Randomized, Double-Blind, Placebo-Controlled Study of Synbiotic Yogurt Effect on the Health of Children

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Objective To assess the effects of daily consumption of a synbiotic yogurt drink on the health, growth, and quality of life of healthy children 12-48 months of age in out-of-home child care.

Study design Healthy children attending child care centers were enrolled in a prospective, double-blind, placebo-controlled clinical trial. The intervention was a yogurt drink containing *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium animalis* subspecies *lactis* (BB-12) (5×10^9 cfu/100 mL serving), and 1 g of inulin (synbiotic group) vs a similar nonsynbiotic-containing acidified milk drink (placebo group) once daily for 16 weeks. The end points were days of diarrhea, fever, vomiting, symptoms of upper respiratory tract infection, use of antibiotics, physician visits, child care absenteeism, parental work absenteeism, and quality of life (PedsQL 4.0; Mapi Research Trust, Lyon, France).

Results Compared with placebo (n = 73), children receiving synbiotic (n = 76) had significantly fewer days of reported fever (1.85 vs 1.95, $P < .05$), significant improvement in social functioning ($P < .035$; pre-to-end intervention), and school functioning ($P < .045$; pre-to-mid intervention). More days with ≥3 loose/watery stools were reported in the synbiotic group ($P < .05$).

Conclusions Daily supplementation of children's diet with yogurt containing probiotic bacteria BB-12 and inulin significantly reduced days of fever and improved social and school functioning. The increased frequency of bowel movements may be explained by an accelerating effect of BB-12 and inulin on intestinal transit. Further research on the possible benefits of synbiotics on children's health is advised. *(J Pediatr 2015;166:1475-81)*.

Trial registration ClinicalTrials.gov: NCT00653705.

Dietary prebiotics are nondigestible food components that improve host health by selectively stimulating growth and/or activity of beneficial intestinal bacteria.[1,2] Examples include inulin, fructose oligosaccharides, and galacto-oligosaccharides.[3] Probiotics are live, nonpathogenic microorganisms that, when taken in adequate amounts, may confer individual health benefits in addition to their nutritional value. A combination of both probiotics and prebiotics in the same product is referred to as a synbiotic.[4] The availability and use of probiotics has increased over the past few years.[5] Although the mechanisms by which they contribute to human health are not completely understood, probiotics generally are thought to have beneficial effects both in children and adults; however, the beneficial effects of probiotics are strain- and condition-specific, and different probiotics have shown varying effects on human health, including prevention and treatment of acute diarrhea,[6-8] reduced duration of antibiotic-associated diarrhea,[9] and treatment benefits for ulcerative colitis, infantile colic, and irritable bowel syndrome.[3] In children, *Bifidobacterium animalis* subspecies *lactis* BB-12 is one of the most widely studied probiotic strains. Consumption of BB-12 has exhibited mixed effects relating to the frequency, duration of, and recovery from diarrhea episodes, in addition to effects on growth and missed days of school.[10-15]

Most reported probiotic and synbiotic studies in children have been in relation to specific diseases; only in a limited number studies have investigators evaluated their effect on healthy children.[7,11-13,15-25] In addition, these studies varied with respect to study population, probiotic/synbiotic composition, and intervention. They showed mixed results, thus warranting additional investigation.

Approximately 36% of US children younger than 5 years of age receive care in child care centers (CCCs), Head Start programs, preschools, and other early childhood programs.[26] Given that the transmission of infectious agents is enhanced in such settings, this setting is conducive to studying the effects of probiotics on healthy children.[27] The purpose of this study was to investigate the effect of daily consumption of a synbiotic yogurt drink on days of illness and child

BB-12	*Bifidobacterium animalis* subspecies *lactis*
BMI	Body mass index
CCC	Child care center
QOL	Quality of life

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care absenteeism in children participating in regular, out-of-home, center-based child care.

Methods

We conducted a prospective, 16 week, parallel, 2-arm, randomized, double-blind, placebo-controlled study over 13 consecutive months. Children were assigned to study arms through a permuted block-randomization procedure, with block sizes of 2 and 4. Investigators and surveyors/interviewers were blinded to the assignment. The study was approved by the Biomedical Institutional Review Board of University of North Carolina at Chapel Hill. The study was begun before the US Food and Drug Administration statute requiring clinical trial registration was in favor. Therefore, the study was registered after patient enrollment (ClinicalTrials.gov: NCT00653705).

One hundred eighty-one healthy children, ages 12-48 months, were recruited from 29 CCCs in 3 contiguous counties in central North Carolina. Recruitment was carried out through the CCCs by child care health consultants and study research assistants. All parents signed a consent form before participation.

To be eligible, a child must have attended the CCC at least 5 days a week for >4 hours a day. Given the increase in infectious illness experienced by children in child care settings, restricting to children enrolled 5 days a week increased the potential for observing an effect of synbiotics on incidence or duration of illness. Children were required to be healthy at the time of enrollment. Exclusion criteria included prematurity (<37 weeks' gestation), low birth weight (<5.5 pounds at birth), presence of any congenital anomalies, structural abnormality of the digestive tract or previous gastrointestinal surgery, chronic disease or malignancy, serious or unstable medical condition, failure to thrive, allergy or atopic disease, milk intolerance or lactase deficiency, and having received antibiotic treatment within 4 weeks or intentionally consuming probiotic products within 2 weeks before the screening interview. At the time of recruitment, parents and CCC teachers were instructed to not provide participants with yogurts, prebiotic/probiotic/synbiotic foods, supplements, or products other than the study product throughout the 16 weeks of the study.

The intervention consisted of a 91 mL/97 g synbiotic yogurt drink containing 2 probiotic yogurt starters, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (1×10^8 colony-forming units/g), the probiotic *Bifidobacterium animalis* subspecies *lactis* (BB-12) (5×10^9 colony-forming units/serving), and 1 g of inulin. This dose of inulin is used in commercially available dairy products because it provides mouth feel, foam stability, and is used as a replacement for sugar and fat. The study placebo was an acidified, flavored milk, without bacteria starters, probiotics, or inulin. Both products were manufactured by General Mills, Inc (Minneapolis, Minnesota), according to Good Manufacturing Practice, were similar to each other in appearance and taste, and were provided in unmarked containers. Participating parents were instructed to provide their child with one bottle of the dairy drink, each day, for 16 weeks, at a time of day convenient for the family.

Participants received biweekly supplies of dairy product through their CCCs. Parent/guardians were provided diary cards for daily documentation of use of the yogurt product, use of medications, and any episodes of fever, diarrhea, or other illnesses. Other symptoms reported included constipation, abdominal pain, gas, vomiting, loss of appetite, lethargy, cold symptoms, cough, runny nose, fever, congestion, bronchitis, respiratory infection, ear infection, and swollen gums. Most of these symptoms, with the possible exception of gastrointestinal symptoms (ie, constipation, abdominal pain, gas), are extremely unlikely to be related to probiotic use but rather represent common disorders of childhood.

The Survey Research Unit of the University of North Carolina at Chapel Hill contacted parents/guardians biweekly by telephone to collect the aforementioned information. The PedsQL (version 4.0 short form [SF15] Parent Report for Toddlers 2-4; Mapi Research Trust, Lyon, France) was administered by Survey Research Unit at the screening interview, at 8 weeks, and at end of intervention. Weight and height were measured at the CCC at baseline, 8 weeks, and 16 weeks by research staff blinded to each subjects' study arm (**Figure 1**; available at www.jpeds.com).

A set of covariates, including demographic, socioeconomic, and living environment measures are described in **Table I** (available at www.jpeds.com). These covariates served as adjustment variables in the intention-to-treat and dosage models.

Parents were asked to fill out the amount of product consumed by their child in a daily diary. Categories were "none," "part," or "all" of the product consumed. The provisions for a participant to exit the study included completion of the study, parent refusal to continue participation, or participant drop-out. Subjects who no longer attended one of the participating CCCs and/or failed two consecutive biweekly yogurt pick-up or phone interviews were dropped from the study. Furthermore, stopping rules for study termination included reports of adverse events resulting from participation that posed unnecessary risk to the participant. Adverse events that were reported to the study physician were determined to add no serious risk of harm. The institutional research board determined that no changes were needed and that the study could continue as approved. There were no differences in adverse events between the study groups.

Clinical Outcome Measures/Variables

Primary outcome measures included number and duration of illness episodes (diarrhea, upper respiratory infection, and febrile illness), days of child care absenteeism, and number of days of parental absence from work attributable to child illness. Secondary measures included number of physician visits, emergency department visits, hospitalizations, number and duration of antibiotic uses, and changes in quality of life