ORIGINAL ARTICLE

A Microbiota-Directed Food Intervention for Undernourished Children

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ABSTRACT

BACKGROUND

More than 30 million children worldwide have moderate acute malnutrition. Current treatments have limited effectiveness, and much remains unknown about the pathogenesis of this condition. Children with moderate acute malnutrition have perturbed development of their gut microbiota.

METHODS

In this study, we provided a microbiota-directed complementary food prototype (MDCF-2) or a ready-to-use supplementary food (RUSF) to 123 slum-dwelling Bangladeshi children with moderate acute malnutrition between the ages of 12 months and 18 months. The supplementation was given twice daily for 3 months, followed by 1 month of monitoring. We obtained weight-for-length, weight-for-age, and length-for-age z scores and mid–upper-arm circumference values at baseline and every 2 weeks during the intervention period and at 4 months. We compared the rate of change of these related phenotypes between baseline and 3 months and between baseline and 4 months. We also measured levels of 4977 proteins in plasma and 209 bacterial taxa in fecal samples.

RESULTS

A total of 118 children (59 in each study group) completed the intervention. The rates of change in the weight-for-length and weight-for-age z scores are consistent with a benefit of MDCF-2 on growth over the course of the study, including the 1-month follow-up. Receipt of MDCF-2 was linked to the magnitude of change in levels of 70 plasma proteins and of 21 associated bacterial taxa that were positively correlated with the weight-for-length z score ($P<0.001$ for comparisons of both protein and bacterial taxa). These proteins included mediators of bone growth and neurodevelopment.

CONCLUSIONS

These findings provide support for MDCF-2 as a dietary supplement for young children with moderate acute malnutrition and provide insight into mechanisms by which this targeted manipulation of microbiota components may be linked to growth. (Supported by the Bill and Melinda Gates Foundation and the National Institutes of Health; ClinicalTrials.gov number, NCT04015999.)

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Childhood undernutrition is a global health challenge that produces impaired ponderal and linear growth (wasting and stunting), immune and metabolic dysfunction, altered development of the central nervous system (CNS), and other abnormalities.[1,2] Acute malnutrition in children is classified by the degree of wasting. Moderate acute malnutrition is defined as a weight-for-length measurement that is 2 or 3 SD below the median of the cohort that was evaluated in the Multicenter Growth Reference Study by the World Health Organization; severe acute malnutrition is defined as a weight-for-length measurement that is more than 3 SD below the median.[3] Children with these levels of malnutrition have defects in the development of their gut microbiota, which leaves them with microbial communities that appear to be younger than those of their healthy counterparts.[4,5] Current nutritional interventions for both moderate and severe forms of malnutrition have not focused on the microbiota as a therapeutic target. Coincidentally, existing therapies have shown limited efficacy in treating the long-term sequelae that affect undernourished children[6,7] and in repairing their microbiota.[8] Since it has been estimated that the coronavirus 2019 pandemic will increase childhood mortality from wasting by more than 20%,[9] surmounting this already formidable global health challenge has become even more pressing.

We previously identified a network (ecogroup) of 15 bacterial taxa that can be used to describe normal development of the gut microbial community during the first 2 years of postnatal life in healthy members of birth cohorts in several countries designated as low or middle income.[5] Changes in the abundance of ecogroup taxa provide a way of defining the severity of microbiota perturbations in children with untreated moderate or severe malnutrition, as well as characterizing the incomplete nature of microbiota repair that occurs when these children receive existing therapeutic foods.[5,8] Comparisons of gnotobiotic mice that were colonized with fecal microbiota obtained from age-matched healthy children and from children with wasting or stunting have revealed bacteria that are discriminatory for weight gain, including several bacteria in the ecogroup taxa.[5,10] In mice that were colonized with microbiota obtained from a wasted or stunted child, supplementation with five of these strains prevented microbiota-dependent transmission of an impaired weight-gain phenotype.[10] On the basis of these observations, and through the screening of combinations of food staples in gnotobiotic mice and gnotobiotic piglets, we developed several microbiota-directed complementary food (MDCF) prototypes.[8] We compared three of these formulations with an existing ready-to-use supplementary food (RUSF) in a 1-month-long, randomized, controlled trial involving children between the ages of 12 months and 18 months with moderate acute malnutrition who were living in an urban slum known as Mirpur, located in Dhaka, Bangladesh. One of these formulations (MDCF-2) changed the microbiota to a composition similar to that of age-matched healthy Mirpur children and changed the levels of plasma proteins indicative of improved health status.[5,8] Here, we report the results of a larger study conducted over a longer period to compare the effects of MDCF-2 and RUSF on clinical end points.

Methods

Study Design

The study, which was conducted in Mirpur from November 2018 through December 2019, was approved by the ethics review committee at the International Centre for Diarrhoeal Disease Research, Bangladesh. Parents or guardians of all the study participants provided written informed consent.

Boys and girls with moderate acute malnutrition who were between 12 months and 18 months of age and who satisfied the inclusion and exclusion criteria were randomly assigned to receive MDCF-2 or RUSF (Fig. 1A). The caloric density of MDCF-2 is lower than that of RUSF (204 kcal vs. 247 kcal per 50-g daily dose). Anthropometric features were measured every 15 days, and data regarding health complications were documented daily. Field research assistants monitored the children for any adverse events and treated them according to standard of care, if needed.

During the first month, the children's mothers fed them two daily 25-g servings of MDCF-2 or RUSF at a local study center under the supervision of a health care provider; the amount that had not been consumed was determined by weighing. In the second month, one of the two daily feedings occurred at home, and the amount

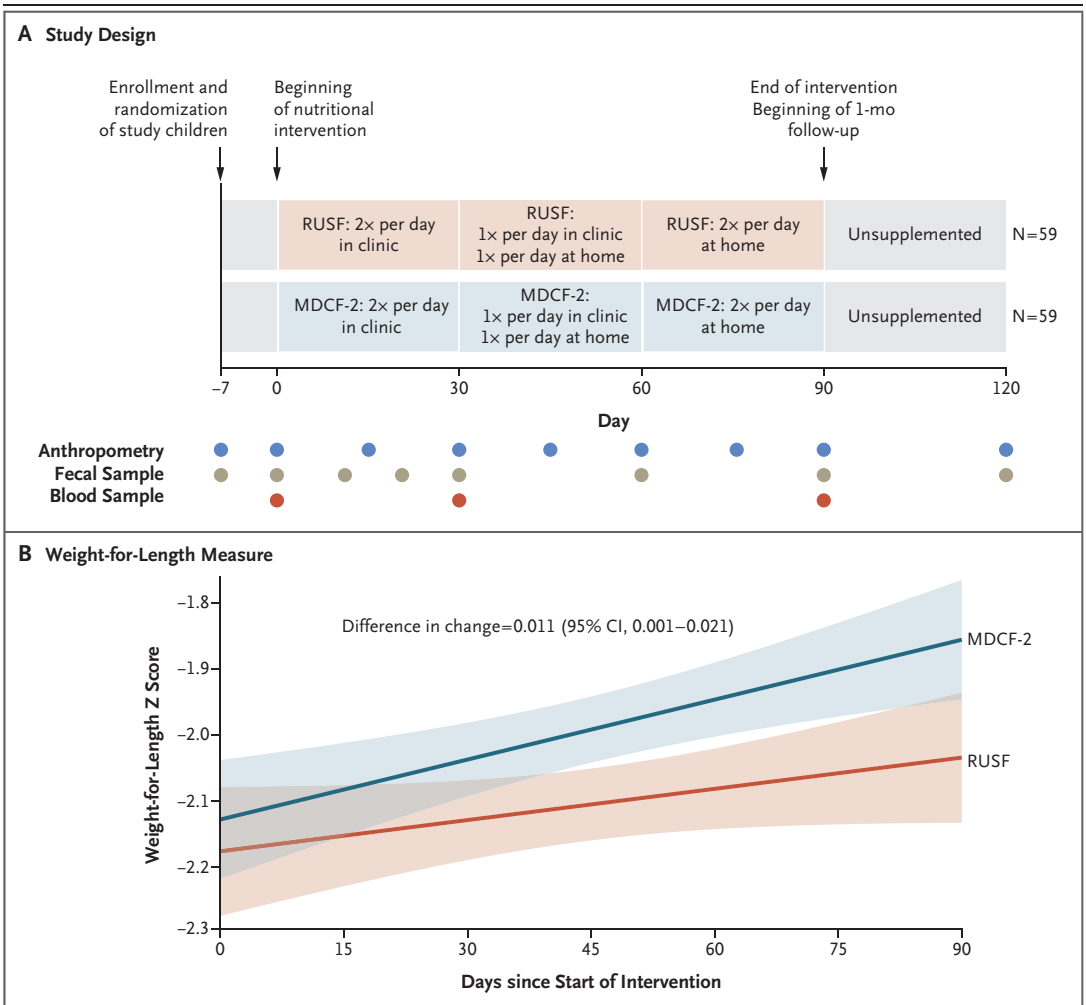

Figure 1. Study Design and Change in Weight-for-Length Measure Associated with MDCF-2 and RUSF Supplementation.
Panel A shows the study design for the evaluation of children between the ages of 12 months and 18 months with moderate acute malnutrition who were treated with microbiota-directed complementary food (MDCF-2) or ready-to-use supplementary food (RUSF). Panel B shows the change in the weight-for-length z score in the two groups during the 3-month treatment period. The 0.011 value is the mean between-group difference in the rate of growth per week, which was faster in the MDCF-2 group. The shaded areas around the curves indicate 95% confidence intervals.

consumed was documented; in the third month, both daily feedings were provided at home. Other than being asked to avoid feeding their children during the 2-hour period before each visit, mothers were advised to continue their usual breast-feeding and complementary feeding practices throughout the study. After completing 3 months of the intervention, the children returned to their normal feeding routine but continued to be monitored; fecal samples and anthropometric data were collected 1 month after the discontinuation of treatment.

Details regarding the composition and nutritional analysis of the diets are provided in Table S1 in Supplementary Appendix 2, which includes all the cited supplementary tables. Additional details regarding the study methods (including the use of food-frequency questionnaires) and all supplementary figures are included in Supplementary Appendix 1; details regarding the inclusion criteria and sample-size calculations are provided in the protocol.[11] All the supplementary materials and the protocol are provided with the full text of this article at NEJM.org.

Table 1. Characteristics of the Children at Baseline.*

Characteristic	MDCF-2 (N=61)	RUSF (N=62)
Demographic		
Age — mo	15.4±1.9	15.5±2.0
Female sex — no. (%)	35 (57)	36 (58)
Anthropometric feature		
Weight-for-length z score	−2.31±0.29	−2.40±0.27
Weight-for-age z score	−2.69±0.67	−2.76±0.62
Length-for-age z score	−2.08±1.16	−2.08±1.12
Mid–upper-arm circumference — cm	12.8±0.53	12.7±0.44
Breast-feeding status — no. (%)		
No breast-feeding since birth	1 (2)	0
Partial breast-feeding	46 (75)	46 (74)
Exclusive breast-feeding	14 (23)	16 (26)
Immunization status — no. (%)		
Complete	53 (87)	52 (84)
Partial	8 (13)	6 (10)
None	0	4 (6)

* Plus–minus values are means ±SD. MDCF-2 denotes microbiota-directed complementary food, and RUSF ready-to-use supplementary food.

END POINTS

Outcome measures were the weekly rate of change in the weight-for-length z score, weight-for-age z score, mid–upper-arm circumference, length-for-age z score, medical complications, plasma proteomic profile, and gut microbiota configuration.

STATISTICAL ANALYSIS

We compared changes in ponderal growth between the two groups using linear mixed-effects models that included fixed effects to control for differences in characteristics between the children at baseline (age, sex, and the occurrence of illness 7 days before starting the intervention), the number of weeks of the intervention, treatment group, the interaction between the number of weeks of the intervention and the treatment group, and a random-effects coefficient for each child to account for the within-participant correlation; other anthropometric measures were assessed in a similar fashion. We used generalized linear mixed-effects models to analyze responses on the food-frequency questionnaire and data regarding medical complications. Because we tested the effects of the supplements on four measures of growth and did not correct for multiple testing, we have provided confidence intervals for each outcome.

Changes in levels of plasma proteins were analyzed with the use of an empirical Bayesian linear model framework[12] and gene-set enrichment analysis,[13] a method for quantifying whether a rank-ordered list of features (e.g., proteins that are ranked according to the changes in levels after treatment or by a correlation coefficient) are enriched for a subgroup of features of interest (e.g., a biologic pathway). We used linear mixed-effects models and gene-set enrichment analysis to quantify the effects of supplementation on microbial community configuration. For all statistical analyses, a P value of less than 0.05 was considered to indicate statistical significance. For analyses requiring adjustment for multiple hypotheses, significance was indicated by a false discovery rate–adjusted P value (Q value) of less than 0.10 for the comparison in the plasma proteomic data set and of less than 0.05 for the comparison in the fecal microbial data set. All reported P and Q values are two-sided.

RESULTS

CLINICAL CHARACTERISTICS

Of the 123 children who underwent randomization, 61 were assigned to receive MDCF-2 and 62 to receive RUSF (Fig. S1 in Supplementary Appendix 1). The mean (±SD) age of the children was 15.4±2.0 months. A total of 59 children in each group completed the 3-month intervention and 1-month follow-up. Five children did not complete the study because of family relocation or withdrawal of consent.

At enrollment, the anthropometric and sociodemographic characteristics of the children were similar in the two groups (Table 1, and Table S3 in Supplementary Appendix 2). During the 3-month intervention period, the mean (±SE) percentage of the 50-g daily dose of supplement that the children actually consumed was similar in the MDCF-2 group and the RUSF group (92.5±0.7% and 92.0±1.2%, respectively; P=0.87). There were no discernible between-group differences in the percentages of children who met the World Health Organization requirements for

Table 2. Clinical Response to MDCF-2 or RUSF Supplementation.*

Anthropometric Feature	MDCF-2 (N = 59)	RUSF (N = 59)	Difference (95% CI)†
At baseline			
Weight-for-length z score	−2.22±0.39	−2.29±0.36	0.086 (−0.056 to 0.228)
Weight-for-age z score	−2.66±0.67	−2.71±0.64	0.036 (−0.213 to 0.285)
Length-for-age z score	−2.14±1.14	−2.13±1.13	−0.044 (−0.467 to 0.380)
MUAC — cm	12.8±0.51	12.7±0.44	0.077 (−0.100 to 0.254)
Mean rate of growth per week during 3-mo treatment (95% CI)‡			
Weight-for-length z score	0.021 (0.014 to 0.029)	0.010 (0.003 to 0.017)	0.011 (0.001 to 0.021)
Weight-for-age z score	0.017 (0.012 to 0.022)	0.010 (0.004 to 0.015)	0.008 (0.001 to 0.015)
Length-for-age z score	0.004 (0.002 to 0.007)	0.005 (0.003 to 0.008)	−0.001 (−0.005 to 0.003)
MUAC — cm	0.031 (0.029 to 0.034)	0.029 (0.025 to 0.032)	0.003 (−0.001 to 0.007)
Mean rate of growth per week during treatment plus 1-mo follow-up (95% CI)‡			
Weight-for-length z score	0.010 (0.005 to 0.016)	0.000 (−0.005 to 0.006)	0.010 (0.002 to 0.018)
Weight-for-age z score	0.009 (0.005 to 0.013)	0.001 (−0.003 to 0.005)	0.008 (0.002 to 0.013)
Length-for-age z score	0.004 (0.002 to 0.006)	0.003 (0.001 to 0.006)	0.000 (−0.003 to 0.003)
MUAC — cm	0.028 (0.026 to 0.031)	0.024 (0.022 to 0.027)	0.004 (0.000 to 0.007)

* Plus–minus values are means ±SD. MUAC denotes mid–upper-arm circumference.

† Values for the between-group difference at baseline were derived from a linear model predicting anthropometric features at the start of treatment as a function of treatment group after adjustment for age, sex, and any illness within 7 days before treatment initiation. Values for the between-group difference in the growth rate per week during the 3-month treatment period and during treatment plus 1-month follow-up were derived from a mixed-effects linear model predicting anthropometric features as a function of the interaction between treatment group and the number of weeks since the initiation of nutritional supplementation after adjustment for the baseline variables plus the number of weeks of treatment, the treatment group, and a random intercept for each participant to account for the within-participant correlation. Positive values indicate a faster growth rate in children receiving MDCF-2.

‡ Values for the rate of growth per week during the treatment period and during treatment plus follow-up were derived from a mixed-effects linear model. This model predicts anthropometric features as a function of the number of weeks since the initiation of nutritional supplementation after adjustment for the baseline variables plus a random intercept for each participant to account for the within-participant correlation.

minimum meal frequency or minimum acceptable diet. (Details regarding the children's dietary practices are provided in Table S4 and in Supplementary Appendix 1.)

RESPONSE TO NUTRITIONAL INTERVENTION

Findings after the 3-month intervention suggest that children in the MDCF-2 group had better outcomes than those in the RUSF group with respect to the mean (±SD) change in two of the four key anthropometric measurements that were evaluated: weight-for-length and weight-for-age z scores (Table 2). The mean weekly change in the weight-for-length z score was 0.021 (95% confidence interval [CI], 0.014 to 0.029) in the MDCF-2 group and 0.010 (95% CI, 0.003 to 0.017) in the RUSF group, for a between-group difference of 0.011 (95% CI, 0.001 to 0.021). The mean weekly change in the weight-for-age z score was 0.017 (95% CI, 0.012 to 0.022) in the MDCF-2 group and 0.010 (95% CI, 0.004 to 0.015) in the RUSF group, for a between-group difference of 0.008 (95% CI, 0.001 to 0.015). For the mid–upper-arm circumference, the mean weekly change was similar in the two groups, with an increase of 0.031 cm (95% CI, 0.029 to 0.034) in the MDCF-2 group and 0.029 cm (95% CI, 0.025 to 0.032) in the RUSF group, for a between-group difference of 0.003 cm (95% CI, −0.001 to 0.007). In the fourth category (length-for-age

z score), the mean weekly change was 0.004 (95% CI, 0.002 to 0.007) in the MDCF-2 group and 0.005 (0.003 to 0.008) in the RUSF group, for a between-group difference of −0.001 (95% CI, −0.005 to 0.003).

In these analyses, a greater positive value indicates a faster growth rate among the children who received MDCF-2 than among those who received RUSF. For example, an extrapolation of the between-group difference of 0.011 per week in the change from baseline in the weight-for-length z score would result in a gain of 0.57 over that in the RUSF group at 1 year. The between-group difference in the change from baseline during the 4-month period encompassing the 3-month intervention and the 1-month follow-up was 0.010 (95% CI, 0.002 to 0.018) for the weight-for-length z score, 0.008 (95% CI, 0.002 to 0.013) for the weight-for-age z score, 0.004 cm (95% CI, 0.000 to 0.007) for the mid–upper-arm circumference, and 0.000 (−0.003 to 0.003) for the length-for-age z score (Table 2, Fig. 1B, and Table S5). Details regarding the changes in medical complications and dietary habits are provided in Figures S2 and S3 and in Tables S4 and S6.

EFFECTS OF INTERVENTIONS ON PLASMA PROTEOME

We quantified the levels of 4977 proteins[14] in plasma samples collected from all 118 children in the study at baseline, at 1 month, and at 3 months (Fig. S4A and S4B and Table S2). For each child, we constructed a linear model relating the duration of supplementation to the child's weight-for-length z score during the 3-month intervention. We then correlated this change with changes in the levels of plasma proteins before and after completion of the intervention (Fig. 2A, 2B, and 2C). A total of 75 proteins had a significant correlation (either positive or negative) with the change in the weight-for-length z score (Q<0.10) (Table S7).

Gene-set enrichment analysis querying Gene Ontology biologic processes revealed that proteins that were positively correlated with the change in the weight-for-length z score were significantly enriched (Q<0.10) for mediators of bone growth and ossification (e.g., insulin-like growth factor 1), cartilage oligomeric matrix protein (an extracellular matrix protein critical for endochondral bone growth),[15,16] and secreted frizzled-related protein 4 (a Wnt inhibitor that prevents excessive osteoclast erosion of bone)[17] (Fig. 2D and 2E and Tables S7 and S8). The correlated proteins were also enriched for CNS development, including the axon guidance protein SLIT and NTRK-like protein 5, BDNF/NT-3 growth factor receptor (NTRK3), and roundabout homologue 2 (an axon guidance receptor with pro-osteoblastic and anti-osteoclastic activities)[18] (Fig. 2F and Tables S7 and S8).

Proteins that had a negative correlation with ponderal growth were significantly enriched for acute phase reactants and actuators of immune activation (e.g., hepcidin, which reduces iron absorption and induces iron sequestration during inflammatory states), RANKL (osteoclast-promoting factor), granulysin (a proinflammatory cytokine produced by activated cytotoxic T cells and natural killer cells), interferon-induced protein with tetratricopeptide repeat-3 (which inhibits the replication of multiple viral pathogens[19]), and immunoglobulin A (Tables S7 and S8).

A total of 714 proteins had significantly (Q<0.10) higher or lower levels after the 3-month MDCF-2 supplementation, in contrast with 82 proteins that showed significant alterations after RUSF treatment (Table S9A and S9B). Proteins that showed increases after 3 months of supplementation with MDCF-2 were significantly enriched for the set of 70 proteins that were positively correlated with the change in the weight-for-length z score (P<0.001), in contrast with the proteins that showed increases after RUSF supplementation (P=0.11) (Fig. S5A and S5B). Comparing the two treatments revealed that proteins with greater increases in the MDCF-2 group were significantly enriched for proteins associated with the weight-for-length z score (P<0.001) (Fig. 2G). Of these proteins that had increased levels after MDCF-2 supplementation, cartilage intermediate layer protein 2 was elevated to the greatest extent. Its levels did not change in children who received RUSF. This protein forms complexes with collagen VI to promote articular cartilage formation and regulates metabolic status.[20] Other proteins that had significant increases in the MDCF-2 group but not in the RUSF group included thrombospondin-4, a multifunctional protein that is involved in the development of bone, skeletal muscle, and vascular and nervous systems,[21] and the osteoclast inhibitor SFRP4.

We performed an analysis of the plasma pro-

teomes among children in the MDCF-2 group according to the quartile of ponderal growth rate (change in the weight-for-length z score); each quartile included 15 children. We found that the children in the upper quartile of change had started off more wasted, had lower levels of mediators of bone growth and neurodevelopment, and had higher levels of proinflammatory proteins than those in the lower quartile of change. By the end of MDCF-2 supplementation, children in the upper quartile had the largest increases in mediators of bone growth and CNS development and the largest decreases in effectors of inflammation (Fig. S6A and S6B and Tables S10 and S11). Together, these results provide evidence that mediators of bone growth, neurodevelopment, and inflammation distinguished the effects of the MDCF-2 nutritional intervention from that of RUSF.

EFFECTS OF SUPPLEMENTATION ON GUT MICROBIOTA

To determine the effects of supplementation on gut microbiota, we obtained fecal samples every 10 days during the first month of the intervention and monthly thereafter. Quantitative polymerase-chain-reaction (PCR) assays showed no significant differences between treatments in the representation of 23 bacterial, viral, and protozoan enteropathogens (Table S12). A more comprehensive analysis was obtained by identifying bacterial taxa through sequencing of PCR amplicons generated from variable region 4 of 16S ribosomal DNA (rDNA) genes present in fecal biospecimens (Fig. S7). We then used linear mixed-effects models to determine the relationships between the levels of bacterial taxa, which were defined by the representation of amplicon sequence variants (ASVs), and the weight-for-length z score for each participant.

An analysis of the ASV data that were generated from 706 fecal samples with temporally matched anthropometric features showed that among the 209 ASVs that satisfied a set threshold for prevalence and abundance, 23 were significantly associated with the weight-for-length z score (called WLZ-associated taxa) in a linear mixed-effects model. Of these taxa, 21 were positively associated; the 2 taxa that were negatively associated were a bifidobacterium species (probably *B. longum*, ASV_1) and *Escherichia coli* (ASV_3) (Fig. 3A and Table S13). Six of the WLZ-associated taxa are members of the ecogroup network of bacteria that had been identified in a previous study involving healthy Bangladeshi infants and children who had normal development of their gut microbiota.[5] In that study, microbiota repair by MDCF-2 was accompanied by changes in 2 of the 6 WLZ-associated ecogroup taxa: increases in *Prevotella copri* and reductions in *B. longum*.[5] Five of the WLZ-associated ASVs that were identified in the present study share taxonomic similarity with bacteria identified as discriminatory for weight gain in our previous studies of gnotobiotic mice colonized with fecal microbiota from healthy versus malnourished children; they include *Faecalibacterium prausnitzii*, *Dorea formicigenerans*, *Ruminococcus gnavus* and a clostridium species. The complementary food ingredients that were included in MDCF-2 were selected on the basis of their abilities to increase the fitness and expressed beneficial functions of these age- and growth-discriminatory taxa.[8] An analysis of covariance between features of the plasma proteome and members of the gut microbiota revealed that the levels of these 21 WLZ-associated taxa had correlation with the 70 plasma proteins that were positively associated with changes in the weight-for-length z scores (Figs. S8, S9, and S10 and Tables S14 and S15). Details regarding the associations between bacterial taxa and dietary practices are provided in the Results section of Supplementary Appendix 1 and in Table S16.

The levels of WLZ-associated taxa increased significantly more in the gut microbiota of children in the MDCF-2 group than in those of children in the RUSF group ($P<0.001$) (Fig. 3B). Data regarding the association between the WLZ-associated taxa and the magnitude of changes in their relative levels during MDCF-2 treatment are provided in Figure S11 and Table S17. The greatest increases occurred with *P. copri* and *F. prausnitzii*, whereas the bifidobacterium species had the greatest decrease. In addition, we compared data from children in the MDCF-2 group and the RUSF group with data from children in Mirpur who had consistently healthy growth phenotypes (weight-for-length z score, ≥1) and normally developing microbiota. In this comparison, children in the MDCF-2 group had a significantly higher rate of change in levels of ASVs assigned to the

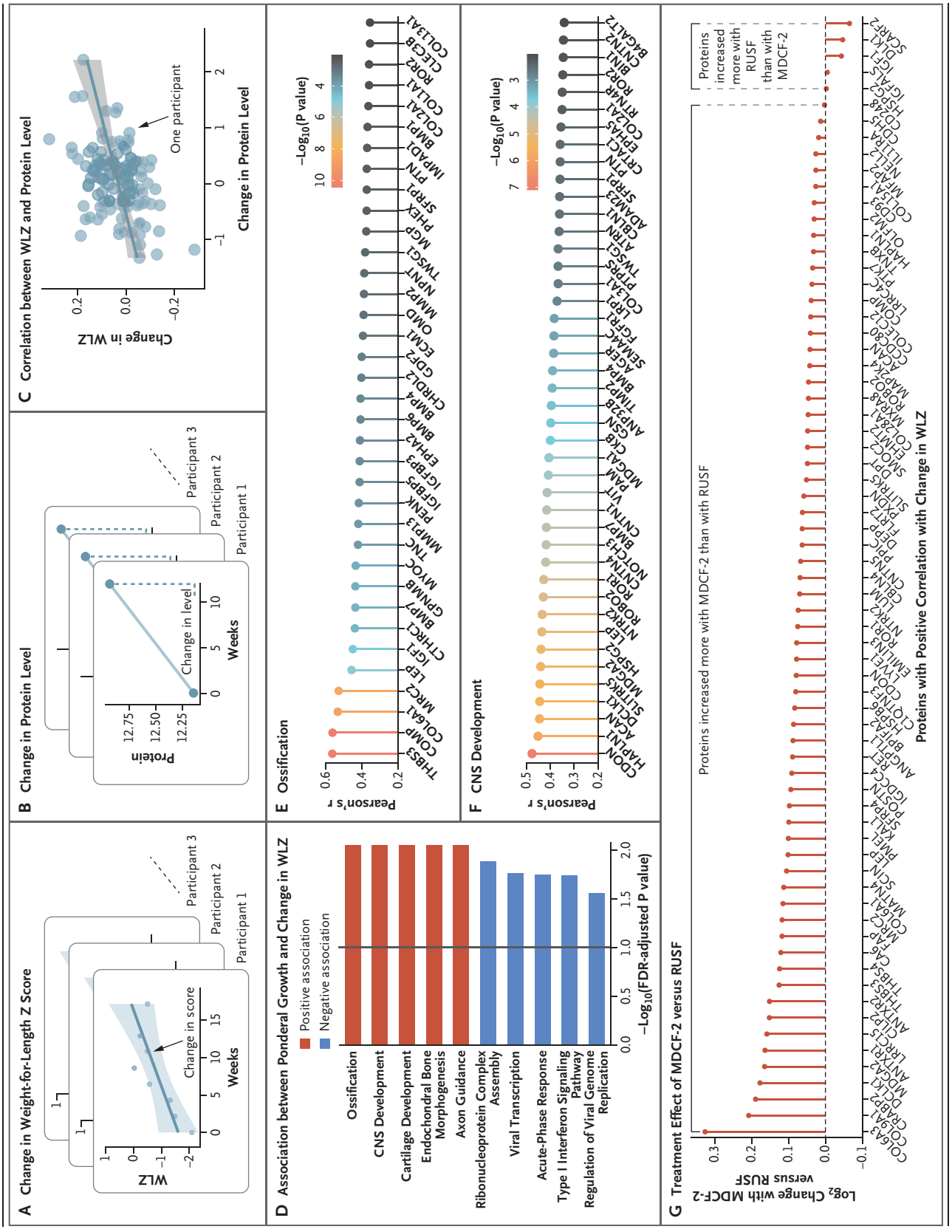

Figure 2 (facing page). Effects of Nutritional Intervention on Plasma Proteins.

Shown are schematic representations of the individual ponderal growth rates expressed as the weight-for-length z score (WLZ) (Panel A), the corresponding changes in protein levels before and after treatment (Panel B), and the overall correlation between these two values for a particular protein (Panel C). In Panels A, B, and C, sample graphs illustrate the calculations that were performed for all the participants. One assay, which simultaneously quantified the levels of 4977 proteins, was performed for each plasma sample. Gene-set enrichment analysis (GSEA) was used to identify biologic processes in the Gene Ontology database that were enriched for the proteins that had a significant correlation with the rate of ponderal growth (Panel D). P values were adjusted for the false discovery rate (FDR) (i.e., Q value) and were transformed to a negative $\log_{10}$ scale, with Q<0.10 indicating significance (vertical gray line). Proteins in which changes in levels were positively correlated with changes in weight-for-length z scores were significantly enriched (Q<0.10) for mediators of ossification (Panel E) and for development of the central nervous system (CNS) (Panel F). In these two panels, only proteins for which the correlation with the change in the weight-for-length z score reached an unadjusted P<0.01 are shown. Proteins are ordered according to the strength of the correlation and colored according to the P value, which was transformed to a negative $\log_{10}$ scale so that decreasing values indicate less statistical significance. Also shown are the differential effects of MDCF-2 or RUSF supplementation on proteins associated with the weight-for-length z score (Panel G). Proteins are ordered according to the $\log_2$ of the ratio of the treatment effect of MDCF-2 as compared with RUSF after 3 months of supplementation. GSEA was used to calculate the enrichment of proteins that had higher levels in the MDCF-2 group than in the RUSF group (P<0.001) for the set of 70 proteins that had a significant positive correlation (Q<0.10) with the change in the weight-for-length z score.

prevotella species (including *P. copri*), *F. prausnitzii*, olsenella species, and bifidobacterium species — taxa that were the most highly positively and negatively correlated with weight-for-length z scores. In contrast, children in the RUSF group did not have significant differences in the rate of change in levels of any of the 23 WLZ-associated ASVs (Fig. S12 and Tables S18 and S19). One month after cessation of supplementation, levels of a majority of MDCF-2–responsive, WLZ-associated ASVs had begun to fall. Although weight-for-length z scores were decreasing in both groups during this period, the between-group disparity in this measurement had become even more pronounced (Table 2 and Fig. S13).

DISCUSSION

We describe the results of a randomized, controlled feeding study that tested the effects of a microbiota-directed complementary food (MDCF-2) against an existing supplementary food (RUSF) on four measures of growth in Bangladeshi children between the ages of 12 months and 18 months who had moderate acute malnutrition. Even though MDCF-2 has a lower caloric density than RUSF, the rates of changes in two of these measures — weight-for-length and weight-for-age z scores — support the hypothesis that MDCF-2 promotes growth. We observed larger changes in plasma protein mediators of bone growth, neurodevelopment, and inflammation and more complete repair of the gut microbiota in children who received MDCF-2 than in those who received RUSF.

We did not attempt to test the effects of MDCF-2 and RUSF on body composition (changes in fat mass vs. lean mass). Chronic undernutrition in early life induces metabolic reprogramming that may enable a child to more efficiently capture and store energy as fat during periods of nutrient scarcity.[22] Although this metabolic shift is adaptive in the short term, it predisposes children to the development of diabetes, hypertension, and cardiovascular disease later in life.[23] MDCF-2 elicits a concerted change in WLZ-associated proteins, a number of which are effectors of bone growth and skeletal-muscle development. Some of these proteins have also been implicated in metabolic disorders.[24-30] However, augmenting the growth of bone and skeletal muscle may promote a rebalancing of the rapid catch-up in fat accretion that has been observed when undernourished children are given standard nutritional interventions. Such augmentation may also promote a more appropriate lean-to-fat mass ratio, which would simultaneously improve growth and provide protection from later obesity.[31,32]

Studies conducted in children with moderate acute malnutrition in Malawi and Ethiopia have shown that increases in fat-free mass during the first 2 years of life are associated with better cognitive and motor development.[33,34] Children who received MDCF-2 had increased plasma levels of proteins that are associated with axonal growth and CNS development; however, future studies are needed to determine whether changes in the circulating levels of these proteins re-

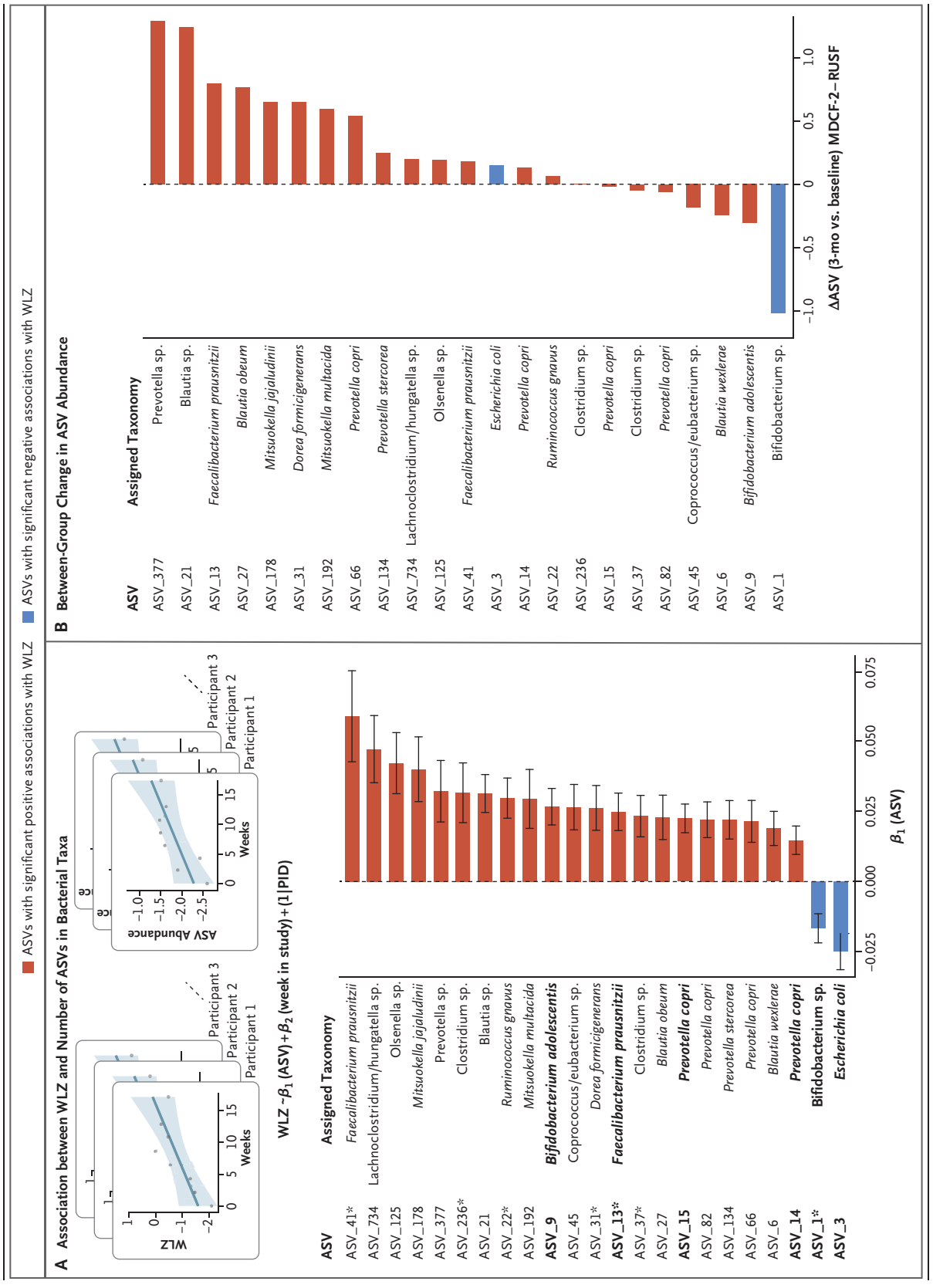

Figure 3 (facing page). Response of the Gut Microbiota to MDCF-2 and RUSF Supplementation.

Panel A shows linear mixed-effects modeling of the relationship between the weight-for-length z score and the number of bacterial taxa (amplicon sequence variants [ASV]) in the two study groups. Each assay simultaneously quantified the abundance of 209 ASVs in each fecal sample. The coefficient β_1 represents the change in the weight-for-length z score for a unit change in the variance-stabilizing, transformed level of an ASV. A random effect (indicated by a vertical bar) for a participant ID (PID) was included in the model to account for repeated measurements obtained from the same participant. Bar graphs indicate β_1 (the linear model coefficients) ±SE for each taxon that was significantly associated with the weight-for-length z score. ASVs in boldface were previously identified as taxa belonging to a so-called eco-group network consisting of 15 bacterial taxa that were detected during the first 2 years of life in children with normal development of the gut microbiota.[5] Asterisks indicate taxa that have previously been described as having associations with weight gain in gnotobiotic mice with gut microbial communities obtained from both healthy and undernourished children.[10] Panel B shows the change in variance-stabilizing transformed ASV counts (ΔASV) over the 3-month course of supplementation in the MDCF-2 group as compared with the RUSF group. A positive value for the difference indicates a greater average increase in a given taxon among the children in the MDCF-2 group, whereas a negative value indicates a greater average decrease. Gene-set enrichment analysis was used to quantify the number of taxa associated with weight gain that increased more in the MDCF-2 group than in the RUSF group ($P<0.001$).

flect changes in the brain itself. In addition, it will be important to follow cohorts of children with moderate acute malnutrition who have been treated with MDCF-2 for longer periods to assess whether the observed changes in these proteins correlate with clinical improvements in cognitive performance.

A hallmark of a successfully executed program of gut-community development in Bangladeshi children (and in those in other resource-poor settings) is the transition from a community dominated by *B. longum* during exclusive or predominant breast-feeding to one in which *Prevotella copri* becomes a major component during weaning.[5] Although *B. longum* has been associated with numerous beneficial outcomes in breast-feeding infants, the level of this organism was negatively associated with the ponderal growth rate in the children enrolled in the present study, which underscores the importance of considering how the timing of nutritional intervention aligns with the state of microbiota development.

Larger trials will need to be performed in disparate geographic regions to further assess the efficacy of this therapeutic approach for treating childhood undernutrition. The plasma and microbiota biomarkers that were identified in the present study should help enable better characterization and stratification of participants in future interventions.

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