A Pilot Randomized Controlled Clinical Trial to Assess Tolerance and Efficacy of Navy Bean and Rice Bran Supplementation for Lowering Cholesterol in Children

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Abstract

Background: Navy beans and rice bran demonstrate efficacy to regulate serum cholesterol in hypercholesterolemic adults; however, the cardiovascular disease (CVD) protective properties of these foods in children are unknown and merit investigation. **Objective:** The objectives were to determine whether cooked navy bean powder (NBP) and/or heat-stabilized rice bran (RB) supplementation is tolerable, improves dietary fiber intake in children, and modulates lipid profiles. **Methods:** Children aged 8 to 13 years at risk for CVD due to abnormal lipids were recruited. Elevated cholesterol levels were defined as total cholesterol ≥180 mg/dL and high-density lipoprotein (HDL) <60 mg/dL; low-density lipoprotein (LDL) ≥100 mg/dL and HDL <60 mg/dL; or non-HDL >100 mg/dL and HDL <60 mg/dL. Participants completed a pilot 4-week, randomized controlled, 4-arm dietary intervention. They consumed study-provided muffins or a smoothie daily that included 0 g NBP or RB (control), 17.5 g NBP, 15 g RB, or a combination 9 g NBP + 8 g RB. Fasting blood was collected at baseline and week 4. Participants also completed 3-day food logs and gastrointestinal health questionnaires. Results: Thirty-eight children completed the trial (n = 9 control, n = 10 NBP, n = 9 RB, and n = 10 NBP + RB groups). Only 3 participants withdrew due to noncompliance of required food consumption. Participants in the intervention groups significantly increased intake of NBP and RB at week 4 (p≤.01). The NBP and NBP + RB groups increased total fiber intake from baseline to week 4 (p=.02 and p=<.01, respectively). HDL-cholesterol was higher in NBP-group participants compared to control at week 4 (P = .02). **Conclusion:** Increasing NBP and/or RB intake is tolerable for children, and our findings suggest higher daily intakes are needed for a longer duration to induce favorable changes across multiple serum lipid parameters.

Keywords

hypercholesterolemia, dietary fiber, rice bran, navy beans, cardiovascular disease prevention

Introduction

In the United States, approximately 21% of children have at least one abnormal lipid measure. The prevalence of abnormal lipid measures increases almost 3-fold in children with obesity compared to those who are normal weight.[1] For overweight or obese children, there is a need for prevention interventions to reduce

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risk of cardiovascular disease (CVD). Increasing evidence indicates that childhood nutrition plays a significant role in CVD progression, and eating behavior changes from childhood to adulthood may exacerbate these youth CVD risk factors.[2-4] A report from the National Heart, Lung and Blood Institute Expert Panel recommends implementing healthier lifestyles in children and foresees childhood as an important window of opportunity to focus on CVD prevention.[5]

Lifestyle interventions that include a combination of a healthy diet, exercise, and/or behavior modification remain the primary treatment for children with CVD risk factors (eg, childhood obesity, hypercholesterolemia, and hypertension).[6] Nutrition research indicates that increasing intake of plant-based foods can protect against CVD.[7,8] Whole grains have shown to improve CVD risk factors,[9] whereby rice bran (a byproduct of whole grain rice) and its bioactive components (eg, γ-oryzanol and tocotrienols) have shown to lower serum cholesterol and triglyceride levels in both animal and human trials.[10] γ-Oryzanol derived from rice bran has been shown to increase fecal excretion of bile acids and cholesterol.[10] Additionally, it has been demonstrated that tocotrienols in rice bran may increase gene expression of Cyp7a1, the rate-limiting step in bile synthesis from cholesterol, and Cpt-1a, the rate-limiting step in beta-oxidation, which has demonstrated triglyceride lowering capabilities.[11] Dry beans incorporated into the diet have also been shown to modulate liver gene expressions of Cyp3a11, Cyp7a1, Fmo1, Gstm1, Mif and Ugt1a6.[12] The overall fiber content of rice bran and navy beans (both soluble and insoluble fibers) may help reduce cholesterol by binding bile acids, leading to decreased reabsorption in the small intestine and increased fecal excretion, lowering hepatic stimulation of cholesterol synthesis secondary to modifying the glycemic response, and increasing satiety, which may decrease oral intake of cholesterol.[13,14] Legumes have cardiometabolic protective properties against excess body weight, hypercholesterolemia, inflammation, oxidative stress, among others.[15] Individuals who regularly eat dry beans have improved nutrient intake levels (eg, fiber, potassium, zinc, iron), weight maintenance, and blood pressure readings compared to non–bean consumers.[16] Additional information for the serum lipid regulatory properties of dry beans and rice bran and the effects in animals and humans have been extensively reviewed.[8,10,15]

Although these foods may elicit promising CVD risk modifications by improving cholesterol, nearly all rice bran and dry beans research have been completed in adults.[8-10,15,16] Little is known if these foods have CVD protective outcomes in children with risk factors.

Approximately 99% of children in the United States between the ages of 9 and 18 years do not meet the adequate intake for whole grains and about 96% do not meet adequate intake for legumes to promote health.[17] The compelling findings for whole grains and legumes to reduce risk for developing major chronic diseases provided strong rationale for implementing a dietary intervention trial to assess tolerance and feasibility of increasing intake of navy beans and rice bran in children.

The Healthy Hearts program has been identifying elementary school–age students in northern Colorado with modifiable CVD risk factors since 1992 through an interactive school-based health education program and health screenings.[18,19] The program screens more than 3300 elementary school–age students yearly and reports approximately 23% with borderline or high cholesterol each year.[18,19] A pilot clinical trial was developed to evaluate whether increased cooked navy bean powder (NBP) and/or heat-stabilized rice bran (RB) can be incorporated into the daily diets of children over a short period of time. Our main objectives were to determine whether increasing intake of NBP and/or RB is tolerable, improves dietary fiber intake, and modulates serum lipid panels. Due to limited dietary intervention studies to lower cholesterol in children, findings from this pilot trial are critical for future, larger-scale interventions in school-aged children as a preferred method for lowering cholesterol. We hypothesized that consuming NBP and RB daily was tolerable for 8- to 13-year-old children and these amounts would promote a healthier overall dietary intake composition, particularly for total dietary fiber, and improved lipid profiles.

Methods

Pilot Trial Design and Participant Eligibility

A pilot 4-week, randomized controlled, 4-arm dietary intervention clinical trial was developed as part of a community-academic research collaboration (NCT01911390). This pilot trial was implemented alongside a school-based health education program (Healthy Hearts) that educates elementary students about cardiac health and CVD prevention to promote heart-healthy lifestyles in the northern Colorado region, as well as provide cholesterol screenings.[18-20]

Eligibility to participate in the clinical trial included children between the ages of 8 and 13 years who had their cholesterol screened by the Healthy Hearts program and were considered at risk for CVD due to abnormal lipids. Children were required to have one of the following: total cholesterol ≥180 mg/dL and high-density lipoprotein (HDL) <60 mg/dL; low-density

lipoprotein (LDL) ≥100mg/dL and HDL <60 mg/dL; or non-HDL >100 mg/dL and HDL <60 mg/dL. Total cholesterol was set 10 mg/dL higher than the national pediatric guidelines for borderline cholesterol, LDL-cholesterol at 10 mg/dL lower, non-HDL-cholesterol at 20 mg/dL lower, and HDL-cholesterol at 15 mg/dL higher.[21] The cholesterol eligibility criterion was used since our objective was to assess modulations in cholesterol levels as opposed to treatment of hyperlipidemia in children; 34% of children screened by the Healthy Hearts program qualified for eligibility based on this criterion. Children had to be healthy, which we defined as not having any ongoing medical illness or taking medications. In addition, children had to be willing to consume the study-provided food for 28 consecutive days, they could not suffer from food allergies, or have major dietary restrictions.

The University of Colorado Health-North Institutional Review Board and the Colorado State University Research Integrity and Compliance Review Office approved the study (Protocols 13-1263 and 13-4390, respectively). Written informed consent from participants' guardians and written informed assent from participants were completed before participation. The study coordinator then collected a blood sample via a nonfasting finger stick. This finger stick was completed to ensure that participants still met study criteria for cholesterol levels. Participants were randomized by sex to 1 of the 4 study arms.

Dietary Intervention

The dietary intervention included 4 study arms: control (0 g NBP or RB), NBP (17.5 g/day), RB (15 g/day), and a combination NBP (9 g/day) + RB (8 g/day). Archer Daniels Midland (ADM) Edible Bean Specialties, Inc (Decatur, IL) supplied the VegeFull cooked NBP. Navy beans were prewashed, presoaked, precooked, ground, and dehydrated to allow for easy incorporation into snacks and to blind participants to their intervention group. Processing the beans does not diminish the nutritional value and was optimal for incorporating legumes into the diet of children who may not be regular consumers.[22] The US Department of Agriculture Agricultural Research Service (USDA-ARS) Dale Bumpers National Rice Research Center provided heat-stabilized RB polished from a Calrose rice variety from US rice mills.

Two study-provided snacks, banana nut muffins and strawberry pineapple banana smoothies, were developed for each study arm and were modified to meet similar caloric content across groups. A 2-week supply of study snacks was provided to participants at baseline and 2-week visits. The study snacks were coded to ensure participants remained blind to their respective intervention group. Participants were instructed to consume either 2 muffins or 1 smoothie daily for a total of 4 weeks.

Sample Collection and Analysis

Two clinic visits were completed during the 4-week study: one at baseline and the other at 4 weeks. Fasting blood samples, height, and weight were collected at these visits. Body mass index (BMI) percentile was then calculated using the participant's weight, height, age, and sex and plotted on the Centers for Disease Control and Prevention BMI-for-age growth chart and categorized as underweight, healthy weight, overweight, or obese based on the percentile.[23] Fasting blood samples were completed by venipuncture and were processed for serum lipid panel, plasma, and peripheral blood mononuclear cell analyses. The lipid panel included total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. Lipopolysaccharide (LPS) concentrations were measured using endpoint chromogenic reaction following a protocol from Lonza (Basel, Switzerland). Samples were thawed on ice, diluted in endotoxin-free reagent water, and heated to 75°C for 5 minutes to heat inactivate proteases. Following incubation with Limulus Amebocyte Lysate for 10 minutes at 37°C, a chromogenic substrate was added, and the reaction was stopped using 25% glacial acetic acid. Color change relative to the standards was determined using the Cytation3 plate reader with an optic density reading at 405 nm. LPS concentrations were interpolated using GraphPad Prism (GraphPad Software, La Jolla, CA).

Additionally, participants completed a gastrointestinal (GI) health questionnaire at these visits to assess GI discomforts or issues while on study. A mid-study visit (week 2) was completed for participants and their families to check-in with the study coordinator and assess how the study was going. Supplemental Figure 1 (available at http://gph.sagepub.com/supplemental) illustrates the pilot study design overview.

Tolerance Assessment to Intervention

To verify tolerance and compliance to the dietary intervention, participants recorded the study snack and the amount that was consumed daily in increments of none (0%), half (50%), or all (100%). Participants completed 3-day food logs each week on study (eg, baseline, week 1, week 2, week 3, and week 4) to record all food and drink consumed on 2 weekdays (Monday to Thursday) and 1 weekend day (Friday to Sunday). The study-provided snacks were included on these forms to record
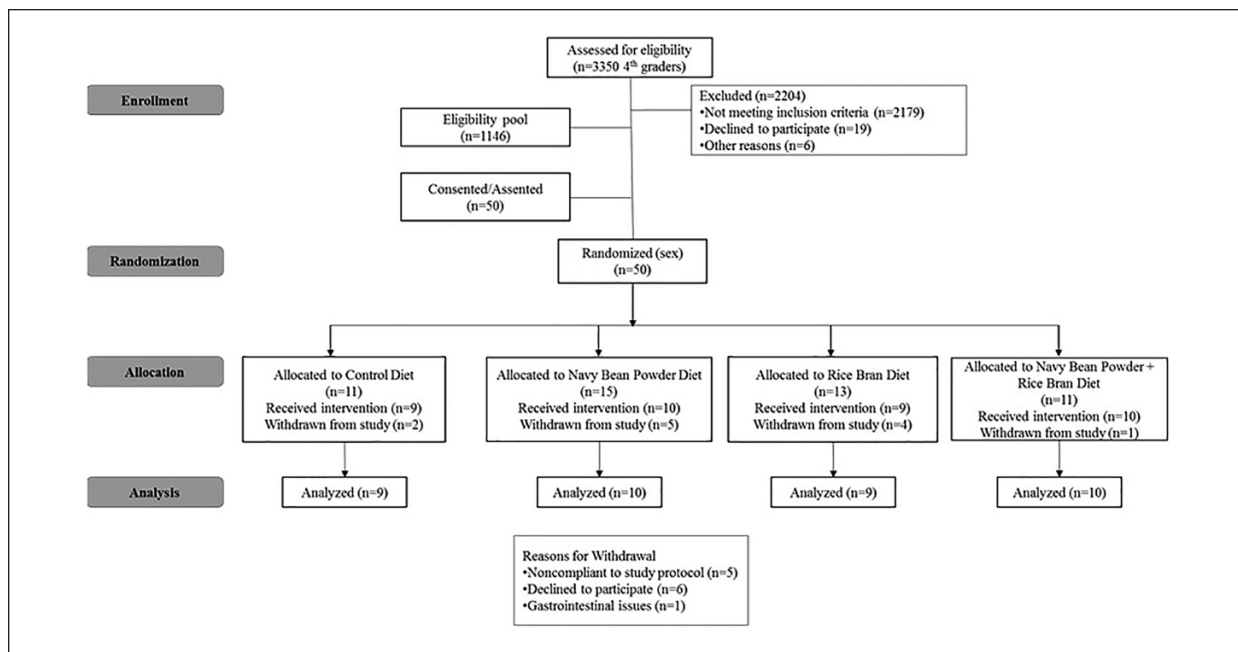
Figure 1. Study recruitment and participation based on CONSORT statement guidelines.

during the intervention. The food logs were analyzed using Nutritionist Pro for average macronutrient and micronutrient intakes and for calculating food group intakes (eg, fruit and vegetable).

Additionally, we calculated how much NBP and RB contributed to each participant's total daily caloric intake. Food logs provided information on whether beans and brown rice/rice bran were consumed outside of the intervention. The dietary intervention provided 55 kcal from 17.5 g NBP, 48 kcal from 15 g RB, 28 kcal from 9 g NBP, and 25 kcal from 8 g RB.[24,25] The following equations were used to calculate percent intake of these foods for each participant (*X* equals the amount of calories based on intervention group):

$$\% \text{ bean consumed} = \left[\left(\frac{X \text{ kcal cooked NBP} + \text{regular}}{\text{bean consumption from diet}} \right) \div \text{average daily kcal} \right] \times 100$$

$$\% \text{ RB consumed} = \left[\left(\frac{X \text{ kcal RB} + \text{brown rice/RB}}{\text{consumption from diet}} \right) \div \text{average daily kcal} \right] \times 100$$

To verify GI tolerance, a questionnaire included 5 items that were adapted from prior studies measuring perceptions on GI symptoms during a dry bean dietary intervention.[26,27] Participants filled out the questionnaire to quantify any GI concerns including changes in flatulence frequency, stool frequency, stool consistency, and bloating. Participants recorded how these symptoms

changed (eg, increase/decrease) and rated the severity of the change (1 = little change, 5 = a lot of change). Additionally, participants recorded if any of these symptoms interfered with their daily activities.

Statistical Analysis

Statistical analyses were completed on characteristics that included age, sex, BMI percentile, caloric intake, lipid panel, as well as macronutrient, micronutrient, and food group (fruit, vegetable, dry bean, and rice bran) intakes. Due to a sample size of n = 9 to 10 in each group, the continuous data were converted into "ranks" to perform a linear regression analysis to make comparisons between "diet" and "time point" and medians were reported. The data on binary outcomes, such as "sex," were evaluated using a logistic regression analysis and odds ratios were reported. The analysis was adjusted for repeated measures on the same individual over time. SAS v9.4 (SAS Institute Inc, Cary, NC) was used to perform analysis. A *P* value of .05 was set to determine statistical significance.

Results

A total of 50 children with abnormal cholesterol were recruited in this pilot 4-week dietary intervention trial between August 2013 and June 2015. Twelve participants were withdrawn from the trial. Six of these participants declined to participate because of not wanting to

Table 1. Baseline participant characteristics across diet groups[1].

Characteristic	Control (n = 9)	Navy Bean Powder (n = 10)	Rice Bran (n = 9)	Navy Bean Powder + Rice Bran (n = 10)	P
Age (years)	10 ± 1 (10)	10 ± 1 (10)	10 ± 1 (10)	10 ± 1 (10)	.68
Sex[2]					
Males (%)	5 (56%)	5 (50%)	4 (44%)	5 (50%)	.97
Females (%)	4 (44%)	5 (50%)	5 (56%)	5 (50%)	
BMI percentile[2]					
Underweight	0 (0%)	0 (0%)	0 (0%)	0 (0%)	.78
Healthy weight	3 (33%)	5 (50%)	3 (33%)	5 (50%)	
Overweight	2 (23%)	3 (30%)	3 (33%)	2 (20%)	
Obese	4 (44%)	2 (20%)	3 (33%)	3 (30%)	
Total cholesterol (mg/dL)	166 ± 13 (169)	178 ± 30 (171)	167 ± 16 (170)	169 ± 26 (167)	.84
LDL (mg/dL)	103 ± 15 (107)	110 ± 35 (96)	104 ± 20 (106)	106 ± 25 (100)	.99
HDL (mg/dL)	44 ± 7 (44)[a,b]	47 ± 7 (48)[a]	42 ± 4 (42)[b]	43 ± 9 (45)[a,b]	.37
Triglycerides (mg/dL)	100 ± 22 (104)	106 ± 53 (86)	104 ± 42 (104)	104 ± 44 (114)	.99
LPS (EU/mL)	0.19 ± 0.01 (0.19)	0.19 ± 0.02 (0.19)	0.18 ± 0.01 (0.18)	0.18 ± 0.02 (0.18)	.76
Fruit (cups/day)	0.6 ± 0.5 (0.5)[a]	0.6 ± 0.4 (0.5)[a]	1.0 ± 0.5 (1.0)[a,b]	1.5 ± 1.1 (1.5)[b]	**.02**
Vegetable (cups/day)	1.4 ± 1.4 (1.0)[a]	0.4 ± 0.3 (0.5)[b]	1.1 ± 0.6 (1.0)[a]	1.2 ± 0.9 (1.0)[a]	**<.01**
Dry beans (g/day)	2.1 ± 6.2 (0)	7.1 ± 16.3 (0)	7.2 ± 12 (0)	9.3 ± 16.7 (0)	.59
Rice bran (g/day)	0 ± 0 (0)	0.3 ± 0.9 (0)	0.3 ± 1.0 (0)	0 ± 0 (0)	.53
Calories (kcal)	1480 ± 381 (1438)	1736 ± 287 (1690)	1645 ± 385 (1606)	1787 ± 432 (1851)	.34
Protein (g)	60 ± 17 (55)	64 ± 15 (63)	66 ± 21 (62)	74 ± 23 (69)	.39
Fat (g)	54 ± 17 (54)	64 ± 15 (62)	60 ± 19 (56)	69 ± 23 (68)	.41
Saturated fat (g)	20 ± 8 (18)	24 ± 7 (25)	23 ± 9 (19)	23 ± 9 (22)	.62
Dietary cholesterol (mg)	181 ± 96 (147)	180 ± 95 (178)	244 ± 186 (167)	260 ± 212 (173)	.84
Carbohydrates (g)	192 ± 58 (181)	231 ± 40 (227)	214 ± 44 (207)	223 ± 41 (234)	.48
Total fiber (g)	12 ± 6 (10)[a]	16 ± 8 (16)[a,b]	17 ± 3 (17)[b]	17 ± 5 (17)[b]	.20
Sodium (mg)	2478 ± 473 (2400)	2980 ± 691 (3003)	2509 ± 635 (2406)	3065 ± 1197 (2905)	.28
Iron (mg)	10 ± 3 (9)	10 ± 2 (10)	12 ± 4 (12)	11 ± 3 (10)	.61
Magnesium (mg)	132 ± 50 (131)[a]	131 ± 63 (130)[a,b]	183 ± 63 (174)[b,c]	195 ± 78 (192)[c]	.08
Zinc (mg)	6 ± 2 (6)	5 ± 3 (5)	7 ± 3 (8)	7 ± 3 (7)	.22
Vitamin B$_1$ (thiamin) (mg)	0.8 ± 0.3 (0.8)[a]	1.0 ± 0.4 (1.0)[a,b]	1.0 ± 0.5 (1.0)[a,b]	1.2 ± 0.6 (1.1)[b]	.26
Vitamin B$_3$ (niacin) (mg)	14 ± 5 (15)	12 ± 6 (12)	14 ± 6 (12)	15 ± 9 (12)	.83
Vitamin B$_6$ (mg)	1.0 ± 0.4 (1.0)[a,b]	0.8 ± 0.3 (0.8)[a]	1.3 ± 0.5 (1.4)[b]	1.3 ± 0.7 (1.0)[b]	.06
Total folate (μg)	224 ± 153 (153)[a]	203 ± 95 (204)[a]	289 ± 164 (256)[a,b]	295 ± 110 (257)[b]	.13
Alpha-tocopherol (mg)	3 ± 2 (2)	4 ± 4 (2)	4 ± 2 (2)	4 ± 3 (4)	.70

Abbreviations: BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPS, lipopolysaccharide.
[1]Values are reported as average ± standard deviation; (Median); unless noted. Bold *P* values indicate significance, $P \leq .05$.
[2]Values are number of participants (percentage).
[a,b,c]Medians in a row with superscripts without a common letter significantly differ, $P \leq .05$.

complete a venipuncture blood draw (n = 4) or were not interested (n = 2). Five participants were noncompliant and were withdrawn from the final analysis. Noncompliance included not eating the study food for the complete 4-week trial (n = 3) and not completing the final blood draw (n = 2). Additionally, one participant withdrew for GI issues. The GI case was reported to the institutional review boards due to possible connection to the study intervention, and no adverse events occurred. Thirty-eight children successfully completed the trial and had complete data for analysis. This included n = 9 in the control group, n = 10 in the NBP group, n = 9 in the RB group, and n = 10 in the NBP + RB group. Figure 1 illustrates the study participation flow. Baseline participant characteristics are reported in Table 1.

Tolerance to Increase NBP and/or RB Consumption in Children

This pilot study resulted in an average of 89% compliance to daily intake of NBP and/or RB across the study groups (67% to 100% range). Table 2 illustrates the nutritional information of the study snacks using Nutritionist Pro Diet Analysis Module (Axxya Systems,

Table 2. Nutrient analysis of dietary intervention for each snack across study arms[a].

	Banana Nut Muffin (114 g/Serving)			
Nutrient (% DRI)[b]	Control	Navy Bean Powder	Rice Bran	Navy Bean Powder + Rice Bran
Calories (kcal)	250 (14-16)	255 (14-16)	253 (14-16)	245 (13-15)
Protein (g)	7 (21)	9 (26)	7 (21)	8 (24)
Fat (g)	7 (11-15)	8 (12-17)	10 (15-21)	9 (14-19)
Saturated fat (g)	2 (n/a)	2 (n/a)	3 (n/a)	3 (n/a)
Dietary cholesterol (mg)	67 (n/a)	67 (n/a)	67 (n/a)	67 (n/a)
Carbohydrates (g)	42 (32)	42 (32)	39 (30)	39 (30)
Total fiber (g)	3 (10-12)	7 (23-27)	6 (19-23)	6 (19-23)
Sodium (mg)	123 (8)	127 (8)	220 (15)	110 (7)
Iron (mg)	2 (25)	2 (25)	4 (50)	3 (38)
Magnesium (mg)	45 (19)	55 (23)	151 (63)	103 (43)
Zinc (mg)	1 (13)	1 (13)	2 (25)	1 (13)
Vitamin B_1 (thiamin) (mg)	0.2 (22)	0.2 (22)	0.5 (56)	0.3 (33)
Vitamin B_3 (niacin) (mg)	1.4 (12)	1.0 (8)	5.9 (49)	3.3 (28)
Vitamin B_6 (mg)	0.2 (20)	0.3 (30)	0.8 (80)	0.5 (50)
Total folate (µg)	45 (15)	59 (20)	42 (14)	47 (16)
Alpha-tocopherol (mg)	0.4 (4)	0.3 (3)	1.1 (12)	0.7 (8)

|	Strawberry Pineapple Smoothie (247 g/Serving)			
Nutrient (% DRI)[b]	Control	Navy Bean Powder	Rice Bran	Navy Bean Powder + Rice Bran
Calories (kcal)	141 (7-9)	196 (11-13)	188 (10-12)	195 (11-13)
Protein (g)	3 (9)	7 (21)	5 (15)	6 (18)
Fat (g)	1 (1-2)	1 (1-2)	4 (6-8)	3 (4-6)
Saturated fat (g)	0.4 (n/a)	0.5 (n/a)	1 (n/a)	0.8 (n/a)
Dietary cholesterol (mg)	2.5 (n/a)	2.5 (n/a)	2.5 (n/a)	2.5 (n/a)
Carbohydrates (g)	32 (25)	44 (33)	40 (31)	42 (32)
Total fiber (g)	3 (10-12)	7 (23-27)	6 (19-23)	6 (19-23)
Sodium (mg)	32 (2)	36 (2)	33 (2)	35 (2)
Iron (mg)	1 (13)	2 (25)	4 (50)	3 (38)
Magnesium (mg)	27 (11)	48 (20)	144 (60%)	100 (42)
Zinc (mg)	0.4 (5)	0.8 (10)	1.3 (16)	1.1 (14)
Vitamin B_1 (thiamin) (mg)	0.1 (11)	0.1 (11)	0.5 (56)	0.3 (33)
Vitamin B_3 (niacin) (mg)	0.6 (5)	0.8 (7)	5.7 (48)	3.4 (28)
Vitamin B_6 (mg)	0.2 (20)	0.3 (30)	0.8 (80)	0.6 (60)
Total folate (µg)	25 (8)	51 (17)	35 (12)	43 (14)
Alpha-tocopherol (mg)	0.2 (2)	0.2 (2)	1.0 (11)	0.6 (7)

Abbreviation: n/a, not applicable.
[a]Dietary intervention snacks were analyzed using Nutritionist Pro diet analysis module (Axxya Systems, Redmond, WA).
[b]DRI (Dietary Reference Intakes) for boys and girls between the ages of 9 and 13 years.

Redmond, WA). The nutrient analysis of our study foods show that we provided a low-fat, low-sodium, high-fiber, heart-healthy snack. Our intervention groups delivered higher amounts of daily dietary reference intakes (DRI) for important nutrients, such as total fiber, magnesium, vitamin B_1 (thiamin), vitamin B_3 (niacin), and vitamin B_6.

Figure 2 illustrates the calculated percent intake of navy bean and rice bran per participant. By consuming NBP, RB, or NBP + RB, participants consumed between 1.7% and 5.2% of total caloric intake with the study snacks. No participants were consuming measurable intake levels of bean and rice bran at baseline (Table 1). At week 4, these consumption levels were significantly
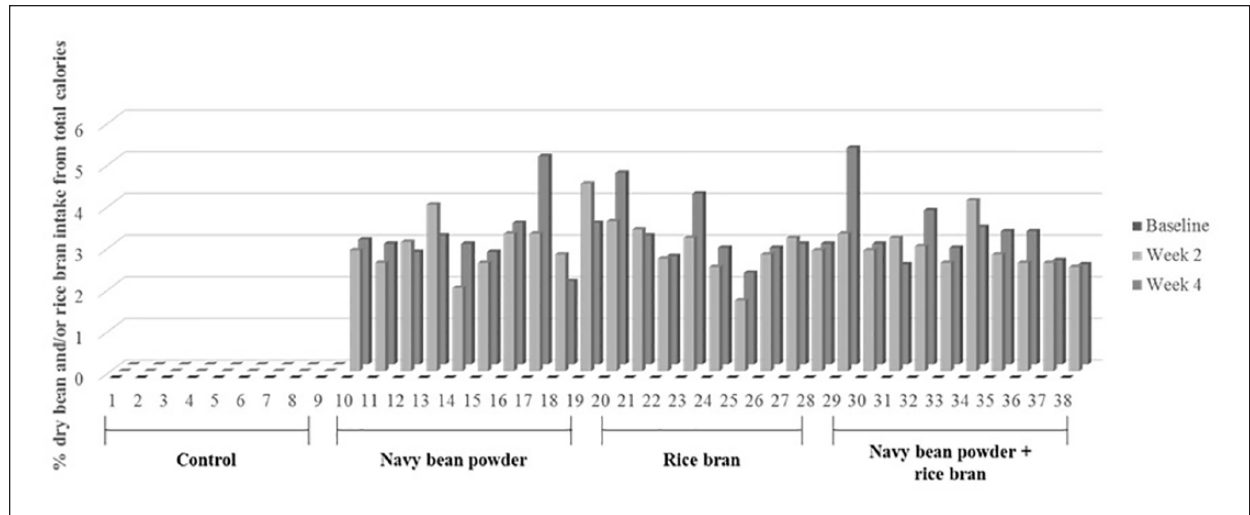
Figure 2. Participant tolerance to daily consumption of cooked navy bean powder and/or heat-stabilized rice bran. Calculated percent caloric intake of navy bean and/or rice bran averaged from total dietary calories based on weekly 3-day diet for each participant across diet groups.

higher in the intervention groups when compared to control and baseline levels (Table 3). No participants had regularly consumed additional beans or rice bran/brown rice throughout the intervention.

The criteria used to quantify GI discomforts or issues included participants who responded "yes" to any GI discomfort and rated the discomfort level ≥3 out of a 5-point scale. A majority of participants in control (n = 9/9), NBP (n = 10/10), RB (n = 8/9), and NBP + RB (n = 9/10) groups reported no major GI discomforts at baseline. At week 2, 3 (control), 1 (NBP), 3 (RB), and 1 (NBP + RB) participants reported GI discomforts. The GI discomforts included changes in flatulence (n = 1, NBP group), stool consistency (n = 5, control, RB, and NBP + RB groups), bloating (n = 2, control and NBP + RB groups), or 2 or more of these symptoms (n = 3, control, RB, and NBP + RB groups). At week 4, 10 participants in the NBP group and 8 in the RB group reported no GI symptoms, which were the same reporting levels measured at baseline. Three participants in the control group, 1 participant in the RB group, and 1 in the NBP + RB group reported 2 or more GI symptoms at week 4. Two participants in the combination of NBP + RB group reported looser stool. Of the participants who reported at least one GI discomfort across the 4 weeks (n = 17, 45%), no participant reported that these discomforts majorly interfered with their daily activities.

Nutrient Modulations Following Dietary Intervention Supplementation

Baseline food group and nutrient levels had some notable differences between groups as reported in Table 1.

Fruit and vegetable intakes were different across groups, with participants in the RB and NBP + RB groups consuming more fruit and control, RB, and NBP + RB groups consuming more vegetables ($P = .02$ and $P < .01$, respectively). Total fiber intake was lowest in the control group at baseline, with a median of 10 g and was significant compared to RB and NBP + RB groups, who had median intake levels of 17 g ($P = .02$ and $P = .03$, respectively). Magnesium, vitamin B_1 (thiamin), vitamin B_6, and total folate were significantly different across groups. The NBP + RB group consumed higher amounts of magnesium, thiamin, and total folate at baseline, while the RB group consumed higher amounts of vitamin B_6 (Table 1). All other nutrients were not significantly different across groups.

Table 3 reports the macronutrients and selected micronutrients across diet groups at week 4, and findings are reported across groups, as well as from baseline levels. Fruit and vegetable intake decreased from baseline across groups; however, navy beans and rice bran significantly increased in the intervention groups compared to control and these increases were significantly higher than baseline levels ($P = .03$ and $P < .01$, respectively). Additionally, the NBP group and the combination NBP + RB group significantly increased fiber levels at week 4 compared to baseline ($P = .02$ and $P < .01$, respectively), as were also significantly higher than the control group at week 4.

For micronutrients, magnesium levels significantly increased across all groups at week 4, with NBP, RB, and combination NBP + RB groups having the greatest increase ($P \leq .01$). Vitamin B_6 significantly increased from

Table 3. Participant characteristic values across diet groups after 4-week dietary intervention[1].

Characteristic	Control (n = 9)	P	Navy Bean Powder (n = 10)	P	Rice Bran (n = 9)	P	Navy Bean Power + Rice Bran (n = 10)	P
BMI percentile[2]								
Underweight	0 (0%)	1	0 (0%)	1	0 (0%)	1	0 (0%)	1
Healthy weight	3 (33%)		6 (60%)		3 (33%)		5 (50%)	
Overweight	2 (22%)		2 (20%)		3 (33%)		2 (20%)	
Obese	4 (45%)		2 (20%)		3 (33%)		3 (30%)	
Total cholesterol (mg/dL)	165 ± 20 (156)	.75	172 ± 33 (167)	.23	171 ± 18 (164)	.80	168 ± 22 (173)	.50
LDL (mg/dL)	103 ± 22 (97)	.64	106 ± 35 (100)	.54	105 ± 23 (95)	.60	102 ± 16 (105)	.88
HDL (mg/dL)	42 ± 8 (41)^a	.22	47 ± 4 (49)^b	.52	42 ± 6 (43)^a	.82	42 ± 7 (41)^{a,b}	.91
Triglycerides (mg/dL)	98 ± 18 (92)	.62	97 ±42 (89)	.63	120 ± 45 (98)	.36	117 ± 38 (116)	.35
LPS (EU/mL)	0.19 ± 0.01 (0.19)	.85	0.18 ± 0.01 (0.19)	.34	0.18 ± 0.01 (0.18)	.59	0.19 ± 0.01 (0.19)	.25
Fruit (cups/day)	0.7 ± 0.5 (0.5)	.50	0.9 ± 0.7 (0.8)	.14	0.7 ± 0.5 (0.5)	**.02**	0.8 ± 0.7 (0.7)	**<.01**
Vegetable (cups/day)	0.9 ± 0.7 (0.8)	**<.01**	0.8 ± 0.4 (0.9)	**<.01**	0.5 ± 0.5 (0.5)	**.03**	0.9 ± 0.8 (0.5)	.08
Dry beans (g/day)	2.7 ± 6.1 (0)^a	.11	17.5 ± 0 (17.5)^b	**<.01**	1.2 ± 2.8 (0)^a	.15	15.1 ± 16.4 (8.8)^b	**.03**
Rice bran (g/day)	0 ± 0 (0)^a	—	0 ± 0 (0)^a	.29	16 ± 2 (15)^b	**<.01**	7.9 ± 1.3 (7.5)^c	**<.01**
Calories (kcal)	1799 ± 402 (1730)	.09	1864 ± 426 (1891)	.21	1624 ± 333 (1665)	.15	1773 ± 382 (1765)	.89
Protein (g)	70 ± 22 (59)	.19	70 ± 14 (69)	.08	64 ± 18 (63)	.99	68 ± 19 (68)	.29
Fat (g)	70 ± 15 (67)	.08	66 ± 11 (69)	.44	61 ± 15 (61)	.37	65 ± 21 (71)	.71
Saturated fat (g)	23 ± 5 (22)	.44	25 ± 6 (25)	.27	22 ± 6 (23)	.75	23 ± 8 (24)	.51
Dietary cholesterol (mg)	219 ± 96 (217)	.13	233 ± 91 (210)	**.03**	205 ± 108 (167)	.92	234 ± 144 (221)	.81
Carbohydrates (g)	230 ± 65 (219)	.14	258 ± 92 (264)	.35	217 ± 43 (222)	.48	242 ± 46 (250)	**<.01**
Total fiber (g)	16 ± 6 (10)^a	.12	20 ± 5 (21)^b	**.02**	20 ± 7 (18)^{a,b}	.33	22 ± 7 (23)^b	**<.01**
Sodium (mg)	2627 ± 642 (2616)	.72	2534 ± 643 (2652)	.08	2666 ± 837 (2413)	.50	2930 ± 953 (3036)	.98
Iron (mg)	11 ± 4 (10)	.13	13 ± 5 (12)	.32	13 ± 3 (13)	.06	13 ± 4 (12)	.17
Magnesium (mg)	171 ± 78 (140)^a	.05	189 ± 40 (169)^a	**<.01**	301 ± 68 (288)^b	**<.01**	244 ± 65 (236)^c	**.01**
Zinc (mg)	7 ± 4 (6)	.29	9 ± 4 (9)	**<.01**	7 ± 2 (8)	.89	8 ± 4 (8)	.78
Vitamin B$_1$ (thiamin) (mg)	0.9 ± 0.5 (0.8)^a	.38	1.5 ± 1.3 (1.1)^{a,c}	.36	1.6 ± 0.5 (1.5)^b	**<.01**	1.2 ± 0.2 (1.2)^c	.27
Vitamin B$_3$ (niacin) (mg)	15 ± 9 (14)	.77	16 ± 7 (15)	.09	19 ± 7 (18)	**<.01**	15 ± 4 (15)	.41
Vitamin B$_6$ (mg)	1.2 ± 0.5 (1.0)^a	.56	1.5 ± 0.6 (1.5)^{a,b}	**<.01**	1.8 ± 0.5 (1.7)^b	**<.01**	1.5 ± 0.4 (1.5)^{a,b}	.17
Total folate (µg)	232 ± 129 (184)	.19	321 ± 169 (288)	.09	269 ± 109 (254)	.88	251 ± 94 (223)	.08
Alpha-tocopherol (mg)	4 ± 3 (3)	.91	5 ± 4 (4)	**.05**	3 ± 1 (3)	.49	4 ± 2 (4)	.53

Abbreviations: BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPS, lipopolysaccharide.
[1]Values are reported as average ± standard deviation; ( ) = median; unless noted. Bold P values indicate significance from baseline value, $P \leq .05$.
[2]Values are number of participants (percentage).
^{a,b,c}Medians in a row with superscripts without a common letter differ significantly, $P \leq .05$.

baseline to week 4 in the NBP and RB groups ($P < .01$) but not in the NBP + RB group. The NBP group increased zinc levels at week 4 compared to baseline ($P < .01$).

Serum Lipid Panel and LPS Modulations From Increased NBP and/or RB Intake

Table 1 shows the fasting serum lipid profiles across the study groups at baseline. No significant modulations were observed in total cholesterol, LDL-cholesterol, tri-glycerides, or LPS levels.

The NBP group had higher HDL levels (median 48 mg/dL) compared to the RB group (median 42 mg/dL; $P = .05$) at baseline. This trend continued to week 4 where the HDL level in the NBP group (median 49 mg/dL) was higher than the RB group (median 43 mg/dL; $P = .02$) and was also notably higher than the control group at week 4 ($P = .02$; Table 3).

We analyzed each participant's lipid panel at week 4 to identify individual responders to the diet intervention within each group. We determined if the child still had abnormal cholesterol levels at the end of study using the same cholesterol eligibility criteria. Of the 38 participants, 17 (45%) improved their lipid panels and no longer met study eligibility criteria for cholesterol (n = 5 in control group, n = 5 in the NBP group, n = 5 in the RB group, and n = 2 in the combination NBP + RB group).

Discussion

This pilot clinical trial established tolerance and efficacy of supplementing NBP and/or RB in children with abnormal cholesterol levels. While there has been numerous studies of the cardioprotective effects of these foods in adults, to our knowledge, this is the first randomized-controlled study to investigate NBP and/or RB consumption

in children. Providing NBP, RB, or a combination NBP + RB was found to have minimal GI health concerns in a 4-week period with similar GI symptoms reported in the control group not consuming these food ingredients. Our findings support even higher percent daily intakes approaching 10% to 20% of NBP and/or RB may be needed to modulate serum lipid panels in children, as indicated in adult and animal research.[10,28-30]

Our analysis indicated that children with abnormal cholesterol levels consume a Western diet that includes high total fat and saturated fat, high sodium, and low fiber. Children participants in the intervention groups significantly increased their dietary fiber intake levels, yet these increased amounts did not meet current dietary fiber recommendations. Previous research has shown that high-fiber foods are acceptable in children and making these foods and snacks more available will improve total fiber intake.[31] Micronutrients such as vitamin B$_6$, potassium, and magnesium improved over time in groups that were consuming NBP and/or RB, which are nutrients that may be protective against heart disease.[8]

Assessing the lipid panels of participants with abnormal cholesterol levels was important to determine if lipid modulations begin to occur after a 4-week dietary intervention. Our findings indicate that 4 weeks may not be long enough to observe statistical changes in overall cholesterol levels. Seventeen participants improved their lipid profiles after 4 weeks and were no longer considered to have abnormal cholesterol levels. Reasons that improvements were observed in the control group may include the presence of a placebo effect, the delivery of important nutrients by a healthy snack option aided in lipid modulation, or fluctuations in lipid levels over time. LPS levels were measured as this serum inflammatory biomarker has limited research findings in children,[32,33] and further studies are needed to understand the relationships between LPS and abnormal cholesterol in children.

There are multiple strengths from this pilot clinical trial, which included the randomized controlled blinded design, standardized intervention snacks, regular communication by trial staff with participants to ensure study compliance, and tracking GI health and dietary intakes throughout the study duration. Furthermore, we were able to utilize an integrated database with at-risk elementary school–age children for CVD from the Healthy Hearts cholesterol screening program in northern Colorado. The limitations included the low number of total participants and short dietary intervention period of 4 weeks that was used to observe changes in serum lipids. The next steps for advancing our knowledge of dietary regulation of serum lipids in at-risk children will be to statistically power this analysis with a larger sample size with 2 intervention groups (placebo control powder vs a combination of NBP + RB) for 12 weeks.

This pilot study showed positive outcomes for tolerance to a NBP and/or RB dietary intervention, which included minimal GI issues and improved dietary fiber intake levels, in children at risk for hypercholesterolemia. Addressing physical activity and other lifestyle behaviors are also important for CVD risk reduction in children.[34-36] A focus on whole grains and legume consumption warrants dietary prevention research attention for serum lipid regulation in children that may lead to increased consumption into adulthood.

Author Contributions

ECB, N J-P, MW, TLN, GL, and EPR designed the research, AP and SR analyzed the data, ECB, NJ-P, KS, DGB, AF, and TLN wrote the paper. GL and EPR had responsibility for final content. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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