Randomized Control Trial of Human Recombinant Lactoferrin: A Substudy Reveals Effects on the Fecal Microbiome of Very Low Birth Weight Infants

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The purpose of this study is to evaluate the effects of enteral lactoferrin on the fecal microbiome and contrast those influences with the neonatal intensive care unit (NICU) environment. We theorized that lactoferrin and the NICU habitat shape the fecal microbial composition of very preterm infants. Although functions attributed to lactoferrin include intestinal immune system development and emergence of a healthy gut microbiota, evidence is limited. Twenty-one very low birth weight (VLBW <1500 g) infants received twice-daily talactoferrin (TLf, a drug designation for recombinant human lactoferrin) or its excipient by gastric gavage from day 1-28 of life. Twenty-four-hour fecal samples were collected on day 21 of life and compared with fecal operational taxonomy units (OTUs) in treated and control infants in 2 NICUs. Workflow included fecal DNA isolation, generation of amplicons for the V1-V3 region of bacterial 16S ribosomal RNA, and sequencing of a gel-purified multiplex amplicon library using a Roche 454 GS FLX Titanium (Roche, Branford, Connecticut) platform and protocols. Fecal OTUs per infant were higher in NICU 1 vs NICU 2 ($P < .001$), consistent with fewer antibiotic days ($P < .02$) and a shorter duration of parenteral nutrition ($P < .007$) in NICU 1. Proteobacteria and Firmicutes were the major phyla in infants treated with TLf and placebo. Among Enterobacteriaceae, TLf prophylaxis reduced Enterobacter and Klebsiella, but increased Citrobacter in feces of VLBW infants. Citrobacter caused no neonatal infections in the study population. OTUs for Clostridiaceae increased in NICU 1 among infants treated with TLf. Importantly, OTUs of staphylococci were barely detectable in both NICUs among infants fed TLf. Fewer hospital-acquired infections occurred in infants treated with TLf vs controls, although the reduction was seen mostly in coagulase-negative staphylococci-related bloodstream and central line infections ($P = .06$). TLf modified the fecal microbiome in VLBW infants, but care practices in the NICU habitat also contributed. Future research must establish whether elimination vs enrichment of gut-related microbiota reduces clinically significant hospital-acquired infections and promotes a healthy commensal microflora in the intestines of VLBW infants. *(J Pediatr 2016;173S:S37-42)*.

Trial registration ClinicalTrials.gov: NCT00854633.

During and after a normal vaginal birth, term infants acquire microbiota from the mother. Colonization of an infant with the mother's gut-related microflora matches the genetic relationships in the intestine.[1] The infant has a genetic make-up of enterocytes similar to that of the mother. Maternal oligosaccharides in breast milk are uniquely matched to the infant so that bacterial adherence to enterocytes occurs or is inhibited in the infant. Hence, intestinal bacteria in maternal feces more readily colonize the infant's intestine. Establishing commensal communities of intestinal bacteria that promote epithelial health and immune system development is a crucial aspect of neonatal care.[2] To their detriment, very low birth weight (VLBW <1500 g) are often delivered by cesarean, leave their mothers, and reside in the threatening microbiologic habitat of the neonatal intensive care unit (NICU). Low levels of organ colonization at birth enable rapid colonization by opportunistic microbiota in these infants. Therefore, antibiotic-resistant bacteria found in the NICU shape the composition and balance of commensal and pathogenic bacteria on the mucosal surfaces of the infants being treated there.[3] This is the reason VLBW infants experience increased risk of hospital-acquired infections (HAIs).[4] If pathogenic microbiota triumph over commensal bacteria during organ colonization, life-threatening bloodstream infections and necrotizing enterocolitis (NEC) result. Intestinal "dysbiosis" underlies mechanisms associated with an increased risk of HAI.[5-7] VLBW infants fed breast milk develop fewer HAIs.[8] Maternal milk confers protection against infection through anti-infective factors such as antimicrobial peptides, antibodies, and effectors that prevent mucosal invasion.[9] Despite in vitro and in vivo research on the antimicrobial, immunostimulatory,

bLf	Bovine lactoferrin
HAI	Hospital-acquired infection
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
OTU	Operational taxonomy unit
PCR	Polymerase chain reaction
TLf	Talactoferrin
VLBW	Very low birth weight

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and anti-inflammatory effects of lactoferrin in mammalian milk,[10] physicians have limited knowledge about how lactoferrin affects the intestinal microbiota of VLBW infants. Caregivers need this information so they can understand the mechanisms whereby lactoferrin reduces costly HAIs in VLBW infants. Caregivers anticipate that eliminating HAIs and undesirable neurodevelopmental outcomes in this patient population.

Methods

The Institutional Review Boards of the University of Missouri–Columbia and the University of Southern California approved the randomized, blinded, and placebo-controlled trial of oral talactoferrin (TLf, a drug designation for recombinant human lactoferrin) solution and the associated substudy of the fecal microbiome (ClinicalTrials.gov: NCT00854633). Parents gave written consent for both studies. One hundred twenty infants, with birth weights ranging from 750-1500 g, received enteral TLf[11] or the excipient for the biologic agent, from the day of birth through 28 days of life. Infants were blindly randomized by computer to the treatment or placebo group. Each arm had 60 infants. The dose of TLf was 150 mg/kg given enterally every 12 hours and simulated the intake of mature maternal milk containing ~2 mg/mL of lactoferrin. A subset of 23 of the 120 infants had a 24-hour fecal collection on the 21st day of life. Researchers recovered demographic and infection-related data on these infants (Figure 1). Feces were transferred with a sterile spatula from the diaper into a sterile, DNA-free container. The container was stored at −20°C during the collection period and then stored at −80°C until analyzed. Investigators collaborated with MOgene, LC (St. Louis, Missouri) for the molecular analyses of bacteria in the fecal samples. Bacteria were classified in fecal samples using the following work flow.[12] For each fecal sample, the genomic DNA of bacteria was isolated from ~200 mg of feces by bead beating. Thereafter, DNA was separated with a QIAamp DNA Stool Mini Kit (Qiagen - #51504; Valencia, California). The bacterial 16S gene variable region (V1-V3) was amplified via polymerase chain reaction (PCR) using a unique bar code primer sequence for each sample (amplicon size

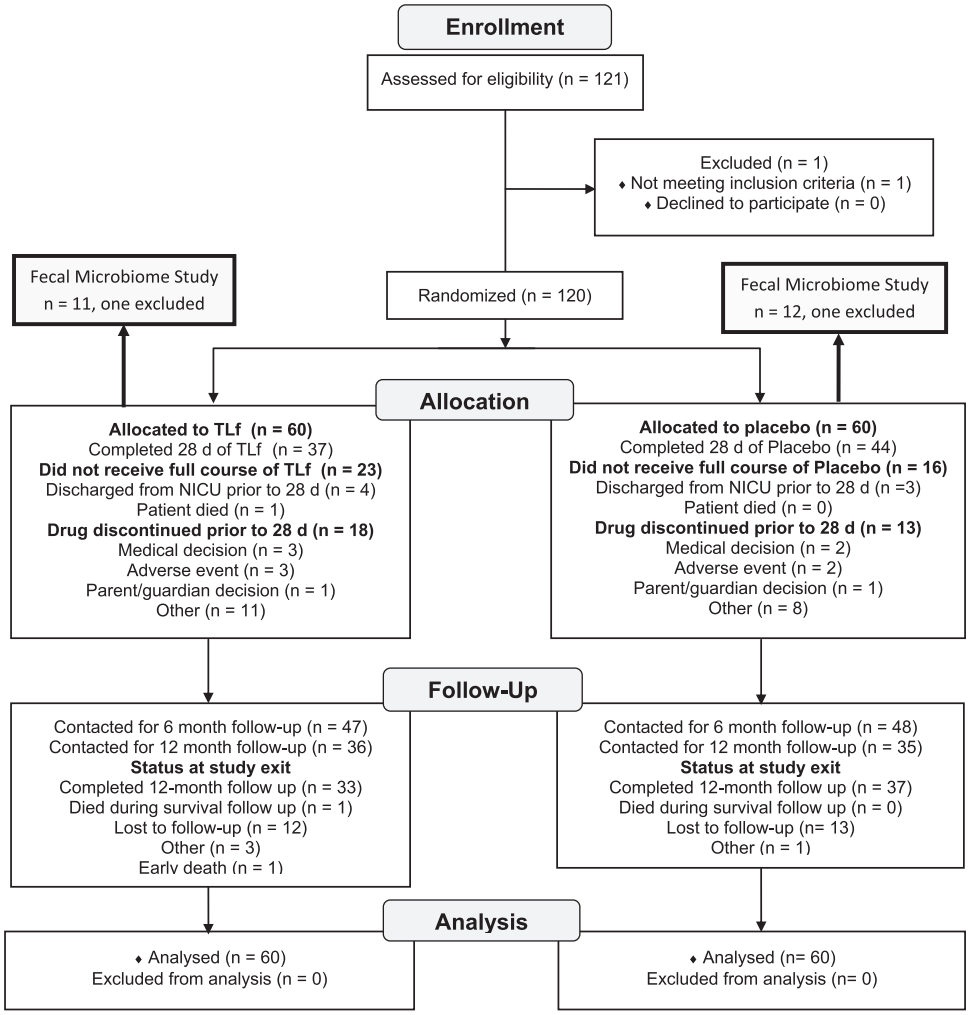

Figure 1. CONSORT diagram.