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Plasma and Urine Metabolite Profiles Impacted by Increased Dietary Navy Bean Intake in Colorectal Cancer Survivors: a randomized-controlled trial

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Abstract

Navy beans contain bioactive phytochemicals with colon cancer prevention properties as demonstrated in carcinogen-induced animal models. Human studies support that dietary navy bean intake modulates metabolism by the gut microbiome. This study investigated the effect of navy bean ingestion on plasma and urine metabolite profiles of overweight and obese colorectal cancer (CRC) survivors. Twenty participants completed a single-blinded, randomized-controlled dietary intervention with pre-cooked navy beans (35g bean powder/day) or control (0g/day) for 4 weeks. Plasma and urine were collected at baseline, 2 weeks and 4 weeks following consumption. Non-targeted metabolomics was applied to study meals and snacks, navy beans, plasma, and urine. Increased navy bean consumption was hypothesized to a) delineate dietary biomarkers and b) promote metabolic shifts relevant for cancer protection in the plasma and urine metabolome. At 4 weeks, 16 plasma and 16 urine metabolites were significantly different in the navy bean intervention group compared to placebo-control ($p<0.05$). Increased plasma 2,3-dihydroxy-2-methylbutyrate (1.34-fold), S-methylcysteine (1.92-fold), and pipecolate (3.89-fold), and urine S-adenosylhomocysteine (2.09-fold) and cysteine (1.60-fold) represent metabolites with cancer

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Authors' Contributions

EPR, RJB and ECB designed and conducted the research; BAB, IZ, RCO, and EPR conducted the metabolomics analysis and wrote the manuscript. All authors read and approved the final manuscript.

Clinical trial registry numbers: Beans/Bran Enriching Nutritional Eating For Intestinal health Trial (BENEFIT) (clinical trial # NCT01929122).

Prevention Relevance Statement: This clinical study suggests that increased consumption of navy beans would deliver bioactive metabolites to individuals at high-risk for colorectal cancer recurrence and produce metabolic shifts in plasma and urine profiles.

Disclosure of Potential Conflict of Interest

The authors declare that they have no potential conflicts of interest.

The datasets used and/or analyzed during the current study are available as supplementary material along with this article.

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standards of the Colorado State University and University of Colorado Health-North Institutional Review Board, and with the 1964 Helsinki Declaration and its 2013 amendments. Informed consent was obtained from all individual participants before included in the study. This trial was registered at clinicaltrials.gov under NCT01929122.

protective actions following navy bean consumption. Diet-derived metabolites were detected in plasma or urine and confirmed for presence in the navy bean intervention meals and snacks. These included 3-(4-hydroxyphenyl)propionate, betaine, pipecolate, S-methylcysteine, choline, eicosapentaenoate (20:5n3), benzoate, S-adenosylhomocysteine, N-delta-acetylornithine, cysteine, 3-(4-hydroxyphenyl)lactate, gentisate, hippurate, 4-hydroxyhippurate, and salicylate. The navy bean dietary intervention for 4 weeks showed changes to pathways of metabolic importance to CRC prevention and merit continued attention for dietary modulation in future high-risk cohort investigations.

Keywords

Navy beans; Metabolomics; Colorectal Cancer; Bioactive Food Components

Introduction

Legumes are globally recognized as high-quality sources of dietary fibers with pre-biotic functions, and are sustainable sources of essential amino acids and many other bioactive plant secondary metabolites [1–3]. Epidemiological studies and multiple clinical cohort investigations now support regular consumption of common beans for colorectal cancer (CRC) prevention [4–7], and additionally promote lower risk of chronic diseases such as obesity [8–13], cardiovascular disease [14, 15], and type II diabetes [16]. Hughes *et al.* reported that 59g of pinto beans per 100g of diet reduced colon tumor (azomethane-induced) incidence by 50% in rats compared to 24% by casein-fed rats [17]. Similar to the findings of previous studies, Hangen and Bennink found that diets fed to rats containing 75% navy beans resulted in 44% decreased colon tumor and adenocarcinoma incidence [18]. The consumption of ~ ½ cup of cooked dry beans per day for adults was practical and significantly increased intake of fiber, protein, and minerals such as folate, zinc, iron, and magnesium, while lowers the intake of saturated fats and total fat [19–21].

Emerging evidence exists for diet-derived small molecules to signal as modifiers of cancer risk and tumor behavior. Reliable dietary intake assessment methods are required to understand the associations between diet, nutritional status, and colon cancer risk. Non-targeted metabolomics is a well-established and sensitive tool for the comprehensive and concomitant characterization of multiple metabolites within a biological matrix, and is increasingly applied to cancer research to screen and discover shifts in metabolism [22, 23]. In recent years, metabolomics has also been used for identifying putative biomarkers of food consumption and dietary patterns [24, 25]. Less is known about whether bioactive food components reach effective cancer protective concentrations in people due to extensive inter-individual variability that can occur in host digestion and gut metabolism. Some mechanisms linking common dry bean consumption and cancer prevention include, but are not limited to, induction of apoptosis and modulation of cell cycle and proliferation [26–28]. Other mechanisms for common bean intake to inhibit colon carcinogenesis involve changes to energy metabolism [29] and phase II detoxification [30] as shown by tissue microarray analysis in animals. Metabolomics has utility for measuring downstream products of

metabolic disturbances shown by gene expression and for associations with environmental exposures involved in cancer risk [31, 32].

Plasma and urine are ideal biological fluids to examine the physiological effects of dietary exposures and have shown sensitivity to detect subtle and major changes in metabolite abundance over time [33, 34]. A non-targeted metabolite approach was applied herein to assess metabolic modulation of CRC risk following increased navy bean intake. The purpose of this study was to assess the impact of navy bean consumption on the plasma and urine metabolome for CRC prevention when compared to placebo-control foods, and to identify a suite of metabolites that originated from navy beans. It was hypothesized that increased consumption of navy beans would deliver bioactive metabolites to individuals at high-risk for CRC recurrence and produce metabolic shifts in plasma and urine profiles.

Materials and Methods

Study design and dietary interventions

Twenty overweight or obese CRC survivors that were a minimum of 4 months post-cancer treatment were recruited for this single-blinded, placebo-controlled, randomized intervention trial as previously described [3, 35, 36]. All the participants were blinded to the study arms, and only the study coordinator was aware of the participant study group allocation group. The study design adhered to CONSORT guidelines (http://www.consort-statement.org/). The recruitment took place through the University of Colorado Health-North Cancer Center Network (Fort Collins, CO). Supplementary Figure 1 illustrates the eligibility criteria and the allocation of 20 CRC survivors that completed a study titled Beans/Bran Enriching Nutritional Eating For Intestinal health Trial (BENEFIT) (NCT01929122). Participants were randomized by body mass index (BMI between 25–35), sex, and daily caloric intake [37] prior to dietary intervention. Supplementary Table 1 shows study population characteristics at baseline. In this 4-week intervention, participants assigned to the navy bean intervention consumed 35g of cooked whole navy beans in powder form (daily intake of one meal and one snack that each contained 17.5g of cooked navy bean powder) [37]. The control study arm received placebo meals and snacks without the addition of navy bean powder as previously described [35, 37]. The study protocol and written informed consent was approved by the Colorado State University Research Integrity and Compliance Review Board and the University Colorado Cancer Center-North Institutional Review Board (Protocol # 09–1530H and 10–1038, respectively) in accordance with the 1964 Helsinki Declaration and its 2013 amendments. The 20 individuals completed the 4-week pilot dietary intervention trial between August 2010 and December 2014.

Blood and urine sample collection for metabolomics

Fasting blood samples were collected into 4 ml ethylene-diamine-tetra-acetic acid (EDTA) blood collection tubes from the 20 participants who completed study visits at baseline, 2 weeks, and week 4 and kept immediately on ice until the centrifugation step to extract the plasma (1500 rpm for 10 minutes). Plasma was further stored at −80°C until processed for metabolomics analysis using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). At each study visit (baseline, 2 weeks, and 4 weeks),

participants were given study-labeled containers for self-collection of first void of the morning urine. Urine was stored at −80°C until extracted for analysis by UHPLC-MS/MS. Urine samples were subjected to the osmolality normalization using a Fiske™ 210 Micro-Sample Osmometer to correct for the fluid intake variability between participants.

Plasma and urine extraction for metabolomics

The global non-targeted metabolomics was performed through Metabolon, Inc. (Durham, NC, USA). The extraction method has been described previously [34]. Briefly, 80% methanol was added on plasma and urine in a ratio of 300 μL solvent per 100 mg plasma or urine. The samples were shaken for 2 min, and then centrifuged for 10 min at 4 °C (12000 rpm). The metabolite extracts from plasma and urine were analyzed using UHPLC-MS/MS with positive and negative ion mode electrospray ionization (ESI).

UHPLC-MS/MS analysis

Samples were analyzed using a Waters ACQUITY UHPLC 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. Prior to analysis, samples were resuspended in acidic or basic UHPLC-compatible solvents. The acidic and basic solutions were analyzed using positive and negative ESI, and both HILIC (hydrophilic interaction chromatography) and reverse phase (RP) chromatography [38]. In HILIC mode, the extracts were injected into a BEH Amide column (2.1 mm × 100 mm, 1.7 μm; Waters Corporation, Milford, MA). The mobile phase was delivered at 0.35mL/min consist of water, acetonitrile, and 10mM ammonium formate at pH 10.8 (HILIC positive), methanol + water, and 6.5mM ammonium bicarbonate at pH 8.0 (HILIC negative). In RP positive and negative ion optimized conditions, extracts were gradient eluted from a C18 column (2.1 mm × 150 mm, 1.7 μm; Corporation, Milford, MA). In RP positive, extracts were eluted from the column with water + methanol, containing 0.05% perfluoropentanoic acid and 0.1% formic acid, while in RP negative, water + methanol, containing 0.05% perfluoropentanoic, 0.01% formic acid, and acetonitrile was used as mobile phase. For the MS data acquisition of study meals and snacks, plasma and urine, an extended dynamic exclusion was used with the instrumental ion acquirement of 70–1000 m/z. The peak picking process was performed by Metabolon through in-house peak detection and integration software. Compound identification (level 1 identification) was completed using library of over 3,300 purified standards and based on the experimentally MS-MS spectra matching the accurate mass the standard within 8 ppm, retention time, and m/z ratio.

The non-targeted metabolomics analysis of cooked navy bean powder has been previously described [3]. The plasma and urine metabolites from participants in both dietary groups were cross-referenced with metabolites identified in the navy beans and in the one meal and three snacks metabolome after 4 weeks of either control or navy bean consumption.

Statistical Analyses

Plasma and urine metabolite intensity levels underwent median-scaled normalization as previously described [34]. A Welch’s two-sample t-test and two-way ANOVA were performed for between group comparisons. Repeated measures ANOVA was used for

comparisons at 2 and 4 weeks from baseline and within each study group. An estimate of false discovery rate (q-value) was calculated. Statistical significance was defined as a p-values of <0.05 and q-values below the threshold of <0.2 for within group comparisons.

Results

Metabolome profile of study provided meals and snacks

The metabolomes of one meal and three snacks used in this study comprised of 653 metabolites with confirmed identifications. Eight groups were used for the overarching metabolite classifications: amino acids, carbohydrates, cofactors and vitamins, energy metabolism, lipids, nucleotides, peptides, and xenobiotics. The meals and snacks metabolome contained 143 amino acids, 41 carbohydrates, 27 cofactors and vitamins, 14 energy metabolism related metabolites, 226 lipids, 61 nucleotides, 32 peptide, and 111 xenobiotics. Supplementary Table 2 shows the metabolite raw abundance for one meal and three snacks. Composition of meals and snacks was analyzed by chemical classes for placebo-control (0g/serving navy bean powder) and navy bean intervention (17.5g/serving navy bean powder). Supplementary Figure 2A displays the meals and snacks metabolite profiles for placebo-control and navy bean intervention, whereby each bar represents total number of identified metabolites in the chemical class, and the shaded portion represents the number of metabolites that appeared in navy bean intervention meals and snacks but not in the control. The navy bean source of metabolites in meals and snacks were determined by overlapping presence of metabolite in the navy bean powder metabolome. Supplementary Figure 2B-D displays normalized relative abundance values in placebo-control and navy bean intervention meals and snacks for the following metabolites: pipecolate, S-methylcysteine, and N-delta-acetylornithine. Pipecolate, S-methylcysteine, and N-delta-acetylornithine had higher abundance in the bean intervention meals and snacks compared to control that was due to the high relative abundance shown for these compounds in the navy bean powder.

Dietary modulation of plasma metabolome in CRC survivors after 2 and 4 weeks

There were 854 identified metabolites from plasma (N = 20 participants) across all time points, including baseline, 2 weeks, and 4 weeks post intervention. Supplementary Table 3 shows the relative abundance of plasma metabolites classified into metabolic pathways for all participants. Table 1 lists 22 plasma metabolites that were significantly modulated in either the control, navy bean, or both study groups for 20 participants at 2 and/or 4 weeks compared to baseline ($p < 0.05$, $q < 0.2$). All data are presented as the mean fold-change in the median-scaled relative abundance of each metabolite. After consuming navy beans for 2 weeks, 21 metabolites including two gut microbial metabolites, four amino acids, one cofactors & vitamins, 11 lipids, and three xenobiotics were significantly modulated in plasma when compared to baseline.

Navy bean powder significantly increased 3 amino acid metabolites in plasma of CRC survivors at week 2 and at week 4 when compared to the baseline levels (Table 1). Figure 1A–C illustrates median-scaled relative abundance for pipecolate, S-methylcysteine, and N-delta-acetylornithine. Individual responses over time are shown in Supplementary Figure 3.

Notably, these three metabolites were highlighted for abundance in navy bean powder and study meals and snacks metabolomes.

Table 2 lists 16 plasma metabolites with significant differences in the abundance between intervention and control groups for the 20 participants at 4 weeks ($p < 0.05$, $q < 0.2$. For all participants, data are presented as the mean fold-difference between intervention and control groups at 4 weeks. The five plasma metabolites increased in abundance for the navy bean group were 3-(4-hydroxyphenyl)propionate (4.48-fold), S-allylcysteine (4.08-fold), pipecolate (3.88-fold), S-methylcysteine (1.92-fold), and 2,3-dihydroxy-2-methylbutyrate (1.34-fold). The 11 plasma metabolites with significant decreased levels compared to control at 4 weeks were betaine (0.84-fold), 1-arachidonoyl-GPC (20:4n6) (0.80-fold), 5alpha-pregnan-3beta-20alpha-diol monosulfate (0.12-fold), choline (0.80-fold), DHA (0.74-fold), docosahexaenoylcarnitine (C22:6) (0.63-fold), eicosapentaenoate (EPA) (0.77-fold), linoleoylcarnitine (C18:2) (0.71-fold), 5,6-dihydrothymine (0.71-fold), gamma-glutamyl-2-aminobutyrate (0.30-fild), and benzoate (0.77-fold). The eight metabolites identified in the plasma that were also present in the cooked navy beans include pipecolate, S-methylcysteine, betaine, EPA, docosahexaenoate (DHA), choline, gamma-glutamyl-2-aminobutyrate, and benzoate (see Table 2, footnote [a]) and 6 metabolites were identified in the study meals and snacks metabolome including 3-(4-hydroxyphenyl)propionate, betaine, pipecolate, S-methylcysteine, choline, and benzoate (see Table 2, footnote [b]). Figure 1D illustrates median-scaled relative abundance for 3-(4-hydroxyphenyl)propionate. This phenolic acid derivative was significantly increased after consuming navy beans for 4 weeks when compared to the control group. This phenolic acid was also found in the study meals and snacks metabolome (Table 2). Figure 1E and 1F show the median-scaled relative abundances for EPA and benzoate. These endogenous and/or diet-derived metabolites were significantly decreased after consuming navy beans for 4 weeks when compared to the control group (Table 2). Both metabolites were identified in the navy bean metabolome, while benzoate was also identified in the study meals and snacks. Figure 1G shows a Venn diagram of 33 plasma metabolites with fold-change or fold-differences identified at 2 or 4 weeks post intervention. The center metabolites in the Venn diagram illustrate those with overlapping significance from each of the statistical comparisons applied, and were Pipecolate, S-methylcyteine, and 2,3-dihydroxy-2methylbutyrate.

Dietary modulation of urine metabolome in CRC survivors after 2 and 4 weeks

There were 703 identified metabolites from urine of 20 participants at all time points. The urine metabolites were classified in the metabolic pathways as shown for plasma. Supplementary Table 4 lists the relative abundance of all the identified urine metabolites and the respective metabolic pathways for the control and navy bean groups. Table 3 lists 10 urine metabolites that were significantly modulated in either the control, navy bean, or both study groups for 20 participants at 2 and/or 4 weeks compared to baseline ($p < 0.05$, $q < 0.2$). All data are presented as the mean fold-change in the median-scaled relative abundance of each metabolite. Figure 2A–D illustrates median-scaled relative abundance for gentisate, hippurate, salicylate, and N2,N5-diacetlyornithine that were significantly increased at week 4 when compared to their baseline in the intervention group. Individual change over time are shown in Supplementary Figure 4. These metabolites were chosen for their potential CRC

protection and the four metabolites were identified in navy bean and study meals and snacks metabolomes.

Table 4 lists 16 urine metabolites with significant differences in the abundance between intervention and control for the 20 participants at 4 weeks ($p < 0.05$, $q < 0.2$. For all participants, data are presented as the mean fold-difference between intervention and control at 4 weeks. The 14 urine metabolites increased in abundance for the navy bean group were 1-methylhistamine (1.72-fold), cysteine (1.6-fold), N2,N5-diacetylornithine (1.55-fold), N-acetyltaurine (1.57-fold), pyroglutamine (1.74-fold), S-adenosylhomocysteine (SAH) (2.09-fold), N6-carbamoylthreonyladenosine (1.44-fold), guanosine (2.81-fold), 2-keto-3-deoxy-gluconate (2.18-fold), 4-hydroxyhippurate (1.92-fold), alliin (3.34-fold), O-sulfo-L-tyrosine (1.33-fold), and thioproline (2.66-fold). The three urine metabolites that decreased in abundance for the navy bean group were dopamine 3-O-sulfate (0.45-fold), indolepropionate (0.35-fold), and isovalerylglutamine (0.53-fold). Among these 17 urine metabolites, eight metabolites were identified in the navy beans, including cysteine, N2,N5-diacetylornithine, pyroglutamine, SAH, N6-carbamoylthreonyladenosine. guanosine, 4-hydroxyhippurate, and O-sulfo-L-tyrosin (see Table 4, footnote [a]) and five urine metabolites were identified in the study meals and snacks metabolome, including cysteine, pyroglutamine, SAH, guanosine and O-sulfo-L-tyrosine (see Table 4, footnote [b]). Navy bean derived metabolites were distinguished for presence and abundance in urine of the intervention group compared to control. Figure 2D–F illustrates the median-scaled relative abundance for amino acids with anti-inflammatory potential; N2, N5-diacetylornithine, cysteine, and SAH which were identified in the navy bean metabolome. The Venn diagram shown in Figure 2G highlights 23 urine metabolites (statistical significance by fold-change or fold-difference) in urine at 2 or 4 weeks after dietary bean intervention. N2,N5-diacetylornithine was the only urine metabolite with overlap in the analyses at 4 weeks in the intervention group.

Discussion

This study established that increased navy bean consumption for 4 weeks produced metabolic shifts in plasma and urine profiles of overweight and obese CRC survivors at risk for recurrence. The plasma and urine metabolites that showed changes in abundance over time or differences between the two study groups were also compared to the phytochemical compounds that originate from navy beans (including the meals and snacks that contained navy beans). This integration of the navy bean-metabolites with the results from changes in plasma and urine have established novel relationships between bean exposure in the diet and cancer prevention biomarkers in adults. There are also a number of metabolites discussed below from distinct metabolic pathways that support novel associations for bean intake with anti-inflammatory, antioxidant and anti-cancer mechanisms that may reduce cancer risk. Thus, plasma and urine metabolites have potential to dually serve as biomarkers of dietary navy beans exposure and metabolic health to prevent and control colon cancer.

Six plasma metabolites modulated by navy bean consumption and that have cancer control and prevention relevance from this study are S-methylcysteine, pipecolate, 3-(4-hydroxyphenyl)propionate, N-delta-acetylornithine, S-allylcysteine, and 2,3-dihydroxy-2-methylbutyrate (Tables 1 and 2/Figures 2). S-methylcysteine, a water-soluble organosulfur

compound, may be a biomarker of dietary exposure as it was previously identified from common bean (*Phaseolus vulgaris*) [39, 40]. Cysteine related components have also demonstrated suppression of chemical induced carcinogenesis [41]. S-methylcysteine was reported to inhibit the formation of glutathione S-transferase placental form-positive foci (GST-P-positive foci) which are markers for pre-neoplastic lesions [42] and was also found to inhibit ornithine decarboxylase (ODC), an enzyme which is upregulated in a wide variety of cancers [43]. ODC is essential in biosynthesis of polyamines [44], namely antioxidants important for stabilizing DNA structure [45]. Lack of ODC has been found to cause cell apoptosis in embryonic mice, induced by DNA damage [46]. S-allylcysteine was also increased in plasma (Table 2) and has antioxidant properties [47, 48], cholesterol-lowering [49] and anti-cancer effects on clusters of abnormal tube-like glands in the lining of the colon and rectum called aberrant crypt foci. A decreased level of urinary cysteine warranted discussion as cysteine is a unique thiol containing amino acid that can undergo oxidation/reduction (redox) reactions with antioxidant properties and may reduce inflammation [50, 51]. These metabolite changes collectively may reduce cell division to decrease risk for CRC recurrence.

Pipecolate represents another candidate dietary biomarker of bean consumption [39] that has functional relevance to this prospective cohort investigation because this phytochemical serves as a precursor to gut microbial metabolism and has anti-inflammatory, antitumor, and antibiotic properties [52–54]. 3-(4-hydroxyphenyl)propionate was also increased following navy bean intake and is a small phenolic acid produced from biotransformation of large polyphenols by human colonic microflora [55]. Phenolic acids exert effects on carcinogen bioactivation, cell-signaling, cell cycle regulation, angiogenesis, oxidative stress, and inflammation [56, 57]. 2,3-dihydroxy-2-methylbutyrate is a normal organic acid constituent of the gut and the increase measured herein for plasma of navy bean consumers suggested beneficial roles against inflammation and alterations to microbiota. Polyunsaturated fatty acids (PUFA), such as EPA (20:5n3) that is a navy bean metabolite, were shown to exert anticancer activities in various stages of cancer progression, such as cell proliferation, metastasis, inflammation, cell survival, and angiogenesis [58, 59].

This next group of metabolites have mechanistic links to diverse epigenetic modifications involved in cancer progression. Benzoic acid (navy bean metabolite) has naturally occurring derivatives with potential to suppress cancer cell growth by blocking histone deacetylase, a key enzyme involved in tumor regulatory gene expression [60]. The decreased urinary SAH following daily bean intake is important as an inhibitor of methyltransferases, the key enzymes for DNA methylation that may cause aberrant DNA methylation patterns in colon cancer [61]. Higher abundance of SAH could be important to block aberrant DNA methylation and dysregulation of histones and gene expression that stimulate carcinogenesis [62]. Both choline and betaine were decreased in plasma from this study, and the roles in CRC merit attention as they are essential to one-carbon metabolism [63–65]. The lack of choline was shown to promote fatty liver disease, DNA hypomethylation, and tumor development (in the absence of carcinogens) [66] and an epidemiological study showed that the risk of colorectal adenomas statistically increased with increasing choline intake [66]. Betaine is notably an oxidation product of choline, and a methyl donor that has nutrient contributions for the prevention of chronic diseases [67].

There were multiple metabolites identified in this study with associated mechanisms to CRC prevention that relate to metabolism by the gut microbiota. These may include, but were not limited to 3-(4-hydroxyphenyl)lactate, N2,N5-diacetylornithine, hippurate and salicylate. High urinary levels of 3-(4-hydroxyphenyl)lactate was found in individuals with tyrosine malabsorption and bacterial overgrowth [68, 69], and our data suggests improved tyrosine absorption and balanced gut microflora with navy bean intake. Dietary bean intake influences on metabolism by gut microbiota were also shown by changes to indole propionate, 4-hydroxyhippurate and 3-(4-hydroxyphenyl)propionate that have been reported previously in stool [3, 36].

The limitations of this study include the small cohort size. The acute (one-month) study duration of the diet intervention may also be considered a limitation, yet these metabolite changes detected early-on following dietary change may have long-term impacts. The study participants were free-living individuals and the study-provided meals and snacks contributed ~35% of daily intake. Follow up investigations will utilize a larger sample size and a longer duration of elevated navy bean exposure may be needed to observe colon tissue metabolic changes that will improve cancer control, treatment, and prevention outcomes.

Conclusion

This study supports that an elaborate array of interactions between food, host digestion and gut microbial metabolism occurs in overweight and obese CRC survivors. Plasma and urine metabolites that merit attention for validation after the daily consumption of navy beans in adults are 2,3-dihydroxy-2-methylbutyrate, pipecolate, and S-methylcysteine (in plasma), and N2,N5-diacetylornithine and 4-hydroxyhippurate (in urine). The metabolite results shown for blood and urine were integrated with the analysis of phytochemicals originating from the navy beans, and revealed the amino acid and lipid metabolism pathways as mechanistic targets to reduce CRC recurrence following dietary bean intervention. Assessing the bioactivity and bioavailability of navy bean-derived phytochemicals in human colon tissue is one future analytical approach to validate food-exposure biomarkers with primary and secondary CRC prevention outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CRC	colorectal cancer

GC-MS	gas chromatography–mass spectrometry
UHPLC-MS/MS	ultra-high-performance liquid chromatography-tandem mass spectrometry
PUFA	polyunsaturated fatty acid
DGLA	dihomo-linolenoylcarnitine (20:3n3 or 6)
SAH	S-adenosylhomocysteine
DHEA-S	dehydroisoandrosterone sulfate
BCG	β-citrylglutamate
ODC	ornithine decarboxylase
GCP2	glutamate carboxypeptidase 2
DHA	docosahexaenoate
EPA	eicosapentaenoate
SAM	S-adenosylmethionine
GPC	glycerophosphocholine
GPE	glycerophosphoethanolamine
GPG	glycerophosphoglycerol
GPI	glycerophosphoinositol
HMDB	Human Metabolome Database
GABA	γ-aminobutyric acid
CEHC	carboxyethyl-hydroxychromans
HODE	hydroxy-10(E),12(Z)-octadecadienoic acid
HILIC	hydrophilic interaction chromatography
RP	reverse phase chromatography

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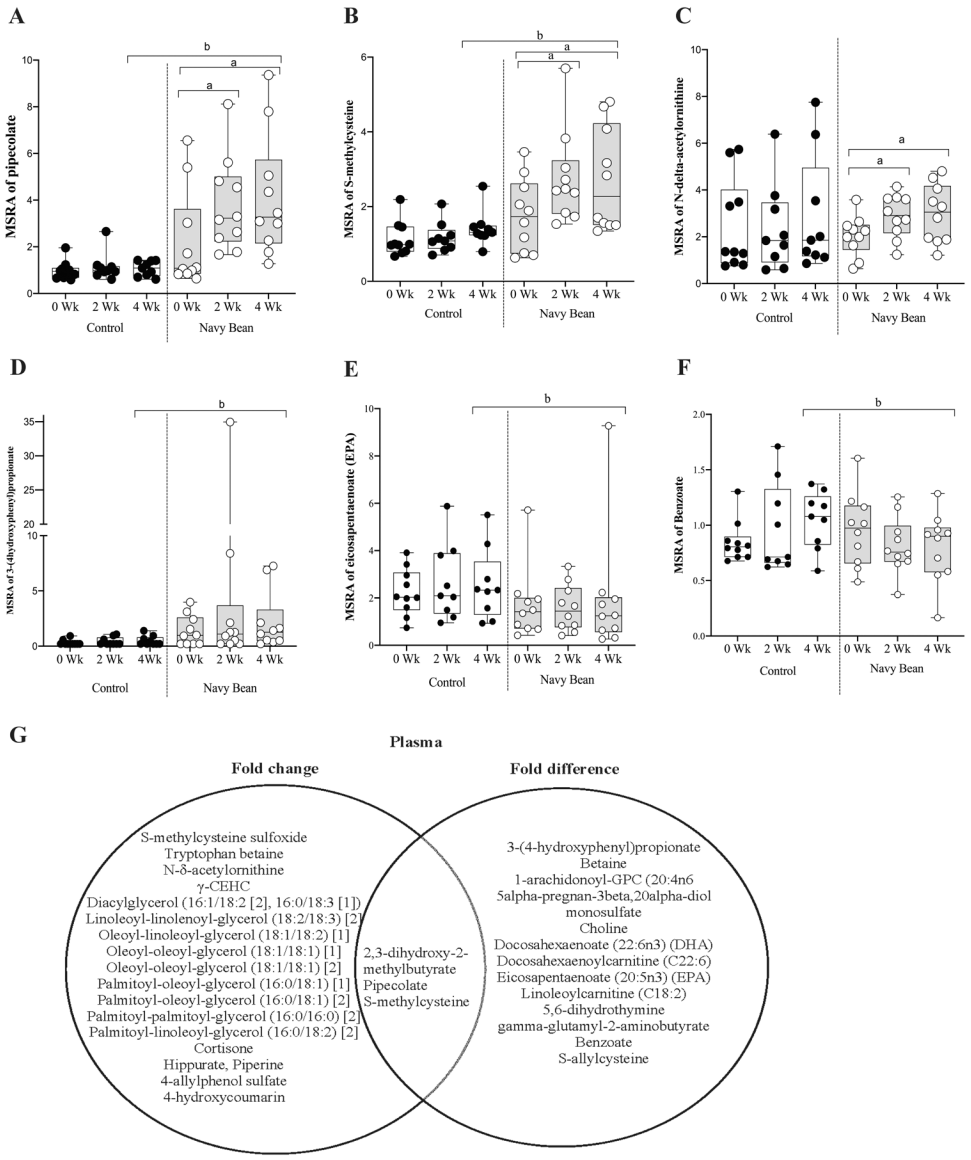

Figure 1.

Median-scaled relative abundance (MSRA) of selected amino acids, gut microbial metabolites, lipid and phytochemicals for control or navy bean at 0, 2 and 4 weeks post dietary intervention in plasma. a = significant fold change, b = significant fold difference ($p < 0.05$).

A. pipecolate, **B**. S-methylcysteine, **C**. N-delta-acetylornithine, **D**. 3–4(hydroxyphenyl)propionate, **E**. eicosapentaenate, **F**. benzoate. **G**. Venn diagram summarizes plasma metabolites with statistical significance to navy bean intake. The left circle includes metabolites with fold changes at two or four weeks of navy bean intake compared to baseline, and the right circle shows metabolites with fold differences between the control and bean groups (4-week timepoint). Overlapping metabolites of statistical significance in each analysis are listed in the center.

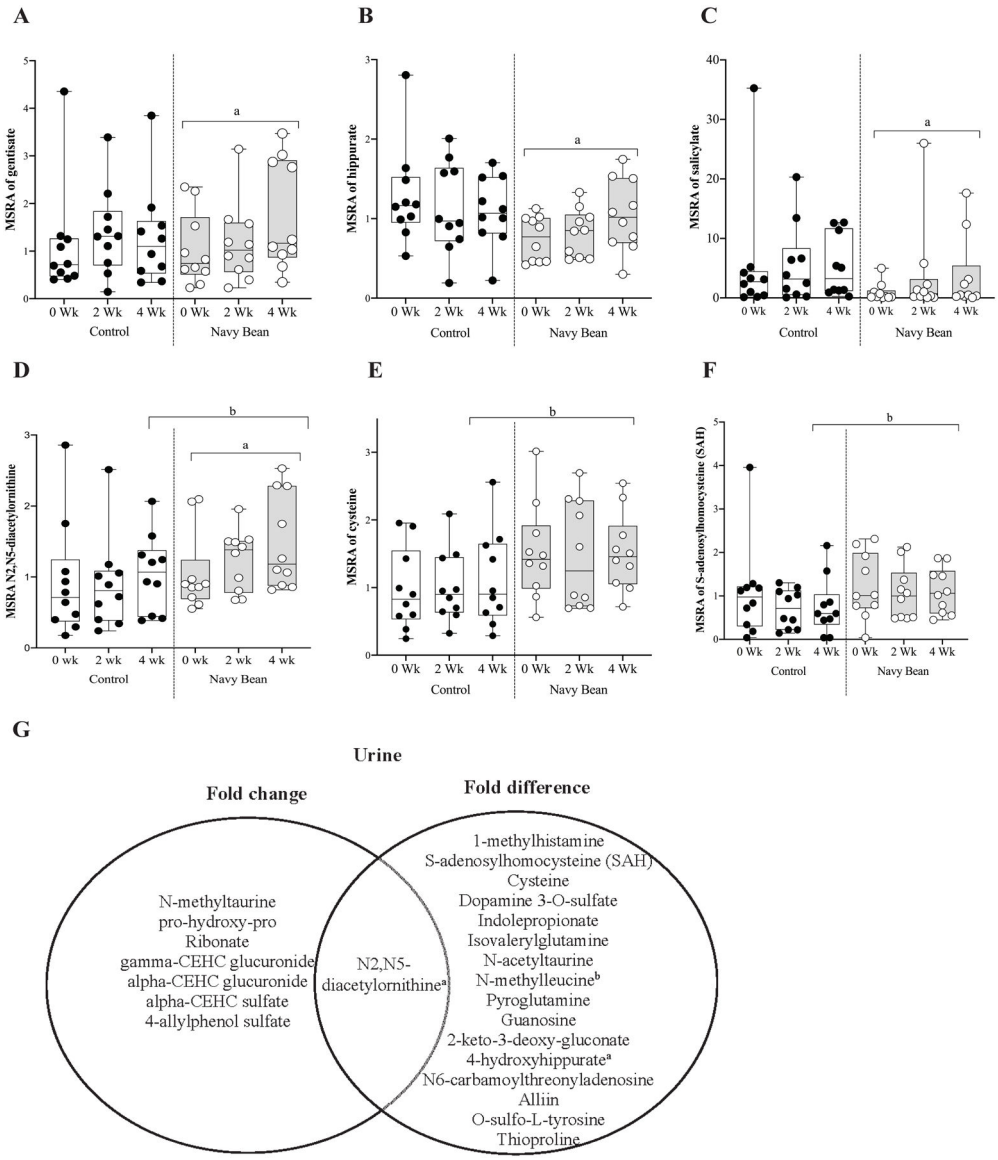

Figure 2.

Median-scaled relative abundance (MSRA) of selected amino acids and phytochemicals for control or navy bean at 0, 2 and 4 weeks post dietary intervention in urine. **A**. gentisate, **B**. hippurate, **C**. salicylate, **D**. N2,N5-diacetlyornithine **E**. cysteine, **F**. S-adenosylhomocysteine a= significant fold-change, b = significant fold-difference ($p < 0.05$). **G**. Venn diagram summarizes urine metabolites with statistical significance to navy bean intake. The left circle includes metabolites with fold changes at two or four weeks of navy bean intake compared to baseline, and the right circle shows metabolites with fold differences between the control and bean groups (4-week timepoint). Overlapping metabolites of statistical significance in each analysis are listed in the center.

Table 1.

Plasma metabolites following consumption of control foods or navy bean-based foods for 2 and 4 weeks. Post intervention metabolite profiles were compared to baseline (0 weeks).

		Fold-change[c]											
		Control group						Navy bean group					
	Metabolite	2wk/ 0wk	*p*-value	*q-value*	4wk 0wk	*p*-value	*q-value*	2wk 0wk	*p*-value	*q-value*	4wk 0wk	*p*-value	*q-value*
Amino Acids													
Leucine, Isoleucine and Valine	2,3-dihydroxy-2-methylbutyrate	1.15	0.676	1	1.16	0.415	1	**1.71**	0.000	0.025	**1.73**	0.000	0.014
Lysine	Pipecolate[a,b]	1.22	0.550	1	1.10	0.677	1	**2.59**	0.000	0.025	**2.67**	0.000	0.014
Methione, Cysteine, SAM and Taurine	S-methylcysteine[a,b]	1.20	0.548	1	1.41	0.059	1	**1.88**	0.000	0.070	**1.88**	0.001	0.173
	S-methylcysteine sulfoxide	**1.53**	0.032	1	**1.66**	0.008	1	**1.86**	0.001	0.123	**2.07**	0.001	0.173
Polyamine	Tryptophan betaine	0.98	0.629	1	1.31	0.183	1	**0.74**	0.002	0.138	**0.68**	0.000	0.017
Urea cycle; Arginine and Proline	N-δ-acetylornithine[a,b]	1.15	0.725	1	1.28	0.091	1	**1.55**	0.002	0.145	**1.69**	0.002	0.255
Cofactors & Vitamins													
Tocopherol	γ-CEHC	**0.81**	0.047	1	1.12	0.37	1	**0.66**	0.002	0.124	**0.72**	0.016	0.567
Lipids													
Diacylglycerol	Diacylglycerol (16:1/18:2 [2], 16:0/18:3 [1])*	1.04	0.961	1	0.92	0.283	1	**1.72**	0.002	0.124	**1.44**	0.021	0.64
	Linoleoyl-linolenoyl-glycerol (18:2/18:3) [2]*	1.02	0.671	1	0.95	0.412	1	**1.73**	0.011	0.296	1.50	0.09	0.698
	Oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	0.95	0.483	1	0.90	0.192	1	**1.47**	0.001	0.124	1.28	0.063	0.688
	Oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	1.01	0.890	1	0.90	0.231	1	**1.49**	0.004	0.176	**1.38**	0.019	0.608
	Oleoyl-oleoyl-glycerol (18:1/18:1) [1]*	1.02	0.930	1	0.88	0.055	1	**1.35**	0.003	0.145	1.14	0.228	0.825
	Oleoyl-oleoyl-glycerol (18:1/18:1) [2]*	1.06	0.654	1	0.89	0.087	1	**1.42**	0.000	0.106	1.21	0.082	0.698

		Fold-change[c]											
		Control group						Navy bean group					
	Metabolite	2wk/ 0wk	*p*-value	*q-value*	4wk 0wk	*p*-value	*q-value*	2wk 0wk	*p*-value	*q-value*	4wk 0wk	*p*-value	*q-value*
	Palmitoyl-linoleoyl-glycerol (16:0/18:2) [2] *[b]	1.05	0.93	1	0.98	0.643	1	**1.59**	0.005	0.196	**1.46**	0.01	0.567
	Palmitoyl-oleoyl-glycerol (16:0/18:1) [1] *	1.07	0.681	1	0.96	0.348	1	**1.45**	0.002	0.139	1.24	0.093	0.698
	Palmitoyl-oleoyl-glycerol (16:0/18:1) [2] *[b]	1.10	0.518	1	0.96	0.38	1	**1.50**	0.000	0.106	**1.30**	0.028	0.678
	Palmitoyl-palmitoyl-glycerol (16:0/16:0) [2] *	1.15	0.513	1	1.00	0.603	1	**1.83**	0.002	0.124	**1.61**	0.015	0.567
Steroid	Cortisone	**0.83**	0.022	1	0.92	0.267	1	**1.43**	0.005	0.19	1.29	0.073	0.688
Xenobiotics													
Benzoate Metabolism	Hippurate[a,b]	1.34	0.860	1	1.19	0.563	1	1.29	0.39	0.964	**2.91**	0.002	0.174
Food Component/Plant	Piperine[a]	1.94	0.451	1	1.91	0.901	1	**13.77**	0.000	0.036	**3.72**	0.001	0.174
	4-allylphenol sulfate	**4.45**	0.000	0.009	**7.58**	0.000	0.000	**3.59**	0.000	0.025	**4.54**	0.000	0.011
Drug	4-hydroxycoumarin	**6.82**	0.030	1	2.22	0.225	1	**8.04**	0.001	0.124	5.68	0.104	0.698

CEHC, carboxyethyl-hydroxychromans

*Indicates that the metabolite identified was not made against a purified chemical standard.

[a]Metabolite also identified from the Navy Bean metabolome.

[b]Metabolite also identified from study food metabolome.

[c]Values presented are fold-change of the mean relative abundance within control, navy bean group at 2 and 4 weeks compared to their baselines ($p<0.05$, are bold) and ($q<0.2$). Statistically-significant fold-changes are bolded and shaded. Light shaded represent metabolites and pathways that showed trends towards significance ($0.05 < p < 0.10$).

Table 2.

Navy bean consumption revealed distinct plasma metabolites after 4 weeks compared to a control intervention for 4 weeks.

		Fold-difference[c]	
Metabolite	**HMDB**	**Navy Bean**	***p*-value**
Gut Microbial Metabolites			
2,3-dihydroxy-2-methylbutyrate	HMDB29576	**1.34**	0.036
3-(4-hydroxyphenyl)propionate[b]	HMDB02199	**4.48**	0.031
Amino Acids			
Betaine[a,b]	HMDB00043	**0.84**	0.032
Pipecolate[a,b]	HMDB00070	**3.88**	0.000
S-methylcysteine[a,b]	HMDB02108	**1.92**	0.006
Lipids			
1-arachidonoyl-GPC (20:4n6)	HMDB10395	**0.80**	0.045
5alpha-pregnan-3beta,20alpha-diol monosulfate	-	**0.12**	0.043
Choline[a,b]	HMDB00097	**0.80**	0.008
Docosahexaenoate (22:6n3) (DHA)[a]	HMDB02183	**0.74**	0.014
Docosahexaenoylcarnitine (C22:6)	-	**0.63**	0.043
Eicosapentaenoate (20:5n3) (EPA)[a]	HMDB01999	**0.77**	0.039
Linoleoylcarnitine (C18:2)	HMDB06469	**0.71**	0.037
Nucleotide			
5,6-dihydrothymine	HMDB00079	**0.71**	0.022
Peptide			
gamma-glutamyl-2-aminobutyrate[a]	-	**0.30**	0.005
Xenobiotics			
Benzoate[a,b]	HMDB01870	**0.77**	0.029
S-allylcysteine	HMDB34323	**4.08**	0.019

GPC, glycerophosphocholine; HMDB, Human Metabolome Database

[a]Metabolite also identified from the Navy Bean metabolome.

[b]Metabolite also identified from the study food metabolome.

[c]Values presented are fold-difference of the mean relative abundance between navy bean compared to control at 4 weeks ($p<0.05$). Statistically-significant fold-differences are bolded.

Table 3.

Urine metabolites following consumption of control foods or navy bean-based foods for 2 and 4 weeks. Post intervention metabolite profiles were compared to baseline (0 weeks).

		Fold-change[c]											
		Control group						Navy bean group					
	Metabolite	2wk/ 0wk	*p*-value	*q*-value	4wk/ 0wk	*p*-value	*q*-value	2wk/ 0wk	*p*-value	*q*-value	4wk/ 0wk	*p*-value	*q*-value
Amino Acids													
Histidine	N-acetyl-1-methylhistidine *	0.78	0.072	0.791	**0.62**	0.000	0.147	1.00	0.998	1	1.00	0.975	1
Lecine, Isoleucine and Valine	N6,N6,N6-trimethyllysine	0.93	0.561	1	**0.64**	0.000	0.147	0.98	0.869	1	0.95	0.650	1
Methionine, Cysteine, SAM and Taurine	N-methyltaurine	**2.70**	0.018	0.584	2.05	0.084	0.534	**4.20**	0.001	0.111	**2.29**	0.048	0.927
Urea cycle; Arginine and Proline	N2,N5-diacetylornithine[a]	1.07	0.511	1	1.19	0.093	0.544	**1.38**	0.003	0.315	**1.62**	0.000	0.007
	pro-hydroxy-pro	0.86	0.207	1	**0.59**	0.000	0.020	0.98	0.880	1	1.13	0.296	1
Carbohydrate													
Pentose	Ribonate[a,b]	**1.52**	0.033	0.684	**1.58**	0.022	0.398	**2.15**	0.000	0.049	**1.83**	**0.002**	0.324
Cofactors & Vitamins													
Tocopherol	gamma-CEHC glucuronide *	0.76	0.137	1	0.86	0.423	0.817	**0.52**	0.000	0.109	**0.58**	0.004	0.405
	alpha-CEHC glucuronide *	0.79	0.196	1	0.97	0.867	0.996	**0.49**	0.000	0.049	0.71	0.061	0.927
	alpha-CEHC sulfate	0.71	0.061	0.751	0.74	0.089	0.536	**0.50**	0.000	0.049	0.73	0.083	0.935
Xenobiotics													
Food Component/Plant	4-allylphenol sulfate	**3.95**	0.000	0.000	**5.09**	0.000	0.000	**4.07**	0.000	0.000	**4.25**	0.000	0.000

CEHC, Carboxyethyl-hydroxychromans

* Indicates that the metabolite identified was not made against a purified chemical standard.

[a] Metabolite also identified from the Navy Bean metabolome.

[b] Metabolite also identified from the study food metabolome.

[c] Values presented are fold-change of the mean relative abundance within control and navy bean at 2 and 4 weeks compared to their baselines (p<0.05) and (q<0.2). Statistically-significant fold-changes are bold and shaded. Light shaded represent metabolites and pathways that showed trends towards significance (0.05 < p < 0.10).

Table 4.

Navy bean consumption revealed distinct urine metabolites after 4 weeks compared to a control intervention for 4 weeks

		Fold-difference*	
Metabolite	**HMDB**	**Navy Bean**	***p*-value**
Amino Acid			
1-methylhistamine	HMDB00898	**1.72**	0.034
Cysteine[a,b]	HMDB00574	**1.60**	0.048
Dopamine 3-O-sulfate	HMDB06275	**0.45**	0.025
Indolepropionate	HMDB02302	**0.35**	0.038
Isovalerylglutamine	-	**0.53**	0.006
N2,N5-diacetylornithine[1]	-	**1.55**	0.020
N-acetyltaurine	-	**1.57**	0.050
N-methylleucine[b]	-	**19.60**	0.000
Pyroglutamine[a,b]	-	**1.74**	0.006
S-adenosylhomocysteine (SAH)[a,b]	HMDB00939	**2.09**	0.027
Nucleotide			
N6-carbamoylthreonyladenosine[a]	HMDB41623	**1.44**	0.037
Guanosine[a,b]	HMDB00133	**2.81**	0.034
Xenobiotics			
2-keto-3-deoxy-gluconate	HMDB01353	**2.18**	0.018
4-hydroxyhippurate[a]	HMDB13678	**1.92**	0.036
Alliin	HMDB33592	**3.34**	0.034
O-sulfo-L-tyrosine[a,b]	-	**1.33**	0.026
Thioproline	-	**2.66**	0.009

HMDB, Human Metabolome Database

[a]Metabolite also identified from the Navy Bean metabolome.

[b]Metabolite also identified from the study food metabolome.

[c]Values presented are fold-difference of the mean relative abundance between navy bean compared to control at 4 weeks ($p<0.05$). Statistically-significantly increased fold-differences are bold.