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Facilitating a high-quality dietary pattern induces shared microbial responses linking diet quality, blood pressure, and microbial sterol metabolism in caregiver-child dyads

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ABSTRACT

Low-resource individuals are at increased risk of obesity and cardiovascular disease (CVD), partially attributable to poor dietary patterns and dysfunctional microbiota. Dietary patterns in childhood play critical roles in physiological development and are shaped by caregivers, making caregiver-child dyads attractive targets for dietary interventions to reduce metabolic disease risk. Herein, we targeted low-resource caregiver-child dyads for a 10-week, randomized, controlled, multifaceted lifestyle intervention including: nutrition and physical activity education, produce harvesting, cooking demonstrations, nutrition counseling, and kinetic activites; to evaluate its effects on dietary patterns, CVD risk factors, and microbiome composition. Subjects in the lifestyle intervention group improved total diet quality, increased whole grain intake, decreased energy intake, and enhanced fecal elimination of the microbe-derived metabolite lithocholic acid (LCA) in contrast to control subjects. Microbiomes were highly personalized, similar within dyads, and altered by lifestyle intervention. Differential modeling of microbiome composition identified taxa associated with total diet quality, whole grain intake, and LCA elimination including recognized fiber-degrading bacteria such as *Subdoligranulum*, and bile acid metabolizing organisms like *Bifidobacterium*. Inclusion of taxa identified in diet and metabolite modeling within blood pressure models improved prediction accuracy of microbiome-blood pressure associations. Importantly, microbiota-blood pressure relationships were shared between dyads, implying shared host-microbiota responses to lifestyle intervention. Overall, these outcomes provide insight into mechanisms by which dietary interventions impact the gut-cardiovascular axis to reduce future CVD risk. Registered at clinicaltrials.gov: NCT05367674

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Introduction

Shared individual and ecological factors, including the built environment, socioeconomic barriers, and behavioral patterns related to diet and physical activity influence the development of obesity and related chronic conditions including cardiovascular disease (CVD).[1,2] Minorities and those residing in low-resource communities are therefore at increased risk for CVD, in part due to lack of access to healthy foods and food environments promoting obesity.[3–6] As onset of CVD starts in childhood, children with obesity are at significantly increased risk, and 70% of obese children display at least one CVD risk factor.[7,8] This highlights the need for early-life nutrition and lifestyle interventions to reduce the risk of future CVD.

Dietary interventions that increase consumption of fiber-rich plant foods mitigate development of CVD in several ways, including modulation of enteric microbial metabolism and sterol absorption.

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Indeed, diet and other environmental factors impact the composition and function of the gut microbiome, accounting for greater inter-individual microbiome variability than genetics.[9,10] Additionally, high-quality dietary patterns rich in fiber-containing food sources are associated with more diverse and metabolically flexible enteric microbial populations, which are associated with reduced risk of obesity and enhanced cardiovascular function.[11–14] In this context, the intestinal microbiota modulate host cardiovascular health through the production of vasoactive metabolites including short-chain fatty acids (SCFA), biogenic amines, and sterols.[15–18] Modulation of microbial sterol metabolites, particularly bile acids (BA), represents a promising target to improve cardiovascular health. Through diverse yet understudied mechanisms, enteric microbiota modify host-derived (primary) BA to microbial (secondary) BA that are less soluble and thus resistant to intestinal reabsorption.[19] Synergistically, higher consumption of dietary fibers, such as those found in fruits, vegetables, and whole grains, also reduces absorption of BA and other sterols through physical interactions with the fiber matrix.[20,21] Ultimately, reduced absorption of these compounds enhances hepatic removal of circulating cholesterol, improving circulating lipid profiles and reducing CVD risk.[22] Improvement of dietary patterns through targeted interventions may therefore improve indicators of cardiovascular health and structure and function of the microbiome, reciprocally reducing CVD risk.[23]

Several interventions providing nutrition education and promoting lifestyle behavior change to children have been developed to mitigate weight gain during childhood. School-based gardening and cooking interventions result in significant improvements in dietary behaviors, including increased vegetable and total fiber intake, as well as clinical indicators of health such as reduced weight gain.[24–26] However, children are particularly vulnerable to suboptimal dietary and physical activity patterns during the summer months, when access to nutritious foods and opportunities to partake in organized physical activity are limited.[27–29] Further, many existing interventions do not involve family members who directly regulate food purchasing and preparation (i.e., nutrition gatekeepers) within the home. Inclusion of these nutrition gatekeepers, who are often parents and adult caregivers, is vital, as these individuals control the household nutrition environment.[30–33] Finally, caregivers highly influence eating behaviors formed in childhood, indicating early efforts have lifelong impacts.[34,35] Consequently, interventions targeting caregiver-child dyads offered during the summer months may be an effective strategy to improve health outcomes in low-resource child-caregiver dyads.[36,37]

Therefore, the goal of the present study is to assess the impact of a 10-week, summertime, high-fiber dietary intervention targeting low-resource caregiver-child dyads. Specific research questions include: 1) does an intervention that provides fiber-rich foods paired with evidence-based nutrition education to caregiver-child dyads improve caregiver diet quality and blood pressure; 2) are differences in blood pressure potentially mediated by diet-induced changes in microbiota structure and function; and 3) are responses of the microbiome and their relationship to blood pressure shared between caregiver-child dyads? We hypothesized that the intervention would improve diet quality and blood pressure compared to control, these changes would be mediated by microbiota structure and function (particularly through enhanced SCFA and sterol metabolism), and microbiome responses between members of caregiver-child dyads would be shared.

Herein, we establish for the first time in low-resource caregiver-child dyads that a multifaceted lifestyle intervention focusing on a high-fiber dietary pattern improves overall diet quality and fecal sterol elimination compared to control. These differences are underpinned by key alterations in microbiome structure and function, including bile acid metabolizing organisms, with some involved taxa shared between dyads with 100% matched sequence identity. In sum, we provide evidence of both the importance of dietary modulation on the structure and function of the enteric microbiome to reduce factors associated with CVD risk, and the ability of integrated dietary, microbiome, and metabolomic data to accurately identify microbes associated with blood pressure.

Results

Participant characteristics

In total, n = 28 caregivers (n = 13 intervention, n = 15 control) and n = 18 children (n = 10 intervention, n = 8 control) provided complete data and fecal samples for analyses (Table 1). The mean age for caregivers was 37.8 ± 4.8 years, most of which were female (85.7% of total). Approximately half of all caregivers identified as an under-represented minority group, though a greater proportion identified as White/Caucasian in the control group (60.0%) than those in the intervention (30.8%). Nearly three-quarters of those in the control group reported obtaining a college degree compared to 53.8% in the intervention. All caregivers reported status as the nutrition gatekeeper for the household. Mean age for children included in this study was 8.8 ± 0.5 years, with a nearly equal split between sexes in both groups. Similar to caregivers, approximately 50% of children in this study were Black/African American, South African, or Multiracial, with the remainder identifying as White/Caucasian.

Table 1. Sociodemographic Characteristics of Caregivers and Children Participating in a Randomized Controlled High-Fiber Dietary Intervention.

Participant Characteristics		SHA n (%) or Mean (SD)	MSP n (%) or Mean (SD)
Caregivers		**n = 13**	**n = 15**
Number of Children in Household, 18 & Under, Mean (SD)		2.4 (1.0)	2.3 (1.0)
Participating Adult Relationship to Child	Mother	12 (92.3)	11 (73.3)
	Father	1 (7.7)	3 (20.0)
	Other	0 (0.0)	1 (6.7)[a]
Is Participating Adult Nutrition Gatekeeper?	Yes	13 (100.0)	15 (100.0)
	No	0 (0.0)	0 (0.0)
Sex	Female	12 (92.3)	12 (80.0)
	Male	1 (7.7)	3 (20.0)
Age, Mean (SD)		37.1 (4.8)	38.4 (4.9)
Hispanic/Latino	Yes	1 (7.7)	0 (0.0)
	No	11 (84.6)	15 (100.0)
	Prefer Not to Answer	1 (7.7)	0 (0.0)
Race	White or Caucasian Only	4 (30.8)	9 (60.0)
	Black or African American Only	6 (46.1)	3 (20.0)
	South African	0 (0.0)	1 (6.7)
	White or Caucasian and Asian	0 (0.0)	1 (6.7)
	Black or African American, Pacific Islander, and Dutch	1 (7.7)	0 (0.0)
	Black or African American and Mixed	1 (7.7)	0 (0.0)
	Prefer Not to Answer	1 (7.7)	1 (6.7)
Marital Status	Married	6 (46.1)	10 (66.7)
	Never Married	3 (23.1)	4 (26.7)
	Divorced	2 (15.4)	0 (0.0)
	Member of an Unmarried Couple	2 (15.4)	1 (6.7)
Education	College Graduate	7 (53.8)	11 (73.3)
	Some College/Technical School	6 (46.1)	3 (20.0)
	High School Graduate/GED	0 (0.0)	1 (6.7)
Employment	Employed	11 (84.6)	10 (66.7)
	Unemployed	0 (0.0)	3 (20.0)
	Self Employed	2 (15.4)	0 (0.0)
	Student	0 (0.0)	1 (6.7)
	Other	0 (0.0)	1 (6.7)[b]
Household Income	> $50,000	6 (46.1)	9 (60.0)
	$10,000-$49,999	5 (38.5)	4 (26.7)
	< $10,000	0 (0.0)	1 (6.7)
	Unknown	1 (7.7)	1 (6.7)
	Prefer Not to Answer	1 (7.7)	0 (0.0)
Children		**n = 10**	**n = 8**
Age, Mean (SD)		8.9 (0.3)	8.8 (0.7)
Sex	Female	6 (60.0)	4 (50.0)
	Male	4 (40.0)	4 (50.0)
Hispanic/Latino	Yes	0 (0.0)	0 (0.0)
	No	10 (100.0)	8 (100.0)
Race	White or Caucasian Only	5 (50.0)	4 (50.0)
	Black or African American Only	4 (40.0)	2 (25.0)
	South African	0 (0.0)	1 (12.5)
	White or Caucasian and Black or African American	0 (0.0)	1 (12.5)
	Black or African American, Pacific Islander, and Dutch	1 (10.0)	0 (0.0)

SHA, Summer Harvest Adventure; MSP, My Summer Plate
[a]Aunt; [b]Homemaker

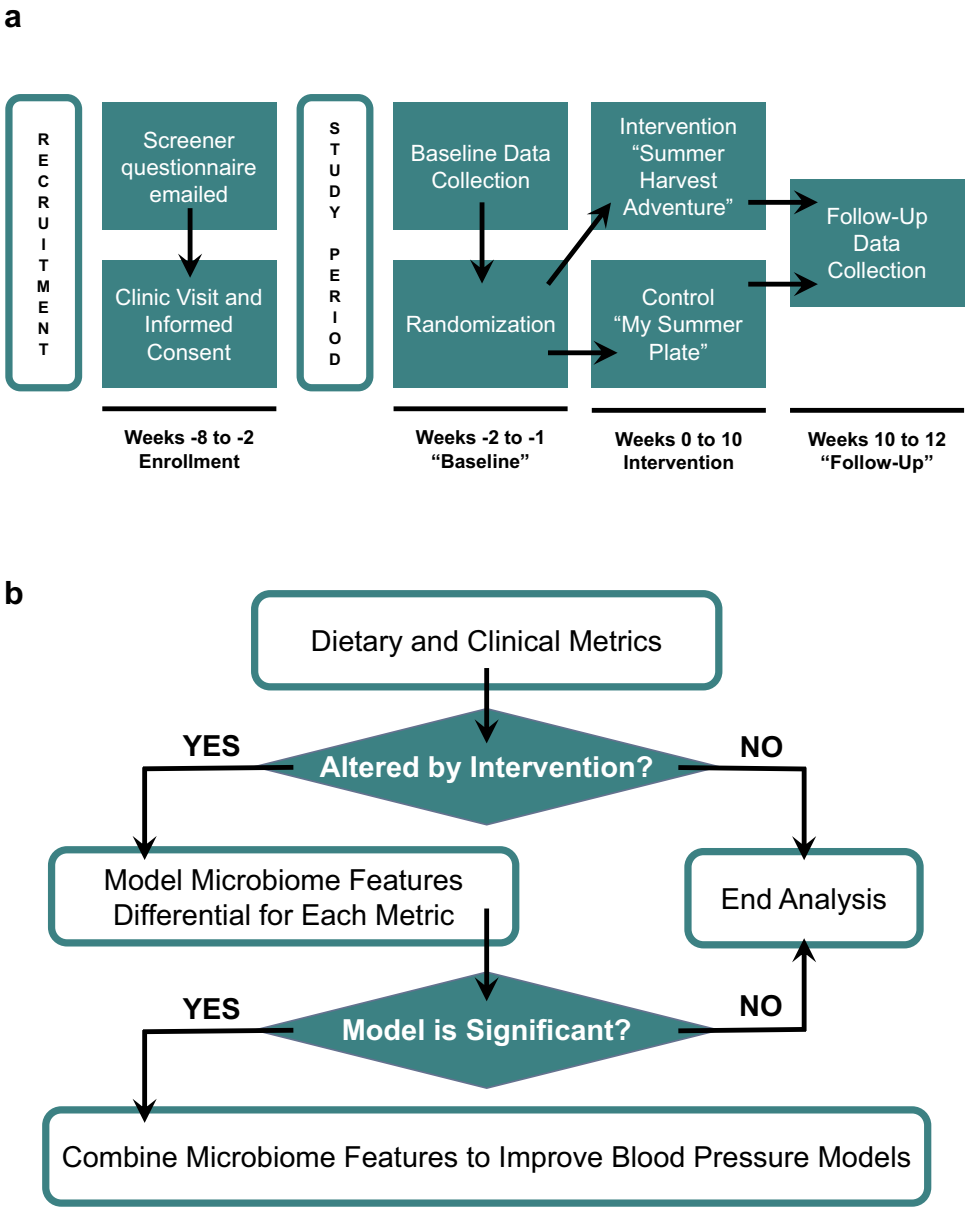

Figure 1. Study design and sequence data analysis workflow. A) Dyads composed of children aged 8–9 years and their adult caregivers were recruited and randomized to either the intervention or control group for a 10-week nutrition and lifestyle intervention. B) Analysis of 16S rRNA gene amplicon sequencing data from caregiver-child dyads was conducted to identify fecal microbes associated with dietary and clinical parameters with the ultimate goal of identifying microbes highly associated with blood pressure.

Dietary intervention improves diet quality and is associated with dyad microbiome composition

To assess the effect of the intervention on diet quality, CVD risk factors, and microbiome composition, 30-day food frequency questionnaires (FFQs) and blood pressure measurements were employed in conjunction with 16S rRNA gene amplicon sequencing analyses. The intervention improved caregiver total Healthy Eating Index (HEI), indicating improved compliance with the Dietary Guidelines for Americans (Figure 2a). Skin carotenoids were likewise improved; the intervention increased caregiver skin carotenoids, which were positively correlated with total HEI in the intervention group ($r = 0.63$, $p < .001$), but not in the control group ($r = 0.21$, $p = .26$, Figure 2b-c). The intervention reduced caregiver energy intake,

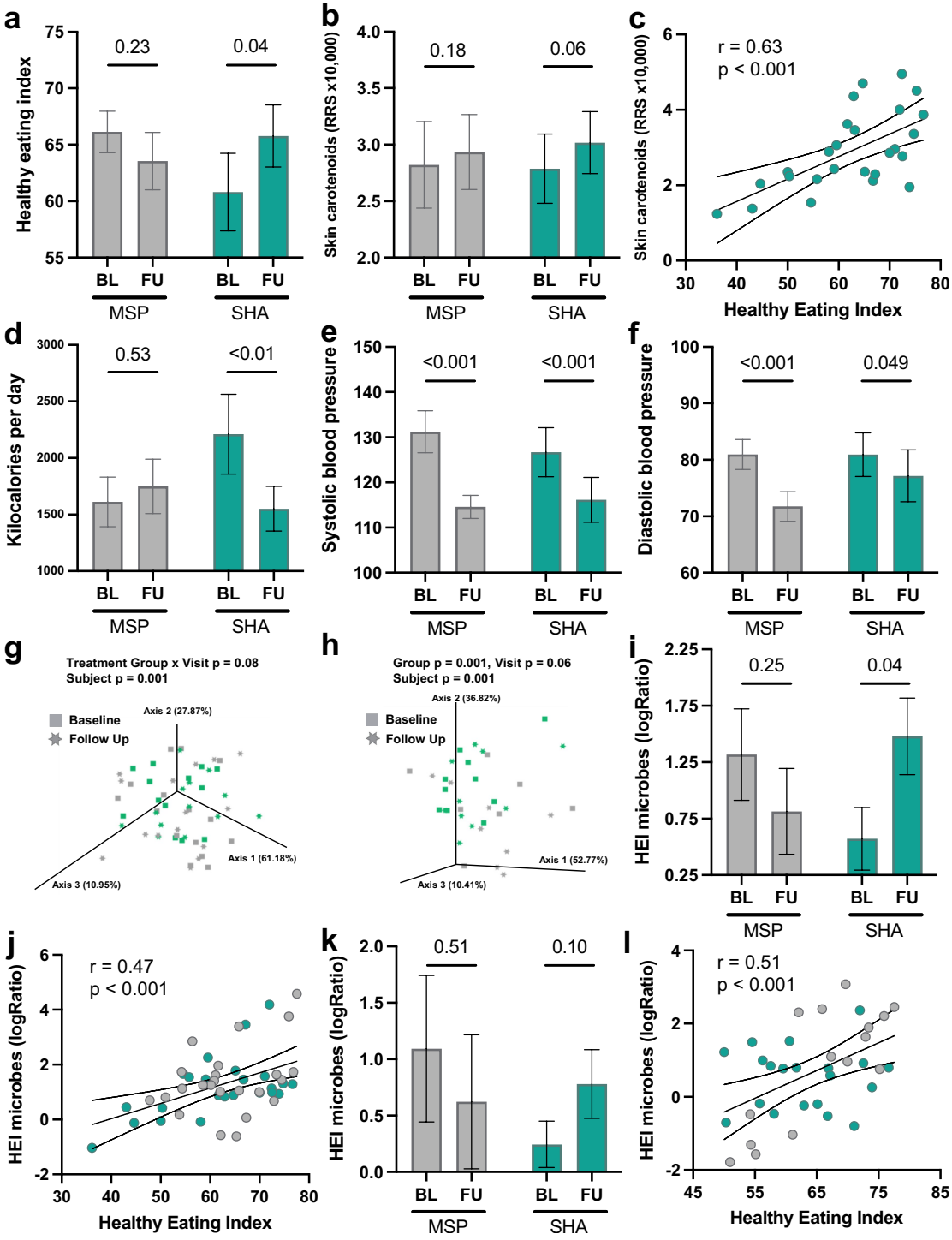

Figure 2. Dietary intervention improves dietary pattern and is associated with caregiver-child dyad microbiome composition. A) Caregiver dietary patterns assessed by 30-day food frequency questionnaires is improved by SHA intervention. B-C) Caregiver skin carotenoids (an indicator of fruit and vegetable intake) tended to be increased by SHA, and were correlated to HEI in the SHA group. D) SHA reduced caregiver energy intake. E-F) SBP and DBP were decreased in both groups during the study period. G) Caregiver microbiomes were highly individualized and tended to cluster as an interaction between treatment group and study time point via Aitchison beta diversity. Each point represents the entire fecal microbiome of a sample and closer points represent more-similar microbiomes. H) Child microbiomes were also highly individualized, clustered by treatment group, and tended to be altered by the intervention. I-J) Microbial cohorts related to HEI via differential modeling were increased by SHA in caregivers, and were correlated to HEI. K-L) Using caregiver diet as a proxy for child diet, child microbial cohorts related to HEI tended to be increased by SHA, and were correlated to HEI.

and both groups experienced a decrease in systolic blood pressure (SBP) and diastolic blood pressure (DBP) from baseline to follow-up (Figure 2d-f). Child blood pressure was slightly higher in the intervention group, but >80% of children at each time point were within the normal range. Summary biometric and diet data are included in Supplementary Tables 1 and 2.

Analyzing caregiver-child dyad microbiomes together, microbiome composition was highly related within dyads (Aitchison beta diversity, ADONIS, family ID variable $p < .001$), but also different between dyad components (Aitchison beta diversity, ADONIS dyad component variable $p < .001$) and treatment group (i.e. intervention vs control) (Aitchison beta diversity, ADONIS group variable $p < .001$). Granted the recognized differences between adult and child microbiomes, further analyses were completed for children and caregivers separately. Caregivers' microbiomes were highly individualized and tended to diverge between the two treatment groups over time (Figure 2g). Notably, children's microbiomes were different by treatment group (main effect $p = .001$) and tended to be different by visit (main effect $p = .06$) (intervention vs control at baseline $p = .17$, intervention vs control at follow-up $p = .08$), while still highly individualized (subject main effect $p = .001$) (Figure 2h). There were no significant differences detected by alpha diversity metrics (data not shown).

Differential multivariate modeling can be utilized to identify relationships between dietary patterns and microbiome composition while accounting for the compositional nature of sequencing data. Leveraging this technique, cohorts of microbiota that shift relative to diet (positively and negatively) are detected and represented as a logRatio of the relative abundances of the amplicon sequence variants (ASVs) involved. Microbial cohorts differential for adult total HEI score (Supplementary Table 3) were increased by the intervention and correlated to total HEI (Figure 2i-j). Using caregiver (i.e., nutrition gatekeeper) diet as a proxy for their child counterparts (see "Assessment of dietary intake" Methods), child microbiomes were also analyzed for their relationship to total HEI. While the microbial cohorts identified as differential for total HEI in children did not significantly change during the study period, they were more strongly correlated to total HEI than their caregiver counterparts (Figure 2k-l).

Importantly, some ASVs (exact sequence matches) responsive to diet were shared by both caregivers and children, implying shared microbial responses to diet (Supplementary Table 3). One ASV from each of the genera *Bifidobacterium* and *Subdoligranulum* were positively associated with total HEI in both dyad components, while one ASV from the genus *Agathobacter* was inversely associated with total HEI by dyad components (positively in caregivers, but negatively in children).

Dyad microbiome composition is related to whole grain intake and systolic blood pressure

To understand what food groups and microbes were related to differences in blood pressure, individual HEI component scores were evaluated from baseline to follow-up, and microbiota differential for food groups of interest and blood pressure were modeled. Caregiver whole grain (WG) HEI component scores were the only subcategory altered by intervention and were improved in the intervention group (Supplementary Table 2, Figure 3a). Similar to total HEI, microbial cohorts differential for WG score were increased by the intervention and correlated to WG score (Figure 3b-c). Unlike total HEI score, cohorts differential for WG score in children were both enhanced by the intervention and correlated to WG score (Figure 3d-e). Consistent with total HEI, some ASVs related to WG score were shared between dyads (Supplementary Table 4). Two ASVs from each of the genera *Clostridium senu stricto 1*, and *Coprococcus* were positively associated with WG score, reinforcing the idea that microbial responses to diet are shared by dyads.

In contrast to dietary parameters, direct modeling of microbes differential for blood pressure yielded modest results. While cohorts of microbes differential for blood pressure were reduced in both adult groups during the study period, they were mildly correlated to SBP (figure 3f-g). Taxa differential for SBP were not altered by intervention in

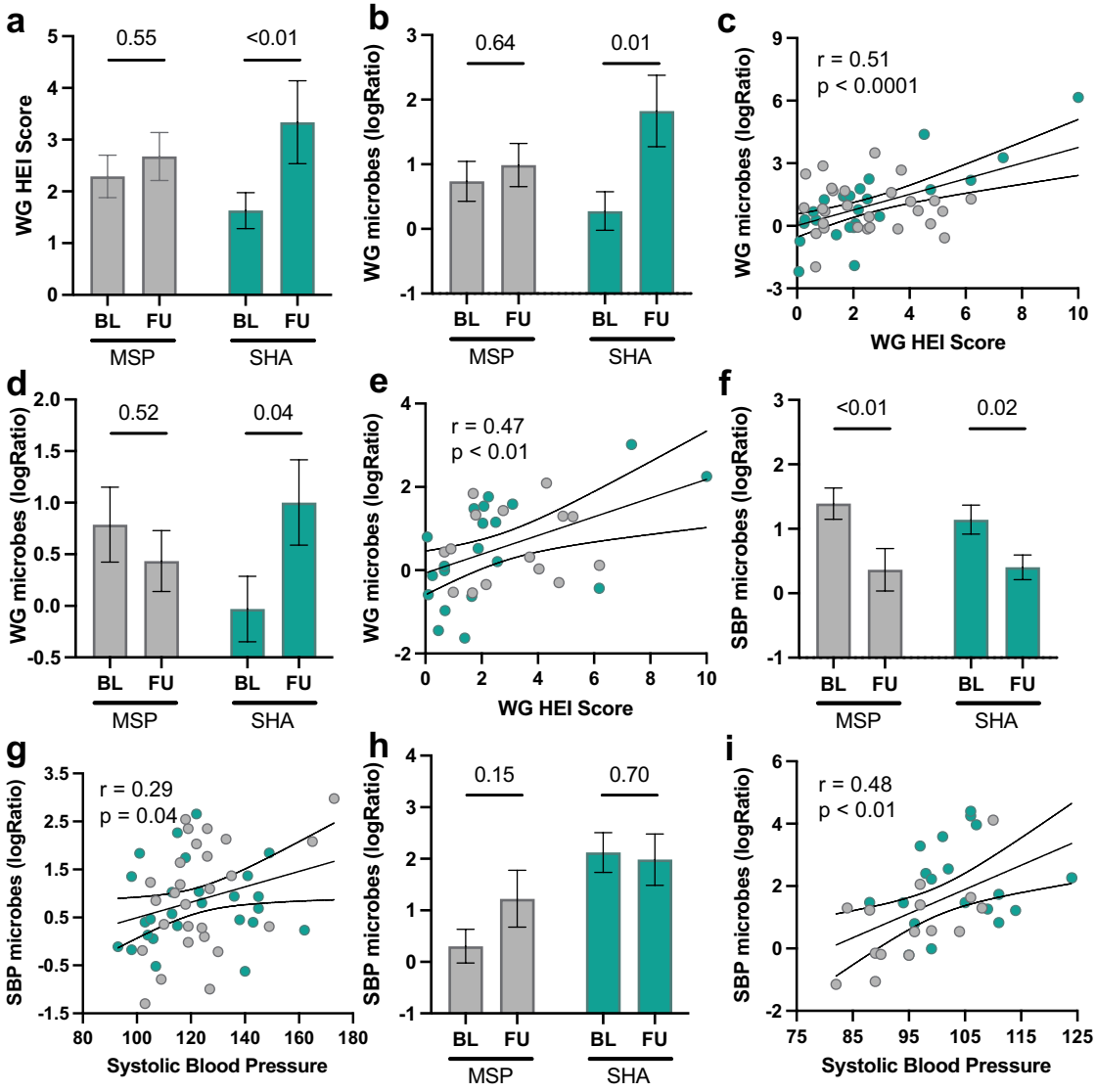

Figure 3. Caregiver-child dyad microbiome composition is related to whole grain intake and systolic blood pressure. A) Caregiver WG component score of HEI was increased by SHA. B-C) Microbial cohorts related to the WG score via differential modeling were increased by SHA in caregivers, and were correlated to WG score. D-E) Using caregiver diet as a proxy for child diet, child microbial cohorts related to WG score were increased by SHA, and were correlated to WG score. F-G) Microbial cohorts related to SBP were decreased in both caregiver groups, and were correlated to SBP. H-I) Although microbial cohort related to SBP in children was not altered by treatment, the cohort was related to SBP.

children but were correlated to SBP (Figure 3h-i). No significant relationships were identified between microbial composition and DBP in either caregivers or children. Once again, some ASVs related to SBP were shared between dyad components (Supplementary Table 5). While two ASVs from the genus *Blautia* were negatively associated with SBP in both dyad components, two ASVs from the genus *Dorea* were inversely associated with SBP by dyad component (positively in caregivers, but negatively in children.)

Dietary intervention enhances elimination of microbial sterol metabolites related to cardiovascular disease

To better understand how diet–microbiota interactions may be influencing blood pressure, we implemented targeted fecal metabolomics of microbial metabolites recognized to influence cardiovascular function. SCFA are implicated in the regulation of blood pressure through modulation of vasodilation, inflammation, and sympathetic neuronal activities.[38]

However, fecal SCFA were not impacted by intervention in this study; no differences were detected in caregivers, and only caproic acid was increased in children in the control group from baseline to follow-up (Supplementary Table 6).

Sterols, namely cholesterol and BA metabolites, are recognized modulators of CVD risk, including blood pressure.[16,39–42] Of all BA surveyed, only caregiver excretion of total lithocholic acids (sum of both conjugated and unconjugated) (LCA) was enhanced by the intervention (Figure 4a, Supplementary Table 7). Additionally, caregiver excretion of cholesterol was enhanced by the intervention despite no changes in dietary cholesterol intake (Figure 4b-c). Unlike blood pressure, microbial cohorts differential for fecal LCA in caregivers were highly enhanced by the intervention and correlated to fecal LCA (Figure 4d-e) (Supplementary Table 8). Changes in BA excretion were more pronounced in children. At the end of the study period, children in the control group reduced fecal excretion of the conjugated BA taurohyodeoxycholic acid, glycohyodeoxycholic acid, taurodeoxycholic acid, and glycodeoxycholic acid, and the unconjugated BA ω-muricholic acid/α-muricholic acid (Supplementary Table 7). Conversely, children in the intervention group had enhanced excretion of total conjugated BA, specifically glycohyocholic acid, glycoursodeoxycholic acid, and glycocholic acid (Supplementary Table 7). Although LCA excretion was not altered in children, microbial cohorts differential for LCA excretion were identified and correlated to fecal LCA, but these cohorts were unchanged during the study period (figure 4f-g) (Supplementary Table 8). Microbial cohorts differential for cholesterol excretion were not altered in caregivers nor children but were correlated to fecal cholesterol (Figure 4h-k) (Supplementary Table 9).

Multiple ASVs differential for LCA and some ASVs differential for cholesterol were shared between dyads (Supplementary Tables 8 and 9). Nine ASVs from the genus *Bifidobacterium* and one ASV from *Terrisporobacter* were positively associated with LCA excretion, while one ASV from the genus *Turicibacter* was negatively associated with LCA excretion in both dyad components. One ASV from the *Ruminococcus torques group* was positively associated with cholesterol excretion, while two ASVs from *Clostridium sensu stricto 1* were negatively associated with cholesterol excretion in both dyad components. One unidentified bacterial ASV and two ASVs from the *Ruminococcus gauvreauii group* were conversely associated with cholesterol excretion in children vs caregivers.

Incorporation of microbes related to diet and microbial metabolites improves modeling of microbes related to blood pressure and reveals shared microbial responses in caregiver-child dyads.

With relationships established between microbial composition and both dietary patterns and microbial metabolites associated with CVD risk, we hypothesized that incorporating information from these models could enhance our ability to identify microbial populations more robustly associated with differences in blood pressure. By combining taxa represented across the diet and metabolite models (described in Methods), combined microbial differentials were generated for caregivers and children (Figure 5a-b, Supplementary Table 10). While more genera were identified in the caregiver combined differential microbial cohort overall, taxa were again shared between dyads including *Bifidobacterium, Subdoligranulum*, and *Ruminococcus Torques group* being negatively associated with blood pressure, and *Turicibacter* being positively associated with blood pressure in both caregivers and children. Caregivers in the intervention group increased representation of this bacterial cohort from baseline to follow-up compared to those in the control group (Figure 5c). As hypothesized, the incorporation of microbial taxa associated with dietary patterns and microbial metabolic responses did generate cohorts of microbiota more robustly associated with SBP and DBP values compared to initial blood pressure modeling (Figure 5d-e). Similarly, in children, the intervention enhanced the combined differential cohort compared to control, which was correlated to SBP but failed to reach significance when correlated to DBP (figure 5f-h). Given the high overlap of ASVs included in the final blood pressure-related child and caregiver microbial cohorts, we wanted to understand the extent to which these shared associations were specific to each dyad pairing vs effects within treatment

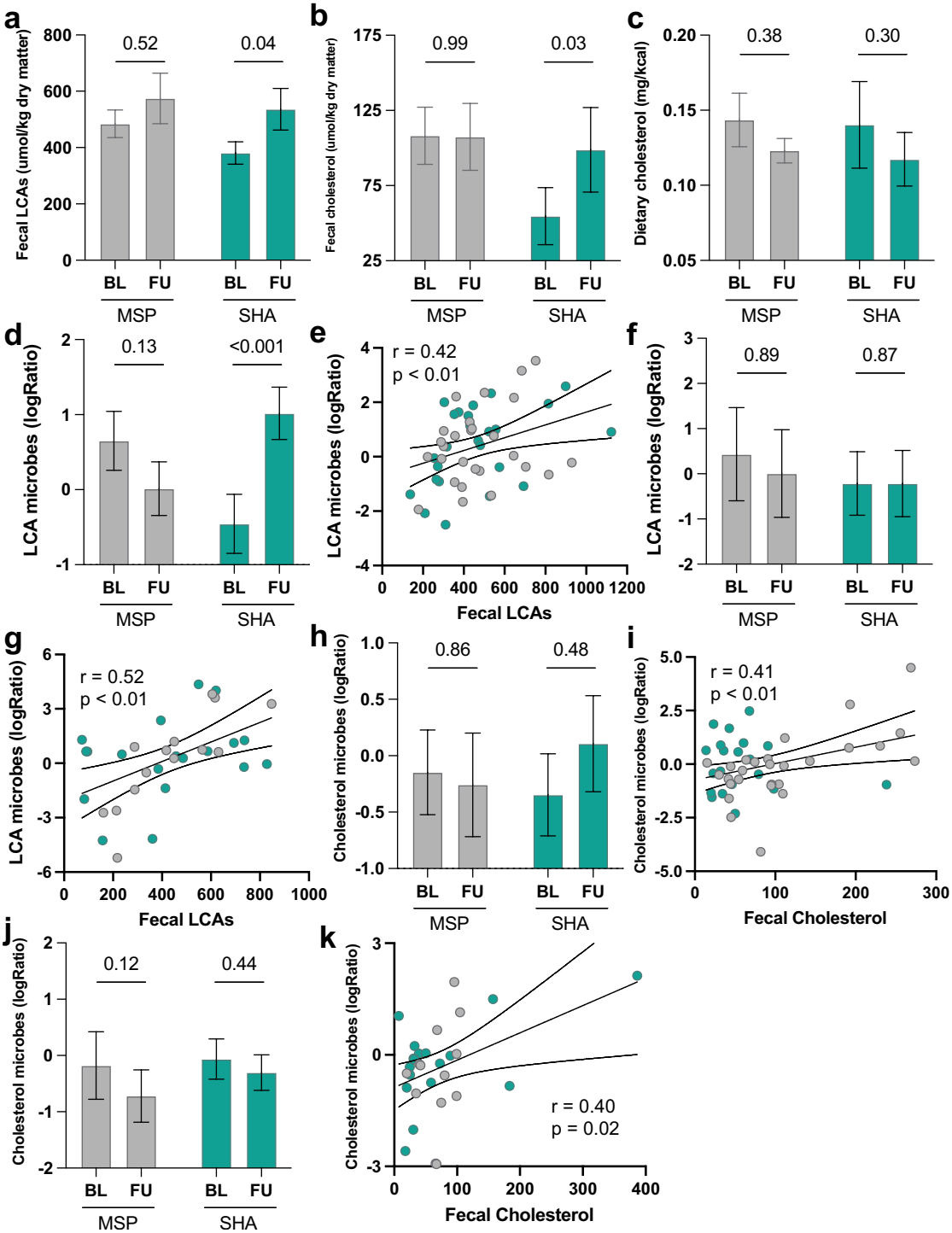

Figure 4. Dietary intervention enhances elimination of microbial sterol metabolites related to cardiovascular disease in caregivers. A) Caregiver fecal elimination of the microbial BA metabolite, LCA, is increased by SHA. B-C) SHA enhances caregiver fecal elimination of cholesterol despite no changes in dietary cholesterol intake. D-E) Microbial cohorts related to fecal LCA via differential modeling were increased by SHA in caregivers, and were correlated to fecal LCAs. F-G) Although microbial cohorts related to fecal LCAs in children was not altered by treatment, the cohort was correlated to fecal LCAs. H-I) Although microbial cohort related to fecal cholesterol in caregivers was not altered by intervention, the cohort was correlated to fecal cholesterol. J-K) Although microbial cohort related to fecal cholesterol in children was not altered by intervention, the cohort was correlated to fecal cholesterol.

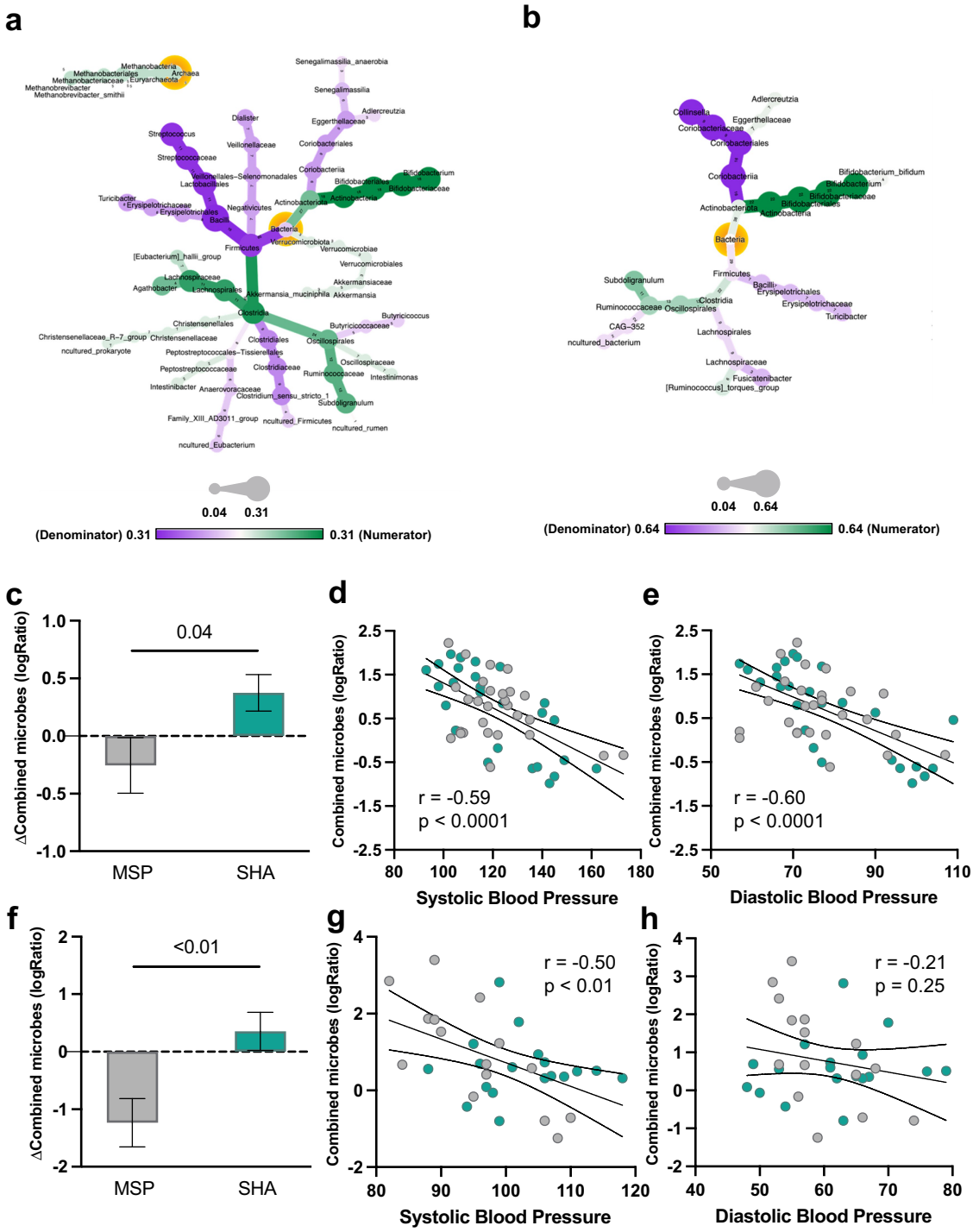

Figure 5. Incorporation of microbes related to diet and microbial metabolites improves modeling of microbes related to blood pressure and reveals shared microbial responses in caregiver-child dyads. A) Microbes shared by caregiver differential models for HEI, WG, SBP, LCA, and cholesterol create a combined differential microbial cohort. Heat trees display phylogenetic trees of taxa included in the combined differential microbial cohorts, with color and size increasing with the relative influence each taxa provides within the numerator (increases logRatio value, negatively associated with blood pressure) or denominator (decreases logRatio value, positively associated with blood pressure) of the differential. Roots are indicated by golden halos, nodes represent each taxonomic level, and edges are labeled with the number of ASVs contributing to each node. B) Combining microbes shared by child differential models reveals shared microbial relationships between caregiver and child microbiomes. C-E) Microbial cohorts in the caregiver combined differential are enhanced by SHA compared to MSP, and display an improved relationship to SBP and DBP than modeling blood pressure alone. F-H) Microbial cohorts in the child combined differential are enhanced by SHA compared to MSP, and display an improved relationship to SBP and DBP than modeling blood pressure alone.

group. Changes in their respective combined microbial cohorts were correlated within dyad pairs (r = 0.52, p = .037), but the probability that a correlation of the same or higher intensity would occur randomly within this population (via bootstrapping) was estimated at 54%. Taken together, this indicates that there is some shared intensity and directionality of the responsing microbiota within dyads, but that these differences are also a shared group response to treatment.

Discussion

Interactions between dietary patterns and the intestinal microbiota play central roles in disease development, particularly in the context of health disparities.[43–45] In this randomized, controlled trial, we establish for the first time that provision of fiber-rich food plus intensive nutrition education improved microbial composition related to CVD risk factors in low-resource caregiver-child dyads. Importantly, some alterations in these microbial cohorts were shared between dyad components, implying that both children and caregivers have some shared responses to the changed nutrition environment and potentially reduced future CVD risk.

Multiple dietary patterns have been associated with lower risk of cardiovascular and metabolic disease, such as the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diets.[46,47] As also recommended by the Dietary Guidelines for Americans, a major component of these dietary patterns is regular consumption of high-fiber foods including fruits, vegetables, and whole grains. High-fiber dietary patterns alter structure and function of the intestinal microbiota through provision of fermentable and structural nutrients, and other recent studies connect consumption of healthy dietary patterns to altered microbiome structure and function.[48] Although the chemical composition, concentration, and duration of fiber consumption can have variable effects on the taxa enhanced or suppressed, relative abundance of *Bifidobacterium spp*. is dependably elevated by fiber consumption in humans.[49,50] Consistently, we found *Bifidobacterium spp*. to be associated with total HEI and WG HEI component score in both children and adults in this population. Other microbial taxa associated with improved diet quality in this study include recognized fiber-degrading bacteria like *Subdoligranulum* and various *Ruminococcus spp.*,[51,52] and the archaea *Methanobrevibacter smithii* which plays a critical role in efficient microbial fermentation of fiber through hydrogen metabolism.[53] While these microbes are generally associated with health, these associations are likely due to production of diverse microbiota-specific metabolites rather than metabolites of a single class. Thus, future studies should investigate the mechanisms by which these taxa may influence community-level metabolism of various fiber-derived substrates.

Microbiota-mediated elimination of several sterol metabolites is linked to improved cardiovascular health.[39,41,42] LCA excretion in particular has been linked to reduced risk of atherosclerosis and coronary artery disease.[39,54] In the present study, improved diet quality in the intervention group was concurrent with enhanced LCA excretion, and microbial taxa associated with higher diet quality were also associated with higher fecal LCA. *Bifidobacterium spp*. were highly associated with LCA excretion (nine positively associated ASVs) and were notably shared between dyads. While many members of the intestinal microbiota have been studied for the ability to modify host-derived BA (and many of the enzymes involved remain uncharacterized),[55,56] *Bifidobacterium spp*. are well-studied for their ability to deconjugate primary BA,[57,58] a critical first step in secondary BA formation.[59] Furthermore, these processes are sensitive to dietary modulation. Through fiber fermentation, generation of organic acids reduces pH of the intestinal lumen.[60] This shift to acidic pH drives accumulation of deconjugated BA and cholesterol in *Bifidobacterium spp*., encouraging sterol elimination as they pass out of the gastrointestinal tract, while also reducing luminal BA solubility and reabsorption.[61,62] Studies in adults habitually consuming low amounts of fiber have demonstrated increased fecal primary BA excretion in response to a high-fiber intervention, particularly those associated with whole grains.[63,64] Notably, no such data exists for children. While caregivers in the intervention group of this study did not enhance primary BA excretion in tandem with increased whole grain intake, children participating in the

intervention exhibited higher excretion of the conjugated primary BA glycocholic acid at follow-up. Dietary intake in children is notoriously difficult to assess accurately,[65] but future studies could directly administer whole grains to children to validate these findings.

Recent investigations reveal intestinal microbiome composition as a key determinant of personalized physiological responses to diet and indicators of cardiovascular health, including blood lipid profiles.[66–68] This was reflected in our improved ability to identify features of the microbiome associated with blood pressure upon inclusion of dietary and microbial metabolite data. Furthermore, this dietary intervention enhanced representation of those microbiota in low-resource caregiver-child dyads in a short 10-week period. Although both caregiver groups experienced reductions in blood pressure over the study period, this is typical during the transition from winter to summer seasons.[69–71] Importantly, while dyads in the intervention group increased representation of microbiota associated with lower blood pressure during the study period, representation of the same microbiota declined in the control group. In this way, providing access to foods encouraged within healthy dietary patterns and related nutrition education may represent a feasible means to reduce CVD risk in low-resource populations as mediated through the intestinal microbiome. However, long-term studies to determine persistence of microbial modulation and longitudinal disease risk are necessary to test this hypothesis.

Several strengths and weaknesses of this trial should be noted. This study covers a diverse, low-resource, and underrepresented population that is rarely studied in nutrition research. Furthermore, it employs the unique setting of caregiver-child dyads, allowing for a family-based understanding of intervention principles and efficacy. These analyses also integrate multi-omics associated with dietary intake, microbiome composition, and quantitative metabolomics to gain a better understanding of how these components interact to influence CVD risk factors. However, this study was limited in several aspects. Although implemented as a randomized, controlled trial, the donation of fecal samples was optional, thus impacting true randomization and potentially biasing toward those subjects with higher socioeconomic status (although still within the target, low-resource population) or an unequal balance between groups on other demographic factors such as race. Also due to the optional nature of sample donation, the resulting small sample size of this cohort limits the generalizability of the results. Finally, there is potential for self-report bias as participants may report healthier behaviors (e.g. healthier dietary patterns) compared to reality; however, the documented improvement over time as well as concomitant improvement in biological values (i.e. skin carotenoids, microbial metabolites, etc.) challenges this phenomenon.

In summary, we demonstrate that consumption of a high-quality diet rich in fiber-containing foods facilitates structural and functional changes in the intestinal microbiome, thus supporting cardiovascular health in low-resource dyads. This is the first study investigating the gut-cardiovascular axis within a clinical trial for low-resource dyads, providing important insights into potential mechanisms by which early dietary intervention may impact the gut microbiome to reduce future disease risk. Additional studies should be conducted to characterize microbial metabolic responses to specific dietary components and determine whether these shifts in the microbiome impact longitudinal cardiovascular disease risk.

Methods

Participants

Caregiver-child dyads residing in low-resource communities from a large Midwestern U.S. city were recruited through local public schools meeting federal eligibility guidelines for free and reduced-priced breakfast and lunch. Eligible participants were: (a) caregiver-child dyads consisting of one child in the summer, aged 8–11 years, and one caregiver residing in the same home; (b) English-speaking; (c) residents of a low-resource community; and (d) with ability to consume fruits and vegetables who consented to participation. Individuals were ineligible if they were diagnosed with mental or physical disabilities that would impair full participation in all components of the intervention; displayed communication difficulties

(e.g., non-English speaking, severe developmental delays); lacked transportation to weekly classes or harvesting activities; reported consumption of herbals, botanicals, or nutritional supplements; were diagnosed with active metabolic or digestive illness that may result in nutrient malabsorption (e.g., Crohn's disease, food allergies); refused to sign informed consent/assent. All participants provided written, informed consent. Participants were enrolled and completed baseline assessments between May and June 2019, and post-intervention assessments were completed in August 2019. All procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983, and the present study was registered with clinicaltrials.gov as NCT05367674 and approved by The Ohio State University Institutional Review Board.

Study design

Participants in this study include a sample of individuals enrolled in a two-arm, parallel-design, randomized controlled trial intended to determine the efficacy of a 10-week multifaceted obesity prevention and lifestyle (nutrition and physical activity) intervention in low-resource caregiver-child dyads called "Summer Harvest Adventure" (Figure 1a).[36,37] The randomization scheme was developed by the study statistician using a pseudorandom number generator employing permuted block randomization of varying block size of four or six, chosen randomly within a sequence with equal probability. Given the nature of the study, intervention allocation could not be concealed from participants or research staff. All participants were assigned a unique alphanumeric study ID to anonymize them for data analyses.

All participants completed data collection upon enrollment, where demographic and health questionnaires and caregiver dietary intake assessments were completed and clinical indicators measured. As part of a nested feasibility pilot within the parent intervention, optional collection of fecal samples was also completed. Participants assigned to the intervention group, Summer Harvest Adventure (SHA), underwent a 10-week intervention. Dietary intervention components included weekly: (a) in-person group nutrition education based on the Dietary Guidelines for Americans; (b) harvesting of fresh fruits, vegetables, and herbs at the study garden; (c) cooking demonstrations with taste-testing; and (d) remote nutrition counseling informed by motivational interviewing from trained registered dietitians for caregivers. Within each weekly session, 15 minutes were allocated to basic education on evidence-based physical activity recommendations and opportunities for kinetic activity (e.g., outdoor games). Those enrolled in the control group, My Summer Plate (MSP), received written nutrition education materials at the enrollment data collection visit but were not provided any other intervention components. After 10 weeks, both intervention and control groups returned for post-intervention data collection, where assessments were repeated. Of those who attended both enrollment and post-intervention visits, only subjects who provided the following samples at both time points were included in these analyses: caregivers who provided biometrics, fecal samples, and dietary intake data; and children who provided biometrics and fecal samples.

Sociodemographic and health questionnaires

Each member of the caregiver-child dyad completed sociodemographic and health questionnaires at enrollment and post-intervention data collection visits using a secure online data collection tool, Research Electronic Data Capture (REDCap). Modified Behavioral Risk Factor Surveillance System (BRFSS) and Supplemental Nutrition Assistance Program Education (SNAP-Ed) questionnaires were employed to assess demographic characteristics and health-related behaviors, respectively.[72,73]

Assessment of clinical indicators

Caregiver and child clinical and anthropometric data were measured by trained study personnel at data collection visits and recorded using REDCap data collection tools. Participants were asked to hydrate before visits and to wear light clothing free of metal and remove shoes prior to assessment of height and weight. Height was measured to the nearest 1 mm using standard protocols via a stadiometer with a movable headboard (Seca 213, Seca North America, Chino, CA, USA). Weight and body fat percentage were measured using a digital body composition monitor equipped with bioelectric impedance analysis (BIA) technology and appropriate

for body fat analysis for adults and children aged 5 to 17 years (Tanita SC-331S Total Body Composition Analyzer, Tanita Corporation, Tokyo, Japan). Weight was recorded to the nearest 0.1 kg. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained by standard clinical methods on the right side of the body using an automated blood pressure cuff (Omron Autocuff, Omron Healthcare Co. Ltd., Lake Forest, IL, USA). All participants were asked to rest in a seated position for 5 min prior to blood pressure assessment.

Assessment of dietary intake

Caregiver dietary intake was assessed at enrollment and post-intervention using an online-delivered food frequency questionnaire (FFQ) with a recall period of 30 days (Diet History Questionnaire III, DHQ III, National Institutes of Health, National Cancer Institute, Epidemiology and Genomics Research Program, Bethesda, MD, USA). Total and component Healthy Eating Index (HEI) scores from output files were used to determine diet quality.[74] As nutrition gatekeepers control the family eating environment and parental dietary intakes have shown to be predictive of child dietary intakes for overall diet quality as well as specific food groups such as fruits and vegetables, caregiver dietary intakes were used as a proxy for child dietary intakes.[75–78]

Assessment of skin carotenoids

Caregiver and child skin carotenoids were assessed on the palm of the hand using a Pharmanex NuSkin Biophotonic Scanner S3 (NuSkin Enterprises, Provo, Utah). This scanner employs resonance Raman spectroscopy to assess dermal carotenoid content and has been shown to be an indirect biomarker of total plasma/serum carotenoid levels and a reliable and reproducible objective indicator of changes in fruit and vegetable consumption among adults and children.[79–81] All measures were taken in triplicate and averaged for analyses.

Fecal collection and processing

At data collection visits, participants were provided a pre-labeled specimen container with collection tool, gloves, a plastic toilet hat, and a cooler with ice pack for at-home fecal sample collection. They were instructed to record the date and time of collection and to place fecal samples immediately in a home freezer after collection. Participants returned samples to study personnel in the provided cooler with ice pack.

Fecal samples were frozen upon receipt at −20°C. Samples were subsequently transported on dry ice to the Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH for storage at −80°C. Samples were thawed at 4°C for 24 hours prior to processing. During processing, samples were maintained on ice and approximately 1.5 mL of each sample was aliquoted into 2-mL cryovials in a class II biological safety cabinet. After aliquoting, all samples were immediately frozen at −80°C until analyzed.

Fecal sterol metabolite quantification

Approximately 50 mg feces were weighted from each sample and added a to a 2 mL conical tube containing 30 mm glass beads. The sample was homogenized with 180 µL methanol and 20 µL 25 µM d4-cholic acid as an internal standard for 10 seconds, repeated three times, using a BioSpec MiniBeadBeater-16. When finished, the samples were immediately transferred to an ice water bath to sonicate for 15 min. After 10 min centrifuge at 14000 rpm and 4°C, the supernatant was then transferred into 2 mL glass vials for ultra-high performance liquid chromatography – high-resolution mass spectrometry (UPLC-HRMS) analysis. A Thermo Vanquish UPLC system coupled with Q-Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization (HESI) probe (Thermo Fisher, CA, USA) was used for bile acids analysis. The fecal sterol quantification method was modified based on a previous study.[82] Chromatographic separations were performed on a reverse phase column (Kinetix C18, 2.6 µm, 150 mm × 4.6 mm ID; Phenomenex, Torrance, CA). Mobile phase A was 1 mM ammonium acetate and 0.1% acetic acid in methanol: acetonitrile: water (1:1:3; v/v/v) and mobile phase B was 0.1% acetic acid in methanol: acetonitrile: 2-propanol (4.5:4.5:1; v/v/v). Samples were injected (5 µl) into a column equilibrated in 100% A. The

separation gradient and flow rate were conducted as follows: 0–2 min 0% B with 0.3 mL/min; 2–20 min 0–100% B with 0.3 mL/min; 20–28 min 100% B with 0.5 mL/min. To eliminate carry over, an extensive washing step was included at the end of each run as follows: 100% A from 28 to 35 min with 0.5 mL/min, then flow rate decreased to 0.3 mL/min from 35 min to 40 min. The mass spectra were recorded in negative ion mode. The PRM mode was used for qualitative determination of bile acids, while t-SIM mode was used for quantitative analysis. The QE mass parameters were as follows: sheath gas flow rate 50 mL/min, spray voltage 2.75 kV, capillary temperature 350°C, Aux gas heater temperature 425°C. For t-SIM mode, it was set as resolution 70,000 FWHM, automatic gain control target 3e6, maximum IT 100 ms, and isolation window 1.0 m/z. For PRM mode, the instrument was set at resolution 17500 FWHM, automatic gain control target 2e5, fragmentation NCE 35, 50, and 80, maximum IT 10 ms, and isolation window 1.0 m/z were performed. Fecal metabolites were normalized based on dry matter of each sample. Dry matter was determined by drying a fecal aliquot overnight in a convection oven at 70°C.

Microbiome composition and modeling

Approximately 100 mg of each fecal sample was used for DNA extraction using a QIAmp Fast DNA Mini Kit (Qiagen, Hilden, Germany) using the manufacturer's instructions with the following modifications. Contents were incubated for 45 min at 37°C in lysozyme-mutanolysin buffer (22 mg/ml lysozyme, 0.1 U/ml mutanolysin, 20 mM TrisHCl, 2 mM EDTA, 1.2% Triton-x, pH 8.0), before homogenization for 150 s with 0.1 mm zirconia beads. Samples were then incubated at 95°C for 5 min with InhibitEX Buffer and incubated at 70°C for 10 min with proteinase K and buffer AL. Following this step, the QIAmp Fast DNA Stool Mini Kit isolation protocol was followed, beginning with the ethanol step. DNA was quantified with the Qubit 2.0 fluorimeter (Life Technologies, Carlsbad, CA) using the dsDNA Broad Range Assay Kit, and sent to the Genomic Services Core at the Institute for Genomic Medicine at Nationwide Children's Hospital, Columbus, OH, for DNA extraction, library preparation, and high-throughput sequencing. Paired-end (250 nt forward and reverse) sequences of the V4 hypervariable region of the 16S rRNA gene (515 F-806 R) were generated on the Illumina MiSeq. Quantitative Insights into Microbial Ecology (QIIME) 2.0 was utilized for amplicon processing, quality control with DADA2, downstream taxonomic assignment using the SILVAv132 database, and diversity analyses.[83,84] Beta diversity was conducted three separate times for: 1) the entire study population, 2) all caregivers, and 3) all children; using the Aitchison metric and ADONIS test. The regression model included the following factors: dyad component (caregiver or child, entire population only), group (treatment group), visit (baseline or follow-up), family ID (separate ID for each dyad pair), and all potential factor interactions. Sequencing of samples initially resulted in 8,898,805 paired-end sequences (median = 95,888). After quality control, 924,899 high-quality sequences remained (median = 9,991). No rarefaction was performed for the reported microbiome analyses.

Differential modeling of microbial taxa associated with metadata was performed using Songbird v1.0.3 and Qurro (Figure 1b).[85,86] In brief, Songbird produces differentials that describe log-fold changes in relative abundances of microbial taxa (in this case amplicon sequence variants (ASV)) in relation to target metadata values and the effects of the input multinomial regression model. These differentials are calculated to take into account the compositional nature of sequencing data by comparing how all taxa change relative to one-another between samples, since their absolute abundance is unknown. Each differential is ranked based on its relative association with a given covariate or model, which can be utilized when selecting differentials of interest (conducted in Qurro and described for this study in more detail below). For this study, differentials were created separately for dyad counterparts (caregivers vs children) in Songbird using sequencing data from both time points, and an individual model for each metadata parameter containing the metadata in question and the unique subject identifier for each individual (i.e., controlling for individual). For example, the model for caregiver total HEI utilized all adult sequencing data with the model argument "Total_HEI+Subject_ID." All

models were required to have lower error and loss compared to a null model for inclusion. Differentials created in Songbird were processed in Qurro; the top 10% of positively and negatively differential taxa at the ASV level (differential rankings) were selected to create logRatios of microbial taxa relative abundances (calculated by taking the log of the quotient of the sum of positively differential taxa relative abundances by the sum of negatively differential taxa relative abundances, referred to as "microbial cohorts" for the purposes of this manuscript), which were then compared back to the metadata value in question via Spearman correlation to assess strength of the association, and compared by two-way ANOVA (treatment group and time point) with post-hoc paired t-test to compare within-treatment time points. The final, combined differentials for SBP and DBP were created by comparing all ASVs across models (Total HEI, whole grain HEI component score, SBP, DBP, fecal LCA, and fecal cholesterol) within dyad counterpart. If an ASV was present in ≥2 logRatios, the representing genus was considered in the final, combined model. Since lower SBP was a desirable outcome in this study, numerator and denominator taxa were switched (numerator to denominator, and denominator to numerator) for consideration in the final, combined model. For taxa that existed in both numerator and denominator across models, each taxa was included in the term in which it was more-highly represented. Each taxa was tested for final inclusion in the combined multivariate model using leave-one-out cross-validation. For the final differential microbial cohorts, the relationship between changes in these cohorts from baseline to follow-up was evaluated within dyad pairings (caregiver and child of the same family) via spearman correlation, and the probability of the same or greater correlation occurring within this population was determined via bootstrapping for 1000 random combinations with replacement.

Statistical analysis

Descriptive statistics (mean and standard deviation for continuous variables and proportion for categorical variables) were generated for all outcomes. Clinical parameters, dietary intake data, and were compared by two-way ANOVA (treatment group and time point) with post-hoc paired t-tests to compare within-treatment time points in JMP Pro v16.0.0.

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Disclosure statement

The authors report there are no competing interests to declare.

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Data availability statement

The sequencing data that support the findings of this study are available from the following link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA847619?reviewer=i8d1bbem9mcvun9g0sjvou570l

Authors' contributions

BRL, EBH, MB, ASK, KK, and CKS designed the research; BRL, EBH, LC, and CKS conducted the research; JZ, MB, ASK, RM, and KK provided essential materials; BL performed statistical analysis; BRL and EBH wrote the paper; BRL had primary responsibility for final content. All authors read and approved the final manuscript.

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