








ORIGINAL RESEARCH

Increases in Circulating and Fecal Butyrate are Associated With Reduced Blood Pressure and Hypertension: Results From the SPIRIT Trial

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BACKGROUND: Short chain fatty acids (SCFAs) are microbially derived end products of dietary fiber fermentation. The SCFA butyrate reduces blood pressure (BP) in mouse models. The association of SCFAs, including butyrate, with BP in humans is unclear, due in part to predominantly cross-sectional analyses and different biospecimens (blood versus fecal) for SCFA measurement. Longitudinal studies including both circulating and fecal SCFAs are lacking.

METHODS AND RESULTS: We leveraged existing data from the SPIRIT (Survivorship Promotion In Reducing IGF-1 Trial), which randomized 121 adult cancer survivors with overweight/obesity to a behavioral weight-loss intervention, metformin, or self-directed weight-loss. Of participants with baseline serum and fecal SCFAs measured (n=111), a subset had serum (n=93) and fecal (n=89) SCFA measurements 12 months later. We used Poisson regression with robust error variance to estimate baseline associations of SCFAs with hypertension, and we assessed the percent change in SCFAs from baseline with corresponding 12-month changes in BP using multiple linear regression. Baseline fecal butyrate was inversely associated with prevalent hypertension (standardized PR [95%CI]: 0.71 [0.54, 0.92]). A 10% increase in fecal butyrate from baseline was associated with decreased systolic BP (β [95%CI]: -0.56 [-1.01, -0.10] mm Hg), and a 10% increase in serum butyrate was associated with decreased systolic (β [95%CI]: -1.39 [-2.15, -0.63] mm Hg) and diastolic (β [95%CI]: -0.55 [-1.03, -0.08] mm Hg) BPs. Butyrate associations with systolic BP were linear and not modified by sex, race, or intervention arm.

CONCLUSIONS: Increased serum or fecal butyrate is associated with lowered BP. Butyrate may be a target for SCFA-centered BP-lowering interventions.

REGISTRATION: URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT02431676.

Key Words: acetic acid ■ blood pressure ■ butyric acid ■ fatty acids, volatile ■ hypertension

Short chain fatty acids (SCFAs)—molecules produced by microbial fermentation of dietary fiber—are postulated to mediate associations of gut microbiota and health. Of increasing interest is the role of the SCFA butyrate in hypertension risk. Butyrate has been shown to lower blood pressure in rodent models, and these effects

are thought to be through indirect effects that derive in the gut (eg, reducing inflammation through increased intestinal barrier function¹ or by acting on afferent vagal nerve signaling²) or through more direct effects in the periphery (eg, through action on G protein-coupled receptors or by acting as a histone deacetylase inhibitor³⁻⁶).

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CLINICAL PERSPECTIVE

What Is New?

- Higher fecal butyrate was associated with lower prevalence of hypertension and 1-year increases in both fecal butyrate and serum butyrate were associated with reductions in blood pressure.
- One-year changes in other short chain fatty acids were also associated with 1-year changes in blood pressure, however associations differed by the type of biospecimen in which the fatty acid was measured.
- Sex significantly modified the associations between changes in several short chain fatty acids and changes in blood pressure, but associations between butyrate and blood pressure were not modified by sex.

What Are the Clinical Implications?

- Butyrate may be a target for SCFA-centered blood pressure lowering interventions.

Nonstandard Abbreviations and Acronyms

BKMR	Bayes kernel machine regression
DBP	diastolic blood pressure
SBP	systolic blood pressure
SCFA	short chain fatty acid
SPIRIT	Survivorship Promotion In Reducing IGF-1 Trial

While there is a robust literature linking butyrate and blood pressure in rodents, evidence in humans has been mixed,^{1,7–14} possibly due to differences in the biological specimens (blood or fecal) used for SCFA measurement. This postulate is supported by results from cross-sectional studies^{8,15} which examined both circulating (blood) and excreted (fecal) SCFAs with cardiometabolic health risk factors and identified biospecimen-specific associations. Few longitudinal analyses report associations between changes in SCFAs and blood pressure; those that do were relatively short in length (6–18 weeks) and have focused solely on SCFAs measured in blood, not fecal SCFAs.^{9,13,14} No longitudinal studies to date have simultaneously assessed how changes in both circulating (blood) and excreted (fecal) butyrate, or other SCFAs, are associated with changes in blood pressure.

In this secondary analysis of a randomized trial, we examined baseline associations of serum and fecal

SCFA levels with prevalent hypertension, and longitudinal associations of changes in serum and fecal SCFAs with changes in blood pressure over 1 year. Consistent with prior evidence in murine models, we hypothesized that increasing butyrate levels in humans is associated with decreases in blood pressure.

METHODS

Data Availability Statement

Data described in the manuscript, codebook, and analytic code will be made available upon reasonable request.

Study Population

The SPIRIT (Survivorship Promotion In Reducing IGF-1 Trial) was a 3 arm randomized trial with a parallel design in adult cancer survivors with a body mass index (BMI) corresponding to overweight or obese categories.¹⁶ The primary outcome was insulin-like growth factor-1. The full protocol for SPIRIT can be found at ClinicalTrials.gov (identifier: NCT02431676). We recruited and enrolled participants at the Johns Hopkins ProHealth clinical research unit (Baltimore, MD) between August 2015 and December 2016. Relevant inclusion criteria for SPIRIT were: age ≥ 18 years, a previous solid tumor diagnosis and treatment-free survival of at least 1 year and a BMI ≥ 25 kg/m² with a weight ≤ 400 pounds. Relevant exclusion criteria were: current pregnancy or breastfeeding; antidiabetic medication use, fasting glucose ≥ 200 mg/dL, or fasting glucose ≥ 126 mg/dL and hemoglobin A1c $\geq 7\%$; chemotherapy, radiation treatment, or metformin use within past 3 months; an estimated glomerular filtration rate < 45 ; hepatic dysfunction, defined as Aspartate aminotransferase (AST)/Alanine transaminase (ALT) ≥ 2 times the upper limit of normal or reported liver disease; alcohol consumption > 14 drinks per week; and prior or planned bariatric surgery.

Of the 121 participants included in SPIRIT, 111 participants had data for both serum and fecal SCFAs, blood pressure, and relevant covariates at baseline. A subset of these participants also had complete baseline and 12-month post-randomization serum (n=93) and fecal (n=89) SCFA measures. The Institutional Review Board at Johns Hopkins University approved the study protocol. All participants provided written informed consent.

Treatment Groups

We randomized participants 1:1:1 to (1) coach-directed weight loss, (2) metformin treatment arm, or (3) self-directed weight loss, with stratification by race and BMI with variable block sizes. Participants in the

coach-directed arm received a remotely delivered life-style intervention consisting of increased physical activity, reduced calorie intake, and guidance to eat the Dietary Approaches to Stopping Hypertension (DASH) diet; the weight-loss goal for this arm was 5% weight loss by the 6-month timepoint, with maintenance of weight loss through the remainder of the study. The metformin arm received metformin up to 2000 mg per day, as tolerated by participants. The self-directed weight loss control participants received printed materials on weight loss at baseline.

Collection and Storage of Biospecimens

We collected stool and fasting serum at baseline, 6 months, and 12 months. We stored aliquots of serum at -80°C . At each study visit, we provided participants with an OMNIgene kit (DNA Genotek Inc., Ontario, Canada) to self-collect their stool at home. We instructed participants to refrigerate the stool samples at home and return them to the clinical center within 1 day, where the stool samples were stored at -80°C .

Primary Exposure: Fecal and Serum Short Chain Fatty Acids

Fecal SCFAs were measured by Microbiome Insights using gas chromatography following a previously published protocol.¹⁷ In brief, fecal samples were suspended in MilliQ-grade water, homogenized (MP Bio FastPrep), and acidified to a pH of 2.0 using 5 M HCl. These suspensions were centrifuged at 14 257g, whereafter the supernatants were extracted and spiked with 2-Ethylbutyric acid (to a 1 mmol/L concentration) before being stored in 2-mL GC vials with glass inserts. SCFAs were then measured by gas chromatography (Thermo Trace 1310) coupled with flame ionization detection (Thermo). Results were reported in mmol SCFA/kg feces. The SCFAs acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and hexanoate were measured in fecal samples; however, a large proportion of participants showed measures of 0 mmol/kg at baseline for fecal SCFAs isobutyrate, valerate, and hexanoate (58.1%, 30.1%, and 75.3% of participants, respectively). Because our primary analysis was focused on percent change in SCFAs from baseline and the co-modeling of SCFAs within a biospecimen, we excluded these 3 fecal SCFAs from both cross-sectional and longitudinal analyses to maintain a sufficient sample size and consistency between analyses. The sum of acetate, propionate, butyrate, and isovalerate were used to calculate a “total” fecal SCFA level. The lower limit of quantitation was 30.1 $\mu\text{mol/L}$ for acetate, 15.2 $\mu\text{mol/L}$ for propionate, 11.2 $\mu\text{mol/L}$ for butyrate, and 7.7 $\mu\text{mol/L}$ for isovalerate. The inter-assay coefficient of variation was $\approx 10\%$ for all fecal SCFAs.

Serum SCFAs were measured by Metabolon using liquid chromatography as previously described.¹⁸ In brief, fasting serum samples were spiked with a solution containing stable labeled internal standards for each SCFA. Following protein precipitation and centrifugation, an aliquot of the supernatant was derivatized and analyzed by liquid chromatography mass spectrometry (Agilent 1290/AB Sciex 5500). The peak area for each SCFA was measured against its corresponding internal standard peak. SCFAs were quantified using a weighted least squares regression analysis based on calibration standards, and SCFA measures outside the limits of quantitation were further extrapolated beyond these limits. Results are reported in ng SCFA/mL serum. The SCFAs acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and hexanoate were measured in serum samples, and the sum of all SCFAs was used to calculate a “total” serum SCFA level. The lower limit of quantitation was 25 ng/mL (butyrate, isobutyrate, methylbutyrate, valerate, and isovalerate), 50 ng/mL (hexanoate), 100 ng/mL (propionate), and 1000 ng/mL (acetate). The coefficients of variation were $<7\%$ for serum SCFAs.

Blood Pressure and Hypertension

We measured systolic (SBP) and diastolic (DBP) blood pressure using an automated oscillometric device (Omron HEM 907XL). Participants rested for 5 minutes in a seated position with feet flat on the floor, their right arm positioned with the elbow and forearm resting comfortably on a table, and their palm turned upwards; the left arm was used in participants with a history of breast cancer with lymph node dissection in the right arm. A trained and certified data collector measured the blood pressure from the positioned arm of participants using an appropriately sized cuff. Three consecutive measures were obtained (with 30 seconds between measurements), and an average blood pressure was reported. We defined hypertension [yes/no] using the AHA/ACC blood pressure cutoffs for stage 1 or 2 hypertension (SBP ≥ 130 mm Hg or DBP ≥ 80 mm Hg),¹⁹ or use of an antihypertensive medication regardless of measured blood pressure.

Other Covariates

Participants self-reported age, sex, and race. We collected data on antihypertensive medication use at each study visit and we dichotomized antihypertensive medication use [yes/no]. We measured weight at each visit using a digital scale without shoes. We measured height was measured at the study entry, and calculated BMI using height and weight (kg/m^2). Participants filled out a fruit and vegetable screening questionnaire (National Cancer Institute Eating at America's Table

Study Quick Food Scan) at each study visit. We estimated fiber intake (in grams) using a regression model based on self-reported fruit/vegetable/legume intake, age, and sex.

Statistical Analysis

In cross-sectional analyses, to determine the presence of nonlinear and nonadditive associations of serum or fecal SCFAs with hypertension, we used a Bayesian kernel machine regression (BKMR) approach. BKMR applies a kernel machine regression model to estimate a multivariable exposure-response function.²⁰ It then allows for graphical visualizations of the estimated exposure-response function by setting some exposures to specific quantile values and examining the associations of the remaining exposures to the outcome. For dichotomous outcomes, BKMR uses probit regression. We standardized SCFAs prior to model entry, and models were run separately for inclusion of all serum SCFAs or for all fecal SCFAs. We adjusted all models for age (continuous), BMI (continuous), sex (male or female), and fiber intake (continuous). We fit BKMR models using 100 000 iterations and without variable selection. Using BKMR, we visualized the exposure-response functions in 3 different ways: (1) the overall joint effect of SCFA exposures, in which we compared the exposure-response function when all SCFAs are at a particular quantile to when they are all at their 50th percentile levels; (2) the univariate effect of each SCFA, in which we compared the association of one SCFA with an outcome while holding the remaining SCFAs at their median values; and (3) potential 2-way interaction effects between SCFAs, in which we plotted the effect of one SCFA at fixed levels (10th, 50th, and 90th percentiles) of a second, while holding the remaining SCFAs at their 50th percentile values.

To corroborate the baseline BKMR findings and to provide statistical test assessments, we used Poisson regression models with robust error variances to estimate associations of a 1 SD increase in total or individual SCFAs with the prevalence ratio of hypertension at baseline. We adjusted all models for age, sex, BMI, and fiber intake, and we ran models separately for serum and fecal SCFAs.

For our 12-month longitudinal change analyses, we included only participants with complete change data and non-zero baseline SCFA values, resulting in 93 participants with changes in serum SCFAs and 89 participants with changes in fecal SCFAs. We presented 12-month changes in blood pressure, weight, and fiber as mean change (95% CI) and in SCFAs as median change (95% CI). To examine potential nonlinear relations, we used restricted cubic splines with 3 knots for the percent changes in each of the SCFAs. Nonlinearity was assessed graphically and using an F

test for the nonlinear term. We used linear regression models to assess the effects of a percent change in serum or fecal SCFAs from baseline with corresponding 12-month absolute changes in blood pressure. We adjusted models for baseline age, sex, baseline BMI, baseline blood pressure (SBP or DBP), intervention group, baseline antihypertensive medication use, baseline fiber intake, change in weight, and percent change in other serum or fecal SCFAs. We used heteroscedasticity-consistent White standard errors if heteroscedasticity was present.

We examined effect measure modification by sex, race, or treatment group in separate models using interaction terms for all variables as suggested in Buckley et al.²¹ As we identified sex modification for several SCFAs, we report main effects (with sex interaction terms included for remaining variables) for our primary longitudinal results; stratified results for sex, race, and treatment group are reported in the supplemental material.

We performed BKMR models using the *bkmr* package²² and restricted cubic spline models with the *rms* package²³ in R version 4.0.2; we performed all remaining analyses using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina). We based statistical significance on an $\alpha < 0.05$.

RESULTS

Baseline Characteristics and Cross-Sectional Associations of SCFAs With Hypertension

Nearly 68% of participants had hypertension at baseline (Table 1). Of those who were classified as having hypertension, a majority were taking an antihypertensive medication. A majority of those taking an antihypertensive medication were on a diuretic, beta-blocker, ACE inhibitor, Angiotensin II receptor blocker, or calcium channel blocker (Table S1). Participants were several years from a past cancer treatment (median 6 years), with most participants having a history of breast cancer.

We used BKMR (see Methods) to examine the mixture effects of serum and fecal SCFAs on prevalent hypertension. BKMR plots indicated an inverse association between the mixture of fecal SCFAs with prevalent hypertension and a null association for the mixture of serum SCFAs and hypertension (Figure S1). Univariable associations of individual SCFAs (in both serum and stool) with hypertension appeared to be mostly linear (Figure S2). Among the SCFAs examined in BKMR, serum propionate and serum valerate showed modest positive associations with hypertension, while fecal butyrate showed an inverse association with

Table 1. Baseline Characteristics of Sample (n=111)

Variable	Mean (SD), Median (interquartile range), or n (%)
Age, y	60.2 (8.9)
Female sex, n (%)	87 (78.4%)
Black or African American, n (%)	52 (46.9%)
Treatment group assignment, n (%)	
Self-directed	38 (34.2%)
Coach-directed	34 (30.6%)
Metformin	39 (35.1%)
Fiber, g/d	11.1 (4.8)
Weight, kg	95.5 (16.9)
BMI, kg/m ²	34.9 (5.4)
Type of cancer, n (%)	
Breast	62 (55.9%)
Colorectal	11 (9.9%)
Prostate	10 (9.0%)
Thyroid	9 (8.1%)
Time since last cancer treatment, y*	6.0 (3.0, 13.0)
Blood pressure	
SBP, mm Hg	118.5 (15.7)
DBP, mm Hg	69.8 (9.5)
Antihypertensive Medication, n (%)	67 (60.4%)
Hypertension, n (%)	75 (67.6%)
Short chain fatty acids	
Total serum SCFAs, ng/mL	2359.2 (1923.8, 3076.7)
Serum acetate, ng/mL	1270.0 (958.0, 2150.0)
Serum propionate, ng/mL	149.0 (110.0, 204.0)
Serum butyrate, ng/mL	62.7 (51.7, 79.7)
Serum isobutyrate, ng/mL	530.0 (426.0, 650.0)
Serum methylbutyrate, ng/mL	82.1 (62.7, 110.0)
Serum isovalerate, ng/mL	68.1 (49.5, 99.2)
Serum valerate, ng/mL	19.5 (15.7, 24.4)
Serum hexanoate, ng/mL	66.9 (59.1, 76.3)
Total fecal SCFAs, mmol/kg	14.0 (11.2, 18.1)
Fecal acetate, mmol/kg	7.6 (5.7, 10.0)
Fecal propionate, mmol/kg	4.2 (3.3, 5.3)
Fecal butyrate, mmol/kg	1.7 (1.2, 2.3)
Fecal isovalerate, mmol/kg	0.4 (0.3, 0.6)

BMI indicates body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; and SCFA, short chain fatty acid.

*n=107.

hypertension. BKMR visualizations of bivariable interaction plots did not indicate strong interaction effects between SCFAs on hypertension (Figures S3 and S4).

We next corroborated BKMR findings using traditional linear regression models (Table 2). After adjustment for covariates, we found that among serum SCFAs, valerate trended toward a positive association (albeit not statistically significant) with hypertension

while other serum SCFAs were null. Among fecal SCFAs, butyrate was significantly inversely associated with hypertension, and the association of total fecal SCFAs with hypertension trended inverse but was not statistically significant.

Longitudinal Changes in SCFAs and BP

We report 12-month changes in SCFAs and blood pressure, along with changes in body weight and dietary fiber, overall and by treatment group (Table S2). We found no treatment group differences in changes in blood pressure, fiber, or SCFAs. In Table 3 we demonstrate how a 10% increase in SCFAs is associated with change in SBP and DBP over 12 months. Notably, increases in serum acetate were positively associated with increases in SBP and DBP over 12 months. In contrast, increases in serum and fecal butyrate were significantly associated with decreases in SBP over 12 months, and an increase in serum butyrate was also associated with a decrease in DBP over 12 months. We did not find evidence for nonlinearity of associations using restricted cubic spline models (all *P* values >0.05).

Sex, Race, and Intervention Arm Interactions

Associations of several SCFAs, particularly those measured in serum, differed by sex (Figure and Table S3). Of note, in males there was an inverse association between change in serum propionate and SBP and positive association between change in serum isobutyrate and SBP. Moreover, in males there was a positive association between change in fecal propionate and SBP. Of note, the association of serum butyrate and fecal butyrate with SBP were consistent by sex.

Some race interactions were identified. Notably, serum hexanoate and fecal butyrate were inversely associated with change in DBP in Black participants only; and associations of percent change in total fecal SCFAs with blood pressure trended towards an inverse association with SBP or DBP in Black participants only (Table S4). We also report evidence for treatment group-specific effects of several serum SCFAs with blood pressure (Tables S5 and S6). Association of serum and fecal butyrate with changes in systolic BP, however, were not modified by treatment group.

DISCUSSION

In a racially diverse population of adult cancer survivors with overweight and obesity, we found that higher levels of fecal butyrate were inversely associated with prevalent hypertension and that increases in serum or fecal butyrate over 1 year of follow up were

Table 2. Baseline Associations of a 1-SD Increment in SCFAs With Hypertension Status (n=111)

SCFA predictors	Serum SCFAs		Fecal SCFAs	
	SCFA SD (ng/mL)	Hypertension prevalence ratio (95% CI)	SCFA SD (mmol/kg)	Hypertension prevalence ratio (95% CI)
Total SCFAs	969.1	0.99 (0.88, 1.11)	6.1	0.87 (0.75, 1.01)
Acetate	885.3	1.02 (0.87, 1.19)	3.6	1.10 (0.87, 1.38)
Propionate	71.3	1.06 (0.88, 1.29)	1.9	1.02 (0.84, 1.24)
Butyrate	34.1	0.90 (0.72, 1.12)	1.0	0.71 (0.54, 0.92)*
Isobutyrate	158.0	1.02 (0.85, 1.22)
Methylbutyrate	34.3	1.07 (0.93, 1.24)
Isovalerate	36.3	0.94 (0.77, 1.15)	0.2	1.04 (0.89, 1.22)
Valerate	8.3	1.15 (0.98, 1.36)
Hexanoate	15.3	0.91 (0.78, 1.07)

Adjusted covariates: age, sex, BMI, and fiber intake. Models including individual SCFAs additionally adjust for remaining specimen SCFAs. BMI indicates body mass index; and SCFA, short chain fatty acid.

*is statistically significant at $P < 0.05$.

associated with lower blood pressure. We also identified effect modification by sex and by intervention arm for several SCFAs, but notably not for serum or fecal butyrate. Based on these data, we conclude that that butyrate may be a potential target for SCFA-centered blood pressure lowering interventions, however the clinical significance of changes in butyrate still need to be determined.

Table 3. Main Effect (β [95% CI]) of a 10% Increase in 12-Month SCFA on 12-Month Change in Blood Pressure

Predictor	12-month Δ SBP (mm Hg)	12-month Δ DBP (mm Hg)
% Δ Serum total SCFAs	0.57 (0.01, 1.12) [†]	0.19 (−0.12, 0.51)
% Δ Serum acetate	0.62 (0.32, 0.91) [†]	0.26 (0.08, 0.45) [†]
% Δ Serum propionate	−0.04 (−1.11, 1.04)	−0.05 (−0.57, 0.46)
% Δ Serum butyrate	−1.39 (−2.15, −0.63) [†]	−0.55 (−1.03, −0.08) [†]
% Δ Serum isobutyrate	0.76 (−0.73, 2.25)	0.59 (−0.33, 1.51)
% Δ Serum methylbutyrate	−0.91 (−1.86, 0.03)	−0.06 (−0.68, 0.56)
% Δ Serum isovalerate	−0.30 (−0.86, 0.26)	−0.20 (−0.54, 0.14)
% Δ Serum valerate	0.59 (−0.97, 2.14)	−0.17 (−1.12, 0.77)
% Δ Serum hexanoate	−0.62 (−2.33, 1.10)	0.23 (−0.95, 1.40)
% Δ Fecal total SCFAs*	−0.03 (−0.22, 0.15)	0.07 (−0.07, 0.20)
% Δ Fecal acetate*	0.22 (−0.32, 0.75)	−0.00 (−0.31, 0.30)
% Δ Fecal propionate*	−0.00 (−0.53, 0.52)	0.12 (−0.16, 0.41)
% Δ Fecal butyrate*	−0.56 (−1.01, −0.10) [†]	−0.16 (−0.43, 0.11)
% Δ Fecal isovalerate*	0.12 (−0.24, 0.47)	0.03 (−0.21, 0.28)

Models adjusted for treatment assignment, baseline age, sex, baseline BMI, baseline blood pressure (SBP or DBP), baseline (yes/no) antihypertensive medication use, baseline fiber intake, change in weight, and sex interaction terms. Models with individual SCFAs also adjust for the percent change in remaining specimen SCFAs. BMI indicates body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; and SCFA, short chain fatty acid.

*n=89 for fecal SCFA.

[†]indicates statistical significance at $P < 0.05$.

Previous cross-sectional human studies have generally indicated that circulating butyrate (ie, measured in blood) is inversely associated,^{1,8} and fecal butyrate is positively associated,^{7,8,10,12} with blood pressure and hypertension. In contrast, dietary intervention studies have reported no associations of changes in circulating butyrate with changes in blood pressure.^{9,13,14} Divergent results across studies may be due to differences in study exclusion criteria related to blood pressure or BMI categories. Additionally, some prior studies did not account for BMI in their modeling or study design.^{1,7,8,10} Individuals with obesity have higher levels of fecal SCFAs compared with lean individuals,^{24,25} which may reflect an increased ability of the microbiome to harvest energy.²⁶ However, studies have also reported that SCFA supplementation protects against a high-fat diet-induced obesity in mice^{27,28} and against weight gain in humans with overweight status.²⁹ Our study population comprised individuals with overweight or obesity by design, and we adjusted for BMI and weight change in our models; thus, we may be better able to disentangle some of the confounding or mediating effects of body habitus on SCFA-blood pressure associations. Our study population also had relatively well-controlled blood pressure; it is possible that our findings may be even more pronounced in populations with poorly controlled blood pressure. We also cannot rule out the possibility that differences across studies arose from differences in mass spectrometry measurement techniques; residual or unmeasured confounding by factors such as dietary intake; or by differences in the composition of our study population (compared to others) that are not related to BMI or blood pressure.

There is biologic plausibility to the observed inverse association between butyrate and blood pressure in our study. In murine models, supplemental oral butyrate reduces blood pressure.^{1,11,30,31} In addition, in human studies, individuals with hypertension

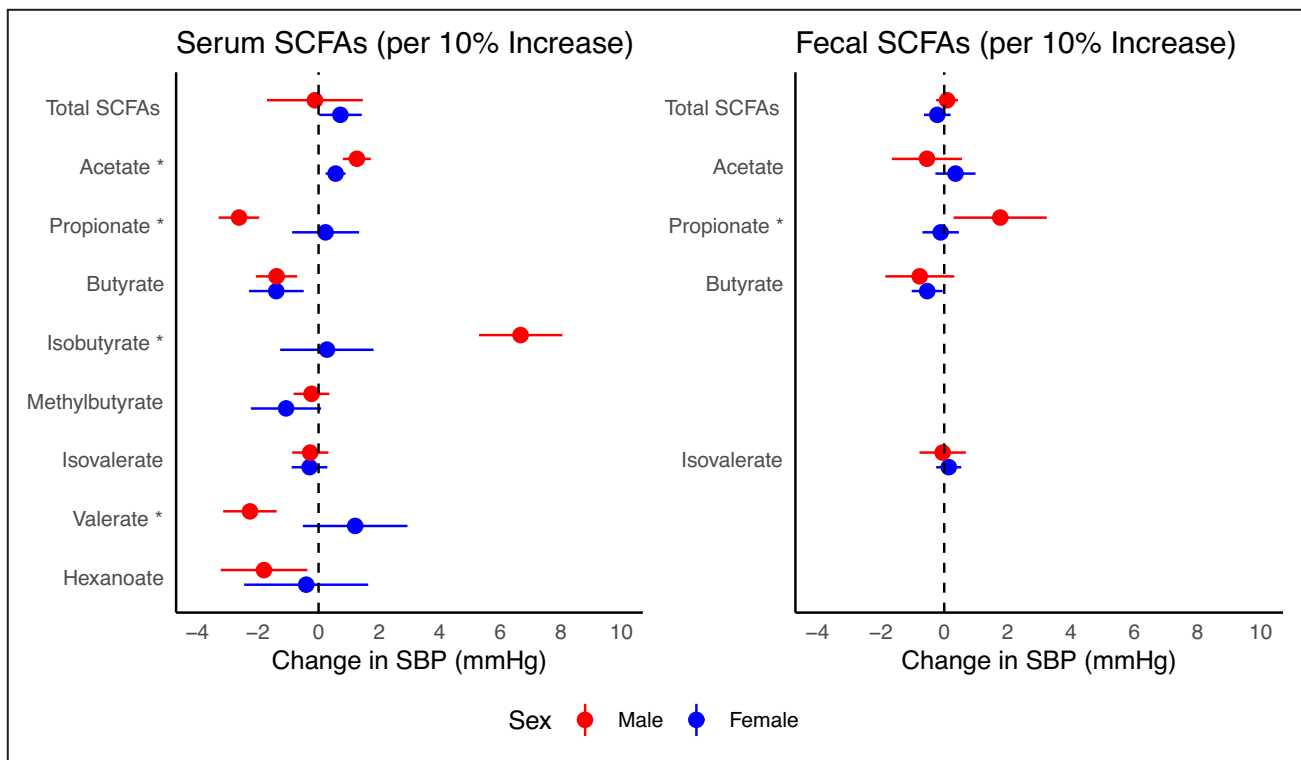


Figure. Forest plots of sex-stratified β (95% CI) for effect of a 10% increase in SCFAs from baseline on change in SBP.

Coefficients obtained from sex-stratified multivariable regression models adjusting for baseline age, baseline BMI, baseline blood pressure (SBP or DBP), intervention group, baseline antihypertensive medication use, baseline fiber intake, change in weight, and percent change in other serum or fecal SCFAs. BMI indicates body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; and SCFA, short chain fatty acid. *SCFAs significantly modified by sex.

have been reported to have lower levels of butyrate-producing bacteria.^{1,8} Butyrate is the primary energy source for colonocytes and promotes intestinal barrier integrity,³² which can prevent the translocation of pro-inflammatory products. Butyrate also exhibits anti-inflammatory effects through histone deacetylase inhibition and cytokine inhibition.¹² Further, butyrate may lower blood pressure through afferent vagal nerve signaling.² Regardless of the exact mechanism(s) of action underlying butyrate's effects on blood pressure, our findings support increasing butyrate as a potential intervention to lower blood pressure.

We found that the association of several SCFAs with blood pressure differed by sex, suggesting that the effects of the microbes and their metabolites may differ by sex. There is a growing literature base that has documented sex differences in the microbiome^{33,34} and sex differences in hypertension.^{35,36} Mechanisms involving diet and the immune system may underly sex differences in both the microbiome and blood pressure. Diet has been shown to differentially impact the microbiome of males compared with females,³⁷ and there are immune response differences between males and females which may be further impacted by microbiome differences.³⁸ Increased sodium intake

can lead to reductions in salt-sensitive *Lactobacillus spp.* and increases in T helper 17 cells and blood pressure,³⁹ and a recent randomized trial also identified sex-specific changes in circulating SCFAs in response to sodium reduction.¹³ Future work is needed to replicate our findings of sex differences in associations of SCFAs with blood pressure.

We also report differences in associations of several SCFAs with blood pressure by intervention group. This suggests that the way in which some SCFAs are changed may influence their association with blood pressure. Our group previously reported that individuals in the SPIRIT metformin intervention arm showed increased acetate-producing pathways in their gut microbiome.¹⁸ Interestingly, the metformin intervention group in our analysis showed a (non-significant) greater reduction in daily fiber intake compared with the other intervention arms. Thus, the differential impacts of interventions on lifestyle characteristics, SCFA precursors, and SCFA-generating capacity may counteract each other; the mechanisms underlying these differences require replication and further investigation.

Our study has limitations. First, our measures of serum and fecal SCFAs were derived using different methodologies (liquid versus gas chromatography, respectively).

While these assessments did allow for direct quantitation of SCFAs within a specimen, potential differences in measurement error preclude us from directly comparing SCFAs across specimen types. Second, our fecal analyses were limited to the 4 most abundant SCFAs in our sample, though the other SCFAs could also play a role in the etiology of hypertension. Third, we cannot rule out the possibility that collection or storage of biospecimens influenced SCFA measurements, or the possibility of reverse causation (eg, individuals undergo medication or lifestyle changes following a high blood pressure diagnosis and subsequently alter their microbiome/SCFA production). Fourth, our analytic sample size is relatively small for our sex and treatment group stratified results, which may lead to false positive or false negative subgroup findings. Fifth, our measurements of diet did not allow us to assess the effects of specific food groups, sodium, or total energy intake. Sixth, our study population consists of adult cancer survivors with overweight and obesity, and thus our findings may not be generalizable to younger individuals, those with normal weights, or those without a history of cancer. While treatments for cancer can affect the microbiome, the median time since last cancer treatment for participants in this analysis was ≈ 6 years, with only 3 individuals reporting a last treatment under 1 year prior to enrollment. Finally, it is possible that the relationship between SCFAs, including butyrate, and blood pressure may be underestimated in our study because of the high proportion of participants on antihypertensive medications.

Our study also has several strengths. Our study population was diverse and included individuals of both sexes and of both Black and White race identification. Furthermore, there were high rates of retention and data collection at 1 year of follow-up. With longitudinal follow-up of a year, this analysis is the longest longitudinal assessment of both serum and fecal SCFAs with blood pressure in humans. To our knowledge, this is the first study to also assess the effects of changes in SCFAs independent of changes in other specimen SCFAs. Additionally, this is the first study to our knowledge to apply BKMR methods to SCFA data, allowing for the co-modeling of SCFAs within a specimen.

Perspectives

In our longitudinal study, in which we model the independent effects of changes in serum or fecal SCFAs, we identified butyrate as a potential target for SCFA-centered blood pressure lowering interventions. Future analyses investigating the mechanisms underlying these relations are warranted.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S6

Figures S1–S4

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Supplemental Material

Supplemental Material

Table S1: Classes of Antihypertensive Medication Usage at Baseline

Table S2: Twelve-month changes in characteristics and SCFAs from baseline, overall and by treatment group

Table S3: Sex-stratified effects β (95%CI) for a 10% increase in 12-month SCFA predicting change in blood pressure

Table S4: Race-stratified effects β (95%CI) for a 10% increase in 12-month SCFA predicting change in blood pressure

Table S5: Treatment Group Stratified β (95% Confidence Interval) for the 10% increase in 12-month SCFA predicting 12-month change in SBP

Table S6: Treatment Group Stratified β (95% Confidence Interval) for the 10% increase in 12-month SCFA predicting 12-month change in DBP

Figure S1: Overall joint effects of Serum or Fecal SCFAs with hypertension using BKMR approach

Figure S2: Univariate SCFA associations for hypertension using BKMR approach

Figure S3: Two-way interaction plots for serum SCFA associations with hypertension using BKMR approach

Figure S4: Two-way interaction plots for fecal SCFA associations with hypertension using BKMR approach

Table S1: Classes of Antihypertensive Medication Usage at Baseline

Antihypertensive Medication Class	Prevalence among those taking any antihypertensive medication (N=67)
Diuretics	28 (41.8%)
Beta-blockers	17 (25.4%)
ACE inhibitors	19 (28.4%)
Angiotensin II receptor blockers	20 (29.9%)
Calcium channel blockers	25 (37.3%)
Alpha blockers	1 (1.5%)
Combined alpha and beta-blockers	4 (6.0%)
Peripheral adrenergic inhibitors	1 (1.5%)
Vasodilators	3 (4.5%)

Table S2: Twelve-month changes in characteristics and SCFAs from baseline, overall and by treatment group (N=93)

Variable	Overall (N=93)	Coach-directed (N=29)	Metformin (N=29)	Self-directed (N=35)	P value
	<i>Mean (95% CL)</i>	<i>Mean (95% CL)</i>	<i>Mean (95% CL)</i>	<i>Mean (95% CL)</i>	
Weight (kg)	-2.4 (-3.5, -1.3)	-3.3 (-5.2, -1.3)	-3.8 (-6.2, -1.4)	-0.5 (-1.8, 0.8)	0.0213 MF vs SD Mean (95%CI): -3.3 (-6.4, -0.3)
SBP (mmHg)	1.4 (-1.7, 4.5)	-3.3 (-8.3, 1.8)	3.7 (-2.9, 10.3)	3.4 (-1.4, 8.2)	0.1276
DBP (mmHg)	0.6 (-1.3, 2.5)	-2.2 (-5.1, 0.7)	1.9 (-2.3, 6.1)	1.9 (-1.1, 4.8)	0.1427
Fiber (g/day)	-0.5 (-1.3, 0.3)	0.4 (-0.9, 1.7)	-1.7 (-3.0, -0.3)	-0.3 (-1.8, 1.3)	0.0977
	<i>Median (95% CL)</i>	<i>Median (95% CL)</i>	<i>Median (95% CL)</i>	<i>Median (95% CL)</i>	
Total Serum SCFAs (ng/mL)	299.9 (37.0, 656.5)	283.5 (-544.6, 722.2)	387.6 (-98.2, 881.0)	299.9 (-261.1, 739.5)	0.8457
Serum Acetate (ng/mL)	320.0 (100.0, 590.0)	314.0 (-403.0, 891.0)	460.0 (40.0, 910.0)	229.0 (18.0, 930.0)	0.7674
Serum Propionate (ng/mL)	0.0 (-6.0, 11.0)	10.0 (-12.0, 39.0)	-5.0 (-14.0, 28.0)	-2.0 (-16.0, 7.0)	0.7690
Serum Butyrate (ng/mL)	-5.9 (-11.3, 1.6)	-9.1 (-18.7, 10.1)	-3.5 (-11.3, 17.3)	-3.9 (-17.5, 3.4)	0.2901
Serum Isobutyrate (ng/mL)	-36.0 (-80.0, 5.0)	-39.0 (-87.0, 72.0)	-34.0 (-100.0, 52.0)	-43.0 (-165.0, 5.0)	0.7115
Serum Methylbutyrate (ng/mL)	-2.7 (-7.0, 3.0)	-4.1 (-16.0, 4.0)	-5.0 (-8.1, 8.2)	2.3 (-7.0, 8.1)	0.2086
Serum Isovalerate (ng/mL)	-6.5 (-9.8, -2.0)	-10.4 (-28.3, 2.8)	0.5 (-7.5, 11.8)	-6.4 (-15.1, -2.0)	0.2663

Serum Valerate (ng/mL)	-2.9 (-5.0, -0.7)	-4.3 (-6.8, -0.9)	-1.7 (-5.5, 1.3)	-2.6 (-5.1, 0.9)	0.4472
Serum Hexanoate (ng/mL)	-2.3 (-6.4, -0.5)	-2.6 (-7.7, 3.5)	-0.8 (-9.1, 2.5)	-4.2 (-10.5, 0.6)	0.7677
	<i>N</i> =89	<i>N</i> =26	<i>N</i> =29	<i>N</i> =34	
Total Fecal SCFAs (mmol/kg) *	2.2 (-0.4, 4.2)	3.7 (-1.8, 6.2)	2.9 (-1.4, 5.3)	1.1 (-2.4, 7.4)	0.9297
Fecal Acetate (mmol/kg) *	1.1 (-0.2, 2.6)	1.5 (-0.8, 2.9)	1.6 (-1.2, 3.2)	0.3 (-2.0, 4.7)	0.9852
Fecal Propionate (mmol/kg) *	0.6 (-0.0, 1.2)	0.7 (-0.4, 2.3)	0.6 (-0.1, 1.3)	0.7 (-0.5, 3.2)	0.9509
Fecal Butyrate (mmol/kg) *	0.3 (-0.1, 0.7)	0.4 (-0.0, 0.9)	0.3 (-0.2, 0.8)	0.2 (-0.5, 1.1)	0.7896
Fecal Isovalerate (mmol/kg) *	0.0 (-0.0, 0.1)	0.1 (-0.1, 0.2)	0.0 (-0.1, 0.1)	-0.0 (-0.1, 0.2)	0.8809

*: *N*=89

Table S3: Sex-stratified effects β (95%CI) for a 10% increase in 12-month SCFA predicting change in blood pressure

	12-month Δ SBP (mmHg)		P sex interaction	12-month Δ DBP (mmHg)		P sex interaction
	Female	Male		Female	Male	
<i>Serum SCFAs</i>	<i>N=72</i>	<i>N=21</i>		<i>N=72</i>	<i>N=21</i>	
% Δ Total Serum	0.72 (0.02, 1.43)	-0.12 (-1.70, 1.46)	0.1135	0.30 (-0.10, 0.71)	-0.27 (-1.29, 0.75)	0.1302
% Δ Acetate	0.56 (0.23, 0.89)	1.26 (0.80, 1.72)	0.0039	0.24 (0.04, 0.44)	0.59 (0.12, 1.06)	0.0791
% Δ Propionate	0.23 (-0.87, 1.34)	-2.63 (-3.30, -1.96)	<.0001	0.15 (-0.36, 0.66)	-1.93 (-2.79, -1.08)	<.0001
% Δ Butyrate	-1.39 (-2.29, -0.49)	-1.39 (-2.07, -0.71)	0.9929	-0.69 (-1.24, -0.14)	0.21 (-0.54, 0.96)	0.0226
% Δ Isobutyrate	0.27 (-1.27, 1.81)	6.67 (5.29, 8.04)	<.0001	0.52 (-0.48, 1.51)	1.36 (0.35, 2.38)	0.1751
% Δ Methylbutyrate	-1.08 (-2.23, 0.08)	-0.23 (-0.82, 0.36)	0.1770	-0.10 (-0.86, 0.65)	0.12 (-0.53, 0.78)	0.6109
% Δ Isovalerate	-0.30 (-0.89, 0.29)	-0.27 (-0.87, 0.332)	0.9400	-0.21 (-0.57, 0.15)	-0.01 (-0.74, 0.72)	0.5355
% Δ Valerate	1.21 (-0.52, 2.93)	-2.27 (-3.15, -1.38)	0.0004	0.13 (-0.94, 1.20)	-1.54 (-2.47, -0.61)	0.0101
% Δ Hexanoate	-0.41 (-2.46, 1.64)	-1.80 (-3.23, -0.37)	0.2287	0.07 (-1.26, 1.40)	1.16 (-0.27, 2.59)	0.1994
<i>Fecal SCFAs</i>	<i>N=68</i>	<i>N=21</i>		<i>N=68</i>	<i>N=21</i>	
% Δ Total Fecal	-0.22 (-0.64, 0.20)	0.09 (-0.26, 0.44)	0.1510	-0.06 (-0.30, 0.19)	0.15 (-0.07, 0.37)	0.1276
% Δ Acetate	0.36 (-0.28, 0.99)	-0.55 (-1.66, 0.56)	0.1215	0.03 (-0.30, 0.37)	-0.21 (-1.19, 0.76)	0.6160
% Δ Propionate	-0.11 (-0.69, 0.46)	1.77 (0.30, 3.24)	0.0092	0.11 (-0.18, 0.41)	0.31 (-0.85, 1.46)	0.5421
% Δ Butyrate	-0.54 (-1.03, -0.05)	-0.77 (-1.86, 0.32)	0.6648	-0.06 (-0.30, 0.19)	0.18 (-0.41, 0.77)	0.4925
% Δ Isovalerate	0.14 (-0.25, 0.54)	-0.05 (-0.78, 0.68)	0.6097	-0.19 (-0.48, 0.10)	0.10 (-0.47, 0.66)	0.6950

Models adjusted for treatment assignment, baseline age, baseline BMI, baseline blood pressure (SBP or DBP), baseline [yes/no] antihypertensive medication use, baseline fiber intake, and change in weight. Models with % changes in an individual SCFA as a predictor additionally adjust for % change in remaining specimen SCFAs.

Table S4: Race-stratified effects β (95%CI) for a 10% increase in 12-month SCFA predicting change in blood pressure

	12-month Δ SBP (mmHg)		P sex interaction	12-month Δ DBP (mmHg)		P sex interaction
	White	Black		White	Black	
<i>Serum SCFAs</i>	N=49	N=44		N=49	N=44	
% Δ Total Serum	0.02 (-0.79, 0.84)	0.45 (-0.40, 1.29)	0.3826	0.17 (-0.27, 0.61)	0.09 (-0.40, 0.58)	0.7853
% Δ Acetate	0.24 (-0.07, 0.56)	0.39 (0.01, 0.77)	0.5474	0.26 (0.08, 0.43)	0.06 (-0.22, 0.34)	0.2261
% Δ Propionate	-0.67 (-2.15, 0.82)	0.31 (-1.39, 2.01)	0.3798	-0.41 (-1.08, 0.26)	0.31 (-0.38, 1.00)	0.1338
% Δ Butyrate	-0.37 (-1.49, 0.75)	-0.89 (-1.96, 0.17)	0.4905	-0.08 (-0.52, 0.36)	-0.40 (-1.18, 0.39)	0.4741
% Δ Isobutyrate	0.97 (-1.07, 3.01)	1.28 (-1.10, 3.66)	0.8412	0.99 (-0.17, 2.15)	0.98 (-0.34, 2.29)	0.9901
% Δ Methylbutyrate	-0.78 (-2.05, 0.50)	-1.25 (-2.71, 0.20)	0.4321	-0.24 (-0.92, 0.44)	0.22 (-0.37, 0.80)	0.3026
% Δ Isovalerate	-0.24 (-0.78, 0.31)	-0.13 (-1.21, 0.96)	0.1840	-0.33 (-0.62, -0.03)	-0.38 (-1.36, 0.61)	0.9206
% Δ Valerate	0.24 (-1.30, 1.78)	0.64 (-1.24, 2.53)	0.7349	-0.13 (-0.91, 0.66)	0.47 (-0.90, 1.84)	0.4404
% Δ Hexanoate	0.37 (-1.46, 2.20)	-2.10 (-4.71, 0.51)	0.1176	0.32 (-0.76, 1.40)	-1.86 (-3.63, -0.09)	0.0351
<i>Fecal SCFAs</i>	N=46	N=43		N=46	N=43	
% Δ Total Fecal	0.04 (-0.22, 0.29)	-0.51 (-1.07, 0.05)	0.0123	0.10 (-0.04, 0.23)	-0.23 (-0.56, 0.10)	0.0121
% Δ Acetate	0.35 (-0.20, 0.89)	0.52 (0.06, 0.98)	0.6162	0.18 (-0.08, 0.44)	0.16 (-0.19, 0.50)	0.9284
% Δ Propionate	-0.09 (-0.61, 0.42)	-0.49 (-1.11, 0.12)	0.3121	-0.09 (-0.38, 0.20)	-0.07 (-0.48, 0.34)	0.9156
% Δ Butyrate	-0.20 (-0.79, 0.38)	-0.82 (-1.31, -0.33)	0.1023	-0.01 (-0.30, 0.27)	-0.40 (-0.74, -0.06)	0.0810
% Δ Isovalerate	-0.23 (-0.72, 0.25)	0.37 (-0.10, 0.84)	0.0735	-0.04 (-0.26, 0.17)	0.18 (-0.11, 0.46)	0.2141

Models adjusted for treatment assignment, sex, baseline age, baseline BMI, baseline blood pressure (SBP or DBP), baseline [yes/no] antihypertensive medication use, baseline fiber intake, and change in weight. Models with % changes in an individual SCFA as a predictor additionally adjust for % change in remaining specimen SCFAs.

Table S5: Treatment Group Stratified β (95% Confidence Interval) for the 10% increase in 12-month SCFA predicting 12-month change in SBP

	Self-Directed (N=35)	Coach-Directed (N=29)	Metformin (N=29)	P treatment group interaction
<i>Serum SCFAs</i>				
% Δ Total Serum SCFAs	0.86 (0.03, 1.70)	0.55 (-0.15, 1.25)	-1.13 (-2.85, 0.60)	0.0420
% Δ Serum Acetate	0.57 (0.24, 0.91)	0.68 (0.20, 1.16)	0.42 (-0.62, 1.47)	0.8643
% Δ Serum Propionate	1.07 (-0.04, 2.18)	-0.77 (-1.92, 0.37)	0.08 (-1.54, 1.70)	0.0484
% Δ Serum Butyrate	-0.96 (-2.44, 0.51)	-0.86 (-2.12, 0.40)	-1.58 (-4.31, 1.16)	0.8757
% Δ Serum Isobutyrate	0.93 (-1.02, 2.87)	-0.31 (-1.65, 1.03)	-1.43 (-5.62, 2.75)	0.4087
% Δ Serum Methylbutyrate	-1.04 (-2.75, 0.67)	-0.40 (-1.10, 0.29)	3.35 (0.82, 5.88)	0.0049
% Δ Serum Isovalerate	-1.30 (-2.27, -0.34)	0.01 (-0.31, 0.33)	-1.27 (-3.55, 1.00)	0.0136
% Δ Serum Valerate	-0.75 (-2.73, 1.24)	2.01 (0.88, 3.13)	0.82 (-5.97, 7.61)	0.0385
% Δ Serum Hexanoate	0.39 (-2.04, 2.82)	-1.71 (-3.28, -0.14)	0.33 (-3.34, 4.00)	0.2251
<i>Fecal SCFAs</i>				
% Δ Total Fecal SCFAs	0.13 (-0.42, 0.67)	-0.09 (-0.52, 0.35)	0.04 (-0.39, 0.47)	0.9092
% Δ Fecal Acetate	0.55 (-0.93, 2.04)	0.68 (-0.55, 1.90)	-0.25 (-2.25, 1.75)	0.5505
% Δ Fecal Propionate	-0.26 (-1.80, 1.28)	-0.12 (-1.27, 1.02)	0.56 (-2.75, 3.87)	0.7845
% Δ Fecal Butyrate	-0.30 (-1.19, 0.60)	-0.77 (-1.93, 0.40)	-0.49 (-2.49, 1.52)	0.5557
% Δ Fecal Isovalerate	0.10 (-0.74, 0.94)	0.59 (-0.32, 1.50)	0.32 (-1.16, 1.80)	0.5153

Models adjusted for baseline age, sex, baseline BMI, baseline blood pressure (SBP), baseline [yes/no] antihypertensive medication use, baseline fiber intake, and change in weight. Models with individual SCFAs also adjust for the percent change in remaining specimen SCFAs.

Table S6: Treatment Group Stratified β (95% Confidence Interval) for the 10% increase in 12-month SCFA predicting 12-month change in DBP

	Self-Directed (N=35)	Coach-Directed (N=29)	Metformin (N=29)	Interaction p-value
<i>Serum SCFAs</i>				
% Δ Total Serum SCFAs	0.55 (0.13, 0.98)	0.01 (-0.40, 0.42)	-0.72 (-1.79, 0.34)	0.0053
% Δ Serum Acetate	0.37 (0.23, 0.51)	-0.10 (-0.39, 0.20)	0.01 (-0.73, 0.76)	0.0070
% Δ Serum Propionate	0.45 (-0.06, 0.96)	0.68 (-0.00, 1.37)	-0.32 (-1.24, 0.61)	0.1644
% Δ Serum Butyrate	-0.74 (-1.26, -0.22)	-0.21 (-0.90, 0.49)	-1.41 (-2.80, -0.03)	0.1786
% Δ Serum Isobutyrate	1.64 (0.79, 2.50)	-0.69 (-1.60, 0.21)	-2.91 (-5.30, -0.51)	<.0001
% Δ Serum Methylbutyrate	-0.87 (-1.59, -0.15)	0.26 (-0.23, 0.74)	1.32 (-0.19, 2.83)	0.0037
% Δ Serum Isovalerate	-0.45 (-1.03, 0.13)	0.00 (-0.22, 0.23)	0.29 (-1.58, 2.16)	0.2855
% Δ Serum Valerate	-0.58 (-1.60, 0.43)	0.90 (0.14, 1.65)	2.42 (-1.28, 6.11)	0.0234
% Δ Serum Hexanoate	-0.18 (-1.18, 0.83)	-1.30 (-2.70, 0.09)	0.18 (-1.04, 1.39)	0.2038
<i>Fecal SCFAs</i>				
% Δ Total Fecal SCFAs *	0.15 (-0.14, 0.44)	0.00 (-0.34, 0.34)	0.09 (-0.17, 0.35)	0.4548
% Δ Fecal Acetate *	0.32 (-0.48, 1.12)	0.14 (-0.59, 0.87)	-0.32 (-1.50, 0.87)	0.5036
% Δ Fecal Propionate *	-0.24 (-1.07, 0.59)	-0.18 (-0.82, 0.47)	0.56 (-1.38, 2.49)	0.5464
% Δ Fecal Butyrate *	-0.00 (-0.48, 0.47)	-0.12 (-0.80, 0.57)	-0.36 (-1.46, 0.74)	0.5610
% Δ Fecal Isovalerate *	0.13 (-0.32, 0.57)	0.21 (-0.32, 0.74)	0.39 (-0.50, 1.28)	0.7976

Models adjusted for baseline age, sex, baseline BMI, baseline blood pressure (DBP), baseline [yes/no] antihypertensive medication use, baseline fiber intake, and change in weight. Models with individual SCFAs also adjust for the percent change in remaining specimen SCFAs.

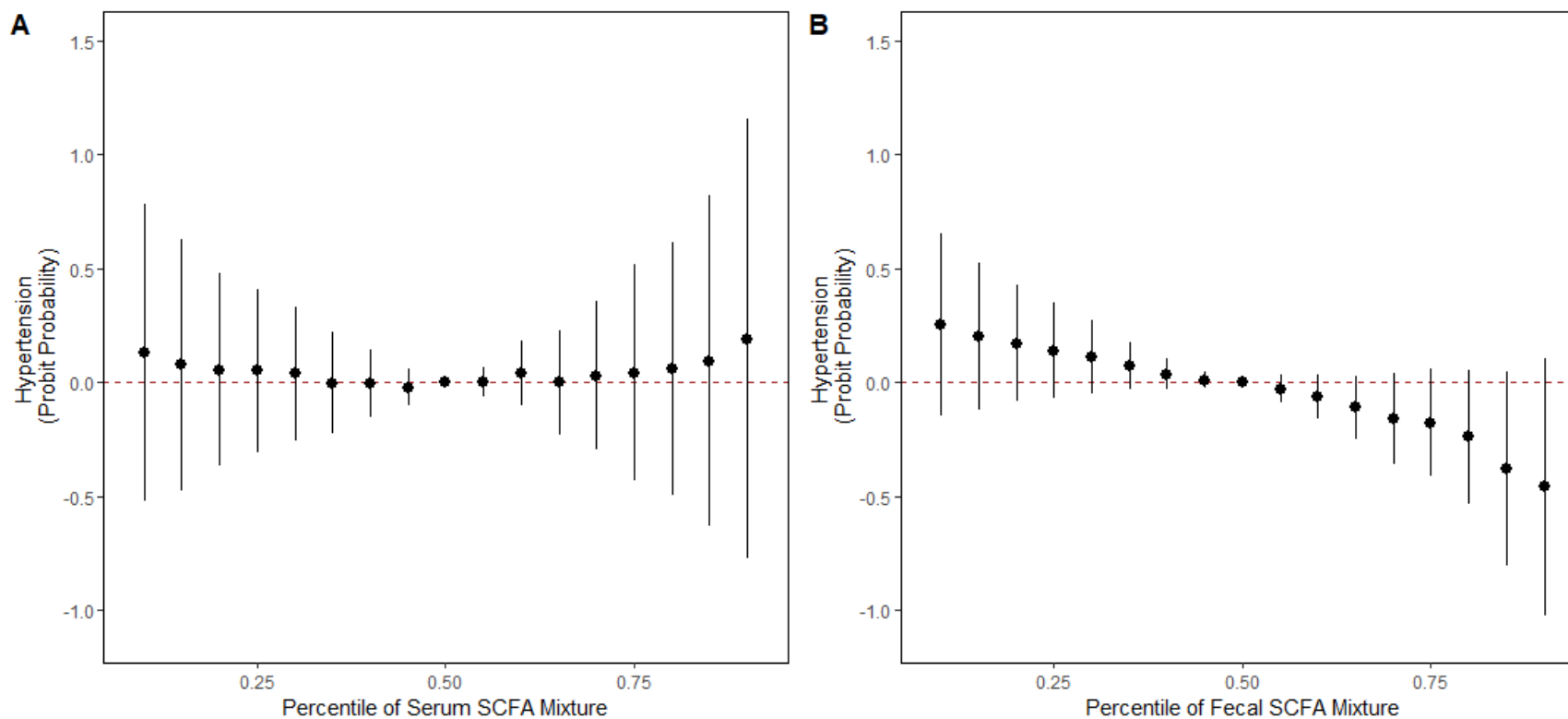


Figure S1: Overall joint effects of Serum or Fecal SCFAs with hypertension using BKMR approach. Plots show the estimates ($\pm 1.96 \times \text{SD}$) from the exposure-response function, comparing the effects when all SCFAs are at the specified quantile to when they are all at their 50th percentile levels. Plots (A) and (B) show hypertension outcomes for joint serum or joint fecal SCFA exposures, respectively.

Abbreviations: SD = standard deviation; SCFA = short chain fatty acid

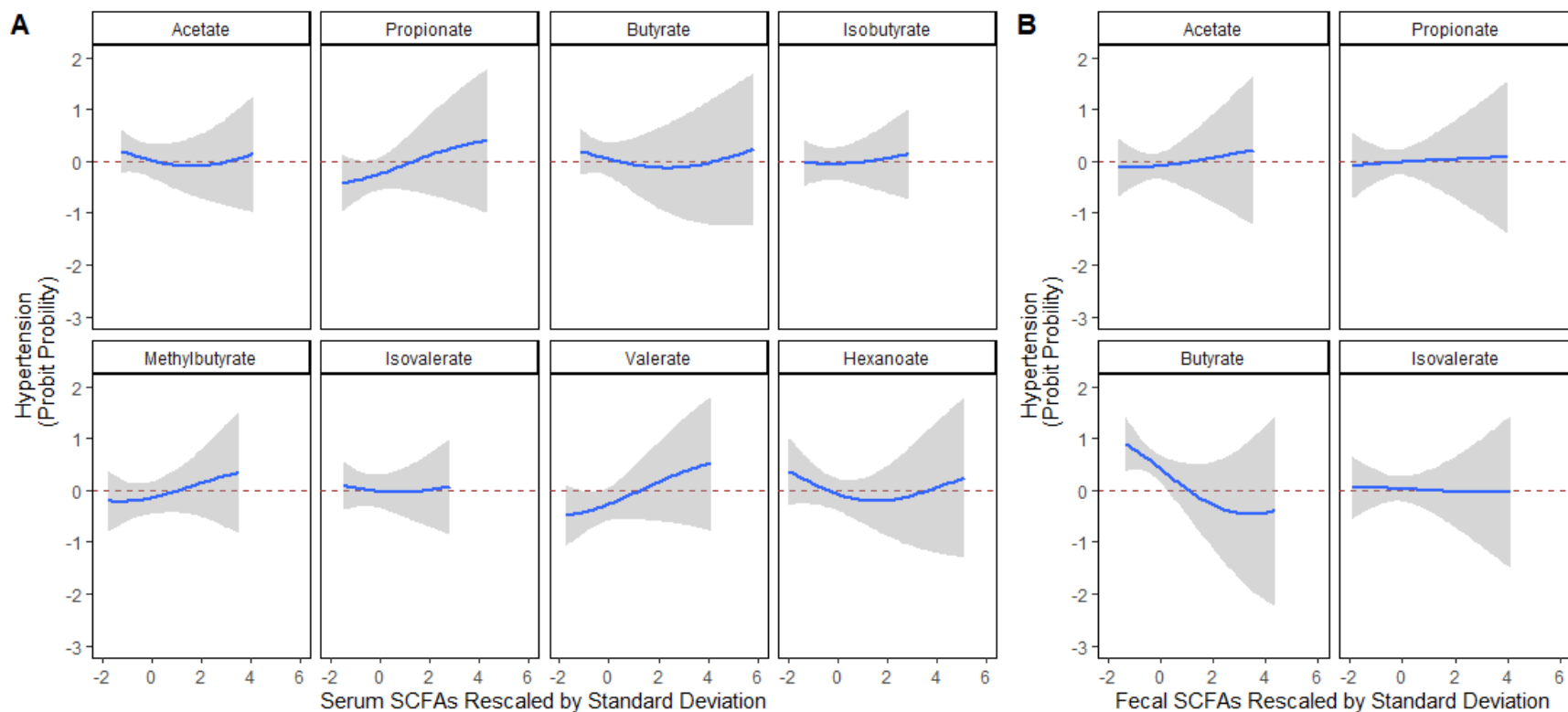


Figure S2: Univariate SCFA associations for hypertension using BKMR approach. Plots show the estimates and 95% CI for the association of an SCFA with hypertension, while other specimen SCFAs are held at their 50th percentile values. Plot (A) shows associations with serum SCFAs. Plot (B) shows associations with fecal SCFAs.

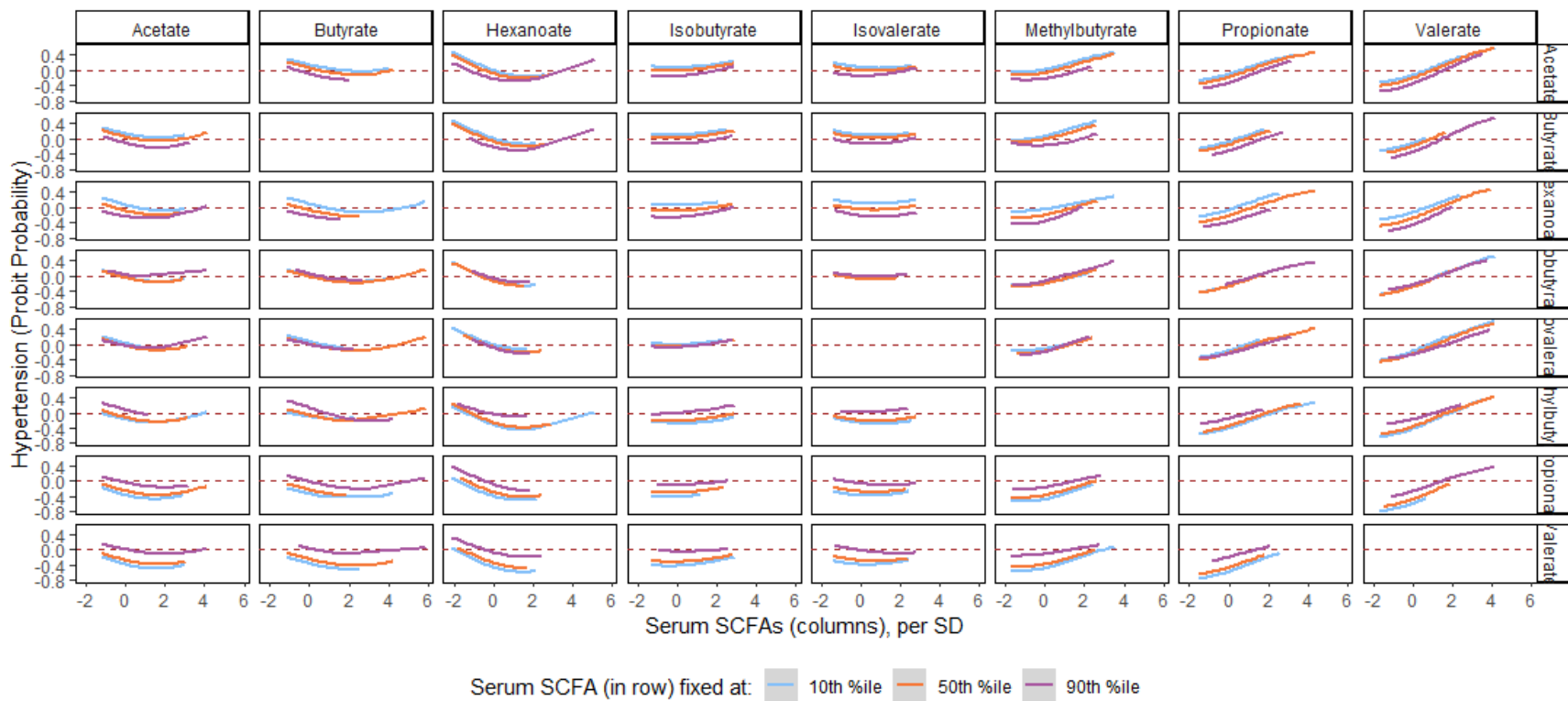


Figure S3: Two-way interaction plots for serum SCFA associations with hypertension using BKMR approach. Plots show associations of a particular serum SCFA (standardized, in columns) with hypertension at differing quantiles of a second serum SCFA (in rows), with the remaining serum SCFA held at its 50th percentile. Abbreviations: SCFA = short chain fatty acid, SD = standard deviation

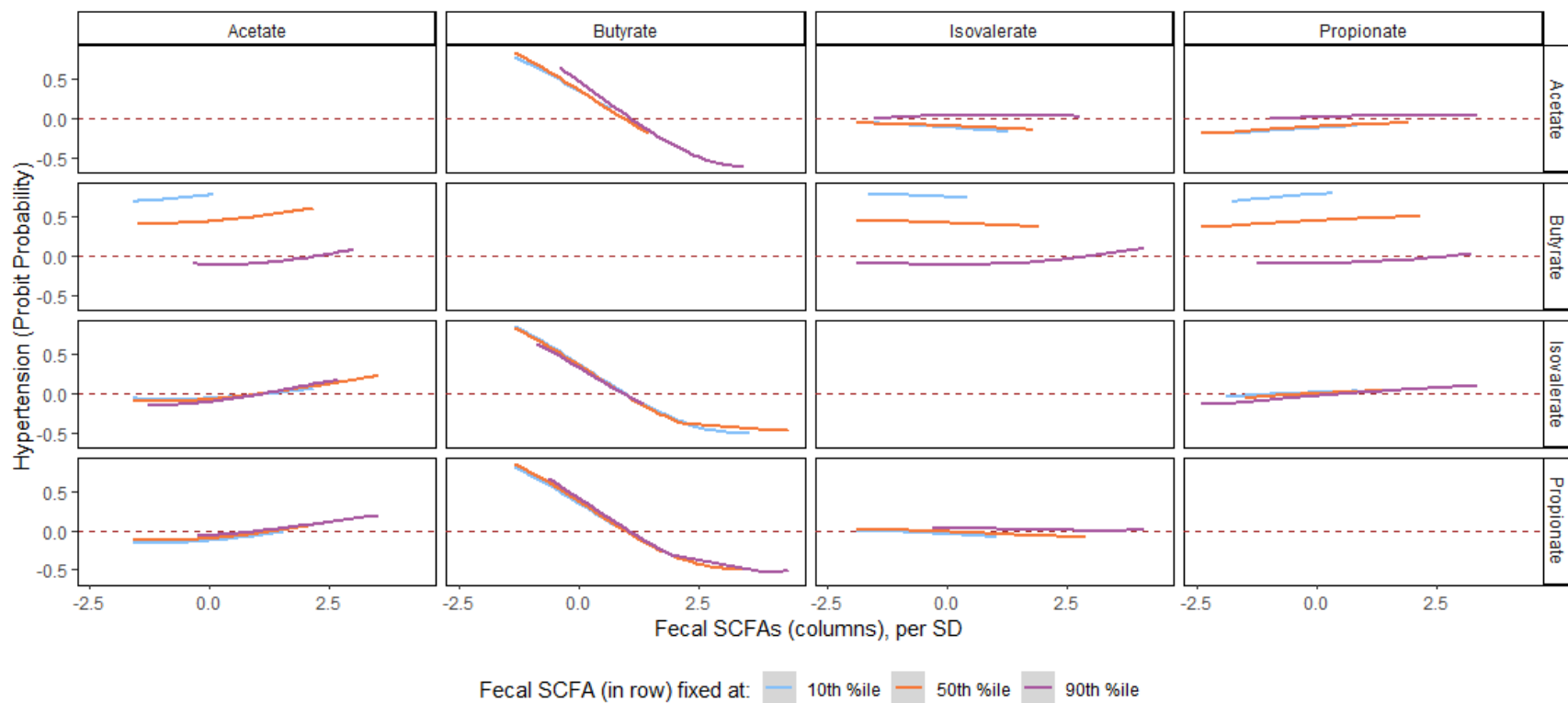


Figure S4: Two-way interaction plots for fecal SCFAs associations with hypertension using BKMR approach. Plots show associations of a particular fecal SCFA (standardized, in columns) with hypertension at differing quantiles of a second fecal SCFA (in rows), with the remaining fecal SCFA held at its 50th percentile.

Abbreviations: SCFA = short chain fatty acid, SD = standard deviation