9–33.8)	32.4 (30.8–33.5)	.66
Week 28	33.4 (31.8–35.1)	33.3 (32–34.9)	.90
Baseline to wk 28[b]	0.9 (0.19)	1.1 (0.19)	.42
Red blood cells, $\times 10^9$/mL			
Baseline	4.0 (3.8–4.1)	3.9 (3.7–4.1)	.33
Week 28	4.0 (3.8–4.2)	4.0 (3.8–4.1)	.25
Baseline to wk 28[b]	0.0 (0.02)	0.0 (0.02)	.80

[a] IQR = 25% to 75% interquartile range.
[b] Changes from baseline to study week 28 were analyzed by using analysis of covariance, with baseline values as the covariate; the values listed are Adjusted Mean (SE).

shorter than the duration of upper respiratory infections reported in a previous study in 2- to 3-year-old day-care children (7.8 days); this difference may be explained by the different age range in the 2 studies, because shorter duration of respiratory infection has been demonstrated as a child grows older.[35] Notably, the decrease in duration of ARI in the FUF group when compared with control (mean of 0.8 days) is of a similar magnitude as the decrease in duration reported with use of antiviral treatment (1 day).[36] A dietary approach, however, does not have the risks of adverse events linked to the use of antiviral drugs.[37]

The prevalence of allergic diseases reported in 3- to 4-year-old children in China is lower than the prevalence in some Western countries, especially for asthma (2.2%–9.7%), although it is increasing.[38] It has been a matter of debate whether exposure to early infections is a risk or a protective factor against later allergic disease. The existence of siblings or day-care attendance are proxy variables for early exposure to infections and have been identified as protective against allergy and asthma at 3 to 4 years of age, according to the hygiene hypothesis.[39,40] Contrary to the hygiene hypothesis, some studies showed that attending day care in the first year of life was a risk factor for wheezing and allergic rhinitis at 1 to 4 years of age[32,33,41]; likewise, ARI caused by rhinovirus have been identified as the strongest predictor of wheezing in the third year of life.[42] In the current study, the absence of asthma-like wheezing and allergy symptoms (only 1 child with food allergy reported) is noteworthy. This may be partially explained by the low prevalence of childhood allergy in China and seems to conflict with the hygiene hypothesis because both groups had low exposure to siblings and no early day-care attendance that could explain the low incidence of allergy. Nonetheless, our findings are consistent with data showing that absence of siblings or of early day-care exposure are associated with decreased wheezing and allergy at 3 to 4 years of age.[32,33]

A notably high prevalence of anemia was observed in the overall study population. Anemia was higher than the 48% prevalence reported in preschool children in Asia[43] (65% at start and 58% at end of study), with no response to the supplemental amount of iron in the FUF (9 mg/day), suggesting that this dose was too low compared with the recommended dose to treat anemia (3 mg/kg/day)[28] and/or other causes of anemia could be operating. Similarly, we observed a 41% prevalence of iron deficiency in the study population, which is higher than a prevalence of 24%, reported in a mixed urban and rural preschool cohort in China,[44] and was not reverted by the FUF, remaining at 42% at the end of the study. Zinc status was normal, with no differences between groups at onset and end of study.

Given the contribution of direct transmission of pathogens to the prevalence of ARI in children in a day care, as well as the limited ability to reduce incidence of ARI through infection control techniques,[45] alternative measures such as nutritional supplementation should be considered. Cow's milk is a regularly consumed beverage after 1 year of age. A recent survey in France showed that 1- to 2-year-old children consuming regular cow's milk were at increased risk of insufficient intake of nutrients, such as essential fatty acid, iron, and vitamins C and D, compared with those consuming a fortified cow's milk–based formula.[46] The higher amount of micronutrients in FUF compared with the control may have contributed to improved respiratory health in our study, with DHA, prebiotics, and yeast β-glucan contributing to specific immune effects. To identify the contribution of individual nutrients to the respiratory findings, subsequent studies are warranted.

The higher levels of IL-10 in the FUF group suggest an antiinflammatory mechanism, and the higher WBC values in the FUF group, albeit within the normal range, suggest an increase in effector immune cells, both of which may have contributed to decreased ARIs. DHA can reduce production of pro-inflammatory cytokines,[47,48] and antiinflammatory effects have been demonstrated for DHA and its metabolites such as resolvins and protectins.[49] For example, protectin D1 (PD1) reduces allergic pulmonary inflammation,[50] and inhibits production of pro-inflammatory cytokines such as IL-1β, TNFα, and IFNγ.[49] DHA can also exert antiinflammatory effects via G protein-coupled receptors in macrophages, thereby inhibiting pro-inflammatory signaling pathways.[51] Our data are also consistent with a study in which subcutaneous injections of yeast β-glucan for 8 weeks increased serum IL-10 levels and improved asthma symptoms in children.[52] Additionally, administration of a combination of colloidal silver and β-glucan relieved viral rhinitis and ARI in children.[53] β-glucan polymers are pathogen-associated molecular patterns that are recognized by multildren.

in those children whose dietary intake meets the minimal nutrient requirements but may not be of the highest quality necessary to promote improved health. The findings in the current study suggest that regular consumption of a follow-up formula enriched with DHA, the prebiotics PDX and GOS, yeast β-glucan, and essential nutrients may improve respiratory health.

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