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Evaluation of brewers' spent grain on cardiovascular disease risk factors in adults: Lessons learned from a pilot study

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ABSTRACT

The beer industry generates large amounts of leftover barley called brewers' spent grain (BSG). Fiber-rich grains like barley are associated with ameliorating cardiovascular disease (CVD) risk factors. This randomized pilot study investigated the influence of daily BSG consumption for 8 weeks on lipid profiles, inflammation, and metabolic functions in healthy adults. Subjects (n = 37, 26 ± 4 y; BMI 23 ± 3 kg/m^2) received muffins containing 8.3 g BSG or 0 g BSG daily for 8 weeks. Body composition, blood pressure, and fasting blood were collected at baseline and week 8. Gastrointestinal symptoms and Bristol stool scale ratings remained stable throughout the study in both groups. Dietary fiber intake increased in the BSG group (5 g/day; 26%; p = 0.003); however, there were no significant between-group effects on blood lipids, glucose, insulin sensitivity, C-reactive protein, body composition, or blood pressure. Consuming 8.3 g BSG for 8 weeks is well tolerated and improves dietary fiber intake but does not significantly impact CVD risk factors in this sample of healthy adults. Subject health status, sample size, and BSG flour substitution rate may support the lack of effect in the current study. Larger controlled trials are needed to understand the potential of BSG as a value-added ingredient and its impact on human health.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death globally, accounting for an estimated 17.9 million mortalities each year (World Health Organization, 2019). Adhering to a heart-healthy diet that emphasizes whole grains has been shown to attenuate the risk of developing CVD (Barrett et al., 2019; Fatahi et al., 2018; Garutti et al., 2022). Whole grains contain numerous bioactive nutrients and phytochemicals within the bran, germ, and endosperm layers. Barley is a less common whole grain that is predominantly used in Western countries for animal feed and malting but has been shown to exert a cardioprotective effect as it contains roughly 17% total dietary fiber (US Department of Agriculture, 2019; Djurle et al., 2016). Controlled human interventions evaluating barley intake on CVD risk factors have resulted in improved postprandial glucose and insulin responses (Ames et al., 2015) and reduced lipids associated with plaque development (Soong et al., 2015). However, most human diet interventions have only evaluated the health benefits of whole grains and minimally processed barley (Ho et al., 2016; Tovar et al., 2014). In response to a growing global population and a world cereal equivalent food demand expected to reach 10.094 million tons in 2030, researchers are focusing on the valorization of agro-industrial cereal by-products as a source of bioactive compounds for value-added products (Farcas et al., 2021).

Brewers' spent grain (BSG) are the leftover malted barley and

Abbreviations: BMI, body mass index; CV, coefficients of variation; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; HEPA, health-enhancing physical activity; IPAQ, international activity questionnaire; LDL-C, low-density lipoprotein cholesterol; LSD, least significant difference; WHO, World Health Organization.

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additional cereal grains or adjuncts from beer production that represent around 85% of the more than 30 million tons produced worldwide each year (Gutiérrez-Barrutia et al., 2022; Karlović et al., 2020). Scanning electron microscopy images of BSG show that it consists primarily of barley grain husks with pericarp and endosperm fragments (Steiner et al., 2015). Approximate chemical composition analyses of BSG have reported the following: hemicellulose 19–29% (w/w), cellulose 16–33% (w/w), total carbohydrates 42–60% (w/w), fiber 19–41% (w/w), protein 14–24% (w/w), lignin 8–22% (w/w), lipids 3–10% (w/w), and ash 1–4% (w/w) (Lynch et al., 2016; Naibaho & Korzeniowska, 2021). The lignocellulosic nature of BSG lends structural dietary fibers such as lignin and arabinoxylans as common bioactive compounds of interest. Arabinoxylans act as prebiotics with butyrogenic effects (Riviere et al., 2016), attenuate post-prandial glucose (Giulia Falchi et al., 2016), and improve lipid profiles (Schioldan et al., 2018). Lignin is another structural fiber comprised of antioxidant polymers shown to improve cardiometabolic outcomes in obese rats (Raza et al., 2019). Additionally, BSG has been incorporated in baked goods between 5 and 50% dry w/w basis of the flour content resulting in dose-depended increases of dietary fiber, protein, and phenolic acids, which could contribute to CVD risk factors (Amoriello et al., 2020; Heredia-Sandoval et al., 2020; Nocente et al., 2019; Shih et al., 2020; Torbica et al., 2019). Therefore, utilizing BSG for food fortification provides opportunities to reduce brewing industry waste, improve nutritional profile, and create sustainable foods that promote heart health benefits.

Few studies have explored the impact of BSG on human health in human subjects as most are in vitro cell models in vivo animal models. Cell models evaluating BSG protein isolates have resulted in decreased proinflammatory cytokine production (McCarthy et al., 2015) and significant angiotensin converting enzyme (ACE) inhibitory activity suggesting the use BSG as a dietary ingredient for management of hypertension (Connolly et al., 2015). Phenolic extracts from BSG are shown to provide protection against oxidant-induce DNA damage and inhibit glucose metabolism enzymes, thereby potentially lowering blood glucose levels (Becker et al., 2021). Supplementation of a BSG-diet to obesity- or hyperlipidemia-induced rodents resulted in weight loss, hypolipidemic effects, improved insulin resistance, and increased short-chain fatty acids and abundance of probiotic bacteria (Ferreira et al., 2022; Pei et al., 2022; Raza et al., 2019). Zhang et al. (1991) is the only trial to our knowledge to demonstrate the effect of BSG on blood lipids in humans. However, the study involved ileostomy subjects and used a crossover design with a small sample size and short treatment duration (Zhang et al., 1991). Therefore, the primary aim of the pilot study was to determine the impact of daily consumption of muffins with 8.3 g BSG (treatment) or 0 g BSG (control) on fasting plasma total cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglycerides at baseline and eight weeks in healthy adults. BSG muffins were hypothesized to reduce total cholesterol, LDL-C, and triglycerides; whereas HDL-C would be maintained or increased. The secondary aim was to determine differences in fasting glucose, insulin, and C-reactive protein between groups at baseline and eight weeks. It was hypothesized that BSG muffins would reduce glucose and insulin and reduce or maintain C-reactive protein. The third aim determined differences in body composition (body weight, waist-hip ratio, fat mass, and fat-free mass) and blood pressure between groups. It was hypothesized that these variables would be reduced or maintained. Findings from this pilot study will also aid in effect size interpretations of BSG on CVD risk factors for conducting power analyses of future human trials.

2. Materials and methods

2.1. Study design

The present study was an 8-week, randomized-controlled, parallel-arm, single-blind human feeding trial. The study was carried out according to the rules of the Declaration of Helsinki, and all procedures involving human subjects were approved by the Texas Woman's University Institutional Review Board, Houston, TX. Written informed consent was obtained from all subjects before any study procedures were performed.

2.2. Subjects

Healthy men and women with a BMI of 18.5–29.9 kg/m^2, able to speak and read English, and between 18 and 60 years of age of any race or ethnicity were recruited from Houston, TX and the surrounding area. Those who experienced ≥5% weight change 3 months prior to entering the study, sensitivity or aversion to whole grain muffins or whole grain baked foods, and known food allergies or intolerances to eggs or wheat were excluded. Individuals diagnosed with diabetes, cancer, chronic inflammatory diseases, cardiovascular disease, high cholesterol (total cholesterol ≥240 mg/dL), high blood pressure (>120/80 mm Hg), or any other disorder known to impact normal metabolism were excluded. Those taking medications, supplements, or substances (e.g., smoking/ tobacco use, excessive alcohol (15 or more drinks per week for men and 8 or more drinks per week for women) and/or caffeine >400 mg/day) known to impact metabolism or those following a prescribed or specialized diet (e.g., high fiber, gluten-free, or ketogenic) were also excluded. Women who were pregnant, lactating, planning to become pregnant during the study period, or women who were post-menopausal were excluded. Lastly, individuals with a condition that could affect their ability to provide informed consent, comply with study protocol, or put the individual at unnecessary risk were excluded at the discretion of the investigator.

2.3. Test foods

Previously, we conducted a consumer acceptance study evaluating muffins enriched with BSG (0, 20, 30% wt:wt flour) on overall, appearance, texture, moistness, sponginess, and taste attributes in adults (Combest & Warren, 2022). Muffins containing 30% BSG maintained consumer acceptance among all attributes. Therefore, the 30% BSG muffin inclusion concentration was chosen for this human intervention as it provides greater opportunity to elicit a biological response.

Brewers' spent grain and study muffins were formulated and prepared in the Texas Woman's University food laboratory (Houston, TX). The treatment muffins were developed to contain 10.4 g BSG (30% wt:wt flour), whereas the control muffins contained 0 g BSG (0% wt:wt flour). Spent grains were prepared using an American adjunct lager recipe comprised of crushed Rahr 2-row malted barley (44.4% wt:wt), crushed Rahr 6-row malted barley (44.4% wt:wt), and flaked maize (corn) adjuncts (11.1% wt:wt) that were all purchased from Northern Brewer LLC, Roseville, MN and represent grains used in popular U.S. light beers like Miller®, Coors®, and Budweiser®. Malted barley grains and flaked corn were added to a mesh bag for ease of filtering and steeped in water for 2 h at 65.5 °C. After 2 h, grains were removed from water and dehydrated overnight (Excalibur, Sacramento, CA, USA) for approximately 14 h at 50 °C ± 5 °C. The following morning, BSG was finely milled (KoMo, Hampton, NE, USA) and sifted through a #35 mesh screen (500 μm) to remove any remaining hulls. Finished BSG was sealed in polyethylene bags and stored at 0 °C until further use. Proximate chemical analyses (Analytical Food Labs, Grande Prairie, TX) of BSG consisted of 69.7% total carbohydrates, 16.1% protein, 30.1% total dietary fiber, 5.3% fat, and 3.0% ash, similar to previously reported compositions (del Río et al., 2013; Ktenioudaki et al., 2012).

Two flavors of muffins were developed, apple cinnamon and cranberry orange, to reduce monotony and improve compliance. Ingredient analyses of each muffin flavor are shown in Table 1. Apple cinnamon and cranberry orange control muffins (0 g/100 g BSG) and treatment muffins (10.4 g/100 g BSG) were developed to contain similar amounts of energy and nutrients except fiber (Table 2). The apple cinnamon and cranberry orange BSG muffins provided 6 g and 7 g dietary fiber per day

Table 1
Ingredient analysis of muffin formulations.

	Apple Cinnamon		Cranberry Orange	
	Ingredient (g/100 g)		Ingredient (g/100 g)	
	0%[1] (Control)	30%[1] BSG	0%[1] (Control)	30%[1] BSG
BSG	n/a	10.4	n/a	10.4
Flour, all-purpose	34.7	24.2	34.7	24.2
Applesauce	28.4	28.3	28.4	28.3
Sugar, granulated	13.3	13.3	13.3	13.3
Dried, diced apples[2]	6.9	6.9	n/a	n/a
Reduced sugar, dried cranberries[3]	n/a	n/a	6.9	6.9
Eggs, raw	5.8	5.8	5.8	5.8
Olive oil, light	5.8	5.8	5.8	5.8
Water	3.5	3.7	3.5	3.7
Cinnamon, ground	0.5	0.5	n/a	n/a
Salt	0.4	0.4	0.4	0.4
Baking soda	0.4	0.4	0.4	0.4
Vanilla extract	0.4[4]	0.4[4]	0.4	0.4
Orange extract	n/a	n/a	0.2	0.2
Orange oil	n/a	n/a	0.2	0.2

Abbreviation: BSG brewers' spent grain.
[1] % refers to the percentage substitution of total all-purpose flour by BSG.
[2] Nuts.com® dried diced apples, unsulphured
[3] Ocean Spray® 50% less sugar dried cranberries.
[4] Control and BSG muffin ingredients total 100 g when using 100th decimal place for all values.

Table 2
Nutritional analysis per serving of muffins[1-4].

	Apple Cinnamon		Cranberry Orange	
	0% (Control)	30%[5] BSG	0% (Control)	30%[5] BSG
Energy (kilocalories)	319	323	319	323
Carbohydrates (g)	57	56	57	57
Total Fat (g)	8	9	8	9
Total Protein (g)	5	6	5	6
Total Dietary Fiber (g)	2	6	4	7

Abbreviation: BSG brewers' spent grain.
[1] Proximate nutrition compositional analysis of BSG performed by Analytical Food Labs, Grand Prairie, TX, USA.
[2] Nutrition Data System for Research (NDSR), version 2020, University of Minnesota Nutrition, Coordination Center, Minneapolis, MN, USA used to determine nutritional analysis per serving of muffins.
[3] Serving size is 80g or 5 mini muffins.
[4] Participants received equal quantities of apple cinnamon and cranberry orange muffin flavors throughout the study to prevent variations in energy, macronutrient, and dietary fiber intra-individual distributions.
[5] % refers to the percentage substitution of total all-purpose flour by BSG.

compared to the control muffins that provided 2 g and 4 g, respectively.

Muffins were prepared by combining all wet ingredients using an electronic mixer at medium speed for approximately 1 min. The dry ingredients were combined in a separate bowl and incrementally added to the wet ingredients until fully combined. Muffin pans were coated with nonstick spray, and 1 tablespoon (16 g ± 3 g) of batter was added to each well. Control muffins were baked using a conventional oven (manufacturer) for 10 ± 1 min and treatment muffins for 13 ± 1 min at 177 °C. Muffins were packaged in polyethylene resealable food storage bags and stored at 0 °C until subject retrieval. Each bag included a daily serving size of 5 muffins (80 g/day) and was labeled with the muffin flavor, production date, storage conditions, and food allergens (wheat and egg). Subjects were directed to store the first week of muffins refrigerated (4 °C) and the second week frozen (0 °C) to maintain optimal quality. In addition, 7 days of each muffin flavor were provided every 2 weeks, so subjects consumed equal quantities of energy, macronutrients, and dietary fiber throughout the 8-week study.

2.4. Protocol

Subjects were recruited from Texas Woman's University (Houston, TX) and the surrounding community through flyers, emails, internet and social media announcements, or word-of-mouth. Interested individuals were screened for eligibility by phone or in-person. Individuals who met the screening criteria and agreed to participate in the study were randomly assigned to either the BSG muffin group (n = 19) or the control muffin group (n = 18). Randomization was performed by the investigator using Microsoft Excel®.

Prior to baseline data collection, subjects met with the investigator to obtain written consent and receive training on completing 3-day diet intake logs and bowel habit logs (Blake et al., 2016; Delgado-Herrera et al., 2017). Subjects were instructed to follow a low-fiber diet throughout the 8-week study by avoiding or replacing high-fiber foods with foods that contain less fiber. The rationale for subjects consuming a low-fiber diet was to control for false-positive biological effects on study outcomes from consumption of high-fiber foods in addition to test foods. If a particular high-fiber food could not be avoided, subjects were instructed to reduce intake by ≤ 3 servings/week to limit fiber consumption. A spreadsheet of common high-fiber fruits, vegetables, legumes, nuts, seeds, grains, and pasta was provided to subjects to help guide food decisions and identify foods in their current diet that should be avoided or reduced. Subjects were instructed to maintain physical activity routines during the study and to inform the research team if any lifestyle conditions or medications were changed.

Subjects arrived for baseline data collection following an overnight 8 h fast (water only). Baseline anthropometric and blood pressure measurements were obtained in triplicate followed by fasting blood collection from a trained phlebotomist. Once baseline data collection was complete, subjects received their 2-week supply of muffins. Body composition analysis, blood pressure measurement, and blood collection were repeated using the same protocol as baseline at the end of the 8-week intervention. Subjects were given $100 cash as incentive for completing the study.

2.5. Anthropometrics and blood pressure

Anthropometric indices and blood pressure were measured in the fasted state with tight-fitted clothing and without shoes. Body composition (body fat and fat-free mass) was determined by air displacement plethysmography using the BOD POD (COSMED USA Inc., Concord, CA). Body weight was measured using the BOD POD integrated digital scale (COSMED USA Inc., Concord, CA), and height was measured with a stadiometer. BMI was calculated using the standardized formula: weight (kg)/height (m^2). Waist and hip circumference were measured using an inelastic tape measure according to the World Health Organization's (WHO) guidelines (World Health Organization, 2008). Waist and hip circumference measurements were used to determine the waist-hip ratio calculated as: waist (cm)/hip (cm) circumferences. Finally, blood pressure was measured in triplicate on the right arm in a seated position using a blood pressure arm cuff (Sun Tech 247, Morrisville, NC).

2.6. Dietary intake, bowel habits, and physical activity

Subjects completed 3-day 24-h dietary intake logs at 3 timepoints during the study. Diet logs were completed 1 week prior to baseline measurements, at midpoint (week 4), and the end of the study (week 8) and included 1 weekend day and 2 weekdays for energy and nutrient intake (Nutrition Data System for Research 2019; University of Minnesota, Minneapolis, MN). In addition, subjects recorded the number of muffins consumed each day during the intervention to assess compliance.

Subjects recorded bowel habits to evaluate tolerance and possible adverse events related to low-fiber diet adherence and study muffin consumption. Stool logs were recorded for 2 consecutive days (48 h) and completed 1 week prior to baseline measurements and at weeks 1, 4, and 8. Stool logs included 4 questions regarding symptoms of gastrointestinal discomfort rated on a 7-point scale (1 = no discomfort at all; 7 = very severe discomfort). Subjects recorded all bowel movements during each 48-h stool log using the Bristol Stool scale, which is a validated assessment of bowel movements using images to help subjects describe changes in intestinal function (Lewis & Heaton, 1997). Subjects were instructed to contact investigators immediately if abnormal changes in bowel habits such as excessive stomach pain, diarrhea, constipation, or related pain occurred. The shortened form of the International Physical Activity Questionnaire (IPAQ) was completed at baseline and final data collections to assess the level of physical activity at the beginning and end of the study (Craig et al., 2003). Estimated total weekly physical activity at baseline and final timepoints was categorized into 3 physical activity levels: category 1 = inactive, category 2 = minimally active, category 3 = health-enhancing physical activity (HEPA) active. Subjects who did not meet categories 2 or 3 were considered inactive. Minimally active was defined as 3 or more days of vigorous activity for at least 20 min, 5 or more days of moderate-intense activity or walking for at least 30 min, or 5 or more days of walking, moderate-intense, or vigorously intense activity. At least 3 days of vigorous-intense activity or 7 days of walking, moderate-intense, or vigorously intense activity was considered HEPA active.

2.7. Blood biomarkers

Approximately 14 mL of blood was collected into 2 pre-labeled EDTA vacutainers (BD Diagnostics, Franklin Lakes, NF). Plasma was separated from whole blood by centrifugation at 4000 rpm for 15 min. Plasma was immediately aliquoted into 1.5 mL microcentrifuge tubes and stored at −80 °C until analysis of biomarkers. Lipids (total cholesterol, HDL-C, and triglycerides) were determined using enzymatic colorimetric assays from commercially available kits (Stanbio, Boerne, TX, USA). Total cholesterol, HDL-C, and triglyceride intra-assay coefficients of variation (CV) were 1.2%, 1.0%, and 2.4%, respectively. Glucose was determined using a glucose oxidase colorimetric assay (Stanbio, Boerne, TX, USA) with an intra-assay CV = 2%. LDL-C was quantified using the Friedewald equation. Insulin (intra-assay CV = 9.2%, inter-assay CV = 3.3%) and CRP (intra-assay CV = 3.1%, inter-assay CV = 3.4%) concentrations were determined using enzyme-linked immunosorbent assays from commercially available kits (Alpco, Salem, NH, USA). All samples were measured in duplicate using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA) for biomarker concentration determination. All assays were performed according to the manufacturer's protocol for each kit.

2.8. Statistical analysis

A priori power analysis was conducted using G*Power version 3.1 to determine the sample size needed to find significance with power (1-β) set at 0.80, α = 0.05, and a moderate effect size of 0.25 (f) using total cholesterol as the primary endpoint while assuming a 5% reduction in total cholesterol resulting from treatment muffin consumption (Faul et al., 2007; Li et al., 2003; Shimizu et al., 2008). A minimum sample size of 34 subjects (17 control and 17 treatment) was necessary to test for mean differences in the primary aim, total cholesterol, between- and within-groups over time.

Preliminary data analyses included descriptive statistics of the final sample, evaluation of missing data, potential baseline differences between the two groups, attrition, and determination of statistical assumptions for the primary analyses. Descriptive statistics were presented as (mean ± SD) for continuous variables and frequencies and percentages for the categorical variables. Age, weight, body mass index, fat and fat-free mass, waist-hip ratio, blood pressure, total cholesterol, HDL-C, LDL-C, triglycerides, glucose, insulin, C-reactive protein, and mean 3-day dietary intake (energy, total carbohydrate, total fat, total protein, total dietary fiber, and percent of calories from carbohydrates, fat, and protein) were treated as continuous variables. Mixed-design 2-way ANOVA was used to evaluate outcome variables at baseline and final study timepoints within the control and treatment muffin groups, between the two muffin groups, and group by time interaction effects. Fisher's Least Significant Difference (LSD) was used for the post hoc analysis to determine which outcome variables achieved significance. All statistical analyses were performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA), and all tests of significance were performed at α = 0.05.

3. Results

3.1. Subjects

After screening all interested individuals, 62 were enrolled in the study. Twenty-five subjects dropped out or were excluded from the study due to time constraints, personal reasons, BMI ineligibility, refusable of blood draw, or lost to COVID-19 campus closures, and 37 (76% female) completed the protocol and were included in the data analysis (Fig. 1). Baseline characteristics of subjects who completed the study did not differ between the control group (n = 18) or the BSG treatment group (n = 19) (Table 3).

3.2. Dietary intake and compliance

Energy and macronutrients were similar between BSG and control groups throughout the 8-week trial except for total dietary fiber and percent of calories from fat (Table 4). Brewers' spent grain group daily dietary fiber intake was 29% (P = 0.026), 28% (P = 0.005), and 22% (P = 0.034) higher than the control group at pre-baseline, week 4, and week 8, respectively. The magnitude of this group main effect is partial $\eta^2 = 0.232$. Habitual dietary fiber intake was considerably higher in the BSG group at pre-baseline but did not differ than the control, at pre-baseline. Dietary fiber remained relatively constant at weeks 4 and 8 in both study groups suggesting subjects adhered to the low-fiber diets. The BSG muffin group consumed a higher fat diet averaging 97 ± 69 g/day (38% of total calories, P = 0.038) at pre-baseline and 84 ± 45 (37% of total calories, P = 0.034) at week 4. The magnitude of this group main effect is partial $\eta^2 = 0.135$. This observation resulted from 2 BSG group subjects consuming more than 45% of total calories from fat at pre-baseline and 3 BSG group subjects at week 4. However, total fat was reduced to non-significant group differences at week 8. There were no significant group by time interactions among any of the nutrient intake measures. Based on daily muffin intake records, the BSG and control groups consumed 96.6% and 97.2% of the study muffins, respectively, over the course of the 8-week trial.

3.3. Tolerance to muffins

Gastrointestinal symptoms of both study groups reported as frequencies and percentages are shown in Table 5. Only 1 subject reported severe-to-very severe diarrhea in week 4 in the control group. This was an isolated illness event not related to the study. Gastrointestinal symptoms were relatively stable within each group over time and between both groups throughout the study. Watery/diarrhea stool consistency increased from weeks 1–4 in the control group with no watery stools observed in the BSG group from beginning to end of study (Table 6). Bowel movement need increased in the BSG group with 3 subjects feeling quite a bit of need to extreme need from pre-baseline to week 1. In addition, BSG group stool consistency changed from hard/constipated stools to normal stool consistency from pre-baseline to week 1. Stool habits appeared to stabilize and remain consistent from midpoint to end of study. Changes in stool habits in the first week may result from subjects adjusting to the low-fiber diet or study muffins. Overall, BSG and control muffins were well tolerated with few changes

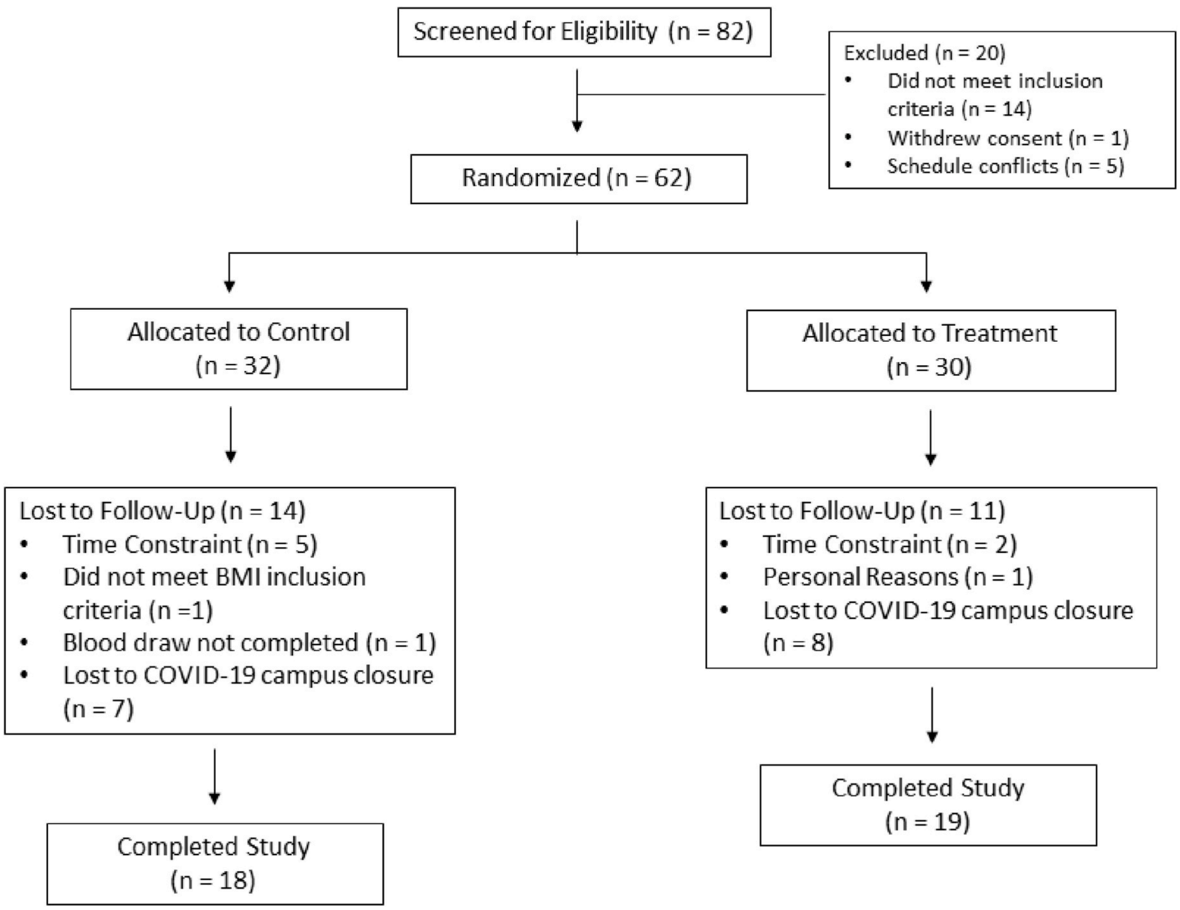

Fig. 1. Consort diagram.

Table 3
Baseline characteristics of participants who completed the study.[1].

Characteristic	Control (n = 18)	BSG (n = 19)	t	df	p-value*
Female, (n) (%)	14 (77.8)	14 (73.7)			
Age, years	26.0 ± 4.0	26.8 ± 3.9	−0.61	35	0.545
Weight (kg)	63.6 ± 12.8	62.1 ± 11.1	0.38	35	0.706
BMI (kg/m^2)	22.9 ± 2.9	22.8 ± 3.0	0.10	35	0.923
Waist-hip Ratio	0.8 ± 0.1	0.8 ± 0.1	−0.23	35	0.822
Fat Mass (kg)	18.4 ± 7.9	15.8 ± 6.9	1.09	35	0.283
Fat-free Mass (kg)	45.2 ± 7.1	46.3 ± 10.1	−0.41	35	0.688

Abbreviation: BSG brewers' spent grain, BMI body mass index.
Data presented as mean ± SD except for qualitative values.
* Between group differences obtained from independent sample *t*-test. Significance set a p ≤ 0.05.
[1] Total number of participants who completed the study (n = 37).

in gastrointestinal symptoms and stool habits.

3.4. Physical activity

Subjects completed the IPAQ shortened form at baseline and week 8 data collections to evaluate their estimated physical activity levels. At baseline 61% of the control group was categorized as inactive with 22% categorized as minimally active (Fig. 2). The group activity level increased at week 8 because only 39% were categorized as inactive and 44% as minimally active with no changes observed in HEPA activity levels. Baseline BSG group activity levels were categorized as 11% inactive, 47% minimally active, and 42% HEPA active. In contrast to the control group, BSG group activity reduced at week 8 with 26% inactive and 37% both minimally and HEPA active.

3.5. Body composition

Systolic blood pressure was significantly lower in the BSG group at baseline (−5.1 mm Hg; P = 0.028) and week 8 (−5.6 mm Hg; P = 0.018) than the control group with a large group effect size ($\eta^2 = 0.216$), but within-group differences were maintained throughout the trial (Table 7). At week 8, BSG group fat mass was significantly lower than the control group (P = 0.034), but there were no significant changes in fat mass within either group throughout the study. Consuming study muffins for 8 weeks did not significantly change body composition or blood pressure within groups or group by time interactions.

3.6. Blood biomarkers

Within-and-between group comparisons for all blood biomarkers at baseline and week 8 time points for each study group are shown in Table 7. There were no significant effects of BSG on blood lipids, glucose, insulin, or C-reactive protein. In the BSG group, total cholesterol and triglycerides were reduced by 3.8 mg/dL and 3.4 mg/dL, respectively; whereas total cholesterol increased by 0.8 mg/dL and triglycerides reduced slightly by 1.4 mg/dL in the control group. At week 8 LDL-C was 16.3 mg/dL lower in the BSG group than the control but was not significantly different between groups. Within-group comparisons show LDL-C increases 5 mg/dL from baseline to week 8 in the control group, while it is maintained in the BSG group throughout the study. In the BSG group HDL-C non-significantly increased 2.3 mg/dL and in contrast, was reduced 2.4 mg/dL in the control group. A near significant difference (P = 0.056) in insulin occurred between groups as BSG group

Table 4
Change in nutrient intakes from pre-baseline to week 4 and week 8[1,2].

	Control (n = 18)			BSG (n = 19)			*p*-value*
	Pre-Baseline	Week 4	Week 8	Pre-Baseline	Week 4	Week 8	
Energy							
kcal	1823 ± 426	1721 ± 382	1749 ± 384	2191 ± 1209	1950 ± 787	1777 ± 550	0.167
kJ	7626 ± 1783	7201 ± 1597	7318 ± 1605	9168 ± 5059	8159 ± 3293	7436 ± 2299	0.167
Total Carbohydrate (g)	220 ± 49	213 ± 54	219 ± 44	255 ± 142	216 ± 68	208 ± 63	0.565
Total Protein (g)	76 ± 29	75 ± 32	72 ± 25	77 ± 18	78 ± 42	74 ± 21	0.744
Total Fat (g)	72 ± 28	64 ± 16	66 ± 20	97 ± 69	84 ± 45	73 ± 33	0.061
Total Dietary Fiber (g)	15 ± 7	13 ± 5	14 ± 5	21 ± 9	18 ± 5	18 ± 6	0.003
% Calories from Carbohydrate	49 ± 10	49 ± 6	50 ± 6	46 ± 5	45 ± 7	47 ± 7	0.079
% Calories from Protein	17 ± 5	18 ± 7	16 ± 4	16 ± 4	16 ± 5	17 ± 2	0.556
% Calories from Fat	34 ± 7	33 ± 4	33 ± 5	38 ± 5	37 ± 8	36 ± 7	0.025

Abbreviation: BSG brewers' spent grain.
Data presented as mean ± SD.
*Between group comparisons obtained from mixed-design 2-way ANOVA for comparison of changes. Adjustment for multiple comparisons using Fisher's Least Significant Difference (LSD). Significance set a $p \leq 0.05$.
Interaction (time x group) main effect size reported as partial η^2 range from 0.000 to 0.024.
Time main effect size reported as partial η^2 range from 0.008 to 0.07.
Group main effect size reported as partial η^2 range from .000 to .232.
[1] Nutrient intake values based on daily averages of 3-day diet logs that included 2 weekdays and 1 weekend day dietary intake records during baseline, week 4, and week 8 of the intervention.
[2] Dietary analysis software used is Nutrition Data System for Research (NDSR), version 2020, University of Minnesota Nutrition Coordination Center, Minneapolis, MN, USA.

Table 5
Frequency of gastrointestinal symptoms from pre-baseline to week 1, week 4, and week 8.

Gastrointestinal Symptoms	Control (n = 18)				BSG (n = 19)			
	Pre-Baseline	Week 1	Week 4	Week 8	Pre-Baseline	Week 1	Week 4	Week 8
Stomachache or pain?[1]								
No-mild discomfort	36 (100)	32 (88.9)	32 (88.9)	34 (94.4)	37 (97.4)	36 (94.7)	36 (94.7)	38 (100)
Moderate-moderately severe discomfort	0 (0)	4 (11.1)	4 (11.1)	2 (5.6)	1 (2.6)	2 (5.3)	2 (5.3)	0 (0)
Severe-very severe discomfort	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gas or flatulence?[2]								
No-mild discomfort	36 (100)	33 (91.7)	33 (91.7)	33 (91.7)	38 (100)	37 (97.4)	36 (94.7)	37 (97.4)
Moderate-moderately severe discomfort	0 (0)	3 (8.3)	3 (8.3)	3 (8.3)	0 (0)	1 (2.6)	2 (5.3)	1 (2.6)
Severe-very severe discomfort	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Constipation[3]								
No-mild discomfort	35 (97.2)	36 (100)	34 (94.4)	36 (100)	37 (97.4)	38 (100)	38 (100)	38 (100)
Moderate-moderately severe discomfort	1 (2.8)	0 (0)	2 (5.6)	0 (0)	1 (2.6)	0 (0)	0 (0)	0 (0)
Severe-very severe discomfort	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhea[4]								
No-mild discomfort	36 (100)	33 (91.7)	35 (97.2)	35 (97.2)	36 (94.7)	38 (100)	36 (94.7)	38 (100)
Moderate-moderately severe discomfort	0 (0)	3 (8.3)	0 (0)	1 (2.8)	2 (5.3)	0 (0)	2 (5.3)	0 (0)
Severe-very severe discomfort	0 (0)	0 (0)	1 (2.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Abbreviation: BSG brewers' spent grain.
Data presented as frequency (percentage)Frequency data includes two 24-h questionnaires conducted on two consecutive week days at each timepoint.
[1-4] Likert scale was used 1 = No discomfort at all; 2 = Slight discomfort; 3 = Mild discomfort, 4 = Moderate discomfort, 5 = Moderately severe discomfort, 6 = severe discomfort, 7 = Very severe discomfort. Scale values grouped where No-mild discomfort = 1–3 ratings, Moderate-moderately severe discomfort = 4–5 ratings, Severe-very severe discomfort = 6–7 ratings.
[1] In the past 24 h, on a scale of 1–7, have you been bothered by stomachache or pain?.
[2] In the past 24 h, on a scale of 1–7, have you been bothered by passing gas or flatulence?.
[3] In the past 24 h, on a scale of 1–7, have you been bothered by constipation?.

insulin concentrations were lower than the control group at baseline and week 8 (−3 μIU/mL and −3.8 μIU/mL, respectively). No significant effects on glucose or C-reactive protein occurred between the BSG and control group or from baseline to week 8 in either study group. Interestingly, inflammation status was slightly more pronounced in the BSG group as C-reactive protein increased 0.5 mg/L from baseline to week 8 compared to 0.2 mg/dL in the control group.

4. Discussion

Brewers' spent grain are the leftover grains from beer manufacturing that contain protein, dietary fiber, minerals, and antioxidants. Spent grains have been incorporated into baked goods to improve the nutritional content in a dose-dependent fashion similarly to that of whole grains (Fărcaş et al., 2015; Petrović et al., 2017; Roth et al., 2016). The primary aim of the present pilot study was to evaluate changes in total cholesterol, HDL-C, LDL-C, and triglycerides after consuming muffins containing BSG for 8 weeks in healthy adults. Additional aims determined changes in fasting glucose, insulin, C-reactive protein, body composition, and blood pressure following BSG consumption. The results indicated that muffin consumption compliance was high and similar between groups. In addition, muffins were well tolerated with minor changes in gastrointestinal symptoms and stool habits during the study. Nutrient intakes between both study groups were comparable and showed no significant differences except for total dietary fiber and the percentage of calories from fat. Although dietary fiber consumption was

Table 6
Frequency of Stool Habits between the control and BSG groups over time.

Stool Habits	Control (n = 18)				BSG (n = 19)			
	Pre-Baseline	Week 1	Week 4	Week 8	Pre-Baseline	Week 1	Week 4	Week 8
Immediate need[1]								
No to slight immediate need	21 (58.3)	22 (61.1)	21 (58.3)	18 (50.0)	15 (39.5)	16 (42.1)	16 (42.1)	15 (39.5)
Moderate immediate need	7 (19.4)	3 (8.3)	8 (22.2)	9 (25.0)	15 (39.5)	11 (28.9)	14 (36.8)	15 (39.5)
Quite-a-bit to extreme immediate need	3 (8.3)	4 (11.1)	3 (8.3)	2 (5.6)	2 (5.3)	5 (13.2)	4 (10.5)	3 (7.9)
No stool passed	5 (13.9)	7 (19.4)	4 (11.1)	7 (19.4)	6 (15.8)	6 (15.8)	4 (10.5)	5 (13.2)
Consistency[2,3]								
Constipation	8 (22.2)	11 (30.6)	12 (33.3)	6 (16.7)	8 (21.1)	3 (7.9)	8 (21.1)	7 (18.4)
Normal	23 (63.9)	17 (47.2)	16 (44.4)	24 (66.7)	23 (60.5)	29 (76.3)	25 (65.8)	25 (65.8)
Diarrhea	2 (5.6)	1 (2.8)	5 (13.9)	0 (0)	2 (5.3)	1 (2.6)	1 (2.6)	0 (0)
No stool passed	3 (8.3)	7 (19.4)	3 (8.3)	6 (16.7)	5 (13.2)	5 (13.2)	4 (10.5)	6 (15.8)

Abbreviation: BSG brewers' spent grain.
Data presented as frequency (percentage).
Frequency data includes two 24-h questionnaires conducted on two consecutive weekdays at each timepoint.
[4] In the past 24 h, on a scale of 1–7, have you been bothered by diarrhea?.
[1] How immediate was your need? Scale used where 1 = No immediate need, 2 = Slight immediate need, 3 = Moderate immediate need, 4 = Quite-a-bit of need, 5 = Extreme immediate need. Scale values grouped where 1–2 = No to slight need, 3 = Moderate immediate need, 4–5 = Quite a bit to extreme immediate need (4–5).
[2] Bristol stool scale to describe any changes in intestinal function and stool consistency (Lewis & Heaton, 1997).
[3] Which best describes what your bowel movement looked like? Scale classifies seven different types of stool where 1 = Like marbles or hard rock, 2 = Single, solid clumpy stool, 3 = Hard, solid, formed, harder to pass, 4 = Smooth, softer stool, 5 = Soft chunks or clumps, 6 = Fluffy pieces, ragged edges, 7 = Watery, no solid pieces, liquid. Scale values grouped where 1–2 = Constipation, 3–5 = Normal, 6–7 = Diarrhea.

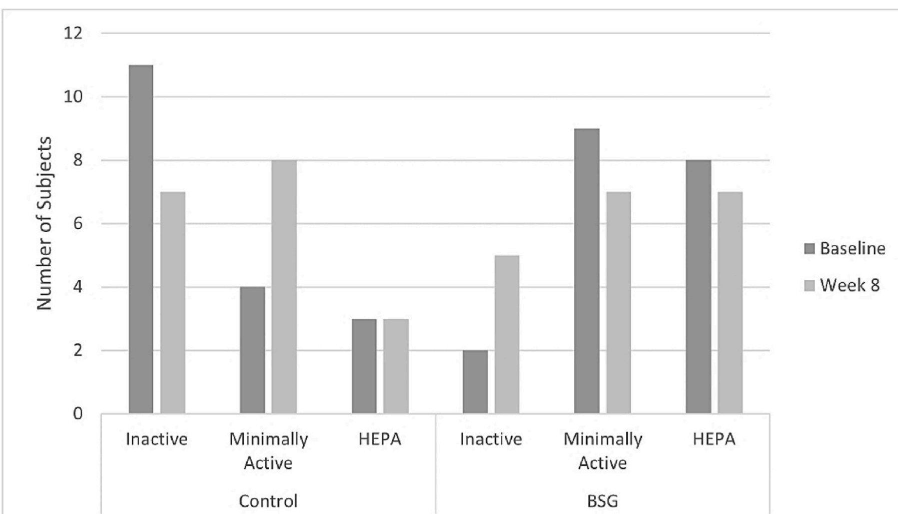

Fig. 2. International Physical Activity Questionnaire (IPAQ) short form score frequencies of subjects within control and BSG groups at baseline and week 8 of intervention. This figure shows the frequency counts of IPAQ scores of subjects at baseline and week 8. Frequencies were determined by the number of participants categorized within each physical activity level: inactive, minimally active, or health-enhancing physical activity (HEPA) active. Categorical IPAQ physical activity level scoring was determined by the estimated total weekly physical activity of each participant at baseline and week 8 data collections (Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) - Short and Long Forms; 2005).

on average 26% (5 g/day) higher in the BSG group, no significant effects on blood lipids, glucose, insulin, inflammatory status, body composition, or blood pressure were observed between groups at the end of the intervention.

Many factors may influence the biological impact of BSG consumption on health outcomes such as whole grain type (i.e., wheat, oat, barley, rice, mixed grains, etc.), solubility of dietary fiber, dosage or daily intake, and study design including duration, sample size, and health status of subjects. A meta-analysis of randomized controlled trials demonstrated whole grain consumption lowered the weighted difference of LDL-C (−0.09 mmol/L, 95% CI: 0.15, −0.03 mmol/L; $p < 0.01$) and total cholesterol (−0.12 mmol/L, 95% CI: 0.19, 0.05 mmol/L; $P < 0.001$) with no effect on HDL-C and a weak tendency to lower triglycerides compared to the control (Hollænder et al., 2015). However, once oat studies were removed from the analysis the effect of whole grains on LDL-C and total cholesterol drastically reduced to (−0.01 mmol/L; 95% CI: 0.08, 0.07 mmol/L; P = 0.83) and (−0.02 mmol/L, 95% CI: 0.10, 0.06 mmol/L; P = 0.64), respectively, revealing oats had a greater effect on lipids than all whole grains (Hollænder et al., 2015). Therefore, soluble fiber and beta-glucan content may partially explain the greater effect that certain wholes grains, such as barley and oats can produce on CVD risk factors.

Soluble fiber can reduce blood lipids and inflammation while promoting glucose homeostasis to a greater extent than insoluble fiber (Caferoglu et al., 2022; Tessari & Lante, 2017). Multiple studies have found barley, which is rich in soluble fiber, significantly lowers total cholesterol (0.26–0.60 mmol/L) and LDL-C (0.21–0.66 mmol/L) with little effect on HDL-C and triglycerides (Ho et al., 2016; Velikonja et al., 2019; Wang et al., 2017; Zhou et al., 2014). Soong et al. (2015) discovered barley muffins produced a lower and more sustained glycemic response compared to wheat, rice, or corn muffins in 12 healthy adults. Due to the cooking process barley undergoes during beer manufacturing, BSG mainly contains insoluble fiber as most soluble fiber is removed with the liquid wort during mashing (Ohra-aho et al., 2016). The lack of effect in our study may result from limited soluble fiber content in BSG or larger dosages are needed to increase concentrations of bioactive BSG compounds.

The Dietary Guidelines for Americans 2020–2025 recommends adults 19–50 years consume three 1-ounce equivalent servings (oz eq/day) of whole grains and 25–34 g/day dietary fiber (US Department of

Table 7
Body composition and CVD risk factor changes in adults following 8-week control and BSG interventions.

Characteristic	Control (n = 18)				BSG (n = 19)				
	Baseline[a]	Week 8	Change	*p*-value*	Baseline[a]	Week 8	Change	*p*-value*	*p*-value**
Body Composition									
Weight (kg)	63.6 ± 12.8	64.4 ± 12.3	0.8	0.085	62.1 ± 11.1	62.3 ± 11.3	0.2	0.643	0.436
Waist-Hip Ratio	0.8 ± 0.1	0.8 ± 0.1	0	0.749	0.8 ± 0.1	0.8 ± 0.1	0	0.574	0.996
Fat Mass (kg)	18.4 ± 7.9	18.9 ± 7.5	0.5	0.123	15.8 ± 6.9	15.8 ± 6.9	0	0.934	0.047
Fat-free Mass (kg)	45.2 ± 7.1	45.4 ± 7.0	0.2	0.29	46.3 ± 10.1	46.6 ± 10.5	0.3	0.326	0.586
Systolic BP (mmHg)	125.6 ± 13.1	124.7 ± 10.9	−0.9	0.486	120.5 ± 8.0	119.1 ± 9.7	−1.4	0.657	0.006
Diastolic BP (mmHg)	76.6 ± 8.6	74.7 ± 7.8	−1.9	0.186	75.2 ± 8.7	73.8 ± 9.6	−1.4	0.517	0.402
Lipids (mg/dL)									
Total Cholesterol	163.5 ± 36.9	164.3 ± 33.1	0.8	0.923	155.0 ± 36.8	151.2 ± 23.4	−3.8	0.558	0.140
LDL-cholesterol	65.8 ± 28.9	70.8 ± 25.3	5.0	0.478	53.6 ± 31.7	54.5 ± 21.2	0.9	0.899	0.062
HDL-cholesterol	82.2 ± 17.9	79.8 ± 14.9	−2.4	0.639	81.2 ± 13	83.5 ± 19.7	2.3	0.639	0.749
Triglycerides[b]	59.3 ± 22.9	57.6 ± 34.2	−1.7	0.790	63.8 ± 27.5	60.4 ± 28.7	−3.4	0.568	0.686
Insulin Sensitivity									
Glucose (mg/dL)	91.4 ± 7.0	91.3 ± 6.2	−0.1	0.906	91.8 ± 13.8	91.6 ± 6.1	−0.2	0.956	0.997
Insulin (μIU/mL)	7.2 ± 7.3	8.2 ± 10.7	1.0	0.628	4.2 ± 3.3	4.4 ± 3.1	0.2	0.901	0.056
Inflammatory Status									
CRP (mg/L)	1.4 ± 1.6	1.6 ± 1.8	0.2	0.815	1.1 ± 1.5	1.6 ± 3.1	0.5	0.358	0.769

Abbreviation: BSG brewers' spent grain, *BP* blood pressure, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *CRP* C-reactive protein.
All values are presented as mean ± SD.
* Within group comparisons obtained from mixed-design 2-way ANOVA for comparison of changes. Adjustment for multiple comparisons using Least Significant Difference. Significance set a $p \leq 0.05$.
** Between group comparisons from baseline to week 8 obtained from mixed-design 2-way ANOVA for comparison of changes. Adjustment for multiple comparisons using Fisher's Least Significant Difference (LSD). Significance set a $p \leq 0.05$.
Interaction (time x group) main effect size reported as partial η^2 range from 0.000 to 0.042.
Time main effect size reported as partial η^2 range from 0.000 to 0.126.
Group main effect size reported as partial η^2 range from 0.000 to 0.216.
[a] Baseline is week 0 of the 8-week study.
[b] Outliers removed from triglyceride analysis. Outliers defined as values outside of the first, second, and third quartiles of the data. Control group (n = 16), BSG group (n = 17).

Agriculture, 2020). Recent analyses found mean whole grain and fiber intakes were 0.97 oz eq/day and 16.1 g/day, respectively, demonstrating most U.S. adults do not consume the recommended quantities (Albertson et al., 2016; McGill et al., 2015). The daily serving size of BSG muffins provided slightly less than the 7.2 g dietary fiber content from daily whole grain recommendations (US Department of Agriculture, 2016). However, due to adherence of the low-fiber diet, the treatment group only consumed on average 19 g/day dietary fiber during the intervention. Future BSG studies should include either higher substitutions of BSG or larger serving sizes of test foods to increase daily dietary fiber intakes to recommended quantities. The lack of effect may also be attributed to high levels of fat consumption (36% of calories from fat) shown in Table 4 as the recommended daily intake of fat should not exceed 30% of total calories. Follow-up studies should consider establishing daily macronutrient distribution requirements. Both groups maintained good muffin consumption compliance indicating the daily serving size was achievable with the potential to increase the dosage in future interventions. In addition, individuals who preferred whole grains and whole-grain baked goods were included in the present analysis to promote study muffin compliance. It is possible the inclusion criteria selected for high habitual whole grain or dietary fiber consumers as whole grain consumption is generally associated to healthy lifestyle behaviors (Maki et al., 2019; McGill et al., 2015). Future BSG dietary interventions in free-living individuals may benefit from higher BSG dosages and more specific inclusion criteria, such as selecting subjects who consume refined grains or low-fiber content in their habitual diets.

Physical activity level differences among subjects, study duration, sample size, and health status are additional factors likely responsible for the lack of effect elicited by BSG on CVD risk factors. Subjects were advised to maintain physical activity levels throughout the trial, but physical activity levels were increased at week 8 compared to baseline in the control group. In contrast, physical activity levels reported by the BSG group were reduced at week 8, which could have contributed to the non-significant increases in HDL-C and non-significant decreases in blood pressure and LDL-C. Even with the minor physical activity variations between groups, there were no significant differences of baseline characteristics (Table 3) nor were any significances reported in blood biomarkers. Most previously conducted whole grain or barley diet intervention durations range from 2 to 16 weeks and 4–12 weeks, but not all studies found a significant effect on the outcome of interest (Marshall et al., 2020). It is possible the duration of the present study was insufficient to produce an effect on blood lipids. However, in dietary interventions evaluating whole grain intake on CVD risk, blood lipids were lowered after 2–6 weeks (Cooper et al., 2017; Zhu et al., 2015) suggesting the 8-week length of the current study was more than enough time to modulate cholesterol.

There are few studies on the impact of BSG intake and impact on risk factors of CVD (Zhang et al., 1991), and the uncertainty of effect size in healthy adults made it challenging to conduct a well-justified power analysis. The sample size for our study was comparable to previous studies (Li et al., 2003; Shimizu et al., 2008) assessing the effect of barley intake to create a 5% reduction in total cholesterol as the primary endpoint. However, the potential for whole grains to improve CVD risk factors may be dependent on the health status of the subjects. Studies demonstrating whole grains significantly improve body composition (Roager et al., 2019), lipids (Li et al., 2016), glucose homeostasis (Malin et al., 2018), blood pressure (Nelson et al., 2016), and inflammation (Hoevenaars et al., 2019) often include individuals at risk for CVD. BSG may have limited or no effect in healthy, normocholesterolemic individuals where biomarkers are at normal concentrations. Healthy adults were deliberately chosen due to the novelty of BSG and lack of human intervention data to verify its tolerability when habitually consumed within individuals without underlying health conditions. Future studies should consider pre-screening for hypertensive, hypercholesterolemic, or overweight/obese clinically high-risk subjects.

The present study had several strengths that increased the reliability of the results. This was the first human diet intervention evaluating the effect of BSG intake on CVD risk factors, and the dosage was well-tolerated by subjects with no side effects reported. However, there were several study limitations as well including low daily BSG-derived fiber intakes, study duration, sample size, and subject health status. As this was the first study evaluating the impact of BSG on risk factors of CVD in humans, there were no similar studies present in the literature to compare outcomes. Therefore, future studies with a higher BSG dosage, longer duration, and larger sample size conducted in a clinical at-risk population are necessary to confirm the tolerability of BSG and its capacity to effect cardiometabolic risk factors.

5. Conclusions

In conclusion, daily consumption of 8.3 g (/100 g) BSG in muffins for 8 weeks increases total dietary fiber intake and is well tolerated. However, BSG does not affect body composition, blood pressure, or blood biomarkers associated with cardiometabolic disease risk in this sample of healthy adults. This is the first study to our knowledge that investigates the tolerability and effect of BSG on CVD risk factors in healthy subjects. Data obtained from this pilot study will be used to interpret the effect size of BSG on CVD risk factors, thereby supporting power analyses for sample size estimation of future human interventions. Health status, BSG dosage, and study design may be key determinants of outcome measures and should be further evaluated in larger controlled trials that address the limitations of the current study.

Author contributions

Shannon Schmidt-Combest, Cynthia Warren, Mindy Patterson: Conceptualization, Methodology, Validation **Shannon Schmidt-Combest, Mindy Patterson:** Formal analysis, investigation, project administration **Mindy Patterson**, **Cynthia Warren:** Resources **Shannon Schmidt-Combest:** Writing—original draft preparation, writing—review and editing, visualization, supervision, funding acquisition **Shannon Schmidt-Combest, Wanyi Wang, Marley Grams, Derek Miketinas:** Data curation.

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Institutional Review Board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Texas Woman's University, Houston, TX (Protocol 20319, Date of Approval on December 19, 2018).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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