Gastrointestinal Tolerance and Microbiome Response to Snacks Fortified with Pea Hull Fiber: A Randomized Trial in Older Adults

Zainab Alyousif,1 Daniela Rivero Mendoza,1 Jérémie Auger,2 Vanessa De Carvalho,2 Samantha Amos,1 Charles Sims,1 and Wendy J Dahl1

1Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA and 2Rosell Institute for Microbiome and Probiotics, Montreal, Quebec, Canada

ABSTRACT

Background: Consuming foods with added fiber may help older adults achieve fiber recommendations; however, many high-fiber ingredients have little effect on laxation and may contribute to unpleasant gastrointestinal side effects.

Objectives: The aim of the study was to determine the effects of consuming snacks fortified with pea hull fiber (PHF) on stool frequency and form, gastrointestinal symptoms, and appetite in older adults. An exploratory aim was to determine if PHF altered the microbiota profile.

Methods: A 10-wk, randomized, blinded, crossover study was carried out. Following a 2-wk baseline period, participants [aged (mean ± SD) 69.7 ± 6.5 y; n = 31; 14 men, 17 women] consumed snacks providing 10 g/d of PHF or a control, each for 2-wk periods followed by 2-wk washouts. Participants used the Bristol Stool Form Scale (BSFS) to record daily stool frequency and gastrointestinal symptoms, and completed the Gastrointestinal Symptom Rating Scale (GSRS) and Simplified Nutritional Appetite Questionnaire (SNAQ) biweekly. One stool was collected per period for 16S ribosomal RNA high-throughput amplicon sequencing of the fecal microbiota profile.

Results: Participants reported 1.63 ± 0.05 stools/d and 76.6% normal transit stool form at baseline and no change with PHF. GSRS syndrome scores were similarly unchanged. Daily abdominal noises and bloating were higher for PHF versus control, and flatulence was higher for PHF versus baseline, suggesting fermentation in some individuals. There was no evidence to suggest a common PHF-induced microbiome response for the group as a whole; however, a subgroup of participants (n = 7) who responded with increased flatulence (fermenters), harbored many different taxa than nonfermenters, and demonstrated lower abundance of Clostridiales with PHF. Appetite was unchanged with PHF.

Conclusions: PHF did not modulate stool form or frequency in older adults with normal bowel habits. Because snacks fortified with PHF did not suppress appetite, PHF may be an appropriate fiber source for older adults at nutritional risk. Microbiome profile may be predictive of gastrointestinal symptom response to PHF. This trial was registered at www.clinicaltrials.gov as NCT02778230. Curr Dev Nutr 2020;4:nzaa005.

Keywords: dietary fiber, pea hull, microbiota, gastrointestinal tolerance, sensory evaluation, GSRS, SNAQ, appetite, fermentation, older adults

Copyright © The Author(s) 2020. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License [http://creativecommons.org/licenses/by-nc/4.0/], which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Supplemental Tables 1 and 2 and Supplemental Figures 1–3 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

Address correspondence to WJD (e-mail: w Dahl@ufl.edu).

Abbreviations used: BSFS, Bristol Stool Form Scale; GSRS, Gastrointestinal Symptom Response Scale; LDA, linear discriminant analysis; LeFSe, linear discriminant analysis effect size; PHF, pea hull fiber; SNAQ, Simplified Nutritional Appetite Questionnaire.

Introduction

Consuming foods with added fiber may help to achieve fiber recommendations (1). This may be particularly important for older adults who may require higher-protein diets to preserve muscle mass or for restoration in times of recovery from unintentional weight loss, illness, or injury (2). Increased intakes of protein from animal-based foods, devoid of naturally occurring fiber, may displace plant-based foods containing fiber, such as whole grains, fruits, and vegetables, and thus may further support the need for fiber fortification. However, fiber ingredients, such as fructans (e.g., fructo-oligosaccharides and inulin), commonly added to foods in North America have been shown to contribute to gastrointestinal complaints including flatulence, bloating, and abdominal pain (3, 4), symptoms that may negatively impact quality of life in older adults. In addition, there is some evidence that higher intakes of fructans may suppress appetite and decrease body weight (5, 6). As appetite suppression and its possible effects on food intake and body weight may be contraindicated in older adults at nutritional risk, exploration of the physiological effects of alternative fibers for the purpose of fortification is needed.
Another consideration when choosing a fiber ingredient for fortification of food intended for older adults is its potential impact on the gut microbiota and associated health effects. Prebiotics such as fructans, given their specific carbohydrate structure, require unique enzymes for hydrolysis and thus, by definition, have specific effects on the gut microbiota or its activity (7). Although these effects, such as the enhancement of Bifidobacterium, are considered positive, isolated fructans have not been shown to impact diversity of the microbiota (8). This is an important consideration, as microbiota diversity is associated with stability—protection from disruption by dietary changes (9) and other stressors (10). As decreased diversity is associated with the onset of frailty (11) and increased risk of Clostridium difficile diarrhea (12), a potentially fatal disease more common in older adults (13), maintaining microbiota diversity is particularly important for older adults. Diets high in complex plant fibers such as whole grains are associated with higher microbial diversity (14,15). Added fibers such as brans, containing a variety of indigestible polysaccharides, also may support microbial diversity (16). It is not known if hull fibers (pulse seed coats) have the potential for such effects. Of note, the nondigestible polysaccharides contained in the complex dietary fiber fractions of brans and hulls may be more slowly fermented than purified, soluble, and highly fermentable oligosaccharides and, thus, may produce less noticeable bloating and flatulence and, perhaps, improved acceptance and feasibility while still inducing beneficial shifts in the microbiota.

Pea hull fiber (PHF), a naturally occurring dietary fiber produced from grinding of the outer hulls of yellow field peas (17), has been shown to benefit laxation in older adults reporting low stool frequency (18). Given its complex dietary fiber constituents (i.e., cellulose, pectin, and hemicelluloses), it potentially may enhance gut microbial diversity and profile. As slow transit, suggestive of constipation, negatively impacts the gut microbiota and its metabolism (19), the potential of PHF to modulate stool form, a proxy for transit time, and stool frequency is also of interest. In addition, given that some older adults may be at risk of weight loss and malnutrition, the impact of PHF fortification on appetite requires exploration. The aims of the study were to determine the effects of daily consumption of snacks fortified with PHF on stool form, stool frequency, gastrointestinal symptoms, and appetite in community-dwelling older adults. As appetite may be impacted by the sensory acceptability of the food vehicle used for fiber fortification, sensory evaluation of the study snacks was carried out prior to the trial. In addition, an exploratory aim was to assess the effect of PHF on the microbiota profile.

Methods

Product development and sensory evaluation
Snacks (cinnamon mixed berry and oatmeal raisin chocolate chip cookies) containing 5 g of PHF (Best Cooking Pulses) and control snacks with no added fiber were developed and evaluated for acceptability in 2 sensory panels, older adults and all ages. Commercial higher fiber cookies (Belvita® Mixed Berry and FiberOne® Sugar Cookie, FiberOne® Oatmeal Raisin Cookie and Belvita® Oats & Chocolate) were used as benchmarks. Panelists (aged ≥60 y and all ages) rated samples using a hedonic 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) with questions regarding the liking of sweetness, texture, flavor, moistness, and overall acceptability of the snacks. Sensory evaluation was approved by the University of Florida Institutional Review Board 2.

Intervention study design
A 10-wk randomized, double-blind, crossover study was carried out in Florida (Figure 1) in 2 cohorts, July to October 2016 and May to July 2017. Following a 2-wk baseline period, participants consumed 2 cookies/d providing 10 g of PHF (9.3 g/d of fiber) or no added fiber (control) each for 2-wk periods separated by 2-wk washouts. The randomization was conducted by an individual unaffiliated with the study using a sealed, stacked-envelope method. All study participants, investigators, staff, and the statistician were blinded to the sequence until the statistical analyses were completed. Although study foods were visually very similar, there was the possibility that participants could detect sensory differences between the control and PHF fiber cookies. To determine if blinding was successful, participants were asked at the end of each intervention period, which treatment, fiber or no fiber, they thought they had been consuming. Participants recorded compliance to study food intake in the daily questionnaire and returned any uneaten study foods at each study visit.

Gastrointestinal symptoms, stool frequency and form, appetite, dietary intake, and compliance were assessed throughout the trial and stool samples were collected for sequencing. The trial was approved by the University of Florida Institutional Review Board 1. The protocol is reported on clinicaltrials.gov (registration no. NCT02778230). All participants provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki.

Participants
Participants (aged ≥60 y) were recruited from the community through posters, flyers, announcements, and community and newspaper advertisements. Participants were included if they were willing to undertake study procedures and excluded if they had any known food allergies, were taking medications for diarrhea, had taken antibiotics within the past 4 wk prior to randomization, were taking probiotics supplements and did not want to discontinue a minimum of 2 wk prior to the study, or had previously been or were being treated for any diseases or illnesses such as gastrointestinal disease (gastric ulcers, Crohn’s, celiac, ulcerative colitis, etc.).

Outcome measures
The primary outcomes of interest were stool form and frequency. Secondary outcomes were gastrointestinal symptoms and appetite. Participants recorded stool frequency and form using the Bristol Stool Form Scale (BSFS) (20) in daily questionnaires. Stools were categorized into slow transit (types 1 and 2), normal transit (types 3–5), and fast transit (types 6 and 7). In the daily questionnaire, participants were asked to rank daily abdominal cramping, abdominal noises, bloating, constipation, diarrhea, and flatulence using a scale from 0 (not at all) to 6 (very severe) and appetite using a scale from 0 (very poor) to 6 (very good) in response to the question, “In general, how was your appetite today?” Any changes in medications, supplement intake, and physical activity also were recorded. The Gastrointestinal Symptom Rating Scale (GSRS), a 7-point Likert scale, where 1 represents no discomfort at all and 7 represents very severe discomfort (21,22), was administered...
biweekly. Symptoms of the GSRS were combined into the 5 syndromes: reflux, abdominal pain, indigestion, diarrhea, and constipation. The Simplified Nutritional Appetite Questionnaire (SNAQ) (23) also was administered biweekly. Total scores range from 4 to 20 with a total SNAQ score ≤14 indicating poor appetite and a significant risk of weight loss for community-dwelling older adults (23). Dietary intake (24-h recall) was assessed by phone interview during each study period. Food Processor Nutrition Analysis Software (ESHA version 11.3.2) was used to analyze dietary intake.

Microbiota analysis
An exploratory outcome was microbiome profile. One stool was collected toward the end of each 2-wk period. Stools were collected using the Fisher Scientific Commode Collection System (Fisher Scientific catalog no. 02-544-208), kept on ice, and processed within 6 h of defecation. Samples were homogenized, placed in aliquots, and stored at −80 °C. Total DNA was extracted from homogenized feces using the QIAamp® Fast DNA Stool Mini Kit (Qiagen) as per the manufacturer’s instructions with modifications as previously described (24). Libraries for sequencing were prepared according to Illumina’s 16S Metagenomic Sequencing Library Preparation guidelines (Part no. 15,044,223 Rev. B), with exceptions as previously reported (24). Template-specific primers targeted the V3–V4 region of the 16S ribosomal RNA gene (PMCID: PMC3592464) (25). Resulting sequence reads were analyzed as previously described (26, 27). Taxonomic summaries and α and β diversity metrics, statistical analysis, and taxonomic classifications were computed using QIIME 2 software (28) and downstream analyses by R scripts were performed as previously reported (29). Linear discriminant analysis (LDA) effect size (LeiSe) was used to compare fecal microbiome abundance profiles between treatment groups (30). The Huttenhower galaxy online platform was used to run LEiSe (http://huttenhower.sph.harvard.edu/galaxy/).

Following the primary microbiome analysis and noting that a subgroup of individuals had experienced significant flatulence (≥1 rating-point increase) during the PHF, we hypothesized that these individuals harbored organisms capable of fermenting PHF (fermenters) and, thus, may exhibit a microbiome profile different from the majority of the participants (nonfermenters). Subgroups were compared using LeiSe analysis (30).

**Statistical analysis**
Sensory data for the any-age panels were collected using Compusense® Five software and older adults by paper ballots. Statistical analysis was carried out using Statistical Analysis Systems® 9.4 (SAS). A 2-factor ANOVA was performed to determine if there were differences in ratings for each attribute. Mean separation was completed using Duncan’s multiple range test with an α level of 0.05.

The sample size of the trial was based on a power of 0.80 and a type I error rate of 0.05. Based on a study testing 10.5 g/d of an insoluble fiber (31), reporting a mean stool frequency per day for placebo of ~1.00 and for treatment of 1.35 (range: 1.15–1.51) with SDs of 0.32 and 0.63, respectively, and a correlation of 0.35, a paired sample size of 26 for a crossover trial was needed to show a significant effect. Estimating a drop-out rate of 25%, a sample of 36 was targeted.

Daily symptoms, GSRS syndromes, and stool frequency were analyzed as intent-to-treat. Unless noted otherwise, data are presented as means ± SEMs. Significance was set at an α level of 0.05. Linear mixed models were used to test differences between treatment groups for the daily questionnaire symptoms, GSRS syndromes, SNAQ scores, and dietary intake. Data were square root transformed, where appropriate, with baseline as a covariate. When the F-value was significant, a multiple-means comparison was performed using Tukey–Kramer at a P value of 0.05. SNAQ was compared as the difference between means and categorically (risk: ≥14 points vs. >14 points). A 2-tailed Fisher’s exact test was used to test the effectiveness of blinding. Paired t test was used to determine if fiber intake was different between the subgroups at baseline. For the microbiome data, the Kruskal–Wallis test was used to compare α diversities. QIIME software suite was used to calculate the metrics corresponding to diversity and taxonomic classification (28).

**Results**

**Product development and sensory evaluation**
Sensory panels of older adults (aged ≥60 y) evaluated the PHF and control cinnamon mixed berry (n = 76) and oatmeal raisin chocolate chip (n = 74) cookies as did 2 panels of all ages (n = 120/panel) in comparison to commercial cookie varieties. Results of the sensory evaluations are shown in Supplemental Table 1. Overall liking was rated highest...
for the FiberOne® sugar cookie and the control cinnamon mixed berry cookie. Belvita® and the cinnamon mixed berry with PHF were rated somewhat lower but similar. Overall liking for the FiberOne® oatmeal raisin cookie and the control oatmeal raisin chocolate chip cookie was not different. Belvita® and the oatmeal raisin chocolate chip cookie with PHF were rated as similar. The nutrient compositions of the PHF and control cookies evaluated for acceptability and subsequently used in the intervention trial are shown in Supplemental Table 2.

**Wellness outcomes.**
The intervention study flow diagram is presented as Figure 2. Participant demographics and characteristics are shown in Table 1. Of the 36 individuals who consented, 3 withdrew prior to baseline, 2 withdrew prior to randomization, 1 withdrew during the first intervention period (fiber), and 1 withdrew during the first washout. Participants were unable to ascertain whether they were consuming the PHF or control snacks (intervention 1, \( P = 0.6 \); intervention 2, \( P = 0.3 \)), confirming the effectiveness of blinding. Participants reported normal stool frequency (1.63 ± 0.05 stools/d) at baseline, with no significant changes with interventions (Table 2). BSFS reporting showed 12.0% slow transit, 76.6% normal transit, and 11.4% fast transit at baseline, with no differences between periods (Table 2). No significant differences were reported for the GRSRS (Table 2). For daily symptoms (Table 2), abdominal noises and bloating were higher for the PHF intervention compared with the control, and flatulence was higher for PHF compared with baseline, whereas there were no differences for daily abdominal cramping, constipation, diarrhea, or appetite. Mean SNAQ score was higher during PHF for the fiber period (15.8 ± 0.4) compared with the control (15.3 ± 0.4); however, when assessed as risk categories (at risk vs. no risk) the apparent difference was not significant (PHF: \( n = 7 \) at risk; control: \( n = 10 \) at risk). At baseline (\( n = 29 \)), total energy intake was 1780 ± 179 kcal/d and did not differ between intervention periods. Similarly, background fiber (18.4 ± 2.0 g/d), protein (71.6 ± 7.6 g/d), carbohydrate (204.7 ± 21.6 g/d), and fat (72.7 ± 8.6 g/d) intakes reported at baseline did not change with the interventions. At baseline, the mean fiber intake of the subgroup of fermenters (18.6 ± 4.5 g/d) was not different from that of the nonfermenters (18.2 ± 2.5 g/d). With the inclusion of study snacks, there was a significant increase in total fiber intake during the PHF period (\( P < 0.0001 \)). Intake of dairy foods, some of which may have contained live cultures, did not significantly differ between baseline (1.6 ± 0.3 servings/d) and interventions (PHF: 1.4 ± 0.1 servings/d; control: 1.4 ± 0.3 servings/d). Body weight did not change between periods or over the duration of the study period (data not shown).

**Microbiota composition.**
The relative abundances of bacteria observed for all participants during the baseline, washouts (pooled), control, and PHF are shown in Figure 3 and as a Krona figure in Supplemental Figure 1. No differences were seen between periods with LeFSe analyses. There was no change in bacterial \( \alpha \) diversity with PHF (Supplemental Figure 2). For the subgroup analysis carried out to compare the microbiome of those participants demonstrating an increase in flatulence severity as an indicator of PHF fermentation, baseline differences between fermenters (\( n = 7 \)) and nonfermenters (\( n = 22 \)) are shown in Figure 4. Taxa such as Methanobrevibacter, Coprococcus, and Peptosteptococcus were higher in the fermenters compared with nonfermenters.
TABLE 1  Participant demographics and characteristics

|                       | Participants (n = 31) | Fermenters1 (n = 7) | Nonfermenters2 (n = 22) |
|-----------------------|-----------------------|---------------------|-------------------------|
| Gender (M/F), n       | 14/17                 | 2/5                 | 11/11                   |
| Age, mean ± SD (range), y | 69.7 ± 6.5 (60–86)   | 68.6 ± 6.0 (61–80) | 70.3 ± 6.9 (60–86)      |
| Race, n (%)           |                       |                     |                         |
| African American      | 4 (12.9)              | 2 (28.6)            | 2 (9.1)                 |
| White                 | 27 (87.1)             | 5 (71.4)            | 20 (90.9)               |
| Ethnicity, n (%)      |                       |                     |                         |
| Hispanic              | 2 (6.4)               | 0                   | 2 (9.1)                 |
| Non-Hispanic          | 27 (87.1)             | 6 (85.7)            | 19 (86.4)               |
| Not reported          | 2 (6.4)               | 1 (14.3)            | 1 (4.5)                 |
| BMI (in kg/m²), n (%) |                       |                     |                         |
| Normal (18.5–24.9)    | 8 (25.8)              | 0                   | 8 (36.3)                |
| Overweight (25–29.9)  | 9 (29.0)              | 3 (42.9)            | 6 (27.3)                |
| Obese (> 30)          | 14 (45.2)             | 4 (57.1)            | 8 (36.4)                |
| Reported compliance3  |                       |                     |                         |
| Pea hull fiber snacks | 98%                   |                     |                         |
| Control               | 96%                   |                     |                         |

1Participants demonstrating an increase of ≥1 point (scale 0 to 6) in flatulence severity reported in the daily questionnaire during the PHF intervention.
2Participants demonstrating a <1 point increase in flatulence severity during the PHF intervention.
3Daily questionnaire reporting.

Nonfermenters were enriched in Proteobacteria. Figure 5 shows a significantly higher abundance of Clostridiales during the control compared with the PHF intervention in fermenters. No significant changes in microbiome profile were detected in the nonfermenter subgroup with the provision of PHF compared with control (n = 22). A comparison of the relative bacterial proportions for all samples from all periods for fermenters versus nonfermenters is shown in Figure 6; using a stringent (LDA = 3) cutoff, differences were demonstrated in numerous taxa. A comparison of baseline with washout demonstrated only an enrichment of Lactobacillus during baseline (Supplemental Figure 3).

Discussion

PHF-fortified snacks evaluated in this clinical trial were assessed as being acceptable by sensory panelists, including older adults. The study snacks provided ~200 kcal/d, but the intake of the snacks did not

TABLE 2  Daily and biweekly reported wellness outcomes1

|                      | Baseline | Fiber | Control | P  |
|----------------------|----------|-------|---------|----|
| Stool frequency      |          |       |         |    |
| Stools/d             | 1.63 ± 0.05 | 1.85 ± 0.05 | 1.76 ± 0.05 | NS |
| BSFS, %              |          |       |         |    |
| Slow transit         | 12.0     | 9.2   | 11.3    | NS |
| Normal transit       | 76.6     | 77.0  | 78.4    | NS |
| Fast transit         | 11.4     | 13.8  | 10.3    | NS |
| Daily symptom scores |          |       |         |    |
| Appetite             | 4.07 ± 0.07 | 3.80 ± 0.07 | 3.94 ± 0.07 | NS |
| Abdominal cramping   | 0.16 ± 0.03 | 0.23 ± 0.03 | 0.10 ± 0.02 | NS |
| Abdominal noises     | 0.27 ± 0.03ab | 0.37 ± 0.04a | 0.18 ± 0.02ab | <0.01 |
| Bloating             | 0.26 ± 0.03ab | 0.34 ± 0.04a | 0.18 ± 0.03ab | <0.05 |
| Flatulence           | 0.74 ± 0.04b | 1.05 ± 0.05a | 0.83 ± 0.05ab | <0.05 |
| Constipation         | 0.12 ± 0.02 | 0.16 ± 0.02 | 0.20 ± 0.04 | NS |
| Diarrhea             | 0.17 ± 0.03 | 0.16 ± 0.02 | 0.12 ± 0.03 | NS |
| GSRS syndrome scores |          |       |         |    |
| Abdominal pain       | 1.21 ± 0.07 | 1.36 ± 0.11 | 1.24 ± 0.12 | NS |
| Reflux               | 1.09 ± 0.05 | 1.07 ± 0.04 | 1.09 ± 0.04 | NS |
| Indigestion          | 1.41 ± 0.08 | 1.59 ± 0.13 | 1.40 ± 0.09 | NS |
| Constipation         | 1.24 ± 0.10 | 1.13 ± 0.04 | 1.29 ± 0.10 | NS |
| Diarrhea             | 1.25 ± 0.07 | 1.24 ± 0.06 | 1.43 ± 0.15 | NS |

1Values are means ± SEs unless otherwise indicated. Stool form were categorized into slow transit (types 1 and 2), normal transit (types 3 to 5), and fast transit (types 6 and 7). Means with different superscript letters differ significantly according to Tukey-Kramer (P < 0.05). BSFS, Bristol Stool Form Scale; GSRS, Gastrointestinal Symptom Response Scale.
result in differences in energy intake during the intervention periods, suggesting participants substituted instead of adding study foods to their usual diet. Appetite as assessed by SNAQ category (23) did not change during the PHF intervention, suggesting no increased risk of unintended future weight loss in this older cohort; and the daily reporting of appetite was similarly unaffected. In most previous studies, consuming PHF-fortified foods did not affect appetite, food intake, or body weight (18, 32–34). In contrast, a study in overweight/obese adults...
(aged 44 ± 15 y) showed weight loss with the consumption of 15 g/d of PHF in the form of wafers over 12 wk and decreased food intake in a single-meal study (35). However, appetite and sensory acceptance were not reported and, thus, it is possible that the wafers were somewhat unappetizing and, as such, food intake may have been negatively impacted independent of any metabolic effect resulting from any PHF fermentation. It may also be possible that age confounds appetite response to foods with added fiber. As SNAQ is validated to predict the future risk of weight loss (23) and poor outcomes in older adults (36), this tool may be useful to assess the appropriateness of added fibers for supplementation of foods intended for older adults. PHF did not suppress appetite; thus, it may be an appropriate fiber for fortification of foods for older adults at risk of unintended weight loss.

Reported GSRS syndrome scores fell below clinical significance (mild discomfort) for participants overall, suggesting they were healthy with respect to gastrointestinal function. Stool form was used as a proxy for transit time, and participants reported primarily normal transit stools at baseline, which remained unchanged with PHF. Participants reported the proportion of slow transit stools at 11%, somewhat higher than a representative sample of the US population considered to have normal bowel habits at 6% slow transit (37), but lower than we have previously reported in younger women (38). The PHF used in this study was finely processed (200 mesh) and, thus, its high surface area compared with intact hulls may have facilitated fermentation by the colonic bacteria, thus lessening its laxative effects, a mechanism that has been previously suggested (39). It has been reported that coarse wheat bran decreased transit time, whereas the same dose of finely ground wheat bran did not (40). Similarly, finely ground oat hull fiber did not impact transit time in young male subjects with relatively fast transit time (41).

Although stool form is strongly associated with microbiota profile (19), the changes in the microbiota observed in some of the participants in the present study are likely due to the fermentation of PHF rather than an effect of altered transit time.

Although the current US FDA considers increased stool frequency as a “beneficial physiological effect on human health” for isolated or synthetic nondigestible carbohydrates (functional fibers) (42), in healthy individuals with normal stool frequency, fibers, fermentable fibers in particular, may not alter laxation (39). Although in the present trial an insoluble dietary fiber versus functional fiber was examined, stool frequency did not change. This finding may not be unexpected given that the participants exhibited normal stool frequency (37) at baseline and displayed no symptoms of constipation. In addition, in nonconstipated individuals, stool frequency is not associated with transit time (43). The results of this study add to the significant literature that refutes stool frequency as an appropriate outcome for the evaluation of fiber supplementation in individuals with normal bowel habits. As increases in stool frequency are more often seen as a response to fiber in those with infrequency (e.g., <3 stools/wk) (44) and functional constipation (45), it is possible that if PHF was tested in community-dwelling older adults with low baseline stool frequency and symptoms of constipation, an increase may have been seen, as has been demonstrated in older adults residing in long-term care (18). Similarly, in a small study in older adults with chronic kidney disease, stool frequency increased with an intake of 10 g/d of PHF (46). These conflicting findings may, in part, be due to the length of the intervention. The studies that have shown an increase in frequency with PHF tested it with intervention periods of 4 wk versus 2 wk in the present trial. Further, differences in resident microbiota, extent of fermentation, and transit time may have affected...

**FIGURE 5** LDA effect size (LefSe) comparing the relative bacterial proportions in the subgroup of fermenters (participants responding to PHF intake with an increase in flatulence severity) ($n = 7$) during PHF versus control periods. c, class; LDA, linear discriminant analysis; o, order; p, phylum; PHF, pea hull fiber.
FIGURE 6 LDA effect size (LefSe) comparing the relative bacterial proportions for all samples from all periods in the subgroup of fermenters \((n = 7)\) (participants responding to PHF with an increase in flatulence severity) versus nonfermenters \((n = 22)\). c, class; f, family; g, genus; k, kingdom; LDA, linear discriminant analysis; o, order; p, phylum; PHF, pea hull fiber.

The outcomes. Beyond stool frequency, quantitative measurements such as stool bulk and examination of in vivo fermentability require exploration.

The participants’ baseline microbiota showed significant intraindividual variation, which was not surprising given the diversity that has been shown in the human microbiome (47) and its response to differing dietary patterns (9). The results of this study did not provide evidence for PHF as a means to increase microbial diversity or any modulation of microbiota in most participants. It has been suggested that a higher-fiber diet may not have remarkable effects on the microbiome profile in the short term (48) unless an extreme change is made in dietary intake, such as the elimination of fiber and other carbohydrates from the diet (49). A longer-term exposure to the fiber source may be necessary to significantly impact microbiota. However, in the present study, a subgroup analysis revealed significant differences in the microbiota of those individuals exhibiting increased flatulence (fermenters), suggesting fermentation of PHF by gas-generating microorganisms. Methanobrevibacter, Coprococcus, and Peptostreptococcaceae were higher in fermenters. A higher abundance of methanogens, primarily Methanobrevibacter, may be unfavorable as methane production has been associated with constipation (50); however, it has been suggested that certain methanogens, at least theoretically, may suppress trimethylamine production and, thus, that of trimethylamine-N-oxide, which is associated with cardiovascular disease risk (51). Legume intake has been shown to depress proteolytic bacteria (52). Consuming snacks containing PHF resulted in decreased abundance of Clostridiales compared with controls in the fermenters subgroup, specifically a suppression of Clostridia, which are implicated in protein fermentation (53). Recently, a suppression of unclassified Clostridiales after feeding vegetables high in fructans (artichokes and leeks) was reported (54). In contrast, feeding 15 g/d of PHF over a 12-wk period showed no treatment effect on the limited number of taxa evaluated (33). In a secondary analysis, Lachnospira increased with PHF (55). However, as subjects also lost weight in the PHF intervention arm, it is possible that the change in Lachnospira was due to weight loss instead of the PHF specifically, as a similar enrichment has been seen in bariatric surgery (56) and nonalcoholic fatty liver disease patients (57) following weight loss. Increased Bifidobacterium and Lactobacillus have been commonly reported in fiber studies, but most often in response to oligosaccharide (e.g., galacto-oligosaccharide, fructo-oligosaccharide) supplementation (8) versus a complex fiber source with cellulose as the major indigestible polysaccharide constituent, such as PHF (58). We saw no changes in these genera. Diets higher in fiber, including legumes, support diversity of gut microbiota and these diets are associated with enhanced Bacteroidetes, specifically the genera Prevotella (59, 60). In addition, consumption of a Mediterranean diet has been associated with increased Prevotella (61). In the present study, no enhancement of Prevotella was seen with the PHF, although the subgroup of fermenters exhibited higher relative abundance of Prevotella, which may reflect
habitual higher fiber intake and possibly legume intake. However, the baseline fiber intakes of the fermenters did not differ from those of non-fermenters and long-term intake was not examined.

This study had limitations. As other research has shown, it is difficult to demonstrate change and conclude improvement in gastrointestinal function in individuals with normal bowel habits (62). The length of the intervention period may have been a limitation. Administration of fiber over many weeks or months, although not necessarily expected to impact stool frequency or form, may modulate microbiota. However, the comparisons of microbiome profiles seem to confirm the adequacy of the 2-wk washouts as they differed from baseline only by *Lactobacillus* abundance.

Current fiber intake in the United States falls below recommendations (63), a concern given that low fiber intakes are strongly associated with increased chronic disease risk (64, 65). Foods with added fiber may improve intakes (1); however, given the high burden of gastrointestinal symptoms in the United States (66), added fibers that do not substantially contribute to gastrointestinal discomfort are needed. The findings of the present study using snacks fortified with PHF, a primarily insoluble dietary fiber, showed that only a minority of individuals experienced an increase in daily symptoms of flatulence, bloating, and abdominal noise, suggesting differential fermentation or possibly differing sensitivity to gas production. Thus, in older adults with normal bowel habits, PHF at 10 g/d is well tolerated. Future trials should test PHF in individuals with low stool frequency and slow transit stool form. Snacks fortified with PHF did not suppress appetite; thus, PHF may be an appropriate fiber source for older adults at risk of unintended weight loss and subsequent malnutrition.

It was suggested recently that controlled-feeding trials are needed to determine the specific effects of fiber sources on microbiome composition (9). We have shown that the baseline microbiome profile, particularly the predominance of methanogens, may be predictive of gastrointestinal symptom response to, and potentially health benefits of, PHF. It is not known if microbiome profile also may be predictive of response to whole pulses and other high-fiber ingredients. As has been suggested, individuals may exhibit individualized bacterial population responses to foods (9), and fiber specifically, and our results support this premise. The results of the present study confirm that baseline microbiome profile confounds symptom outcomes and, thus, also may influence health outcomes related to a fiber source. Further research is needed to explore the potential health effects of *Clostridia* suppression. As the impact of fiber on health is thought to be largely related to its modulation of microbiota and its metabolism (67), and possibly transit time (19), the effects of added fibers on these outcomes in community-dwelling older adults presenting with constipation and dysbiosis, specifically low baseline diversity, require investigation.

Acknowledgments
The authors thank James Colee of the University of Florida Institute of Food and Agricultural Sciences for his assistance with the statistical analyses, Maggie McCall for her assistance with the dietary assessment, and Best Cooking Pulses, Inc., for supplying the pea hull fiber. The authors’ responsibilities were as follows—WJD, VDC, and CS: designed the research; ZA, DRM, VDC, SA, and WJD: conducted the research; ZA, JA, and SA: analyzed data/perform statistical analysis; WJD, ZA, DRM, SA, and JA: wrote the manuscript; and all authors: read and approved the final manuscript.

References
1. Clemens R, Kranz S, Mobley AR, Nicklas TA, Raimondi MP, Rodriguez JC, Slavin JL, Warshaw H. Filling America’s fiber intake gap: summary of a roundtable to probe realistic solutions with a focus on grain-based foods. J Nutr 2012;142(7):1390s–401s.
2. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, Phillips S, Sieber C, Stelhe P, Teta D, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J Am Med Dir Assoc 2013;14(8):542–59.
3. Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal tolerance of chicory inulin products. J Am Diet Assoc 2010;110(6):865–8.
4. Holscher HD, Doigale JL, Bauer LL, Gourineni V, Pelmken CL, Fahey GC, Swanson KS. Gastrointestinal tolerance and utilization of agave inulin by healthy adults. Food Funct 2014;5(6):1142–9.
5. Liber A, Srajewska H. Effects of inulin-type fructans on appetite, energy intake, and body weight in children and adults: systematic review of randomized controlled trials. Ann Nutr Metab 2013;63(1–2):42–54.
6. Korcak R, Slavin JL. Fructooligosaccharides and appetite. Curr Opin Clin Nutr Metab Care 2018;21(5):377–80.
7. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 2017;66(11):1968–74.
8. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, Shanahan ER, Staudacher HM, Campbell KL. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am J Clin Nutr 2018;107(6):965–83.
9. Johnson AJ, Vangay P, Al-Ghaliith GA, Killmann BM, Ward TL, Shields-Cutler RR, Kim AD, Shmagel AK, Syed AN, Walter J, et al. Daily sampling reveals personalized diet-microbiome associations in humans. Cell Host Microbe 2019;25(6):789–802 e5.
10. Jeffery IB, Lynch DB, O’Toole PW. Composition and temporal stability of the gut microbiota in older persons. ISME J 2016;10(1):170–82.
11. Jackson M, Jeffery IB, Beaumont M, Bell JT, Clark AG, Ley RE, O’Toole PW, Spector TD, Steves CJ. Signatures of early frailty in the gut microbiota. Genome Med 2016;8(1):8.
12. Seekatz AM, Rao K, Santhosh K, Young VB. Dynamics of the fecal microbiome in patients with recurrent and nonrecurrent *Clostridium difficile* infection. Genome Med 2016;8(1):47.
13. Donskey CJ. *Clostridium difficile* in older adults. Infect Dis Clin North Am 2017;31(4):743–56.
14. Menzi C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. Int J Obes 2017;41(7):1099–105.
15. Martinez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyreughian G, Peterson DA, et al. Gut microbiome composition is linked to whole grain-induced immunological improvements. ISME J 2013;7(2):269–80.
16. Jefferson A, Adolphus K. The effects of intact cereal grain fibers, including wheat bran on the gut microbiota composition of healthy adults: a systematic review. Front Nutr 2019;6:33.
17. Tyler R, Youngs C, Soslowski F. Air classification of legumes [beans, lentils, peas]. I. Separation efficiency, yield, and composition of the starch and protein fractions. Cereal Chem 1981;58(2):144–8.
18. Dahl WJ, Whiting SJ, Healey A, Zello GA, Hildebrandt SL. Increased stool frequency occurs when finely processed pea hull fiber is added to usual foods consumed by elderly residents in long-term care. J Am Diet Assoc 2003;103(9):1199–202.
19. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joosens M, Raes J. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. Gut 2016;65(1):37–62.

CURRENT DEVELOPMENTS IN NUTRITION
20. Riegler G, Esposito I. Bristol scale stool form: a still valid help in medical practice and clinical research. Tech Coloproctol 2001;5(3):163–4.

21. Svedlund J, Sjödin I, Dotevall G. GSRS—a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci 1988;33(2):129–34.

22. Kulich KR, Madisch A, Pacini F, Piqué JM, Regula J, Van Rensburg CJ, Usszasz L, Carlsson J, Halling K, Wulkund IK. Reliability and validity of the Gastrointestinal Symptom Rating Scale (GSRS) and Quality of Life in Reflex and Dyspepsia (QOLRAD) questionnaire in dyspepsia: a six-country study. Health Qual Life Outcomes 2008;6(1):1.

23. Wilson MM, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, Diebold MR, Morley JE. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. Am J Clin Nutr 2005;82(5):1074–81.

24. MacPherson CW, Mathieu O, Tremblay J, Champagne J, Nantel A, Girard SA, Tompkins TA. Gut bacterial microbiota and its resistome rapidly recover to basal state levels after short-term amoxicillin-clavulanic acid treatment in healthy adults. Sci Rep 2016;8(1):11192.

25. Klinworth A, Preece E, Schweer T, Pepljes J, Quast C, Horn M, Gloeckner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 2013;41(1):e1.

26. Yergeau E, Bell TH, Champagne J, Maynard C, Tardif S, Tremblay J, Singh K, Fern A, Kirton ES, He S, Woyke T, Lee J, Chen F, Dangl JL, Tringe SG. Primer and platform effects on 16S rRNA tag sequencing. Front Microbiol 2015;6:1436.

27. Tremblay J, Singh K, Fern A, Kirton ES, He S, Woyke T, Lee J, Chen F, Dangl JL, Tringe SG. Primer and platform effects on 16S rRNA tag sequencing. Front Microbiol 2015;6:1436.

28. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, AbNet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37(8):852–7.

29. Ford AL, Nagulesapillai V, Piano A, Auger J, Girard SA, Christman M, Dahl WJ. 2020. Gastrointestinal tolerance and microbiota stability with a high protein diet: a randomized, double-blind, placebo-controlled trial in older women. J Acad Nutr Diet. doi: 10.1016/j.jand.2019.12.009.

30. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.

31. Baird IM, Walters RL, Davies PS, Hill MJ, Drasar BS, Southgate DA. The effects of two dietary fiber supplements on gastrointestinal transit, stool weight and frequency, and bacterial flora, and fecal bile acids in normal subjects. Metabolism 1977;26(2):117–28.

32. Salmeen YA, Zello GA, Dahl WJ. Foods with added fiber improve stool frequency in individuals with chronic kidney disease with no impact on appetite or overall quality of life. BMJ Res Notes 2013;6(1):510.

33. Smith CE, Mollard RC, Luohovy BL, Anderson GH. The effect of yellow pea protein and fibre on short-term food intake, subjective appetite and glycaemic response in healthy young men. Br J Nutr 2012;108(S1):S74–80.

34. Mollard RC, Luhovyy BL, Smith C, Anderson GH. Acute effects of pea protein and fibre on short-term food intake, subjective appetite or overall quality of life. BMC Res Notes 2013;6(1):510.

35. Lambert JE, Parnell JA, Tunnicliffe JM, Han J, Sturzenegger T, Reimer RA. Measuring appetite with the Simplified Nutritional Appetite Questionnaire identifies hospitalised older people at risk of worse health outcomes. J Nutr Health Aging 2016;20(1):3–7.

36. Pilgrim AL, Baylis D, Jameson KA, Cooper C, Sayer AA, Robinson SM, Robert HS. Measuring appetite with the Simplified Nutritional Appetite Questionnaire identifies hospitalised older people at risk of worse health outcomes. J Nutr Health Aging 2016;20(1):3–7.

37. Mitsushashi S, Ballou S, Jiang ZG, Hirsch W, Nee J, Iturriano J, Cheng V, Lembo A. Characterizing normal bowel frequency and consistency in a representative sample of adults in the United States (NHANES). Am J Gastroenterol 2018;113(1):115–23.

38. Alyousif Z, Ford AL, Dahl WJ. Calcium supplementation does not contribute to constipation in healthy women. Can J Diet Pract Res 2016;77(2):103–5.

39. McRorie JW, Chey WD. Fermented fiber supplements are no better than placebo for a laxative effect. Dig Dis Sci 2016;61(11):3140–6.

40. Wick K, Robertson J, Van Soest P, Lewis B, Rivers J, Roe D, Hacker LR. The influence of dietary fiber source on human intestinal transit and stool output. J Nutr 1983;113(8):1464–79.

41. Stephen AM, Dahl WJ, Johns DM, Englyst HN. Fermentability of oat hull fibre in the human colon and its effects on colonic function and serum lipids. Cereal Chem 1997;74:379–83.

42. Office of Nutrition and Food Labeling, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, Scientific evaluation of the evidence on the beneficial physiological effects of isolated or synthetic non-digestible carbohydrates submitted as a citizen petition (21 CFR 10.30); guidance for industry [Internet]. College Park (MD): US Food and Drug Administration; 2018. [Accessed 2019 Sep 9]. Available from: https://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/UCM528333.pdf.

43. Saad RJ, Rao SS, Koch KL, Kuo B, Parkman HP, McCallum RW, Sitrin MD, Wilding GE, Semier JR, Chey WD. Do stool form and frequency correlate with whole-gut and colonic transit? Results from a multicenter study in constipated individuals and healthy controls. Am J Gastroenterol 2010;105(2):403–11.

44. Yang J, Wang HP, Zhou L, Xu CF. Effect of dietary fiber on constipation: a meta-analysis. World J Gastroenterol 2012;18(48):7378–83.

45. Christodoulides S, Dimidi E, Fragkos KC, Farmer AD, Whelan K, Scott SM. Systematic review with meta-analysis: effect of fibre supplementation on chronic idiopathic constipation in adults. Aliment Pharmacol Ther 2016;44(2):103–16.

46. Salmeen YA, Segal MS, Palii SP, Dahl WJ. Fiber supplementation lowers plasma p-cresol in chronic kidney disease patients. J Ren Nutr 2015;25(3):316–20.

47. Karkman A, Lehtimaki J, Ruokolainen L. The ecology of human microbiota: dynamics and diversity in health and disease. Ann N Y Acad Sci 2017;1399(1):78–92.

48. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Blevins M, Knights D, Walters WA, Knight R, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334(6052):105–8.

49. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505(7484):559–63.

50. Triantafyllou K, Chang C, Pimentel M. Methanogens, methane and gastrointestinal motility. J Neurogastroenterol Motil 2014;20(1):31–40.

51. Gaci N, Borrel G, Teytay W, O’Toole PW, Brugere JF. Archaea and the human gut: new beginning of an old story. World J Gastroenterol 2014;20(43):16062–78.

52. Fernando W, Hill J, Zello G, Tyler R, Dahl W, Van Kessel A. Diets supplemented with chickpea or its main oligosaccharide component raffinose modify faecal microbial composition in healthy adults. Benef Microbes 2010;1(2):197–207.

53. Diether NE, Willing BP. Microbial fermentation of dietary protein: an important factor in diet-microbe-host interaction. Microorganisms 2018;7(1):1.

54. Hiel S, Bindels LB, Pachikian BD, Kalala G, Broers V, Zamariola G, Chang M, Kambashi B, Rodriguez J, Cani PD, et al. Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. Am J Clin Nutr 2019;109(6):1683–95.

55. Mayengbam S, Lambert JE, Parnell JA, Tunnicliffe JM, Nicolucci AC, Han J, Sturzenegger T, Sander J, Wickwiekewicz B, Vogel HJ, et al. Impact of dietary fiber supplementation on modulating microbiota-host-metabolic axes in obesity. J Nutr Biochem 2019;64:228–36.

56. Gutierrez-Repiso C, Moreno-Indias I, de Hollanda A, Martin-Nunez GM, Vidal J, Tinahones FJ. Gut microbiota specific signatures are related to the successful rate of bariatric surgery. Am J Transl Res 2019;11(2):942–52.

57. Patakzy Z, Genton L, Spahr L, Lazarevic V, Terraz S, Gaia N, Rubbia-Brandt L, Golay A, Schrenzel J, Pichard C, et al. Impact of hypocaloric
hyperproteic diet on gut microbiota in overweight or obese patients with nonalcoholic fatty liver disease: a pilot study. Dig Dis Sci 2016;61(9):2721–31.

58. Reichert R. Quantitative isolation and estimation of cell wall material from dehulled pea (Pisum sativum) flours and concentrates. Cereal Chem 1981;58(4):266–70.

59. Simpson HL, Campbell BJ. Review article: dietary fibre-microbiota interactions. Aliment Pharmacol Ther 2015;42(2):158–79.

60. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 2010;107(33):14691–6.

61. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, Serrazanetti DI, Di Cagno R, Ferrocino I, Lazzi C, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut 2015;65(11):1812–21.

62. Dennis-Wall JC, Burns AM, Solch RJ, Ukhanova M, Dahl WJ, Christman MC, Boileau T, Brauchla M, Shin JE, Nieves C. A beverage containing orange pomace improves laxation and modulates the microbiome in healthy adults: a randomised, blinded, controlled trial. J Funct Foods 2019;60:103438.

63. Reicks M, Jonnalagadda S, Albertson AM, Joshi N. Total dietary fiber intakes in the US population are related to whole grain consumption: results from the National Health and Nutrition Examination Survey 2009 to 2010. Nutr Res 2014;34(3):226–34.

64. McRae MP. Dietary fiber is beneficial for the prevention of cardiovascular disease: an umbrella review of meta-analyses. J Chiropr Med 2017;16(4):289–99.

65. Mirmiran P, Yuzbashian E, Asghari G, Sarverzadeh S, Azizi F. Dietary fibre intake in relation to the risk of incident chronic kidney disease. Br J Nutr 2018;119(5):479–85.

66. Almario CV, Ballal ML, Chey WD, Nordstrom C, Khanna D, Spiegel BMR. Burden of gastrointestinal symptoms in the United States: results of a nationally representative survey of over 71,000 Americans. Am J Gastroenterol 2018;113(11):1701–10.

67. Dahl WJ, Agro NC, Eliasson ÅM, Mialki KL, Olivera JD, Rusch CT, Young CN. Health benefits of fiber fermentation. J Am Coll Nutr 2017;36(2):127–36.