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Modulation of plasma and urine metabolome in colorectal cancer survivors consuming rice bran

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Abstract

Rice bran has bioactive phytochemicals with cancer protective actions that involve metabolism by the host and the gut microbiome. Globally, colorectal cancer (CRC) is the third leading cause of cancer-related death and the increased incidence is largely attributed to poor dietary patterns, including low daily fiber intake. A dietary intervention trial was performed to investigate the impact of rice bran consumption on the plasma and urine metabolome of CRC survivors. Nineteen CRC survivors participated in a randomized-controlled trial that included consumption of heat-stabilized rice bran (30 g/day) or a control diet without rice bran for 4 weeks. A fasting plasma and first void of the morning urine sample were analyzed by non-targeted metabolomics using ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). After 4 weeks of either rice bran or control diets, 12 plasma and 16 urine metabolites were significantly different between the groups ($p \leq 0.05$). Rice bran intake increased relative abundance of plasma mannose (1.373-fold) and beta-citrylglutamate (BCG) (1.593-fold), as well as increased urine N-formylphenylalanine (2.191-fold) and dehydroisoandrosterone sulfate (DHEA-S) (4.488-fold). Diet affected metabolites, such as benzoate, mannose, eicosapentaenoate (20:5n3) (EPA), and N- formylphenylalanine have been previously reported for cancer protection and were identified from the rice bran food metabolome. Nutritional metabolome changes following increased consumption of whole grains such as rice bran warrants continued investigation for colon cancer control and prevention attributes as dietary biomarkers for positive effects are needed to reduce high risk for colorectal cancer recurrence.

Introduction

Despite major advances in cancer screening and treatment, colorectal cancer (CRC) remains the third leading cause of cancer-related death in the world [1] and in the United States [2]. Evidence supports that a plant-based, fiber-rich diet can increase longevity and lower the risk of chronic diseases including obesity [3,4], cardiovascular disease [5,6], diabetes [7-9], and CRC [10-13]. The American Institute for Cancer Research (AICR) estimated that almost half of U.S. CRC incidences can be prevented through appropriate lifestyle behaviors, including regular physical activity and a healthy diet comprised of fruits, vegetables, whole grains, and beans [14].

Rice bran is a functional food that has been recognized as globally accessible, but not well known as food that can prevent and control chronic diseases [15,16]. Bioactive food components from rice bran were identified for cancer protective functions related to energy metabolism [17-19], antioxidant [20], and direct anti-neoplastic activity [21,22]. Rice bran was examined as functional food in research for both CRC treatment and prevention [23-27]. Consumption of brown rice once per week was shown to reduce the incidence of adenomatous polyps by 40% [28] and numerous studies demonstrate the diverse nutritional and medicinal properties of rice bran bioactive compounds [21,29,30]. Given the chronic disease fighting properties established for rice bran from *in vitro* and animal studies, this food was studied in humans and showed feasibility to increase total daily fiber intake in adults [15,26] and children [31]. Notably, rice bran was beneficial to modulate the human stool microbiome [26,27] and metabolome [25], which provided rationale to measure metabolic changes in the plasma and urine.

Metabolomics has been shown to be a promising tool for assessing dietary intakes as well as the influence of dietary patterns in relation to cancer risk [24,25]. Metabolomics is used to measure a suite of metabolites in foods [32] and biofluids [33]. This approach has provided valuable insight into the nutrition and health implications on the host metabolome [34]. Plasma and urine are relevant biological matrices for studying the effects of dietary intakes in humans that may suppress or alter colon carcinogenesis [35]. The objective of this study was to identify metabolites and metabolic pathways affected by rice bran consumption in a cohort of overweight and obese individuals at risk for colon cancer recurrence. We hypothesized that rice bran consumption favorably modulates multiple metabolic pathways in the plasma and urine associated with colorectal cancer prevention. Identification of bioactive compounds in the metabolite profiles may uncover candidate dietary biomarkers of intake and a health beneficial response to rice bran by the host.

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Materials and methods

Study design and dietary intervention

CRC survivors were recruited through the University of Colorado Health-North Cancer Center Network (Fort Collins, CO) for a 4-week, randomized-controlled, single-blinded dietary intervention trial as previously described [15,25,26]. Eligibility included: 1) no history of other malignancies, other than a CRC diagnosis, 2) more than four months post cancer treatment (e.g. chemotherapy or radiation), 3) no history of food allergies or major dietary restrictions, 4) not currently pregnant or lactating, 5) a non-smoker, 6) not taking antibiotics within the month prior to enrollment, and 7) no history of gallstones [15].

Participants who met eligibility criteria were randomized by body mass index (BMI), sex, and daily caloric intake. At the baseline, participants completed a 3-day food log recording all food and drink consumed on two weekdays and one weekend day and the food logs were analyzed using Nutritionist ProTM (Axxya Systems, Redmond, WA) as previously described [36]. Participants completed three study visits at baseline, week 2, and week 4 when the fasting blood and first void urine samples were collected. Participants in the rice bran group consumed one snack and one meal daily that each contained 15 g of rice bran for a total consumption of 30 g/day. The control group was provided with macronutrient matched meals and snacks that did not include rice bran.

The Colorado State University Research Integrity and Compliance Review Board and the UCH-North Institutional Review Board approved this study protocol and informed consent (Protocol #s 09-1530H and 10-1038, respectively). Figure 1 illustrates the 19 participants that completed the study called Bran Enriching Nutritional Eating for Intestinal health Trial (BENEFIT) (NCT01929122).

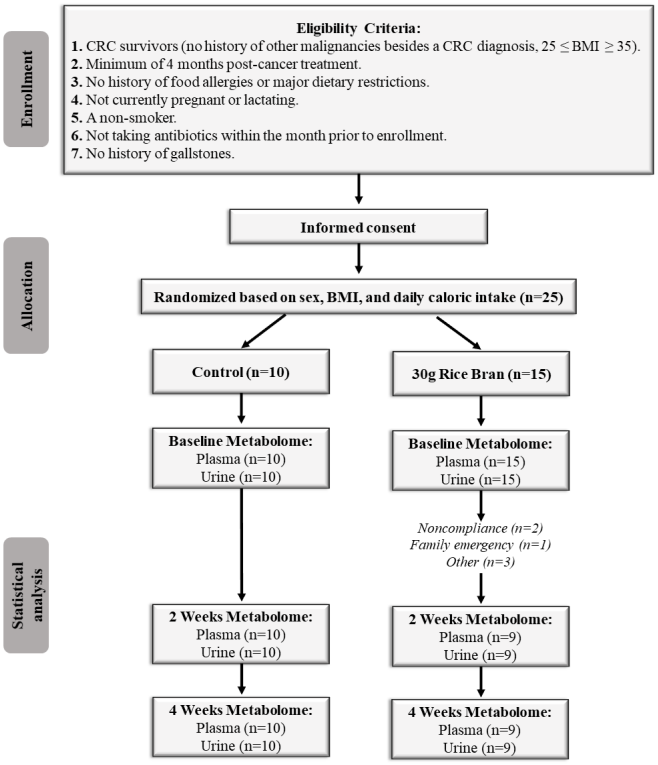

Figure 1. Study design for plasma and urine metabolome analysis in 19 colorectal cancer survivors (CRC) randomized to either control or rice bran intervention. The metabolomics data was analyzed in two comparisons; a) the control group and rice bran group had respective baselines, 2 weeks and 4 weeks post intervention analyses, b) rice bran group and control group were analyzed at 4 weeks post intervention

Blood and urine sample collection for metabolomics

The 19 participants that completed three study visits (baseline, 2 weeks, and week 4) provided fasting blood samples by venipuncture. Plasma was processed from whole blood collected in two 4 ml ethylene-diamine-tetra-acetic acid (EDTA) blood collection tubes, and immediately placed on ice. Whole blood was centrifuged cold at 1500 rpm for 10 minutes, and 3 ml of plasma was aliquoted from the sample and stored in 10 ml tubes at -80°C until processed for metabolomics by ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

First void of the morning urine was self-collected by participants into study-coded specimen containers and transported to the lab within 2-hours of each study visit. Urine was aliquoted by lab personnel and stored at -80°C until analyzed by UPLC-MS/MS. Osmolality normalization was applied to urine samples to correct for variance in fluid intake and urine solute concentrations. Osmolality of urine samples was measured using a Fiske™ 210 Micro-Sample Osmometer. For normalization, the median-scaled ion peak data was divided by the osmolality value to obtain the osmo-corrected value.

Plasma and urine sample extraction for metabolomics

A non-targeted metabolite profiling for all samples was performed by Metabolon, Inc. (Durham, NC, USA). Global metabolic profiles were determined for each participant across both study groups. Each sample was entered into the Metabolon Laboratory Information Management System (LIMS) and was assigned a unique identifier that was associated with the original de-identified study code number. Extraction was performed on plasma and urine using 80% methanol under vigorous shaking for 2 min and was then centrifuged to dissociate metabolites bound to proteins. The resulting extracts from plasma and urine were divided into 3 fractions: 1 for analysis by UPLC-MS/MS with positive ion mode electrospray ionization (ESI), 1 for analysis by UPLC-MS/MS with negative ion mode ESI, and 1 sample was reserved for backup. All samples (plasma and urine) were dried using a concentration evaporator (a TurboVap®, Zymark), and then were stored overnight under liquid nitrogen before preparation for analysis [24].

UPLC-MS/MS analysis

Samples were analyzed using a Waters ACQUITY UPLC system coupled with a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer and interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Prior to analysis, acidic or basic UPLC-compatible solvents was used to re-suspend the dried samples. Eight quality control standards (known metabolites at fixed concentrations) were injected to each sample to guarantee injection and chromatographic consistency. The acidic re-suspension was measured using positive ion conditions, chromatographically optimized for either hydrophilic or hydrophobic compounds. for hydrophilic compounds, the extracts were gradient eluted from a C18 column (Waters UPLC BEH C18-2.1×100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid and 0.1% formic acid. For hydrophobic compounds, the extracts were gradient eluted from another but similar afore mentioned C18 column using methanol, acetonitrile, water, 0.05% perfluoropentanoic and 0.01% formic acid and was operated at an overall higher organic content. The basic solution was analyzed using either basic negative ion extracts or basic positive ion extracts. A C18 column (Waters UPLC

BEH Amide 2.1×150 mm, 1.7 μm) was utilized for both negative and positive ion extracts. The basic extracts were gradient eluted from the column using methanol, water, and 6.5 mM ammonium bicarbonate at pH 8.0. The basic positive ion extracts were gradient eluted from the column with water, acetonitrile, and 10 mM ammonium formate at pH 10.8. The mass spectrometry (MS) analysis utilized both MS and data-dependent MS/MS scans using dynamic exclusion. The scan range covered 70-1000 m/z as previously described [24].

Heat-stabilized rice bran metabolomics

The food metabolome analysis used 100 mg of heat-stabilized rice bran for non-targeted metabolite profiling. Rice bran was extracted with 80% methanol prior to UPLC-MS/MS and gas chromatography-mass spectrometry (GC-MS) platforms [24,25]. The extracts that were allocated for GC-MS analysis, were fist vacuum-dried for 18 hours and then derivatized using bistrimethyl-silyltrifluoroacetamide. Chromatography separation on derivatized extracts was performed using 5% diphenyl/95% dimethyl polysiloxane fused silica column (20 m x 0.18 mm ID; 0.18 um film thickness) with an applicable carrier gas as described previously [24,37]. To verify whether metabolites identified in plasma and/or urine originated from rice bran (exclusively or in part), all the plasma and urine metabolites identified in both dietary groups were cross-referenced with metabolites identified from the rice bran metabolome.

Data extraction and compound identification

Metabolon in-house peak detection and integration software was used to extract and process the raw data (quantitation is based on area under the curve from MS data). Standard industry approaches were used for MS peak detection, including minimum height, signal-to-noise, width and area criteria. Compounds were compared to a library of purified standards and identified according to 3 criteria: the experimentally detected signature matching the accurate mass of the authentic standard within 8 ppm (match to the National Institute of Standards and Technology library within ± 0.005 atomic mass units), retention index match within a defined window (approximately 5 seconds), and the tandem mass spectrometry (MS/MS) forward and reverse scores [24].

Metabolic pathway networks and analysis

The raw abundance of all identified plasma and urine metabolites are shown in Supplementary Table 1. To visualize the networks of metabolic pathways from plasma and urine metabolites, pathway enrichment scores (PES) were generated using Cytoscape (Version 2.8.3). PES was calculated using the following equation:

$$\frac{k/m}{n/N}$$

In the equation, "k" represent number of significant metabolites in a pathway ($p \leq 0{\cdot}05$), "m" the total number of identified metabolites in that pathway, "n" the total number of significant metabolites in the dataset , and "N" the total number of identified metabolites in the complete dataset [37,38]. Metabolic pathways with PES less or greater than one indicated that the pathway contained 1 or more metabolites with a statistically-significant fold-difference (i.e., between rice bran group and control at 4 weeks) compared to all other pathways within the matrix.

Statistical analyses

Plasma and urine metabolite profiles were normalized by dividing the median of each metabolite raw abundance across the entire dataset to get the median-scaled relative abundance. A Welch's two-sample t-test and two-way analysis of variance (ANOVA) were used to compare the scaled values between groups (i.e., rice bran versus control). Repeated measures ANOVA was used to determine statistically-significant metabolites within groups over time. Metabolites that were statistically different at baseline between rice bran group and control were removed from the between-group analyses. False discovery rate (q-value) was calculated to determine false discoveries common to multiple-comparison metabolomics studies. Fold-difference was determined by dividing the relative abundance of the metabolite in the rice bran group by the relative abundance of the metabolite in control. Fold-change was determined by dividing the relative abundance of the metabolite in the rice bran group at 2 or 4 weeks by the relative abundance at baseline (week 0). Significant metabolites had p-values of≤0.05 and q-values below the threshold of≤0.10.

Results

Nutritional metabolome of control or rice bran groups

The plasma metabolome had 854 metabolites and the urine metabolome had 703 metabolites with confirmed identification from 19 participants sampled at baseline, 2 weeks, and 4 weeks post intervention. The metabolites were classified as amino acids, carbohydrates, cofactors & vitamins, energy metabolites, lipids, nucleotides, peptides, and xenobiotics. Supplementary Table 1 shows raw abundance of all metabolites organized into metabolic pathways for the rice bran and groups in both plasma and urine. All rice bran food metabolites that were also detected in the plasma and/or urine metabolome are listed in Supplementary Table 2. Food metabolomics of rice bran showed 453 unique metabolites and 103 rice bran metabolites were detected in plasma and/or urine after 2 or 4 weeks of consumption. Thus, up to 5.5% of the plasma metabolome and 9.7% of the urine metabolome could be classified as derived from rice bran phytochemicals.

Dietary modulation of the plasma metabolome in CRC survivors

Consumption of rice bran for 4 weeks resulted in significant-modulation of 11 metabolites including 8 lipids, 1 amino acid, 1 carbohydrate, and 1 xenobiotic compared to control (n=19). These plasma metabolites with significant differences in the abundance between rice bran group and control are listed in Table 1 and visualized in Figure 2A ($p \leq 0.05$). For all participants, data are presented as the mean fold-difference between rice bran group and control at 4 weeks. Among the 11 plasma metabolites with significant fold-differences in the rice bran group at 4 weeks compared to control, 2 metabolites (mannose and benzoate) were also identified in the rice bran food metabolome (footnote[1] in Table 1). The 2 metabolites that increased in plasma of the rice bran group were β-citrylglutamate (BCG) (1.59-fold) and mannose (1.37-fold). The nine plasma metabolites with a significant decrease compared to control at 4 weeks were linoleoylcholine (0.24-fold), oleoylcholine (0.25-fold), palmitoylcholine (0.25-fold), dihomo-linolenoylcarnitine (C20:3) (DGLA) (0.62-fold), dihomo-linolenoylcarnitine (C20:2) (0.67-fold), arachidonoylcholine (0.27-fold), dihomo-linolenoyl-choline (0.25-fold), docosahexaenoylcholine (0.25-fold), and benzoate (0.70-fold).

Table 2 lists metabolites significantly modulated in both dietary groups over time, with changes reported at 2- and 4-weeks post

Table 1. Rice bran consumption showed different plasma metabolites at 4 weeks compared to control group

		Fold-difference*	
Metabolite	**HMDB**	**Rice Bran**	***p*-value**
Amino Acid			
beta-citrylglutamate (BCG)	HMDB13220	1.60	0.039
Betaine[1]	HMDB00043	0.80	0.057
Carbohydrate			
Mannose[1]	HMDB00169	1.37	0.022
Lipids			
Arachidonoylcholine	-	0.27	0.026
Dihomo-linolenoylcarnitine (20:3n3 or 6) (DGLA)	-	0.62	0.033
Dihomo-linolenoyl-choline	-	0.25	0.029
Dihomo-linoleoylcarnitine (C20:2)	-	0.67	0.026
Docosahexaenoylcholine	-	0.25	0.010
Linoleoylcarnitine (C18:2)	HMDB06469	0.71	0.064
Linoleoylcholine	-	0.24	0.028
Oleoylcholine	-	0.25	0.016
Palmitoylcholine	-	0.25	0.024
Xenobiotics			
Benzoate[1]	HMDB01870	0.69	0.046

GPC: Glycerophosphocholine; **HMDB:** Human metabolome database

[1]Metabolite also identified from the rice bran metabolome

*Values presented are fold-difference of the mean relative abundance between rice bran group compared to control at 4 weeks ($p \leq 0.05$). Statistically-significantly increased fold-differences are highlighted in red and statistically-significantly decreased fold-differences are highlighted in blue. Light blue and light red coloring represent metabolites and pathways that showed trends towards significance ($0.05 \leq p \leq 0.10$)

Table 2. Modulated plasma metabolites following control or rice bran consumption at 2 and 4 weeks post intervention when compared to baseline

			Fold-change*							
			Control group				Rice bran group			
	Metabolite	**HMDB**	**2wk/ 0wk**	**p-value**	**4wk/ 0wk**	**p-value**	**2wk/ 0wk**	**p-value**	**4wk/ 0wk**	**p-value**
Amino acid										
Alanine and Aspartate	Alanine[1]	HMDB00161	0.94	0.119	0.99	0.731	0.96	0.324	0.92	0.049
Creatine	Creatine[1]	HMDB00064	0.99	0.802	0.97	0.465	0.87	0.046	0.97	0.526
Glutamate	β-citrylglutamate (BCG)	-	1.01	0.584	0.82	0.054	1.22	0.862	1.67	0.037
Glutathione	Cysteinylglycine	HMDB00078	0.91	0.040	0.93	0.449	1.21	0.496	1.12	0.797
Leucine, Isoleucine and Valine	3-methyl-2-oxovalerate[1]	HMDB03736	0.90	0.028	0.96	0.368	0.98	0.695	1.01	0.992
	Isoleucine[1]	HMDB00172	0.95	0.050	1.00	0.870	0.99	0.831	1.03	0.572
	Methylsuccinoylcarnitine	-	1.53	0.028	1.30	0.346	1.00	0.807	1.01	0.874
Lysine	Glutarate (pentanedioate)[1]	HMDB00661	1.30	0.431	1.04	0.909	0.77	0.046	1.07	0.596
	N-acetyl-cadaverine		1.84	0.116	1.64	0.114	0.57	0.012	0.68	0.052
Methionine, Cysteine, SAM and Taurine	Cysteine[1]	HMDB00574	0.86	0.034	0.94	0.494	1.13	0.316	1.25	0.075
	S-methylcysteine sulfoxide	-	1.53	0.033	1.66	0.008	1.32	0.164	1.32	0.239
Phenylalanine and Tyrosine	4-hydroxyphenylacetate	HMDB00020	2.88	0.268	3.17	0.116	0.59	0.025	1.47	0.833
	Gentisate[1]	HMDB00152	5.89	0.007	4.68	0.031	1.71	0.239	1.47	0.747
	Phenol sulfate	HMDB60015	1.26	0.307	1.72	0.060	1.56	0.078	1.59	0.048
Polyamine	N-acetylputrescine[1]	HMDB02064	0.95	0.321	1.01	0.981	0.91	0.111	0.89	0.046
Tryptophan	3-indoxyl sulfate	HMDB00682	1.03	0.850	1.13	0.697	0.75	0.012	0.87	0.117
	Indoleacetylglutamine	HMDB13240	1.71	0.144	1.28	0.810	0.74	0.044	1.14	0.736
Urea cycle; Arginine and Proline	2-oxoarginine	HMDB04225	1.37	0.656	1.34	0.409	0.70	0.040	0.91	0.224
	Pro-hydroxy-pro	HMDB06695	1.00	0.639	0.83	0.037	1.10	0.561	1.09	0.809
Carbohydrate										
Fructose, Mannose and Galactose	Mannose[1]	HMDB00169	0.87	0.049	0.92	0.222	1.08	0.396	1.08	0.397
Pentose	Arabinose[1]	HMDB00646	1.03	0.213	1.39	0.463	0.59	0.036	1.00	0.197
	Sedoheptulose	HMDB03219	1.23	0.849	1.17	0.943	2.93	0.017	1.41	0.498
Cofactors & vitamins										
Hemoglobin and Porphyrin	Bilirubin [E,E]	-	0.72	0.002	0.73	0.018	0.99	0.546	0.93	0.335
	Bilirubin [Z,Z]	HMDB00054	0.70	0.034	0.98	0.270	0.92	0.482	0.56	0.098
	Heme	HMDB03178	2.49	0.711	1.13	0.724	2.32	0.173	4.06	0.009
Tocopherol	α-CEHC sulfate	-	0.71	0.040	1.05	0.963	0.72	0.111	0.76	0.108
	δ-tocopherol[1]	HMDB02902	0.67	0.031	0.92	0.205	0.97	0.218	0.89	0.406
	γ-CEHC	HMDB01931	0.81	0.048	1.12	0.378	0.62	0.004	0.71	0.019
	γ-CEHC glucuronide	-	0.78	0.022	1.59	0.874	0.55	0.014	0.66	0.023

	Metabolite	HMDB	Fold-change*							
			Control group				Rice bran group			
			2wk/ 0wk	p-value	4wk/ 0wk	p-value	2wk/ 0wk	p-value	4wk/ 0wk	p-value
Energy										
TCA Cycle	Succinylcarnitine (C4-DC)	-	1.51	0.039	1.05	0.646	1.02	0.884	0.96	0.553
Lipid										
Ceramides	Ceramide (d18:2/24:1, d18:1/24:2)	-	1.31	0.006	1.18	0.072	1.03	0.809	0.94	0.207
	Glycosyl ceramide (d18:2/24:1, d18:1/24:2)	-	1.03	0.993	1.11	0.293	1.07	0.490	0.86	0.030
Diacylglycerol	Palmitoleoyl-oleoyl-glycerol (16:1/18:1) [2]	-	0.92	0.552	0.97	0.478	0.72	0.026	1.08	0.613
	Palmitoyl-linoleoyl-glycerol (16:0/18:2) [1]	HMDB05207 / HMDB07103	0.83	0.170	1.07	0.768	0.71	0.023	1.25	0.888
Endocannabinoid	N-oleoyltaurine	-	1.36	0.716	1.25	0.273	1.00	0.432	0.65	0.046
Fatty Acid (Acyl Choline)	Linoleoylcholine	-	2.06	0.682	2.31	0.159	0.92	0.187	0.37	0.008
	Oleoylcholine	-	2.05	0.605	2.17	0.184	0.92	0.207	0.38	0.006
	Palmitoylcholine	-	1.95	0.666	2.24	0.197	0.95	0.223	0.39	0.009
	Stearoylcholine	-	2.36	0.605	2.63	0.380	0.89	0.110	0.34	0.005
Fatty Acid (Acyl Carnitine)	3-hydroxybutyrylcarnitine	-	1.01	0.262	0.84	0.046	1.26	0.216	1.19	0.525
	Arachidonoylcarnitine (C20:4)	-	1.11	0.296	1.10	0.329	0.98	0.753	0.84	0.031
	Dihomo-linolenoylcarnitine (20:3n3 or 6) (DGLA)	-	1.10	0.341	1.12	0.213	1.02	0.906	0.87	0.040
	Eicosenoylcarnitine (C20:1)	-	1.11	0.272	1.07	0.467	1.22	0.046	1.00	0.841
	Erucoylcarnitine (C22:1)	-	1.01	0.702	1.10	0.329	1.33	0.008	1.04	0.896
	Linolenoylcarnitine (C18:3)	-	1.10	0.592	1.06	0.622	1.02	0.866	0.82	0.029
	Linoleoylcarnitine (C18:2)	HMDB06469	1.10	0.371	1.08	0.417	1.02	0.894	0.85	0.024
	Oleoylcarnitine (C18:1)	HMDB05065	1.12	0.262	1.05	0.574	1.23	0.018	1.00	0.800
	Pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	-	1.10	0.493	1.28	0.215	1.90	0.003	1.42	0.101
	Suberoylcarnitine (C8-DC)	-	1.04	0.736	0.95	0.627	2.03	0.021	1.52	0.128
Fatty Acid, Amino	2-aminoheptanoate[1]	-	1.02	0.741	1.18	0.457	1.50	0.004	1.22	0.162
Fatty Acid, Dicarboxylate	3-methyladipate[1]	HMDB00555	1.77	0.451	1.58	0.878	1.17	0.784	1.11	0.046
Fatty Acid, Monohydroxy	13-HODE + 9-HODE[1]	-	2.02	0.062	2.07	0.030	1.69	0.734	1.13	0.916
	16-hydroxypalmitate	HMDB06294	0.94	0.313	1.08	0.520	1.31	0.038	0.98	0.702
Lysolipid	1-arachidonoyl-GPC (20:4n6)	HMDB10395	1.11	0.396	1.24	0.037	0.97	0.634	0.88	0.139
	1-linolenoyl-GPC (18:3)	-	1.30	0.080	1.35	0.023	0.76	0.005	0.75	0.006
	1-linoleoyl-GPC (18:2)	HMDB10386	1.16	0.242	1.27	0.017	0.93	0.204	0.88	0.103
	1-linoleoyl-GPE (18:2)	HMDB11507	1.23	0.096	1.29	0.034	0.95	0.269	0.90	0.168
	1-oleoyl-GPC (18:1)	HMDB02815	1.12	0.120	1.19	0.014	0.98	0.604	0.92	0.155
	1-palmitoleoyl-GPC (16:1)	HMDB10383	1.20	0.018	1.29	0.001	0.95	0.396	0.90	0.086
	1-palmitoyl-GPC (16:0)	HMDB10382	1.07	0.285	1.11	0.032	0.91	0.056	0.93	0.079
	1-palmitoyl-GPG (16:0)		1.23	0.761	1.32	0.884	0.80	0.212	0.65	0.024
	1-stearoyl-GPC (18:0)	HMDB10384	1.14	0.152	1.20	0.020	0.87	0.040	0.93	0.158
	1-stearoyl-GPI (18:0)	HMDB61696	1.13	0.520	1.41	0.036	1.08	0.908	0.86	0.209
	2-palmitoleoyl-GPC (16:1)	-	1.61	0.239	2.72	0.008	0.75	0.048	0.90	0.471
	2-palmitoyl-GPC (16:0)	HMDB61702	0.98	0.535	1.17	0.155	0.84	0.047	0.90	0.169
Mevalonate	3-hydroxy-3-methylglutarate[1]	HMDB00355	0.75	0.030	0.85	0.168	1.24	0.333	1.09	0.961
Monoacylglycerol	2-myristoylglycerol (14:0)	-	2.73	0.041	1.52	0.329	2.17	0.674	0.80	0.152
Phospholipid	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)	-	1.13	0.162	1.11	0.151	0.88	0.057	0.88	0.046
	1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)	-	1.24	0.550	1.23	0.475	0.81	0.068	0.70	0.009
	1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)	-	1.14	0.375	1.30	0.058	0.92	0.354	0.77	0.012
	1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)	-	1.23	0.027	1.19	0.062	0.94	0.392	0.92	0.225
	1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	-	1.09	0.381	1.09	0.319	0.87	0.066	0.85	0.032
	Arachidonoylcholine	-	1.65	0.927	2.06	0.227	0.95	0.244	0.36	0.007
	Dihomo-linolenoyl-choline	-	2.30	0.416	2.75	0.125	0.88	0.119	0.33	0.003
	Docosahexaenoylcholine	-	2.02	0.352	2.29	0.110	1.05	0.283	0.36	0.001

	Metabolite	HMDB	Fold-change*							
			Control group				Rice bran group			
			2wk/ 0wk	p-value	4wk/ 0wk	p-value	2wk/ 0wk	p-value	4wk/ 0wk	p-value
Plasmalogen	1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)	-	0.91	0.215	1.02	0.837	0.83	0.037	0.94	0.375
	1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)	-	1.00	0.789	0.97	0.442	1.14	0.050	0.95	0.343
	1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)	-	0.92	0.090	0.92	0.073	1.15	0.047	0.93	0.173
	1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)	-	0.97	0.525	0.96	0.464	1.16	0.042	0.95	0.351
Polyunsaturated Fatty Acid (n3 and n6)	Docosatrienoate (22:3n3)	HMDB02823	1.40	0.676	1.42	0.844	0.68	0.027	1.57	0.792
Primary Bile Acid	Cholate	HMDB00619	0.93	0.109	1.23	0.599	0.71	0.008	0.86	0.336
	Glycocholate glucuronide	-	1.41	0.992	1.07	0.932	1.02	0.508	0.82	0.006
Secondary Bile Acid	Deoxycholate[1]	HMDB00626	1.07	0.479	1.35	0.290	0.61	0.002	0.82	0.155
	Glycocholenate sulfate	-	0.81	0.023	0.90	0.240	0.93	0.403	0.88	0.144
	Isoursodeoxycholate	HMDB00686	1.09	0.444	1.16	0.820	0.75	0.031	1.01	0.625
	Taurocholenate sulfate	-	0.85	0.089	0.86	0.164	1.50	0.040	1.05	0.984
	Ursodeoxycholate	HMDB00946	1.39	0.188	1.79	0.822	0.61	0.005	0.71	0.092
Sphingolipid	lactosyl-N-palmitoyl-sphingosine (d18:1/16:0)	-	1.06	0.551	1.05	0.647	1.20	0.023	0.94	0.271
	N-nervonoyl-hexadecasphingosine (d16:1/24:1)	-	1.37	0.010	1.32	0.025	1.07	0.586	1.01	0.494
	Sphingomyelin (d18:1/14:0, d16:1/16:0)	-	1.04	0.429	1.09	0.037	1.01	0.835	0.99	0.653
	Sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	-	1.05	0.583	1.09	0.276	1.19	0.009	0.98	0.596
	Sphingomyelin (d18:1/18:1, d18:2/18:0)	-	1.05	0.548	1.10	0.191	1.14	0.045	1.00	0.885
	Sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	-	1.10	0.554	1.12	0.341	1.24	0.045	0.92	0.213
	Sphingomyelin (d18:2/23:1)	-	1.12	0.275	1.16	0.122	1.26	0.011	0.95	0.333
	Sphingomyelin (d18:2/24:2)	-	1.13	0.416	1.11	0.406	1.26	0.039	0.93	0.256
Steroid	5α-androstan-3β,17β-diol disulfate	HMDB00493	0.71	0.034	0.79	0.155	0.97	0.818	0.92	0.567
	Cortisone	HMDB02802	0.83	0.022	0.92	0.267	0.97	0.712	0.96	0.627
	Epiandrosterone sulfate	HMDB00365	0.60	0.043	0.60	0.044	0.64	0.099	0.71	0.178
Sterol	3β,7α-dihydroxy-5-cholestenoate	-	0.92	0.160	0.89	0.036	0.87	0.014	0.84	0.005
	4-cholesten-3-one[1]	HMDB00921	0.94	0.190	0.95	0.353	1.05	0.825	1.20	0.031
Nucleotide										
Purine, Guanine containing	7-methylguanine[1]	HMDB00897	0.92	0.032	0.92	0.032	1.02	0.685	0.98	0.584
	N2, N2-dimethylguanosine[1]	HMDB04824	0.96	0.350	0.97	0.507	0.92	0.061	0.89	0.008
Pyrimidine, Cytidine containing	3-methylcytidine	-	0.83	0.003	0.86	0.028	0.91	0.137	1.06	0.507
Peptide										
γ-glutamyl Amino Acid	γ-glutamylglycine	HMDB11667	1.04	0.937	1.05	0.848	1.35	0.039	0.94	0.506
Xenobiotics										
Benzoate	3-(2-hydroxyphenyl)propionate	HMDB33752	3.99	0.019	2.00	0.317	2.90	0.205	2.01	0.366
	3-methoxycatechol sulfate	-	2.95	0.021	2.97	0.083	1.24	0.499	0.91	0.642
Chemical	6-hydroxyindole sulfate	-	1.14	0.772	1.24	0.543	0.76	0.049	0.88	0.204
Drugs	4-hydroxycoumarin	-	6.82	0.031	2.22	0.225	8.37	0.043	3.85	0.058
	Hydroquinone sulfate	HMDB02434	1.90	0.061	2.98	0.008	2.67	0.090	3.04	0.013
Phytochemicals	2-piperidinone[1]	-	1.21	0.384	1.09	0.763	0.70	0.005	0.58	0.000
	4-allylphenol sulfate	-	4.45	0.000	7.58	0.000	5.14	0.000	4.44	0.000
	β-guanidinopropanoate	HMDB13222	0.87	0.165	0.70	0.063	1.28	0.717	1.82	0.025
	Dihydroferulic acid	-	3.56	0.025	3.71	0.010	2.19	0.203	2.32	0.207
	Saccharin	HMDB29723	1.06	0.486	1.09	0.845	1.40	0.010	1.74	0.045
	Tartarate[1]	HMDB00956	1.44	0.854	1.33	0.562	0.69	0.067	0.59	0.022
	Theanine	HMDB34365	43.91	0.014	6.53	0.336	7.81	0.618	5.39	0.729
Xanthine	Theobromine	HMDB02825	0.98	0.042	1.01	0.153	0.99	0.666	2.49	0.251

GPC: Glycerophosphocholine; **GPE:** Glycerophosphoethanolamine; **GPG:** Glycerophosphoglycerol; **GPI:** Glycerophosphoinositol; **CEHC:** Carboxyethyl-hydroxychromans; **HODE:** Hydroxy-10(E),12(Z)-octadecadienoic acid; **HMDB:** Human metabolome database

[1]Metabolite also identified from the Rice Bran metabolome

*Values presented are fold-change of the mean relative abundance within control or rice bran group at 2 and 4 weeks compared to their baselines ($p \leq 0.05$). Statistically-significantly increased fold-changes are highlighted in red and statistically-significantly decreased fold-changes are highlighted in blue. Light blue and light red coloring represents metabolites and pathways that showed trends towards significance ($0.05 \leq p \leq 0.10$)

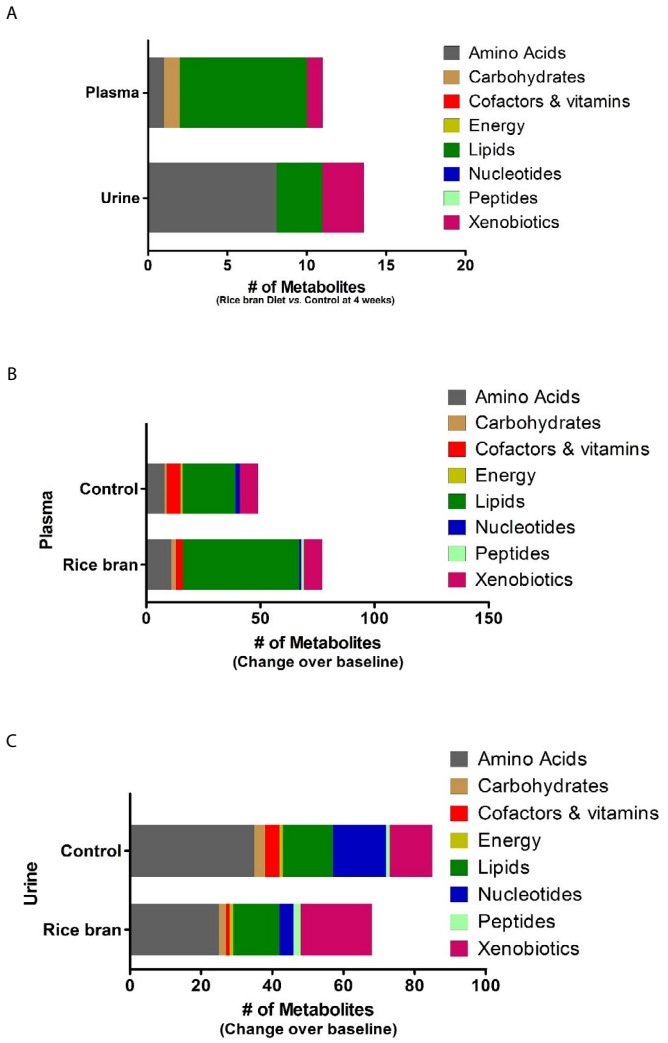

Figure 2. Chemical classes that were significantly modulated after 2 or 4 weeks of rice bran consumption when compared to control group or baseline. **(A)** Total number of metabolites with significant fold-differences in plasma and urine metabolome of CRC survivors at 4 weeks. **(B)** Total number of metabolites with significant fold-changes in plasma metabolome of CRC survivors at 2 and 4 weeks compared to baseline. **(C)** Total number of metabolites with significant fold-changes in urine metabolome of CRC survivors at 2 and 4 weeks compared to baseline. Significance was defined as metabolites with p-value≤ 0.05

intervention compared to baseline. These metabolite changes over time are also illustrated in Figure 2B (p≤0.05). Forty-nine metabolites in the control group and 77 metabolites in the rice bran group showed significant fold-changes compared to baseline. In the control group, the significantly modulated metabolites were 23 lipids, 8 amino acids, 8 xenobiotics, 6 cofactors & vitamins, 2 nucleotides, 1 carbohydrate, and 1 energy metabolite. In the rice bran group, the significantly modulated metabolites were 51 lipids, 11 amino acids, 8 xenobiotics, 3 cofactors & vitamins, 2 carbohydrates, 1 nucleotide, and 1 peptide at 2 or 4 weeks compared to baseline. For all participants, data are presented as the mean fold-change at 2 or 4 weeks compared to baseline. Among the 77 metabolites in the rice bran group that changed at 2 or 4 weeks post intervention compared to baseline, 7 metabolites were also significantly different from between-group analysis (i.e. at 4 weeks compared to the control group). BCG was a metabolite with increased fold difference between groups and significant for changes over time (BCG 1.67-fold), while decreased abundance was measured for linoleoylcholine (0.37-fold), oleoylcholine (0.38-fold), DGLA (0.87-fold), arachidonoylcholine (0.36-fold), dihomo-linolenoyl-choline (0.33-fold), and docosahexaenoylcholine (0.36-fold) at 4 weeks compared to baseline.

Dietary modulation of urine metabolome in CRC survivors

Table 3 lists 14 urine metabolites with significant fold-differences between the rice bran and control participants at 4 weeks (p≤0.05). These urine metabolites were 8 amino acids, 3 xenobiotics, and 3 lipids and are visualized in Figure 2A. Among the 14 urine metabolites with significant fold-differences in the rice bran group at 4 weeks compared to control, 6 metabolites were also identified from the rice bran food metabolome, namely N-acetylisoleucine, N-acetylvaline, N-formylphenylalanine, phenylalanine, O-sulfo-L-tyrosine, and 3-(4-hydroxyphenyl) (footnote[1] in Table 3). The 13 metabolites with significantly increased fold-differences included N-acetylisoleucine (1.56-fold), N-acetylvaline (1.44-fold), N-methylleucine (3.37-fold), 3-(3-hydroxyphenyl)propionate (5.74-fold), N-formylphenylalanine (2.19-fold), phenylalanine (1.41-fold), pro-hydroxy-pro (1.59-fold), N-octanoylglycine (2.92-fold), androstenediol (3β,17β) monosulfate (5.34-fold), dehydroisoandrosterone sulfate (DHEA-S) (4.49-fold), 4-vinylphenol sulfate (2.61-fold), O-sulfo-L-tyrosine (1.37-fold), and theophylline (2.81-fold). The only urine metabolite with significantly decreased fold-difference was 3-(4-hydroxyphenyl)lactate (0.61-fold).

The urine metabolites that had significant fold-changes at 2 or 4 weeks compared to baseline are listed in Table 4 and visualized in Figure 2C (p≤0.05). These included 85 metabolites in the control group and 68 metabolites in the rice bran group. For the control, there were changes in 35 urine amino acids, 15 nucleotides, 14 lipids, 12 xenobiotics, 4 cofactors & vitamins, 3 carbohydrates, 1 energy metabolite, and 1 peptide. Metabolites that were significantly modulated by rice bran

Table 3. Rice bran consumption showed different urine metabolites at 4 weeks compared to control group

		Fold-difference*	
Metabolite	**HMDB**	**Rice Bran**	***p*-value**
Amino Acid			
3-(3-hydroxyphenyl)propionate	HMDB00375	5.74	0.038
3-(4-hydroxyphenyl)lactate[1]	HMDB00755	0.61	0.032
Indolepropionate	HMDB02302	0.37	0.054
Isovalerylglutamine	-	0.68	0.097
N-acetylisoleucine[1]	-	1.56	0.027
N-acetylvaline[1]	HMDB11757	1.44	0.039
N-formylphenylalanine[1]	-	2.19	0.030
N-methylleucine	-	3.37	0.042
Phenylalanine[1]	HMDB00159	1.41	0.037
Pro-hydroxy-pro	HMDB06695	1.59	0.015
Pyroglutamine[1]	-	1.48	0.056
Lipid			
N-octanoylglycine	HMDB00832	2.92	0.028
Androstenediol (3β,17β) monosulfate	HMDB03818	5.34	0.016
Dehydroisoandrosterone sulfate (DHEA-S)	HMDB01032	4.49	0.033
Xenobiotics			
4-vinylphenol sulfate		2.61	0.028
O-sulfo-L-tyrosine[1]	-	1.37	0.019
Theophylline	HMDB01889	2.81	0.040

HMDB: Human metabolome database

[1]Metabolite also identified from the rice bran metabolome

*Values presented are fold-difference of the mean relative abundance between rice bran group compared to control at 4 weeks (p≤0.05). Statistically-significantly increased fold-differences are highlighted in red and statistically-significantly decreased fold-differences are highlighted in blue. Light blue and light red coloring represent metabolites and pathways that showed trends towards significance (0.05≤p≤0.10)

Table 4. Modulated urine metabolites following control or rice bran consumption at 2 and 4 weeks post intervention when compared to baseline

	Metabolite	HMDB	Fold-change*							
			Control group				Rice bran group			
			2wk/ 0wk	p-value	4wk/ 0wk	p-value	2wk/ 0wk	p-value	4wk/ 0wk	p-value
Amino Acid										
Alanine and Aspartate	Alanine[1]	HMDB00161	0.95	0.502	0.99	0.951	0.80	0.016	0.74	0.002
	Asparagine[1]	HMDB00168	0.96	0.664	0.95	0.543	0.76	0.008	0.73	0.003
	N-acetylalanine	HMDB00766	0.72	0.049	0.97	0.850	0.73	0.082	0.68	0.032
	N-acetylaspartate (NAA)[1]	HMDB00812	0.97	0.618	0.88	0.072	1.24	0.007	0.94	0.422
Glutamate	Carboxyethyl-GABA[1]	HMDB02201	0.82	0.157	0.72	0.018	1.14	0.377	0.83	0.219
	N-methyl-GABA	-	0.57	0.049	0.51	0.020	0.62	0.133	0.79	0.445
Glutathione	5-oxoproline[1]	HMDB00267	0.89	0.138	0.89	0.153	1.24	0.016	0.98	0.805
Glycine, Serine and Threonine	N-acetylserine[1]	HMDB02931	0.99	0.923	0.90	0.319	0.72	0.007	0.74	0.015
Guanidino and Acetamido	1-methylguanidine	HMDB01522	0.94	0.604	0.72	0.011	1.29	0.072	1.00	0.972
Histidine	1-methylhistamine	HMDB00898	0.88	0.320	0.77	0.040	0.97	0.808	1.03	0.849
	4-imidazoleacetate[1]	HMDB02024	1.25	0.042	1.13	0.268	1.07	0.569	1.10	0.414
	N-acetyl-1-methylhistidine		0.78	0.072	0.62	0.001	0.90	0.494	0.86	0.312
Leucine, Isoleucine and Valine	3-methyl-2-oxovalerate[1]	HMDB03736	0.45	0.047	0.62	0.221	0.69	0.400	0.57	0.202
	3-methylcrotonylglycine	HMDB00459	1.40	0.031	1.41	0.026	1.35	0.077	1.19	0.303
	Isobutyrylcarnitine (C4)	HMDB00736	1.47	0.009	1.29	0.076	1.16	0.330	1.20	0.248
	Isobutyrylglycine[1]	HMDB00730	1.56	0.004	1.41	0.023	1.19	0.291	1.27	0.140
	Isovalerylglutamine	-	1.18	0.250	1.33	0.047	0.86	0.346	0.80	0.151
	Isovalerylglycine[1]	HMDB00678	1.37	0.043	1.37	0.041	1.07	0.670	1.01	0.976
	Methylsuccinoylcarnitine	-	0.97	0.761	0.85	0.102	1.28	0.031	1.17	0.175
Lysine	Glutarate (pentanedioate)[1]	HMDB00661	1.21	0.145	1.06	0.622	1.36	0.033	1.11	0.440
	N2,N6-diacetyllysine	-	0.95	0.567	0.82	0.025	1.01	0.891	0.94	0.493
	N6,N6,N6-trimethyllysine	HMDB01325	0.93	0.561	0.64	0.001	1.00	0.977	1.18	0.229
Methionine, Cysteine, SAM and Taurine	Cystathionine[1]	HMDB00099	1.29	0.136	1.64	0.004	1.00	0.998	1.47	0.042
	Methionine[1]	HMDB00696	1.09	0.693	1.01	0.951	0.80	0.373	0.58	0.032
	N-methyltaurine	-	2.70	0.019	2.05	0.085	0.90	0.807	0.93	0.871
	S-methylcysteine sulfoxide	-	1.27	0.161	1.58	0.009	1.36	0.106	1.17	0.403
	Taurocyamine	HMDB03584	0.95	0.812	1.04	0.842	0.79	0.326	0.57	0.019
Phenylalanine and Tyrosine	3-(3-hydroxyphenyl)propionate sulfate	-	0.78	0.289	0.69	0.119	1.91	0.015	1.49	0.128
	3-(4-hydroxyphenyl)lactate[1]	HMDB00755	1.14	0.355	1.52	0.004	1.05	0.751	0.86	0.348
	3,4-dihydroxyphenylacetate	HMDB01336	0.82	0.461	0.53	0.022	1.44	0.215	1.16	0.623
	3-hydroxyphenylacetate sulfate	-	0.90	0.645	0.65	0.050	1.72	0.029	1.44	0.136
	4-hydroxycinnamate sulfate	-	1.38	0.265	1.82	0.040	0.90	0.749	0.97	0.922
	4-hydroxyphenylacetatoylcarnitine	-	1.24	0.242	1.48	0.035	1.19	0.382	1.16	0.468
	5-bromotryptophan	-	0.84	0.119	0.80	0.049	1.23	0.098	1.16	0.229
	Catechol glucuronide	-	0.83	0.531	1.00	0.990	2.93	0.002	1.37	0.336
	Homovanillate (HVA)	HMDB00118	1.01	0.959	0.89	0.313	1.31	0.046	1.05	0.687
	Homovanillate sulfate	HMDB11719	0.90	0.438	0.76	0.054	1.44	0.018	1.04	0.801
	Phenol sulfate	HMDB60015	1.08	0.610	0.96	0.787	2.64	0.000	2.31	0.000
	Phenyllactate (PLA)[1]	HMDB00779	1.16	0.473	1.75	0.009	0.75	0.215	0.79	0.306
	Vanillactate	HMDB00913	1.52	0.044	1.32	0.174	1.72	0.018	1.07	0.777
	Vanillylmandelate (VMA)	HMDB00291	0.99	0.889	0.87	0.108	1.29	0.006	1.02	0.847
Polyamine	Acisoga	-	0.90	0.344	0.94	0.581	1.29	0.047	1.15	0.260
	N(4)-acetylspermidine	-	0.88	0.222	0.76	0.012	0.98	0.889	0.91	0.432
	Spermidine[1]	HMDB01257	1.14	0.592	1.05	0.835	1.80	0.038	1.58	0.102
Tryptophan	3-hydroxyanthranilate	HMDB01476	2.09	0.003	1.64	0.037	0.97	0.918	0.93	0.770
	C-glycosyltryptophan	-	0.95	0.612	0.83	0.096	1.30	0.037	1.06	0.606
	Indolelactate	HMDB00671	1.25	0.027	1.08	0.436	1.05	0.665	0.90	0.333
	Indolepropionate	HMDB02302	1.51	0.346	3.05	0.013	1.65	0.295	1.36	0.521
	Kynurenate[1]	HMDB00715	1.31	0.002	1.07	0.407	1.24	0.027	1.03	0.715
	Tryptophan betaine	HMDB61115	0.94	0.730	1.25	0.189	0.68	0.039	0.89	0.521
	Xanthurenate[1]	HMDB00881	1.37	0.005	1.16	0.180	1.37	0.011	1.17	0.201
Urea cycle; Arginine and Proline	Argininate	HMDB03148	1.29	0.034	1.09	0.470	0.95	0.675	0.94	0.640
	Methylurea	-	1.36	0.036	1.12	0.424	1.11	0.492	0.82	0.210
	Pro-hydroxy-pro	HMDB06695	0.86	0.208	0.59	0.000	1.17	0.229	1.11	0.422

	Metabolite	HMDB	Fold-change*							
			Control group				Rice bran group			
			2wk/ 0wk	p-value	4wk/ 0wk	p-value	2wk/ 0wk	p-value	4wk/ 0wk	p-value
Carbohydrate										
Advanced Glycation End-product	N6-carboxymethyllysine[1]	-	1.45	0.019	1.07	0.649	1.24	0.211	0.99	0.960
Aminosugar	6-sialyl-N-acetyllactosamine	HMDB06584	0.95	0.703	0.75	0.039	1.14	0.400	1.05	0.724
	N-acetylneuraminate	HMDB00230	0.93	0.501	0.83	0.065	1.32	0.016	1.08	0.502
Glycolysis, Gluconeogenesis, and Pyruvate	Lactate[1]	HMDB00190	1.07	0.714	1.15	0.437	0.68	0.064	0.64	0.030
Pentose	Ribonate[1]	HMDB00867	1.52	0.034	1.58	0.022	1.49	0.068	1.21	0.381
Cofactors & vitamins										
Hemoglobin and Porphyrin	L-urobilin	HMDB04159	0.49	0.032	0.75	0.369	1.09	0.808	1.33	0.429
Nicotinate and Nicotinamide	Nicotinate[1]	HMDB01488	1.66	0.073	0.91	0.733	1.10	0.764	0.54	0.050
Pterin	Pterin[1]	HMDB00802	0.79	0.154	0.59	0.002	1.15	0.438	1.03	0.852
	Sepiapterin	HMDB00238	0.86	0.632	0.48	0.028	1.32	0.440	0.93	0.830
	Xanthopterin	-	0.58	0.015	0.54	0.007	0.88	0.609	0.77	0.275
Energy										
TCA Cycle	2-methylcitrate	HMDB00379	0.98	0.872	0.90	0.272	1.28	0.029	1.03	0.764
	Citraconate/glutaconate	-	0.54	0.043	0.49	0.018	1.49	0.219	1.02	0.942
Lipid										
Fatty Acid (Acyl Glutamine)	Heptanoylglutamine	-	0.60	0.004	0.65	0.017	1.33	0.136	1.20	0.343
	Hexanoylglutamine	-	0.63	0.011	0.70	0.043	1.40	0.086	1.20	0.351
	N-octanoylglutamine	-	0.68	0.038	0.72	0.071	1.63	0.018	1.42	0.085
Fatty Acid (Acyl Carnitine)	Adipoylcarnitine (C6-DC)	-	0.80	0.175	0.87	0.381	1.84	0.001	1.43	0.051
	Laurylcarnitine (C12)	HMDB02250	0.92	0.690	0.59	0.021	1.23	0.391	1.21	0.423
	Myristoleoylcarnitine (C14:1)	-	0.92	0.734	0.58	0.037	1.47	0.179	1.20	0.531
	Pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)	-	1.04	0.762	1.05	0.668	1.56	0.002	1.13	0.380
	Suberoylcarnitine (C8-DC)	-	0.63	0.009	0.68	0.027	1.53	0.025	1.14	0.474
Fatty Acid (Acyl Glycine)	Hexanoylglycine[1]	HMDB00701	0.94	0.675	0.88	0.408	1.54	0.013	1.34	0.086
Fatty Acid, Dicarboxylate	3-methyladipate[1]	HMDB00555	0.92	0.638	0.86	0.401	1.84	0.003	1.08	0.683
	4-octenedioate	HMDB04982	0.76	0.059	0.73	0.033	1.39	0.041	1.12	0.457
	Dimethylmalonic acid[1]	HMDB02001	1.33	0.025	1.21	0.129	1.22	0.150	1.22	0.155
Fatty Acid, Monohydroxy	3-hydroxysebacate	HMDB00350	0.51	0.005	0.62	0.045	1.16	0.567	1.09	0.726
	3-hydroxysuberate[1]	HMDB00325	0.59	0.014	0.54	0.004	1.38	0.158	1.14	0.577
Ketone Bodies	3-hydroxybutyrate (BHBA)[1]	HMDB00357	0.51	0.021	0.60	0.078	0.89	0.719	0.92	0.803
Mevalonate	Mevalonate	HMDB00227	0.61	0.039	0.70	0.128	0.72	0.207	0.63	0.077
Phospholipid	Glycerophosphoethanolamine[1]	HMDB00114	0.93	0.483	0.77	0.015	1.11	0.380	0.94	0.605
	Trimethylamine N-oxide	HMDB00925	1.31	0.046	1.19	0.185	1.43	0.016	1.14	0.362
Primary Bile Acid	Cholate	HMDB00619	0.77	0.519	1.00	0.995	0.25	0.003	0.52	0.156
Secondary Bile Acid	Deoxycholic acid sulfate	-	0.83	0.643	0.79	0.562	0.37	0.027	0.37	0.024
	Glycolithocholate sulfate	HMDB02639	0.79	0.390	0.70	0.194	1.97	0.029	1.60	0.125
	Taurocholenate sulfate	-	0.76	0.223	0.85	0.484	1.87	0.015	1.43	0.161
	Taurolithocholate 3-sulfate	HMDB02580	0.60	0.106	0.76	0.383	2.25	0.020	1.55	0.202
Nucleotide										
Purine, (Hypo)Xanthine/Inosine containing	Urate[1]	HMDB00289	0.82	0.116	0.72	0.013	0.93	0.580	0.89	0.388
	Xanthine[1]	HMDB00292	0.68	0.020	0.70	0.027	1.13	0.480	0.91	0.578
	Xanthosine[1]	HMDB00299	0.82	0.036	0.80	0.022	1.19	0.105	0.94	0.569
Purine, Adenine containing	Adenosine-3',5'-cyclic monophosphate (cAMP)	HMDB00058	0.88	0.108	0.81	0.011	1.27	0.010	1.07	0.434
	N1-methyladenosine	HMDB03331	0.84	0.086	0.72	0.002	0.97	0.796	0.89	0.274
Purine, Guanine containing	2'-deoxyguanosine[1]	HMDB00085	0.71	0.012	0.82	0.140	1.31	0.065	0.93	0.603
	7-methylguanine[1]	HMDB00897	0.85	0.188	0.76	0.028	1.07	0.598	0.92	0.519
	Guanosine[1]	HMDB00133	0.76	0.350	0.47	0.012	1.30	0.418	0.71	0.292
	Guanosine-3',5'-cyclic monophosphate (cGMP)	HMDB01314	0.68	0.002	0.74	0.016	1.02	0.900	0.92	0.552
	N1-methylguanosine[1]	HMDB01563	0.87	0.513	0.63	0.031	1.13	0.601	0.95	0.817
	N2-methylguanosine	HMDB05862	0.82	0.132	0.76	0.031	1.09	0.520	0.99	0.931
Pyrimidine, Cytidine containing	Cytidine[1]	HMDB00089	0.85	0.299	0.71	0.029	1.00	0.999	0.84	0.315
	N4-acetylcytidine	HMDB05923	0.86	0.158	0.86	0.150	1.29	0.029	0.99	0.921
Pyrimidine, Uracil containing	3-ureidopropionate	HMDB00026	0.89	0.340	0.94	0.611	1.42	0.015	0.99	0.944
	4-ureidobutyrate[1]	-	1.48	0.027	1.23	0.242	1.07	0.720	1.34	0.131
	Pseudouridine[1]	HMDB00767	0.87	0.061	0.84	0.017	1.21	0.019	1.04	0.623
	Uracil[1]	HMDB00300	0.86	0.259	0.76	0.045	1.03	0.846	0.86	0.314

	Metabolite	HMDB	Fold-change*							
			Control group				Rice bran group			
			2wk/ 0wk	p-value	4wk/ 0wk	p-value	2wk/ 0wk	p-value	4wk/ 0wk	p-value
Peptide										
Acetylated Peptides	Phenylacetylhistidine	-	1.35	0.042	1.33	0.054	1.05	0.771	1.08	0.620
γ-glutamyl Amino Acid	γ-glutamylglutamine[1]	HMDB11738	0.77	0.231	1.43	0.099	0.54	0.012	0.72	0.162
	γ-glutamylglycine	HMDB11667	0.99	0.940	1.11	0.472	0.68	0.013	0.74	0.050
Xenobiotics										
Benzoate	3-hydroxyhippurate	HMDB06116	0.78	0.321	0.76	0.273	2.28	0.004	1.74	0.047
	3-methoxycatechol sulfate [1]	-	1.75	0.015	1.19	0.442	1.15	0.562	1.03	0.891
	3-methoxycatechol sulfate [2]	-	1.46	0.191	1.02	0.949	2.42	0.007	1.96	0.036
	4-hydroxymandelate	HMDB00822	1.25	0.127	1.37	0.035	1.20	0.264	1.17	0.323
	4-methylcatechol sulfate	-	0.88	0.466	0.84	0.351	1.69	0.011	1.42	0.086
	Catechol sulfate	HMDB59724	0.89	0.483	0.85	0.321	1.74	0.004	1.20	0.325
	Hippurate	HMDB00714	0.84	0.383	0.75	0.174	1.60	0.041	1.22	0.392
	O-methylcatechol sulfate	-	0.90	0.421	0.86	0.235	1.58	0.002	1.09	0.528
Chemical	2-methoxyresorcinol sulfate	-	2.10	0.021	1.70	0.094	1.27	0.484	1.09	0.802
	3-acetylphenol	-	0.90	0.758	0.68	0.280	2.20	0.049	1.89	0.107
	3-acetylphenol sulfate	-	0.79	0.280	0.96	0.838	1.82	0.016	1.21	0.430
	3-hydroxypyridine sulfate	-	0.58	0.102	0.60	0.122	2.08	0.049	1.20	0.612
	4'-hydroxypropiophenone sulfate	-	2.00	0.024	2.49	0.003	2.05	0.033	1.89	0.058
	6-hydroxyindole sulfate	-	1.01	0.959	1.39	0.035	0.91	0.560	0.98	0.895
	Benzoylcarnitine	-	0.81	0.104	0.72	0.011	1.27	0.083	1.28	0.076
	Dimethyl sulfone	HMDB04983	1.23	0.285	1.68	0.010	1.37	0.147	1.43	0.099
	Lanthionine	-	1.08	0.589	0.93	0.585	0.90	0.503	0.68	0.012
	O-sulfo-L-tyrosine[1]	-	0.91	0.236	0.81	0.012	1.18	0.063	1.04	0.674
Drug	Hydroquinone sulfate	HMDB02434	1.22	0.444	1.37	0.217	3.52	0.000	3.33	0.000
Phytochemicals	2,3-dihydroxypyridine	-	0.67	0.237	0.54	0.075	3.12	0.003	1.49	0.285
	3-hydroxycinnamate sulfate	-	0.61	0.059	0.53	0.019	2.07	0.014	1.48	0.177
	3-hydroxyindolin-2-one[1]	-	1.35	0.262	1.29	0.348	0.82	0.503	0.46	0.011
	4-allylphenol sulfate	-	3.95	0.000	5.09	0.000	4.85	0.000	3.86	0.000
	Enterolactone	-	1.63	0.180	1.02	0.950	5.93	0.000	3.74	0.002
	Erythritol[1]	HMDB02994	0.90	0.578	0.92	0.625	1.54	0.034	0.87	0.496
	N-(2-furoyl)glycine	HMDB00439	0.57	0.145	0.71	0.369	2.61	0.024	1.06	0.887
	Tartarate[1]	HMDB00956	0.43	0.322	0.42	0.306	0.15	0.044	0.15	0.050
	Theanine	HMDB34365	2.83	0.040	1.83	0.226	1.00	0.993	1.25	0.681
	Thymol sulfate	HMDB01878	4.24	0.012	6.06	0.002	1.47	0.531	0.91	0.882

GABA: γ-aminobutyric acid; **CEHC:** Carboxyethyl-hydroxychromans; **HMDB:** Human metabolome database

[1]Metabolite also identified from the rice bran metabolome

*Values presented are fold-change of the mean relative abundance within control or rice bran group at 2 and 4 weeks compared to their baselines ($p \leq 0.05$). Statistically-significantly increased fold-changes are highlighted in red and statistically-significantly decreased fold-changes are highlighted in blue. Light blue and light red coloring represent metabolites and pathways that showed trends towards significance ($0.05 \leq p \leq 0.10$).

intake at 2 or 4 weeks post intervention compared to baseline included 25 amino acids, 20 xenobiotics, 13 lipids, 4 nucleotides, 2 carbohydrates, 2 peptides, 1 cofactor & vitamin, and 1 energy metabolite. Among the 68 significantly modulated urine metabolites over time, 20 metabolites were identified from the rice bran food metabolome (footnote[1] in Table 4). N-acetylaspartate (NAA) (1.24-fold), 5-oxoproline (1.24-fold), glutarate (pentanedioate) (1.36-fold), spermidine (1.8-fold), kynurenate (1.24-fold), xanthurenate (1.37-fold), hexanoylglycine (1.54-fold), 3-methyladipate (1.84-fold), pseudouridine (1.21-fold), and erythritol (1.54-fold) increased at 2 weeks. Cystathionine was the only compound increased at 4 weeks (1.47-fold). Alanine (0.8-fold), asparagine (0.76-fold), and N-acetylserine (0.72-fold), γ-glutamylglutamine (0.54-fold), tartarate (0.15-fold) decreased at 2 weeks after intervention. Alanine (0.74-fold), asparagine (0.73-fold), and N-acetylserine (0.74-fold) were decreased at 4 weeks followed by lactate (0.64-fold), nicotinate (0.54-fold), and 3-hydroxyindolin-2-one (0.46-fold).

Metabolic pathways affected by rice bran intake in plasma

Figure 3 visualizes the 11 plasma metabolites that were significantly different in the rice bran group compared to control at 4 weeks, including the pathway enrichment scores which take into consideration all metabolites identified in the plasma for that metabolic pathway (as described in methods). Lipid metabolic pathways modulated following rice bran consumption included acyl choline fatty acids, (PES=42.7), acyl carnitine fatty acids (PES=3.3), and phospholipids (PES=4.6). Additional pathways affected by rice bran consumption were glutamate (PES=7.1), carbohydrate (fructose, mannose, and galactose) (PES=14.2), and xenobiotic (benzoate) (PES=3.6).

Metabolic pathways affected by rice bran intake in urine

Figure 4 visualizes the 14 urine metabolites that were significantly different in the rice bran group compared to control at 4 weeks with the PES for each metabolic pathway involved. The amino acid metabolic

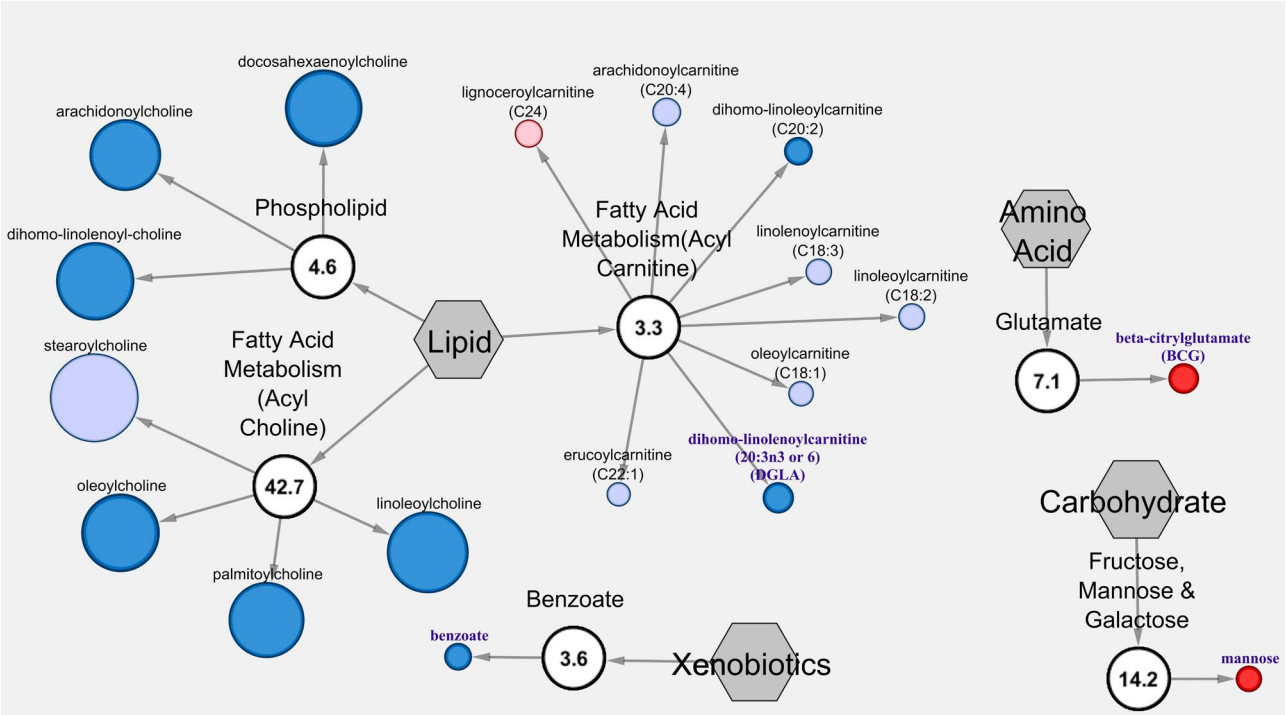

Figure 3. Plasma metabolic pathway visualization of rice bran group compared to control at 4 weeks. There were 6 metabolic pathway enrichment scores showing metabolites that are significantly different between the rice bran group and control at 4 weeks. Each metabolite was represented by a closed, colored node extending from a metabolic pathway node, which connects to a chemical class hexagonal node. A red node represents metabolites with significantly higher expression in rice bran group at 4 weeks compared to control group ($p \leq 0{\cdot}05$). A dark blue node represents metabolites with significantly lower expression in rice bran group at 4 weeks when compared to control group. Nodes colored pink or light blue represent metabolites trending towards significance with higher expression in rice bran group at 4 weeks and lower expression at 4 weeks, respectively ($0{\cdot}05 \leq p \leq 0{\cdot}1$). Node size is proportional to fold-difference magnitude at week 4 for rice bran group compared to control group. Bolded metabolites indicate previously reported evidence for cancer chemoprevention.

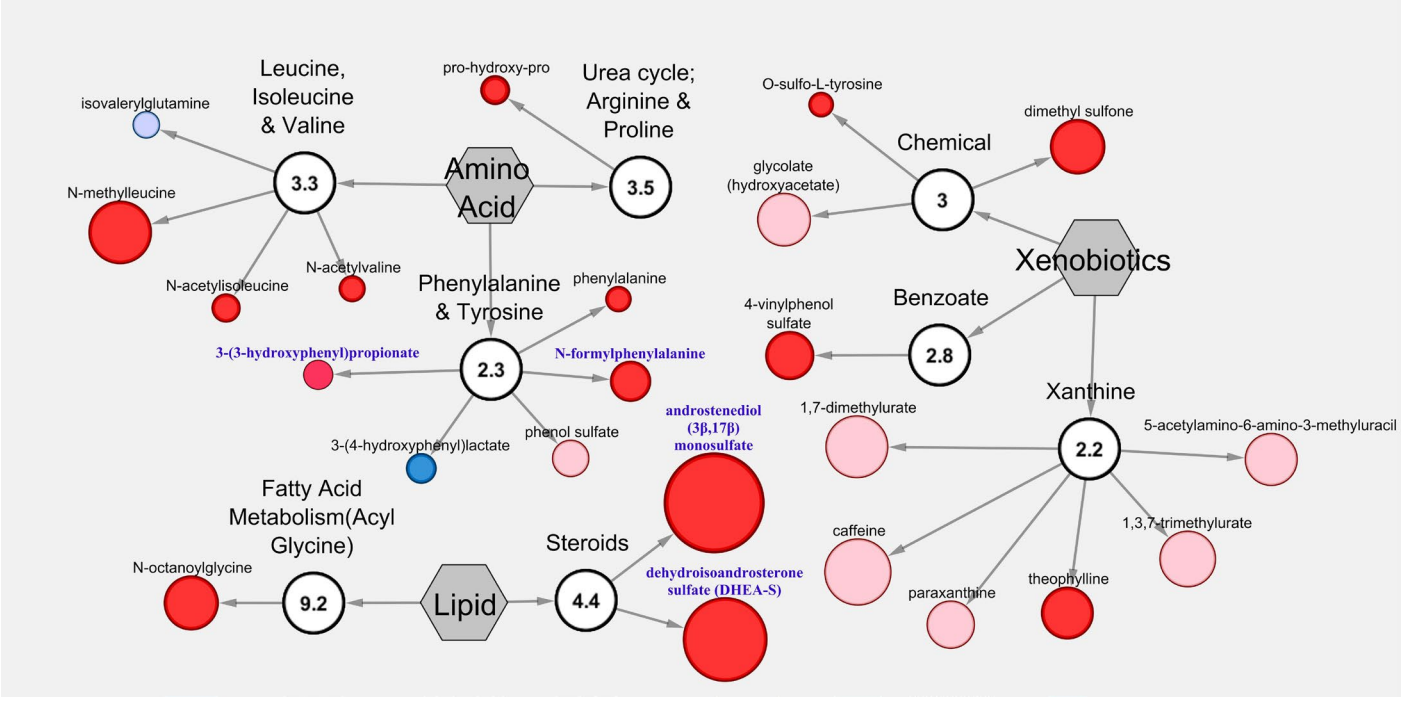

Figure 4. Urine metabolic pathway visualization of rice bran group compared to control at 4 weeks. There were 8 metabolic pathway enrichment scores that show metabolites that are significantly different between the rice bran group and control at 4 weeks. Each metabolite was represented by a closed, colored node extending from a metabolic pathway node, which connects to a chemical class hexagonal node. A red node represents metabolites with significantly higher expression in rice bran group at 4 weeks compared to control group ($p \leq 0{\cdot}05$). A dark blue node represents metabolites with significantly lower expression in rice bran group at 4 weeks when compared to control group. Nodes colored pink or light blue represent metabolites trending towards significance with higher expression in rice bran group at 4 weeks and lower expression at 4 weeks, respectively ($0{\cdot}05 \leq p \leq 0{\cdot}1$). Node size is proportional to fold-difference magnitude at week 4 for rice bran group compared to control group. Bolded metabolites indicate previously reported evidence for cancer chemoprevention.

pathways affected by rice bran were urea cycle; arginine and proline (PES=3.5), leucine, isoleucine, and valine (PES=3.3), and phenylalanine and tyrosine (PES=2.3). The xenobiotic metabolic pathways affected were chemicals (PES=3), benzoate (PES=2.8), and xanthine (PES=2.2). The lipid metabolic pathways affected were acyl glycine fatty acids (PES=9.2) and steroids (PES=4.4).

Discussion

This study established that increased rice bran consumption for 1 month significantly modulated the plasma and urine metabolome of CRC survivors. Multiple plasma and urine metabolites were identified as potentially derived from the rice bran composition [24]. Diet-derived and endogenous metabolites that were affected by rice bran in the diet had previously reported cancer protective actions. This study showed integration of the food and nutritional metabolome using plasma and urine samples collected from a randomized controlled dietary intervention study.

Plasma metabolites with colon cancer prevention properties

In plasma of rice bran participants, the 2 metabolites (mannose and BCG) were examined due to increased fold-differences compared to control participants. Mannose is necessary to activate mannose-binding lectin (MBL), and to initiate the MBL-complement cascade that can reduce cancer cell viability [39]. The MBL complement activation pathway was increased in the blood of participants with CRC [40], supporting that dietary sources of mannose may enhance protection against CRC. BCG also showed increased fold-changes and is structurally close to N-acetylaspartylglutamate (NAAG), a substrate for glutamate carboxypeptidase 2 (GCP2). GCP2 is a prostate specific membrane antigen (PSMA) and highly expressed in prostate cancer [41]. BCG is an inhibitor of GCP2 [42,43] and thus might be useful in the regulation, treatment, and control of cancer [43].

There is relevance to the decreased abundance of plasma lipid metabolites in the rice bran group, such as for linoleoylcholine, oleoylcholine, palmitoylcholine, DGLA, dihomo-linoleoylcarnitine (C20:2), arachidonoylcholine, dihomo-linolenoyl-choline, docosahexaenoylcholine, and benzoate. Among these, linoleoylcholine, oleoylcholine, DGLA, arachidonoylcholine, dihomo-linolenoyl-choline, and docosahexaenoylcholine also showed decreased fold-changes at 4 weeks compared to baseline. The PUFAs in this list, such as linoleoylcholine, DGLA (20:3n3 or 6), dihomo-linoleoylcarnitine (C20:2), arachidonoylcholine, dihomo-linolenoyl-choline and docosahexaenoylcholine were important to consider because a high intake of ω-6-PUFAs, especially in association with a low intake of ω-3 PUFAs, was shown to stimulate mammary and colon carcinogenesis, as well as increase oxidative DNA damage [44], effect cell proliferation [45], and alter free estrogen levels and hormone catabolism [46]. Mostafa *et al.* reported low concentrations of serum PUFAs as a manifestation of gastrointestinal problems [47]. DGLA is a unique ω-6 PUFA with anti-inflammatory and anti-proliferative properties [48]. DGLA competes with arachadonic acid for cyclooxygenase (COX) and lipoxygenase, inhibiting the production of eicosanoids to reduce inflammation and pain [49,50]. DGLA acts by inhibiting motility and invasiveness of human colon cancer cells by increasing the expression of E-cadherin, a cell-to-cell adhesion molecule that acts as a suppressor of metastasis [51,52]. In addition, DGLA reduces tumor-endothelium adhesion, a key factor in the establishment of distant metastases, partly by improving gap junction communication within the endothelium [51,53]. A linkage to the gut-brain axis comes from a report of higher serum DGLA concentrations decreasing risk of depression in elderly men [54]. Benzoic acid was shown to have broad spectrum bacteriostatic and fungistatic properties [55]. A recent study demonstrated that benzoic acid and its naturally occurring derivatives inhibit cancer cell growth by targeting histone deacetylase, a key enzyme in tumor regulatory gene expression [56]. These metabolites merit further investigation in people at risk that will consume rice bran for longer time periods.

Urine metabolites with cancer control and prevention properties

Urine metabolites that had increased fold-differences following rice bran consumption, and that have reported anti-cancer properties included 3-(3-hydroxyphenyl)propionate, DHEA-S, androstenediol (3β,17β) monosulfate, and N-formylphenylalanine. 3-(3-hydroxyphenyl)propionate is a microbial metabolite of ingested caffeic acid, and a product of the phenolic degradation in the colon [57]. 3-(3-hydroxyphenyl)propionate has antioxidative properties and is actively transported by the monocarboxylic acid transporter in human epithelial colorectal adenocarcinoma cells (Caco-2) monolayers [57,58]. DHEA-S is the sulfated form of dehydroepiandrosterone, a natural steroid hormone produced by the adrenal glands [59]. DHEA-S has antioxidant properties and aids in inhibition of weight gain and cellular proliferation, which could contribute to the cancer protective potential [60,61], and DHEA-S was shown to reduce colon cancer incidence in various animal models [61]. Androstenediol (3β,17β) monosulfate or in short, 5-androstenediol, can bind to estrogen receptors to suppress the proliferation signaling pathways in the mammary tumor cells [62]. N-formylphenylalanine, a metabolite of phenylalanine metabolism showed antitumor activity in Dunning Leukemia rats [63].

N-formylphenylalanine and 3-(4-hydroxyphenyl)lactate were identified in the rice bran food metabolome. 3-(4-hydroxyphenyl) lactate, a tyrosine metabolite, was significantly decreased in urine of CRC participants in the rice bran group at 4 weeks compared to control. 3-(4-hydroxyphenyl)lactate is also a bacterial metabolite of non-absorbed tyrosine in intestinal lumen. High urinary concentration of this metabolite was found in individuals with tyrosine malabsorption and bacterial overgrowth [64,65]. Observing a decreased fold-difference of 3-(4-hydroxyphenyl)lactate herein may signify improved tyrosine absorption and balanced gut microflora that occurs with rice bran consumption and for providing protection against CRC progression in people at high risk for recurrence.

Study limitations

The important study limitations to consider in these outcomes were the small cohort size and short study duration of the diet intervention. However, the sample size (N=19) is comparable with other interventions using metabolomics approaches [66]. There was complexity in discerning the number of metabolic pathways and metabolites affected by a diet rich in rice bran as the participants were free-living individuals and did consume 1-2 meals/day that were different from the study-provided meals and snacks. The diversity of food compounds which yield to the presence of food metabolites that were not found in rice bran, but present in plasma and urine merit follow up attention in the overall dietary pattern. Future investigations should consider a larger sample size and longer duration of rice bran intake to observe efficacy of rice bran intake for colon cancer control mechanisms and prevention of recurrence.

Conclusion

Daily consumption of rice bran for 1 month favorably modulates the plasma and urine metabolome and metabolites identified from rice bran had supporting evidence for metabolic mechanisms involved in colon cancer control and prevention. The benefits of integrating the host plasma and urine metabolome with rice bran food metabolite analysis allowed for direct insights into the complex interactions between food, host and gut microbial metabolism. This nutritional metabolomics approach also suggests potential utility for the identification of dietary biomarkers that link rice bran intake to long-term health benefits. The health promoting, nutritional and medicinal properties of rice bran bioactive compounds merit further investigation as a combination functional food approach to improve intestinal health, especially with regards to CRC control and prevention.

Declarations

Ethics: This study was carried out in accordance with the recommendations of local and national guidelines and all human study protocols were approved by Colorado State University and University of Colorado Health-North Institutional Review Boards. Prior to the start of the trial, written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. This trial was registered at clinicaltrials.gov under NCT01929122.

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Conflicts of interest: No potential conflict of interest relevant to this article was reported.

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