Research Article

Cancer Prevention Research

Beneficial Regulation of Metabolic Profiles by Black Raspberries in Human Colorectal Cancer Patients

Pan Pan[1], Chad W. Skaer[1], Steven M. Stirdivant[2], Matthew R. Young[3], Gary D. Stoner[1], John F. Lechner[1], Yi-Wen Huang[4], and Li-Shu Wang[1]

Abstract

Dietary intervention of freeze-dried black raspberries (BRBs) in a group of human colorectal cancer patients has demonstrated beneficial effects, including proapoptosis, antiproliferation, and antiangiogenesis. The aim of this study was to investigate BRB-mediated metabolite changes from this same cohort of patients. Twenty-eight colorectal cancer patients were given 60 g BRB powder daily for 1 to 9 weeks. Urine and plasma specimens were collected before and after BRB intervention. A mass spectrometry–based nontargeted metabolomic analysis was conducted on each specimen. A total of more than 400 metabolites were annotated in each specimen. Of these 34 and 6 metabolites were significantly changed by BRBs in urine and plasma, respectively. Increased levels of 4-methylcatechol sulfate in both post-BRB urine and post-BRB plasma were significantly correlated with a higher level of apoptotic marker (TUNEL) in post-BRB tumors. One tricarboxylic acid (TCA) cycle metabolites, *cis*-aconitate, was increased in post-BRB urine. Furthermore, BRB-derived polyphenols were absorbed and metabolized to various benzoate species, which were significantly increased in post-BRB specimens. Increased benzoate levels were positively correlated with enhanced levels of amino acid metabolite. These results suggest that BRBs induce significant metabolic changes and affect energy generating pathways. This study supports the hypothesis that BRBs might be beneficial to colorectal cancer patients through the regulation of multiple metabolites. *Cancer Prev Res; 8(8); 743–50. ©2015 AACR.*

Introduction

Colorectal cancer is preventable with removal of polyps during colonoscopy (1). Nevertheless, it is the third most common cancer in the world with 3.5 million diagnosed patients in 2012 (2). In the United States, an estimated 132,700 new cases and 49,700 death were attributed to colorectal cancer, accounting for approximately 8.4% of all cancer deaths in 2015 (3). Many risk factors have been shown to contribute to colorectal cancer incidence, including aging, obesity, diabetes, smoking, heavy alcohol use, and family history (4). Tumor promoters, such as inflammation, carcinogens, growth factors, and hormones, increase proliferation and progression of initiated cells and precancerous lesions. Various dietary sources have been intensively studied as chemopreventive agents against colorectal cancer (5). Diets rich in fruits and vegetables have been reported to be inversely associated with colorectal cancer risk (6). In addition, consumption of a dry bean diet has been shown to reduce the recurrence of advanced colorectal adenomas (7).

Given the complex nature of both dietary components themselves and the interactions they have with precancer cells, a comprehensive metabolomic approach has been used to investigate the effects of disease and therapeutic prevention/intervention (8, 9). With the advantages of being sensitive, precise, consistent, and quantitative, metabolomics measures the dynamic status of small-molecular-weight metabolites. Thus, metabolomics characterizes the end-products of transcription and translation and is a closer representation of the phenotype (10). Tumor-specific phenotypes have been reported in colorectal cancer using metabolomics, including changes in amino acid metabolism, polyamine metabolism, glycolysis, tricarboxylic acid (TCA) cycle, nucleic acid metabolism, and methylation (8, 11–15). More importantly, metabolomics can identify bioactive dietary components and provide insights to the mechanisms of action these dietary components have on tumor-specific metabolic pathways (16). For instance, black raspberry (BRB)–derived anthocyanin compounds, cyanidin 3-rutinoside and cyanidin 3-xylosylrutinoside, which have showed antiproliferative effects on colon cancer cells, were the predominant bioactive components identified using metabolomics (17). In addition, the concept of "personalized health" has been proposed to characterize the specific profile for each individual (18), which could predict the patient's risk factors, stage of cancer, and response to chemotherapy or dietary

[1]Division of Hematology and Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin. [2]Clinical Research and Development, Metabolon Inc., Durham, North Carolina. [3]Laboratory of Cancer Prevention, Center for Cancer Research, National Cancer Institute, Frederick, Maryland. [4]Department of Obstetrics and Gynecology, Medical College of Wisconsin, Milwaukee, Wisconsin.

P. Pan and C.W. Skaer share first authorship.

Corresponding Author: Li-Shu Wang, Medical College of Wisconsin, Room C4930, 8701 Watertown Plank Road, Milwaukee, WI 53226. Phone: 414-955-2827; Fax: 414-955-2572; E-mail: liswang@mcw.edu

doi: 10.1158/1940-6207.CAPR-15-0065

interventions. Ideally, personalized therapy based on an individual's unique metabolic profile could be combined with genomic and proteomic profiles to provide the greatest efficacy against diseases.

We previously reported that an average of 4 weeks of dietary intervention with freeze-dried BRBs resulted in demethylation of tumor-suppressor genes and modulation of several biomarkers of tumor development in the human colon and rectum tissues (19). This study was conducted to determine whether metabolic pathways are altered by BRBs in these patients. We performed a metabolomic study to identify metabolites whose levels in urine and plasma are changed by BRBs and to assess the biochemical pathways that may be affected by BRBs. Dietary BRB intervention led to significant alterations of benzoates derived from BRB components both in urine and plasma. An increased level of 4-methylcatechol sulfate was significantly correlated with a higher level of apoptotic marker (TUNEL) in post-BRB tumors. Our data suggest that several metabolic pathways, for example, amino acid metabolism, energy, and lipid metabolism, are altered by BRB intervention, which might be beneficial to colorectal cancer patients.

Materials and Methods

Human clinical trials

The present trial was approved by the Institutional Review Boards of the Ohio State University Comprehensive Cancer Center and the University of Texas, San Antonio. Inclusion criteria and exclusion criteria were described in our previous publication (19). Freeze-dried berry powder (20 g) was mixed with 100 mL of water and orally administered three times a day (a total of 60 g/d), 6 hours apart, for 1 to 9 weeks (19). Plasma and urine were collected at baseline and after berry treatment for metabolomic profiling.

Metabolomic profiling

The mass spectrometer platforms, sample extraction and preparation, instrument settings, conditions, and data handling were performed at Metabolon Inc. (Research Triangle Park, NC), and have been previously described in detail (20). Briefly, the major components of the process can be summarized as follows. Osmolality of each urine sample was determined before processing. A cocktail of recovery standards was added to the urine and plasma samples and 100 μL aliquots were extracted in 500 μL methanol. The resulting urine and plasma extracts were divided into three fractions for untargeted metabolic profiling and samples were randomized for analysis. Each sample was dried under vacuum to remove organic solvent. Samples were characterized using three independent platforms: ultrahigh-performance liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) in the negative ion mode, UHPLC-MS/MS in the positive ion mode and gas chromatography-mass spectrometry (GC-MS) after siaylation. The reproducibility of the extraction protocol was assessed

Table 1. List of metabolites significantly changed in post-BRB urine

Class	Metabolic pathway	Metabolite	Fold control	*P*	*q*
Amino acid	Histidine metabolism	3-methylhistidine	3.57	0.0072	0.0458
		N-acetyl-3-methylhistidine	2.58	0.0062	0.0419
	Phenylalanine and tyrosine metabolism	3-hydroxyphenylacetate	9.43	7.64E−05	0.001
		Gentisate	4.92	0.0011	0.0098
		Phenylpropionylglycine	2.42	0.0023	0.0178
		3-[3-(sulfooxy)phenyl]propanoic acid	32.25	1.40E−05	0.0004
		5-hydroxymethyl-2-furoic acid	8.56	0.0071	0.0458
	Tryptophan metabolism	Anthranilate	5.81	3.21E−10	3.48E−08
	Urea cycle; arginine and proline metabolism	N-acetylproline	10.39	4.00E−08	2.89E−06
Carbohydrate	Pentose metabolism	Xylose	2.19	4.10E−05	0.0008
		Xylitol	0.82	0.0043	0.0304
	Fructose, mannose and galactose metabolism	Methyl-beta-glucopyranoside	3.65	0.0016	0.0142
Energy	TCA cycle	*cis*-aconitate	1.36	0.0001	0.0017
Lipid	Inositol metabolism	Chiro-inositol	5.63	0.0035	0.0256
	Glycerolipid metabolism	Glycerol 3-phosphate (G3P)	0.77	0.0018	0.0143
	Steroid	Cortisol	0.76	0.0018	0.0143
		Cortisone	1.63	0.0026	0.0194
Xenobiotics	Benzoate metabolism	Hippurate	3.71	9.02E−05	0.0012
		3-hydroxyhippurate	36.59	3.06E−05	0.0007
		4-hydroxyhippurate	2.45	0.0002	0.0026
		2,4,6-trihydroxybenzoate	21.27	4.92E−11	1.07E−08
		Catechol	3.1	7.05E−05	0.001
		Catechol sulfate	5.97	2.77E−07	1.50E−05
		4-methylcatechol sulfate	4.25	0.0006	0.0064
	Food component/plant	2,5-furandicarboxylic acid	6.45	2.23E−05	0.0005
		Vanillate	5.48	6.79E−07	2.94E−05
		2,3-dihydroxyisovalerate	5.22	0.0008	0.008
		2-isopropylmalate	6.28	1.71E−05	0.0005
		2-oxindole-3-acetate	5.25	0.0001	0.0017
		Abscisate	5.23	4.98E−05	0.0008
		Cinnamoylglycine	4.15	5.96E−05	0.0009
		N-(2-furoyl)glycine	5.73	0.001	0.0094
	Drug	4-acetylphenol sulfate	4.71	3.18E−06	0.0001
	Chemical	Succinimide	1.9	4.94E−05	0.0008

NOTE: Fold control is calculated as the ratio of post-BRB urine over pre-BRB urine.

Table 2. List of metabolites significantly changed in post-BRB plasma

Class	Metabolic pathway	Metabolite	Fold control	*P*	*q*
Amino acid	Phenylalanine and tyrosine metabolism	3-phenylpropionate (hydrocinnamate)	2.25	0.0004	0.0367
		5-hydroxymethyl-2-furoic acid	3.44	0.0006	0.0413
Carbohydrate	Fructose, mannose, galactose, starch, and sucrose metabolism	Methyl-beta-glucopyranoside	6.92	1.00E−15	3.15E−13
	Nucleotide sugars, pentose metabolism	Xylose	2.67	5.77E−11	1.22E−08
Xenobiotics	Benzoate metabolism	4-methylcatechol sulfate	2.72	0.0004	0.0367
		Catechol sulfate	3.78	6.05E−08	8.49E−06

NOTE: Fold control is calculated as the ratio of post-BRB plasma over pre-BRB plasma.

by the recovery of the xenobiotic compounds spiked in every urine sample before extraction. Urine and plasma extracts were analyzed using a platform consisting of a Waters ACQUITY UHPLC (Waters Corporation) and a Thermo-Finnigan LTQ mass spectrometer (Thermo Fisher Scientific Inc.). The derivatized samples for GC-MS were analyzed on a Thermo-Finnigan Trace DSQ single—quanrupole MS (Thermo Finnegan). Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison with metabolomic library entries of purified standards based on chromatographic properties and mass spectra.

A selection of QC compounds was added to every sample, including the experimental test samples. The internal standard compounds were carefully chosen so as not to interfere with the measurement of the endogenous compounds. These internal standards were used to assess instrument variability. In addition, a matrix pool was assembled from aliquots of all or most of the test samples and endogenous biochemicals in the matrix pool were measured in six replicates per set of 32 test samples. Each test sample was analyzed once with instrument variability determined by calculating the median relative standard deviation (RSD) for the internal standards that were added to each sample before injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., noninstrument standards) present in 100% of the Client Matrix samples, which are technical replicates of pooled client samples. Plasma internal standards and endogenous biochemical median RSDs were 6% and 13%, respectively, whereas urine internal standards and endogenous biochemical median RSDs were 6% and 15%, respectively.

Statistical analysis

Paired *t* tests were performed in R version 2.14.2 (21) to determine the statistical significance of metabolite mean differences between pre- and post-BRB supplementation. The FDR analysis was performed and *q* value was calculated for each metabolite. For all analyses, missing values (if any) were imputed with the observed minimum for that particular compound (imputed values were added after block normalization). The statistical analyses were performed on natural log-transformed data to reduce the effect of any potential outliers in the data. In addition, urine data was normalized to sample osmolality to compensate for differences in urine concentration. Pearson correlation and linear regression tests were performed using SigmaStat3.5 to determine the association between metabolites in post-BRB urine/plasma, and between metabolites and TUNEL in post-BRB tumors. Metabolites with both a *P* value <0.05 and a *q* value <0.05 were considered statistically significant.

Results

To characterize the metabolic profiles induced by BRBs in colorectal cancer patients, we collected urine and plasma specimens before and after BRB treatment and performed MS-based nontargeted metabolomic analysis. A total of more than 400

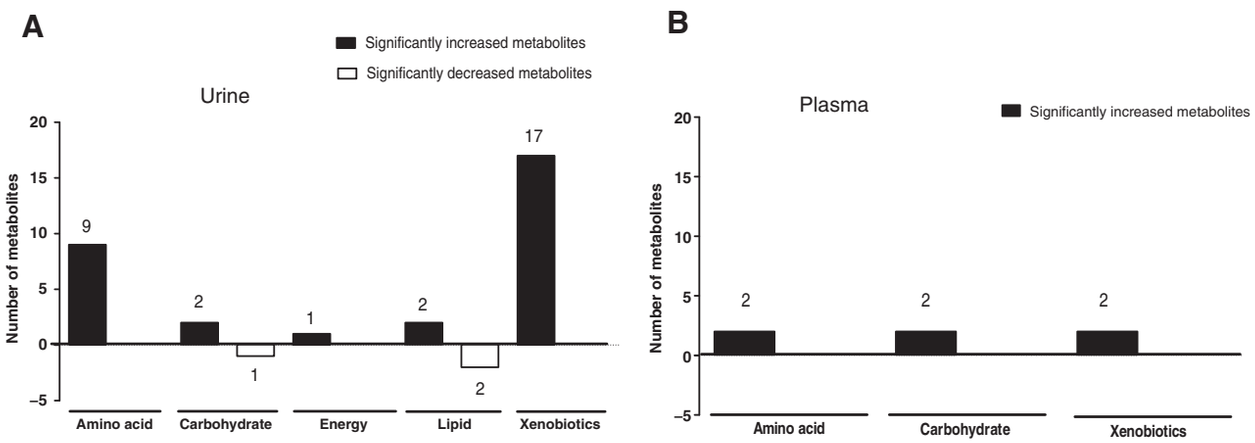

Figure 1.
Metabolic pathway classes for metabolites detected in urine and plasma. Historical graphs represent the number of urinary (A) and plasma (B) metabolites significantly increased (black) and decreased (white) in each metabolic pathway class.

metabolites were annotated in each sample. When data from all 28 patients were combined, 34 urinary metabolites (Table 1) were significantly changed by BRBs, including 31 increased metabolites and 3 decreased metabolites. In addition, BRBs significantly increased 6 plasma metabolites (Table 2). Significantly changed metabolites were referenced with the Koto Encyclopedia of Genes and Genomes (KEGG) database to identify specific metabolic pathways, that is, amino acid metabolism, energy, carbohydrate metabolism, lipid metabolism, and xenobiotics. BRBs significantly increased and decreased multiple urinary (Fig. 1A) and plasma (Fig. 1B) metabolites in various metabolic pathways.

BRBs significantly increased amino acid metabolism

BRBs significantly increased 9 urinary (Table 1) and 2 plasma (Table 2) metabolites in colorectal cancer patients. These increased metabolites were associated with histidine, phenylalanine, tyrosine, tryptophan, and proline metabolism. Particularly, there was a marked increase in several metabolites in post-BRB urine (Table 1), such as 3-[3-(sulfooxy) phenyl] propanoic acid (32.25-fold), N-acetylproline (10.39-fold), 3-hydroxyphenylacetate (9.43-fold), 5-hydroxymethyl-2-furoic acid (8.56-fold), and anthranilate (5.81-fold; Fig. 2A).

BRBs significantly increased TCA cycle intermediate

Regarding tumor cell energy generation, lactate accumulation is frequently associated with an increased dependence on aerobic glycolysis and depletion of TCA cycle intermediates has been reported in nearly every study of colorectal cancer patients at various pathologic stages (11–13, 15, 22–24). Intriguingly, BRBs significantly increased one TCA cycle

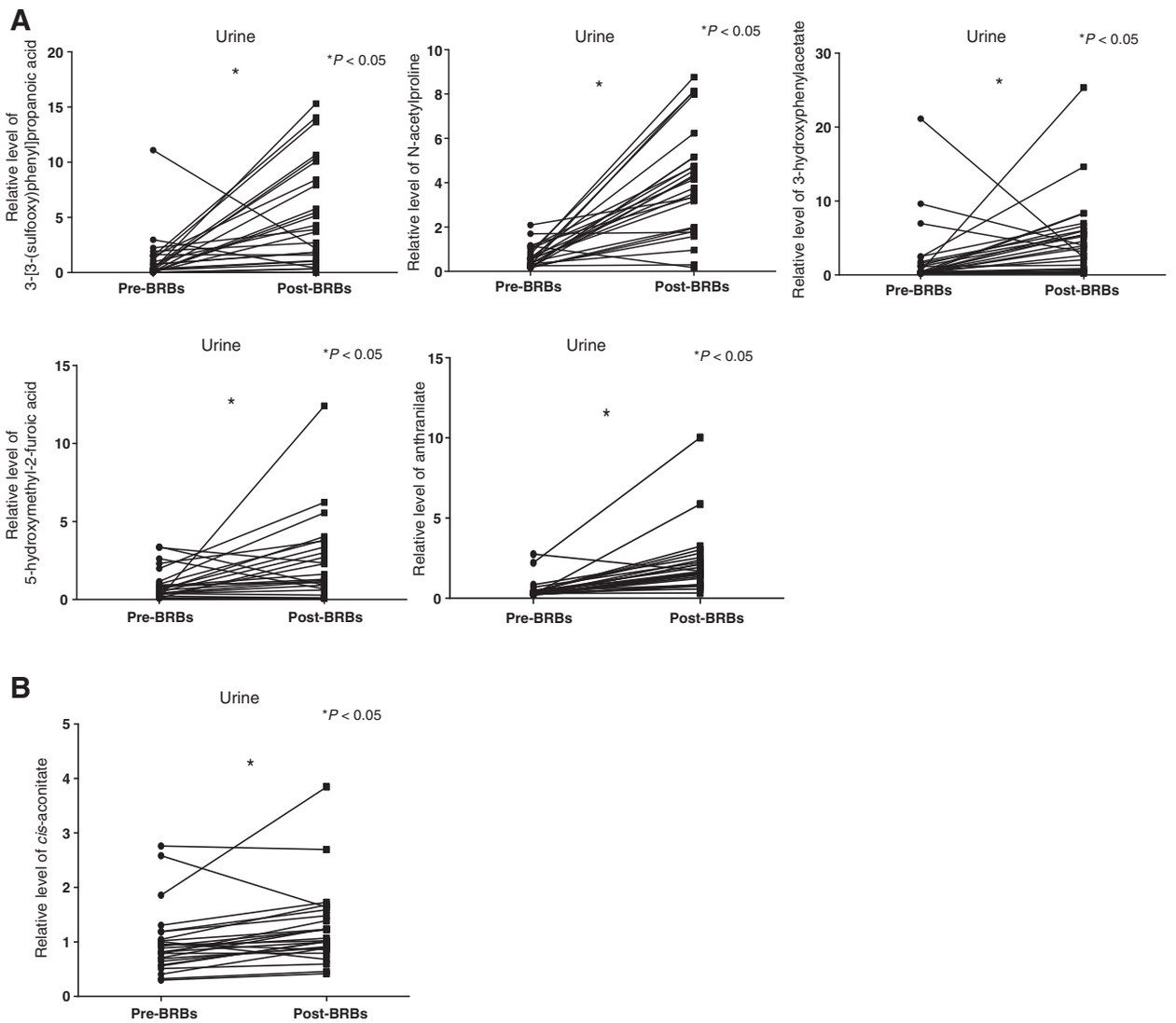

Figure 2.
BRBs significantly increased amino acid metabolism and TCA intermediate. A, significantly increased levels of 3-[3-(sulfooxy) phenyl] propanoic acid, N-acetylproline, 3-hydroxyphenylacetate, 5-hydroxymethyl-2-furoic acid and anthranilate in post-BRB urine. B, significantly increased levels of cis-aconitate in post-BRB urine.

intermediate, *cis*-aconitate in urine (Fig. 2B). However, no significant changes in glycolytic metabolites were detected.

BRBs significantly increased benzoate metabolites

One distinct feature of post-BRB metabolic profiles was the substantially increased levels of benzoate metabolites in urine (Table 1), such as 3-hydroxyhippurate (36.59-fold), 2,4,6-trihydroxybenzoate (21.27-fold), and catechol sulfate (5.97-fold; Fig. 3A). The extent of the increase in benzoates was less in plasma (Table 2). Increased levels of 4-methylcatechol sulfate in post-BRB urine and plasma were correlated with a higher level of apoptotic marker (TUNEL) in post-BRB tumors (Fig. 3B). These benzoate metabolites were most likely produced from the metabolism of BRB polyphenols and anthocyanins and were absorbed in the large intestine (25).

Significant correlations between benzoates and amino acid metabolites

Positive correlations were observed between various benzoate metabolites and amino acid metabolites. For example, in urine, increased levels of hippurate were positively correlated with three increased amino acid metabolites, 3-hydroxyphenylacetate, gentisate and phenylpropionylglycine (Fig. 4A). In plasma, increased levels of catechol sulfate and 4-methylcatechol sulfate were positively correlated with two increased amino acid metabolites, 3-phenylpropionate and 5-hydroxymethyl-2-furoic acid (Fig. 4B).

Discussion

Our prior clinical trial treated 28 colorectal cancer patients with 60 g freeze-dried BRB powder daily for 1 to 9 weeks (19) and presented BRB-induced beneficial effects against colorectal tumors, including antiproliferation, proapoptosis, and antiangiogenesis. Our study performed a comprehensive analysis of metabolic profiles in urine and plasma specimens collected from the same cohort of patients before and after BRB intervention. We detected more than 400 metabolites in each specimen. Of these 34 urinary (Table 1) and 6 plasma (Table 2) metabolites were significantly changed by BRBs. These metabolites are associated with amino acid, energy, carbohydrate, lipid metabolism, and xenobiotics (Fig. 1). Diverse distribution of metabolites suggests that BRBs could induce a systemic impact on colorectal cancer patients. Interestingly, our study showed that increased levels of 4-methylcatechol sulfate in post-BRB urine and plasma were linearly correlated with a higher level of apoptosis (TUNEL) in post-BRB tumors (Fig. 3B; ref. 19). The correlations between metabolites in urine/plasma and tumor markers suggest that BRB-mediated metabolite changes in urine/plasma may be reflective of modulation of tumor metabolism affecting colorectal tumors.

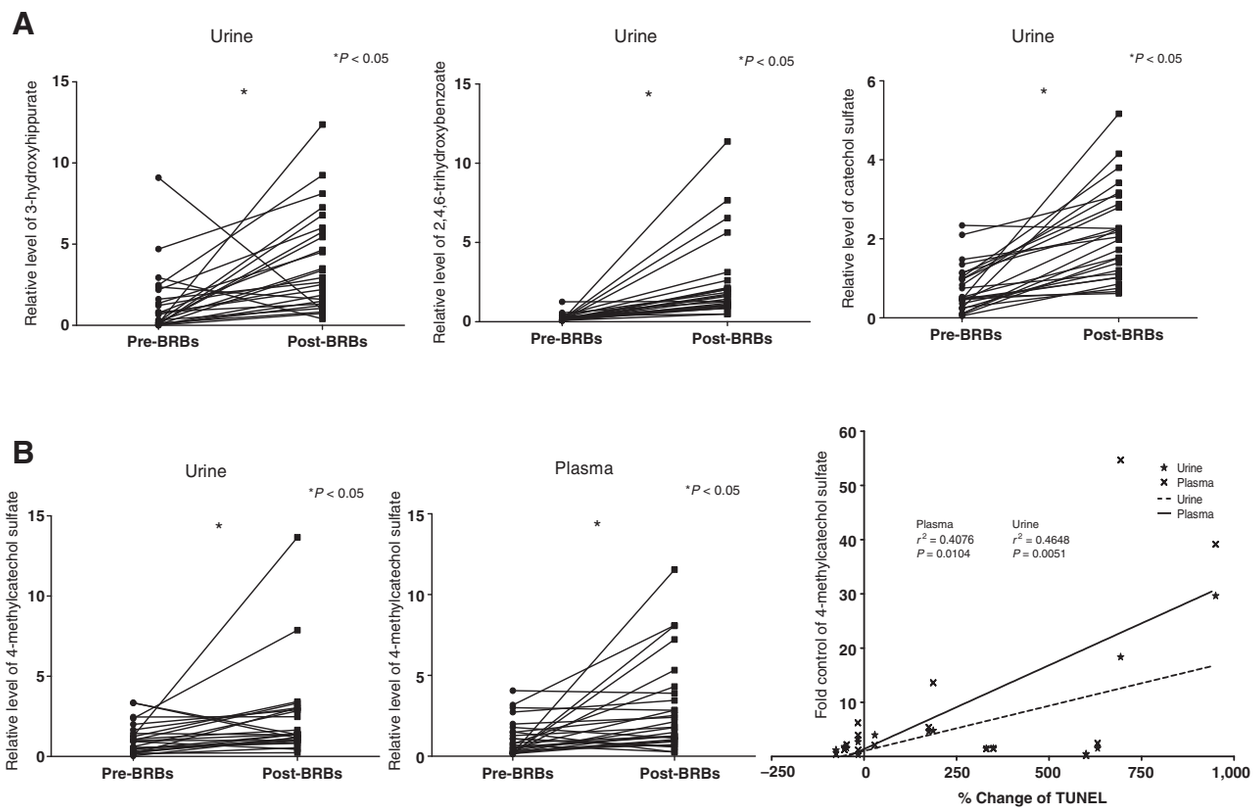

Figure 3.
BRBs significantly increased benzoate metabolites. A, significantly increased levels of 3-hydroxyhippurate, 2,4,6-trihydroxybenzoate, and catechol sulfate in post-BRB urine. B, increased levels of 4-methylcatechol sulfate in post-BRB urine (dash line) and post-BRB plasma (solid line) were significantly correlated with TUNEL staining in post-BRB tumors; *, $P < 0.05$.

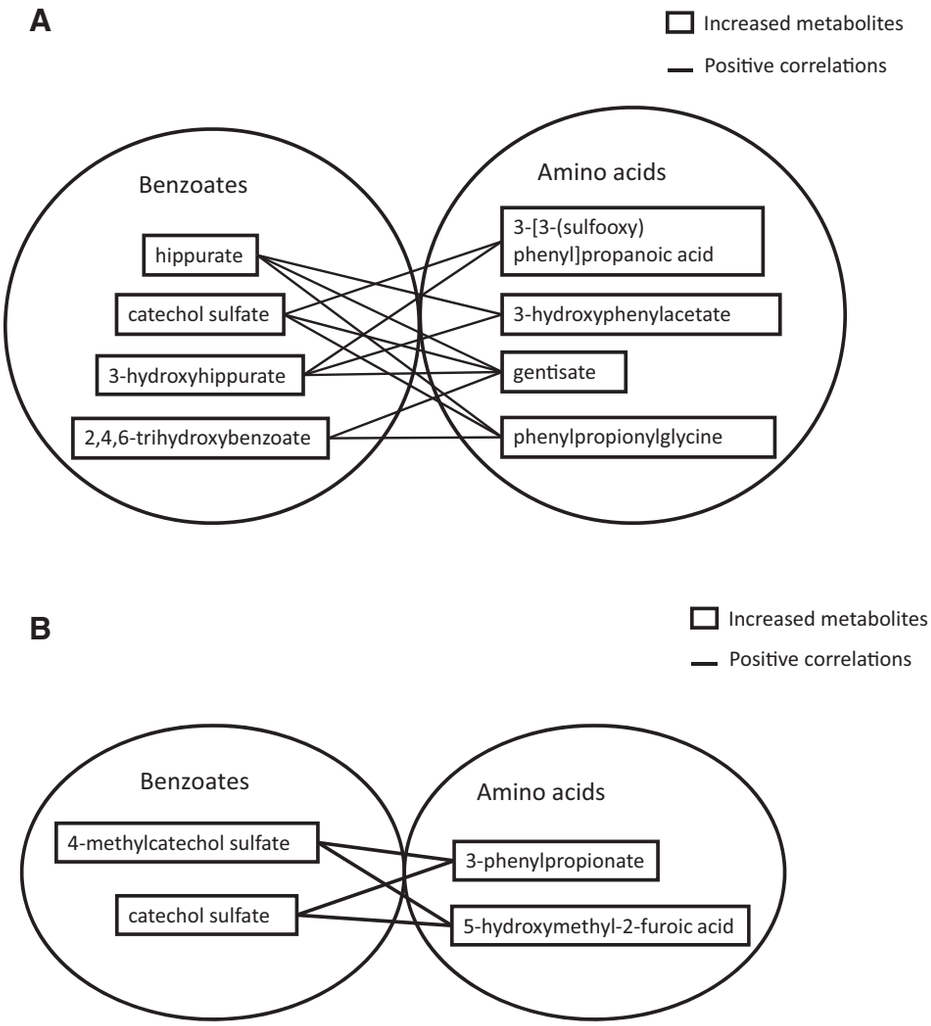

Figure 4.
Positive correlations between benzoates and amino acid metabolites in urine (A) and plasma (B). Metabolites labeled in solid line boxes were upregulated in post-BRB urine (A) and plasma (B). Solid lines indicate significant positive ($P < 0.05$) correlations between benzoates and amino acid metabolites.

Significant changes in amino acid metabolites in colorectal cancer patients have been reported, such as a decrease in histidine (15, 26), lysine (15, 22, 26), tryptophan (15, 22, 27), and valine metabolism (22, 26) in serum, as well as decreased tryptophan, tyrosine (14), and valine metabolism (28) in urine. However, observed alterations varied significantly between different groups. For instance, Leichtle and colleagues (26) detected a decrease in urinary tyrosine, leucine, isoleucine, and methionine metabolites, whereas Nishiumi and colleagues (27) reported enhanced concentrations of these amino acids. This inconsistency might be attributed to sample preparation, diet and patient classification, such as racial population. Leichtle and colleagues conducted a study in German with European patients, whereas Nishiumi and colleagues recruited Asian patients in Japan. In contrast, others have shown that colon tumor tissues presented a highly consistent pattern of changes in amino acid metabolism. Most of the essential amino acids have been reported to be elevated in colon tumors, including alanine, threonine, leucine, isoleucine, valine, lysing, glycine, methionine, and tyrosine (23, 24, 29). It is possible that these tumors increase demand for specific amino acids to support rapid cell proliferation (8). Our study demonstrated numerous amino acid metabolites changed in post-BRB urine and plasma in colorectal cancer patients (Tables 1 and 2). The increase in amino acid metabolites might be attributed directly to BRBs or the metabolism of BRBs.

Numerous studies have consistently confirmed accumulation of lactate and reduced levels of TCA cycle intermediates in many cancer types, including colorectal cancer (11–13, 15, 22–24, 30, 31). This change is commonly referred to as the "Warburg effect" (32). Consistent with our previous studies, which demonstrated antiproliferative effects of BRBs in colorectal cancer patients (19), current metabolomics identified one TCA cycle intermediate, *cis*-aconitate, that was upregulated by BRBs in urine (Fig. 2B). No significant changes were observed in lactate levels or lactate/pyruvate ratio in plasma specimens. However, nucleotide sugars, that is, xylose and xylitol, were increased in post-BRB plasma/urine, which might suggest more glucose was processed through pentose phosphate pathways. Accumulation of TCA cycle intermediates suggests a higher TCA cycle turnover rate and further leads to an enhanced rate of mitochondrial oxidative respiration.

Our study detected several considerably increased polyphenol-derived benzoate metabolites in post-BRB urine specimens, such as 3-hydroxyhippurate (36.59-fold), 2,4,6-trihydroxybenzoate (21.27-fold), and catechol sulfate (5.97-fold; Fig. 3A). Importantly, we observed significant positive correlations between polyphenol-derived benzoate metabolites and amino acid metabolites (Figs. 4A and B). In addition, some amino acid metabolites have been shown to derive from other dietary sources using *in vitro* anaerobic fecal fermentation mode. For example, 3-hydroxyphenylacetate has been reported from rutin (33); 5-hydroxymethyl-2-furoic acid was detected in urine from subjects that consumed dried plum juice (34).

All of the tumor samples from these patients were used to validate the effects of BRBs on inhibition of cell proliferation by Ki67 immunohistochemistry, on apoptosis by TUNEL assays and to determine the levels of gene methylation on tumor-suppressor genes (19). In this study, the urinary and plasma specimens of the colorectal patients before BRB intervention served as their own control in comparison with the specimens collected after BRB intervention; therefore, observed differences could be associated with the BRB intervention, dietary changes during the BRB intervention, or other time-associated differences. We used published information from many groups that have conducted comprehensive metabolomic studies to determine the tumor-specific metabolic profiles in colorectal cancer patients (8, 11–15). Thus, large-scale studies, which include metabolic analysis of tumors, of the microenvironment and of the microbiome are needed to accurately measure the effects of BRBs on colorectal cancer patients in comparison with healthy controls.

Together, our results suggest that BRBs significantly induce metabolic changes in colorectal cancer patients. These BRB-derived metabolites may contribute to an overall beneficial regulation against colorectal tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y.-W. Huang, L.-S. Wang

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y.-W. Huang

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Pan, C.W. Skaer, S.M. Stirdivant, M.R. Young, G.D. Stoner, J.F. Lechner

Writing, review, and/or revision of the manuscript: P. Pan, C.W. Skaer, S.M. Stirdivant, M.R. Young, G.D. Stoner, J.F. Lechner

Study supervision: L.-S. Wang

Acknowledgments

The authors thank Drs. Mark Arnold, Edward Martin, and Christine Sardo, as well as Comprehensive Cancer Center Tissue Procurement Shared Resource at Ohio State University for managing the trial and handling the specimens in this trial at the Ohio State University. They also thank Dr. John Winston III and Sara Olivarri for their help in the trial at the University of Texas, San Antonio. Finally, they thank all patients for their participation in this trial.

Grant Support

This work was supported by NIH grant 5 R01 CA148818 04 and American Cancer Society, RSG-13-138-01—CNE (to L.-S. Wang).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 13, 2015; revised May 19, 2015; accepted May 26, 2015; published OnlineFirst June 8, 2015.

Reference

1. Available from: http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-treating-by-stage-colon.
2. Available from: http://publications.cancerresearchuk.org/downloads/product/CS_REPORT_WORLD.pdf.
3. Available from: http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf.
4. Available from: http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-risk-factors.
5. Miller AB, Linseisen J. Achievements and future of nutritional cancer epidemiology. Int J Cancer 2010;126:1531–7.
6. van Duijnhoven FJ, Bueno-De-Mesquita HB, Ferrari P, Jenab M, Boshuizen HC, Ros MM, et al. Fruit, vegetables, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr 2009;89:1441–52.
7. Bobe G, Sansbury LB, Albert PS, Cross AJ, Kahle L, Ashby J, et al. Dietary flavonoids and colorectal adenoma recurrence in the Polyp Prevention Trial. Cancer Epidemiol Biomarkers Prev 2008;17:1344–53.
8. Wang H, Tso VK, Slupsky CM, Fedorak RN. Metabolomics and detection of colorectal cancer in humans: a systematic review. Future Oncol 2010;6:1395–406.
9. Mamas M, Dunn WB, Neyses L, Goodacre R. The role of metabolites and metabolomics in clinically applicable biomarkers of disease. Arch Toxicol 2011;85:5–17.
10. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999;29:1181–9.
11. Tan B, Qiu Y, Zou X, Chen T, Xie G, Cheng Y, et al. Metabonomics identifies serum metabolite markers of colorectal cancer. J Proteome Res 2013;12:3000–9.
12. Manna SK, Tanaka N, Krausz KW, Haznadar M, Xue X, Matsubara T, et al. Biomarkers of coordinate metabolic reprogramming in colorectal tumors in mice and humans. Gastroenterology 2014;146:1313–24.
13. Qiu Y, Cai G, Su M, Chen T, Liu Y, Xu Y, et al. Urinary metabonomic study on colorectal cancer. J Proteome Res 2010;9:1627–34.
14. Cheng Y, Xie G, Chen T, Qiu Y, Zou X, Zheng M, et al. Distinct urinary metabolic profile of human colorectal cancer. J Proteome Res 2012;11:1354–63.
15. Zhu J, Djukovic D, Deng L, Gu H, Himmati F, Chiorean EG, et al. Colorectal cancer detection using targeted serum metabolic profiling. J Proteome Res 2014;13:4120–30.
16. Kim YS, Milner JA. Bioactive food components and cancer-specific metabonomic profiles. J Biomed Biotechnol 2011;2011:721213.
17. Paudel L, Wyzgoski FJ, Giusti MM, Johnson JL, Rinaldi PL, Scheerens JC, et al. NMR-based metabolomic investigation of bioactivity of chemical constituents in black raspberry (Rubus occidentalis L.) fruit extracts. J Agric Food Chem 2014;62:1989–98.
18. McNiven EM, German JB, Slupsky CM. Analytical metabolomics: nutritional opportunities for personalized health. J Nutr Biochem 2011;22:995–1002.
19. Wang LS, Arnold M, Huang YW, Sardo C, Seguin C, Martin E, et al. Modulation of genetic and epigenetic biomarkers of colorectal cancer in

humans by black raspberries: a phase I pilot study. Clin Cancer Res 2011;17:598–610.
20. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal Chem 2009;81:6656–67.
21. Team RC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
22. Qiu Y, Cai G, Su M, Chen T, Zheng X, Xu Y, et al. Serum metabolite profiling of human colorectal cancer using GC-TOFMS and UPLC-QTOFMS. J Proteome Res 2009;8:4844–50.
23. Chan EC, Koh PK, Mal M, Cheah PY, Eu KW, Backshall A, et al. Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). J Proteome Res 2009; 8:352–61.
24. Denkert C, Budczies J, Weichert W, Wohlgemuth G, Scholz M, Kind T, et al. Metabolite profiling of human colon carcinoma–deregulation of TCA cycle and amino acid turnover. Mol Cancer 2008;7:72.
25. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. Pharmacol Res 2013;69:52–60.
26. Leichtle AB, Nuoffer JM, Ceglarek U, Kase J, Conrad T, Witzigmann H, et al. Serum amino acid profiles and their alterations in colorectal cancer. Metabolomics 2012;8:643–53.
27. Nishiumi S, Kobayashi T, Ikeda A, Yoshie T, Kibi M, Izumi Y, et al. A novel serum metabolomics-based diagnostic approach for colorectal cancer. PLoS ONE 2012;7:e40459.
28. Ma YL, Qin HL, Liu WJ, Peng JY, Huang L, Zhao XP, et al. Ultra-high performance liquid chromatography-mass spectrometry for the metabolomic analysis of urine in colorectal cancer. Dig Dis Sci 2009;54:2655–62.
29. Ong ES, Zou L, Li S, Cheah PY, Eu KW, Ong CN. Metabolic profiling in colorectal cancer reveals signature metabolic shifts during tumorigenesis. Mol Cell Proteomics: MCP, 2010.
30. Ni Y, Xie G, Jia W. Metabonomics of human colorectal cancer: new approaches for early diagnosis and biomarker discovery. J Proteome Res 2014;13:3857–70.
31. Qiu Y, Cai G, Zhou B, Li D, Zhao A, Xie G, et al. A distinct metabolic signature of human colorectal cancer with prognostic potential. Clin Cancer Res 2014;20:2136–46.
32. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 1927;8:519–30.
33. Aura AM, O'Leary KA, Williamson G, Ojala M, Bailey M, Puupponen-Pimia R, et al. Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal flora in vitro. J Agric Food Chem 2002;50:1725–30.
34. Prior RL, Wu X, Gu L. Identification and urinary excretion of metabolites of 5-(hydroxymethyl)-2-furfural in human subjects following consumption of dried plums or dried plum juice. J Agric Food Chem 2006;54:3744–9.