Effects of Different Complementary Feeding Regimens on Iron Status and Enteric Microbiota in Breastfed Infants

Nancy F. Krebs, MD[1], Laurie G. Sherlock, MD[1], Jamie Westcott, MS[1], Diana Culbertson, MS[1], K. Michael Hambidge, MD[1], Leah M. Feazel, MS[2], Charles E. Robertson, PhD[3,4], and Daniel N. Frank, PhD[2,4,5]

Objective To compare iron status in breastfed infants randomized to groups receiving complementary feeding regimens that provided iron from fortified infant cereals or meats, and to examine the development of the enteric microbiota in these groups.

Study design Forty-five exclusively breastfed 5-month-old infants were randomized to 1 of 3 feeding groups (FGs)—commercially available pureed meats, iron- and zinc-fortified infant cereals, or iron-only fortified infant cereals—as the first and primary complementary food through 9-10 months of age. Dietary iron was determined by monthly 3-day diet records. Iron status was assessed at the end of the study by measurements of hemoglobin, serum ferritin, and soluble transferrin receptor levels. In a subsample of 14 infants, enteric microbiota were profiled in monthly stool samples (5-9 months) by 16S ribosomal RNA gene pyrosequencing.

Results Infants in the 2 cereal FGs had 2- to 3-fold greater daily iron intakes versus the meat FG ($P < .0001$). More than one-quarter (27%) of the infants had a low serum ferritin level, and 36% were mildly anemic, with no significant differences by FG; more infants in the meat FG had a high soluble transferrin receptor value ($P = .03$). Sequence analysis identified differences by time and FG in the abundances of several bacterial groups, including significantly more abundant butyrate-producing *Clostridium* group XIVa in the meat FG ($P = .01$)

Conclusion A high percentage of healthy infants who were breastfed-only were iron-deficient, and complementary feeding, including iron exposure, influenced the development of the enteric microbiota. If these findings are confirmed, then reconsideration of strategies to both meet infants' iron requirements and optimize the developing microbiome may be warranted. *(J Pediatr 2013;163:416-23)*.

After approximately 6 months of age, term breastfed infants are increasingly dependent on other sources of iron to avoid iron deficiency, owing to depletion of the iron stores present at birth and to the low concentration of iron in human milk. In the US, iron is most commonly provided to older infants through iron-fortified cereals. The absorption of the electrolytic iron provided in these cereals is <5%.[1] Although meats have been recognized as potentially good sources of more readily absorbable heme iron,[1-4] with absorption of up to 35%,[5] only a small percentage of US infants consume meats during the first year of life.[6,7] Reliance on the poorly bioavailable electrolytic iron in commercial infant cereals, along with infants' relatively high iron requirements, has led to the establishment of an Estimated Average Requirement for iron of 6.9 mg/day[8] and a Recommended Dietary Allowance of 11 mg/day to be applied to individuals' estimated intake needs. Consumption of foods with a more favorable iron bioavailability might be sufficient to meet physiological requirements at a lower dietary intake.

Beyond considerations about dietary strategies to meet iron requirements, studies in animals and older children have implicated iron exposure as a modulator of the enteric microbial profile, including promotion of proinflammatory organisms.[9] In concert with growing recognition of the importance of the enteric microbiome to immunity, these observations provide a link between infants' diets and modulation of the developing immune system.

After birth, the gastrointestinal tract undergoes a transformation from ostensible sterility to robust and adult-like colonization.[10] Numerous factors influence the composition of the microbiome, including mode of delivery at birth, antibiotic use, and breastfeeding versus formula feeding. Given the proclivity of many bacteria for iron, the poorly absorbed electrolytic iron in infant cereals could theoretically influence the enteric microbiome profile.

To date, few studies have examined the potential for meats as a complementary food to meet iron requirements in breastfed infants, and to our knowledge, none has specifically considered the effect of complementary foods with different

FG	Feeding group
rRNA	Ribosomal RNA
sTfR	Soluble transferrin receptor
TDI	Total dietary iron

From the [1]Section of Nutrition, Department of Pediatrics and [2]Division of Infectious Diseases, Department of Medicine, University of Colorado School of Medicine, Aurora, CO; [3]Department of Molecular, Cellular, and Developmental Biology, University of Colorado Boulder, Boulder, CO; and [4]Microbiome Research Consortium and [5]Mucosal and Vaccine Research Colorado, University of Colorado School of Medicine, Aurora, CO

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forms and amounts of iron on development of the gut microbiome. The objective of the present study was to compare iron status in infants who were breastfed only (no formula) and randomized to complementary feeding regimens that provided iron primarily from fortified infant cereals or from meats. In a subset of the infants, we also examined the bacterial profiles in the gastrointestinal tract.

Methods

The iron status and microbiome outcomes were secondary outcomes in a previously reported randomized controlled intervention trial investigating zinc absorption from different complementary feeding regimens.[11] In brief, healthy term infants were assigned at random to 1 of 3 feeding groups (FGs): commercially available pureed meats; iron- and zinc-fortified infant cereals; or organic, whole grain iron-only fortified infant cereals. These foods served as the first complementary food and as a consistent component of the infants' diets throughout the study period from approximately 6 months through 9 months. Fruits, vegetables, teething biscuits, and unfortified cereals and other finger foods were allowed ad libitum. The study foods were provided at monthly visits, and monthly 3-day diet records were obtained. Serologic and hematologic biomarkers of iron status were measured between 9 and 10 months of age.

The last consecutive 14 infants recruited into the primary study were enlisted for analysis of the enteric microbiome. Each mother–infant pair was randomized to a complementary feeding regimen according to overall study randomization procedures. All infants were from the same geographic location and were delivered vaginally; antibiotic use was monitored monthly and recorded. Fecal samples were obtained at monthly intervals from 5 months through 9 months to characterize the longitudinal changes and the effects of different dietary patterns on the composition and diversity of the enteric microbiome.

Sample size for each group in the iron status study was determined by power analysis for the zinc absorption studies reported previously,[11] and the subjects for the microbiome analyses represented the final one-third of subjects enrolled. The entire study, including the microbiome component, was approved by the Colorado Multiple Institutional Review Board, and written and informed consent was obtained from the parents of each infant.

Diets

Details of the nutritional composition of the 3 intervention foods are provided in Table I and have also been reported elsewhere.[11] The intervention foods (cereals or meats) were provided at monthly visits, to encourage compliance. Parents were encouraged to follow responsive feeding practices and were provided monthly guidelines on approximate amounts of the assigned complementary foods to offer the infants. Recommendations included gradually increasing intake from 1 serving (15 g of dry cereal or a 71-g jar of meat) per day by 7 months to 2 servings per day by 9 months.[1] Monthly 3-day diet records were analyzed by a registered dietitian at the Clinical Translational Research Center Bio-Nutrition Unit, using the University of Minnesota's Nutrient Data System for Research dietary analysis program. Iron intake was calculated only from complementary foods, and results do not include estimated intake from breast milk, which contributes a very small amount to daily iron intake. Duplicate diet records were collected at 9 months to determine total dietary iron (TDI) intake for 5 days, in conjunction with 4 days of test weighing to determine human milk intake and metabolic collections for zinc stable isotope studies.[11]

Table I. Nutritional content of study foods

	Iron- and zinc-fortified cereal FG	Iron-fortified cereal FG	Meat FG*
Serving size, g	15 (1/4 cup dry)	14 (1/4 cup dry)	71 (1 jar)
Energy, Kcal†	60	60	70
Protein, g†	1	1	8
Iron, mg‡	7.8	6.2	1.0
Zinc, mg‡	1.2	0.3	2.1
Phytate, mg‡	23	107	0
Phytate:Fe molar ratio	0.25	1.5	-

*Values for pureed beef and gravy.
†From product label.
‡From laboratory analysis.

Anthropometric Measurements

Length and weight were measured at enrollment (5 months) and at each subsequent monthly (±1 week) visit at 6, 7, 8, and 9 months. All measurements were performed in duplicate by a trained researcher (D.C.). Length was measured with the infant recumbent using an infant stadiometer accurate to 0.1 cm (Holtain, Pembrokeshire, United Kingdom). An electronic digital balance that integrates 100 rapid serial measurements to provide a mean weight to the nearest gram (Sartorious, Bohemia, New York) was used to obtain naked weights.

Sample Collection, Laboratory Analyses, and Microbiome Analyses

Iron content of 5-day duplicate diets was measured by atomic absorption spectrophotometry after digestion and quantitative reconstitution, as described previously for determination of zinc concentrations.[11] Blood samples were drawn at approximately 9:00 a.m. in the Pediatric Clinical Translational Research Center. Serum ferritin, C-reactive protein, and soluble transferrin receptor (sTfR) concentrations and hematologic indices were determined in the Clinical Translational Research Center Core Lab by immunonephelometry using a BNII Nephelometer (Siemens Healthcare Diagnostics, Tarrytown, New York) and manufacturer-suggested protocols.

Monthly stool samples for microbiome analysis were obtained from infants in the iron-fortified cereal FG (n = 4), iron- and zinc-fortified cereal FG (n = 6), and meat FG (n = 4). Baseline specimens were obtained at 5 months,