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# Bacteriophage for Gastrointestinal Health (PHAGE) Study: Evaluating the Safety and Tolerability of Supplemental Bacteriophage Consumption

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**ABSTRACT**

**Objective:** The gut microbiota has been recognized as a critical regulator of human health, and novel interventions to selectively modulate the microbiota are actively being sought. Bacteriophages (bacterial viruses) have the potential to selectively eliminate specific detrimental microbes while enhancing beneficial microbe populations. The Bacteriophage for Gastrointestinal Health (PHAGE) study aimed to determine the safety and tolerability of supplemental bacteriophage consumption in a population of healthy adults with mild to moderate gastrointestinal distress.

**Methods:** The PHAGE study was a randomized, double-blind, placebo-controlled crossover intervention. Healthy adults with self-reported gastrointestinal distress were recruited and asked to consume one 15-mg capsule containing 4 strains of bacteriophages (LH01-*Myoviridae*, LL5-*Siphoviridae*, T4D-*Myoviridae*, and LL12-*Myoviridae*) and a placebo, each for 28 days. Participants were randomly assigned to the starting treatment, which was followed by a 2-week washout period before they began the second arm of the intervention. Primary outcome measures included a comprehensive metabolic panel and gastrointestinal health questionnaire. In addition, samples were collected for future analysis of several secondary outcome measures, including global microbiota profiles, plasma lipids, and markers of local and systemic inflammation.

**Results:** Forty-three individuals met all study criteria and consented to participate. Of these participants, 36 completed at least one arm of the trial and 32 completed the study. There were no effects of treatment sequence on comprehensive metabolic panel outcomes, but there were 1- and 2-way carryover effects on gastrointestinal questionnaire data. Levels of aspartate aminotransferase significantly decreased while participants were taking the treatment but not placebo; however, all mean values remained within clinically acceptable ranges. Participants also reported significant improvements in several symptoms of gastrointestinal distress while taking both the treatment and the placebo.

**Conclusions:** Consumption of therapeutic doses of a mixture of 4 bacteriophages was both safe and tolerable in a target human population.

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Bacteriophage; gastrointestinal; microbiome; inflammation; gastrointestinal health

## Introduction

The human microbiome, which encompasses human-associated microorganisms and their functional contributions to their host, is an area of growing interest with regard to health promotion and disease prevention. These microorganisms, particularly those found in the human gastrointestinal tract, are thought to modulate weight, immunity, and development of numerous chronic and inflammatory diseases (1). The gut microbiota contributes to our ability to digest food, acts as a first line of defense against pathogenic organisms, and is important in the development and modulation of the immune system (2). In terms of host energy balance, the gut microbiota is important in assisting with the breakdown of indigestible carbohydrates to produce fermentation by-products that are utilized by human cells. For example, the short-chain fatty acids (SCFAs) produced during fermentation include butyrate, which serves as the primary energy source for colonic epithelial cells, and acetate and propionate, which are metabolized by the liver (2). These SCFAs are also able to interact with free fatty acid receptors in the gut, liver, and adipose tissue to modulate host metabolism of glucose and lipids, regulate intestinal transit time, and increase satiety via increased production of Peptide YY (3).

In addition to its important role in the digestive process, the gut microbiota is one of the first lines of defense against invading pathogens. Humans frequently ingest pathogens, but the microbiota keeps these pathogens from populating

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the gut. Commensal organisms prevent mucosal adhesion of pathogens, secrete antimicrobial peptides and other metabolites that create a hostile environment for pathogens, and interact with elements of the intestinal barrier (4). A well-balanced microbiota will be able to outcompete pathogenic microorganisms for limited resources such as nutrients and space (2). Microbial metabolites produced by commensal organisms are also important defense mechanisms against pathogens and include lactic acid and antimicrobial peptides called bacteriocins. Finally, beneficial bacteria stimulate host defenses against pathogens and are an important part of the innate immune system. The presence of beneficial organisms initiates the conversion of undifferentiated T cells into interleukin 10-producing T regulatory cells to ensure that harmless antigens do not trigger an inflammatory response (4). However, an imbalance in the intestinal lumen that allows an increase in proinflammatory or pathogenic organisms will initiate the production of Th1, Th2, Th17, and B cells that launch an inflammatory response. If this response is great enough, it can lead to a breach in the intestinal epithelial barrier, allowing translocation of luminal contents to the systemic circulation (5).

Disruption to the gut microbiota has been associated with the development of numerous diseases in the intestines and in peripheral tissues (1). Inflammation in the intestines can drive microbial dysbiosis that eventually leads to compromised intestinal barrier function and can ultimately affect peripheral tissues. Factors leading to microbial dysbiosis can also include antibiotic usage, stress, aging, and chronic consumption of a poor diet (6). The gut microbiota also influences disease development through the production of various metabolites. For example, degradation products of proteins, particularly nitrogenous metabolites such as nitrosamine and heterocyclic amines, have been associated with an increased risk of colon cancer, whereas butyrate promotes apoptosis in colorectal cancer cells (7). Thus, modification of the gut microbiota by means of diet or supplementation is an attractive option for the protection against or reversal of microbiota-associated disease.

An emerging interest in modulation of the gut microbiota has resulted in a growing commercial market for dietary supplements and functional foods targeted toward enriching or selectively stimulating populations of beneficial bacteria. In 2014, supplements or functional foods marketed for gut health accounted for approximately US$45 billion in sales, with an expected market increase of 30.5% by 2019 (8). These supplements can typically be classified as probiotics, which are live microorganisms that when administered in adequate amounts confer a health benefit on the host (9), and prebiotics, which are dietary components that stimulate the growth of commensal organisms known to confer beneficial effects to host health (10). Probiotics can be administered in various forms, including supplements, infant formula, medical foods, and some fermented foods (9). There has been evidence to support probiotic use to improve digestive issues, including mild to moderate irritable bowel syndrome and antibiotic-associated diarrhea, reduce blood cholesterol, stimulate the immune system, and act as anticarcinogens (11).

Under the standard definition, prebiotics are indigestible fiber components that are utilized as fermentation substrates by beneficial bacteria residing in the large bowel (10). However, this definition is expanding beyond fiber. For example, a U.S. patent was recently issued for PreforPro (Deerland Enzymes, Kennesaw, GA), a proprietary blend of bacteriophages that target *Escherichia coli* (12). Bacteriophages are highly specific viruses that can target, infect, and destroy pathogenic bacteria. They are believed to be the most abundant type of viruses, accounting for the majority of the $10^{31}$ viruses identified to date. In the 1930s, the use of bacteriophages for the treatment of bacterial diseases (or "phage therapy") was popularized; however, the concept lost momentum with the introduction and widespread use of broad-spectrum antibiotics (13). The specificity of bacteriophages is now viewed advantageously because phages allow selective modulation of the gut microbiota without initiating gut dysbiosis, which occurs with antibiotic use (13,14). The U.S. Food and Drug Administration lists many bacteriophages as Generally Recognized as Safe (GRAS) for human consumption because they are abundant in nature, reside naturally in the human gastrointestinal tract, and are inadvertently consumed by humans on a daily basis (14). They represent good therapeutic agents as long as they are obligately lytic, stable under typical storage conditions and temperatures, subject to appropriate efficacy and safety studies, and ideally fully sequenced to confirm the absence of undesirable genes such as toxins (15,16).

We conducted the Bacteriophages for Gastrointestinal Health (PHAGE) study to explore the safety and tolerability of a mixture of 4 bacteriophages for consumption in humans. Study participants consumed 15-mg capsules containing 4 strains of bacteriophages during one 28-day period and an inert capsule during another 28-day period. The target population included healthy adults with mild to moderate gastrointestinal distress, and participants were asked to report gastrointestinal symptoms throughout the trial to assess tolerability of the treatment. In addition, comprehensive metabolic panels were used to monitor effects on blood chemistry and liver function to determine the safety of bacteriophage consumption.

## Materials and methods

### *Study design*

The PHAGE study was a randomized, double-blind, placebo-controlled crossover trial that aimed to investigate the safety and tolerability of 4 supplemental bacteriophage strains (LH01-*Myoviridae*, LL5-*Siphoviridae*, T4D-*Myoviridae*, and LL12-*Myoviridae*) included in the PreforPro commercial preparation by Deerland Enzymes (Kennesaw, GA USA). Enrolled participants were randomly assigned to either the placebo or treatment starting groups (blinded as treatments A and B) and were asked to consume 1 capsule daily for 28 days. This intervention was followed by a 2-week washout period prior to starting an additional

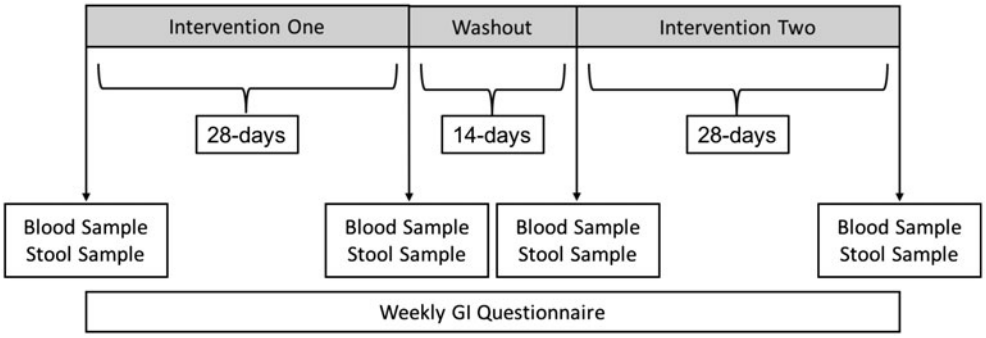


**Figure 1.** Study design schematic. GI = gastrointestinal.

28-day intervention with the opposite treatment (Figure 1). Participants attended a clinic visit at the Colorado State University Human Performance Clinical Research Laboratory at the beginning and end of each 28-day treatment period. During the clinic visits, participants provided a stool sample that was collected using a fecal collection container (Thermo Fisher Scientific, Waltham, MA) provided by the study investigators. Participants were instructed to collect stool within 24 hours of consuming their final capsule and to store samples frozen or refrigerated prior to returning them to the clinic. All collected stool samples were processed and stored at −80 °C to be used for future analysis of gut microbiota populations, stool metabolites, and intestinal inflammatory factors. At the clinic, participants were weighed and subjected to a fasting venous blood draw. Blood was collected in ethylenediamine tetraacetic acid (EDTA) and lithium heparin tubes. Plasma was collected by centrifugation from the EDTA tubes and stored at −80°C for future analyses of circulating inflammatory markers and plasma lipid profiles. Blood from the lithium heparin tubes was used immediately for comprehensive metabolic profiling. Participants were also asked to complete a weekly gastrointestinal assessment published by Metagenics, Inc. (Aliso Viejo, CA) (Supplemental Figure 1) throughout the study. The assessment was provided through a personal, secure Google Docs link. Participants were asked to report any illnesses or adverse events to study personnel. Primary outcome measures included (1) results of comprehensive metabolic panels conducted at each study visit to assess blood chemistry and liver function in order to determine the safety of the treatment and (2) gastrointestinal questionnaire responses to gauge tolerability. Due to low compliance with questionnaire completion, only the questionnaires administered at the beginning and end of each treatment period were evaluated. All participants provided written informed consent prior to study enrollment. The study protocols were approved by the Colorado State University Institutional Review Board (protocol number 16-6666HH). This clinical trial was registered at ClinicalTrials.gov (NCT03269617).

## Study population

For this pilot intervention, we targeted enrollment of a total of 40 participants, based on power calculations conducted on microbiota data from a previously published pilot dietary intervention (17). Although *E. coli* is the target organism of the phage cocktail administered, this organism is often not detected or found only in very low abundance in human stool samples. However, as several synbiotic commercial formulations contain PreforPro to stimulate the growth of probiotic species, we used changes in *Bifidobacterium* spp., which are typically detected in human stool samples, as a basis of our power calculation. We calculated that a total of 26 individuals in a crossover intervention would be sufficient to detect a significant difference ($p = 0.05$) in populations of fecal *Bifidobacterium* with 80% power. Thus, recruitment of 43 individuals allowed us to achieve statistical power, even with predicted 20–25% study attrition. Recruitment for this trial was conducted via flyers, e-mails, and word of mouth through alternative medicine practitioners and other health care providers. Healthy adults, with ages between 18 and 65 years, with mild to moderate gastrointestinal distress but no diagnosed gastrointestinal conditions were recruited. Eligibility was determined through e-mail and phone screening and using an eligibility questionnaire at the study consent visit. Patients who were pregnant or breastfeeding or were previously diagnosed with celiac disease, inflammatory bowel disease, peptic ulcer disease, cancer, or other gastrointestinal or metabolic disorders were excluded from the study. Other exclusion criteria included recent antibiotic use (within 2 months of study enrollment) or the use of other medications that have been reported to alter the gut microbiota or inflammatory cytokines, including metformin (18), statins (19), and nonsteroidal anti-inflammatory drugs (20). Reporting of any dietary supplement use was requested and eligibility was determined on a case-by-case basis (those reporting consistent use of prebiotics or probiotics were excluded from the study). Participants were asked to maintain their typical diet and physical activity levels throughout the study and were required to refrain from recreational drugs or consuming >7 alcoholic beverages/week.

## Intervention

The 4 bacteriophages used in the PHAGE study (LH01-*Myoviridae*, LL5-*Siphoviridae*, T4D-*Myoviridae*, and LL12-*Myoviridae*) were contained within an inert carrier consisting of rice maltodextrin and coconut oil triglycerides.

Treatment and placebo (i.e., rice maltodextrin and coconut oil triglycerides) capsules were coded and provided by Deerland Enzymes. These organisms were shown to be purely lytic and are known to infect a range of *Escherichia coli* strains, including *E. coli* K12, and 16 enterotoxigenic *E. coli* strains and 2 enterohemorrhagic strains (21). The phages used in this study contain no genes encoding any known toxins or antibiotic risk factors, nor did they contain genetic elements shown to be harmful to humans. They are not likely to be able to infect and kill other bacteria outside of the Enterobacteriaceae and were not expected to negatively alter the natural microbiota of the human intestine. On multiple occasions, the U.S. Food and Drug Administration has affirmed GRAS status for other bacteriophage-containing compounds, as long as they were lytic with specific targeted pathogens and specific intended uses. The dose administered during the trial was 10 ng of phage per person per day, which is well within the GRAS quantities found in many conventional foods (e.g., dairy products and fermented foods). Participants were administered one 15-mg capsule per day for 28 days.

### Gastrointestinal health assessment

Gastrointestinal health was evaluated quantitatively at the beginning and end of each treatment period to gain information on the treatment's perceived effects on gastrointestinal symptoms. The gastrointestinal questionnaire had 4 sections: gastric function (section A), gastrointestinal inflammation (section B), small intestine and pancreas (section C), and colon pain (section D). Within each section, participants ranked questions based on symptoms, choosing from 0 (no/rarely), 1 (occasionally), 4 (often), or 8 (frequently). Section A of the questionnaire addressed gastric function and was measured on a scale of 1 (low priority) to 56 (high priority). Section B of the questionnaire addressed gastrointestinal inflammation and was measured from 1 (low priority) to 72 (high priority). Section C of the questionnaire addressed small intestine and pancreas function and was measured from 1 to 80. Finally, section D addressed colon function and was measured on a scale from 1 to 72. Participants were provided with a secure, personal Google Docs link after each clinic visit and were asked to complete the questionnaire.

### Comprehensive metabolic panel

At each clinic visit, a fasting, venous blood sample (~3 mL) was collected in a lithium heparinized tube. Within 1 hour of collection, 120 $\mu$l of whole blood was loaded into a Piccolo Comprehensive Metabolic Panel disk and assayed using a Piccolo Xpress Chemistry Blood Analyzer (Abaxis, Union City, CA). The panel included markers of blood chemistry, metabolism, and liver function, including albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen, calcium, chloride, creatinine, sodium, potassium, total carbon dioxide ($tCO_2$), glucose, total bilirubin, and total protein as the albumin/globulin ratio.

### Statistical analysis

The sequence effect (treatment to placebo or placebo to treatment) was evaluated by determining the significance of the difference between baseline values of both sequences. All continuous data were tested for the assumption of normality prior to linear regression analysis was performed. To control for the effect of sequence and repeated measures on the subject, a linear mixed model approach was taken to compare the treatment effects within each time point. Also a similar approach was taken to compare the time points within treatment and placebo separately. A $p$ value of 0.05 was used to determine statistical significance. SAS software (version 9.4; SAS Institute, Inc., Cary, NC) was used for all statistical analysis.

## Results

### Participant demographics and compliance

We screened 96 individuals who responded to recruitment efforts through e-mail, flyers, or referrals (Figure 2). Twenty participants did not respond after their initial inquiry and an additional 4 respondents were determined to be ineligible due to pregnancy or a diagnosis of celiac disease. An additional 72 respondents were screened through phone or face-to-face conversations; 29 of these individuals were determined to be ineligible or chose not to continue with the study. The most common reasons for ineligibility included recent antibiotic use, current breastfeeding, and consistent use of restricted medications. Forty-three individuals (13 men and 30 women) met all eligibility criteria and provided written consent to participate in the study. Thirty-two individuals completed both study arms, whereas an additional 4 individuals completed the treatment (1 man) or placebo (1 man and 2 women) arm only. An additional 7 individuals dropped out of the study prior to completing either treatment. Of those 7 participants, 4 withdrew participation due to time constraints, 2 had to start antibiotic treatments during the study, and 1 withdrew due to exacerbation of gastrointestinal symptoms (it was later determined that the participant had been taking the placebo capsule, so the complaint was unlikely related to study participation).

Participants ranged in age from 20 to 61 years, with the average age of 40 years (Figure 3A). The average body mass index (BMI) was 25.7 $kg/m^2$, with the majority of participants characterized as normal weight. However, the total participant BMIs ranged from 20 to 35 $kg/m^2$, with approximately one-third of the participants classified as overweight or obese by this metric (Figure 3B). Average participant characteristics are presented in Table 1. With regard to capsule consumption, total study compliance was approximately 95%. For the treatment period, individual compliance ranged from 75% to 100%, with an average compliance of 95.6%. During the placebo period, individual compliance ranged from 61% to 100%, with an overall average of 94.8%. Despite good adherence to study protocols regarding capsule consumption, only 75% of participants (27 of 36) completed the gastrointestinal questionnaire.

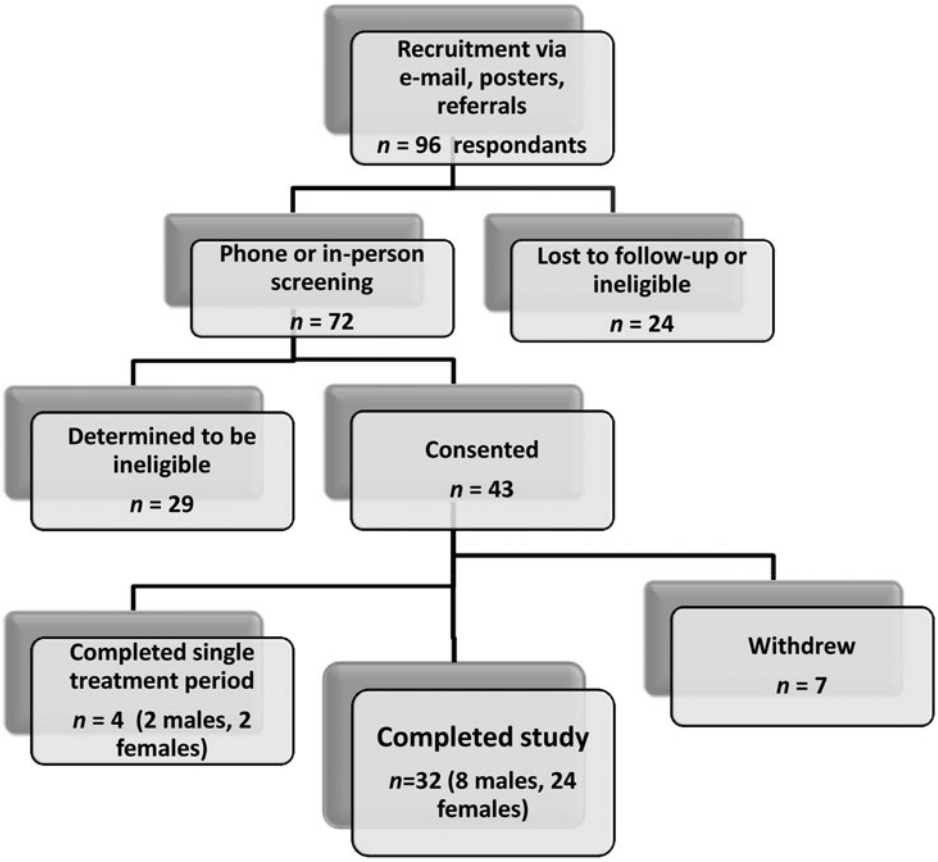


**Figure 2.** Study recruitment, enrollment, and completion.

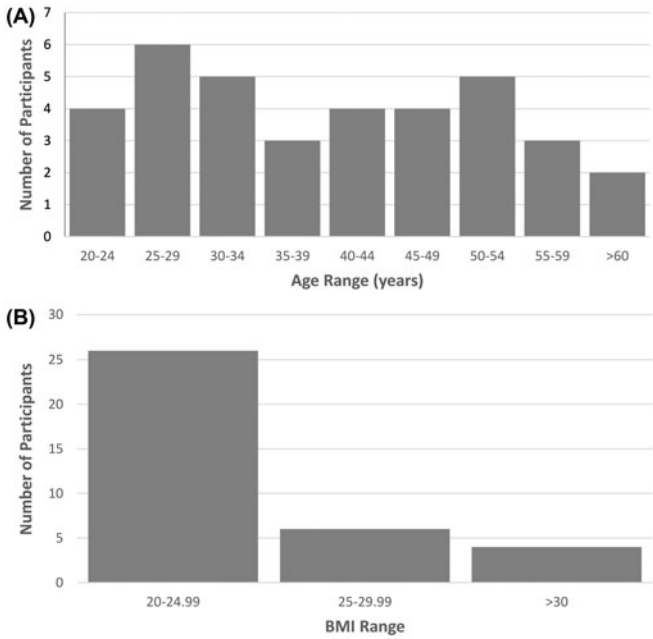


**Figure 3.** Baseline age (A) and body mass index (B) of participants. BMI = body mass index.

### Gastrointestinal health assessment

In total, 27 participants completed the gastrointestinal health questionnaire (Supplemental Figure 1) at the beginning and end of each treatment period. In general, women reported greater baseline symptom severity than men (Table 1). Statistical analysis showed a sequence effect for all 4 sections of the questionnaire. Scores in sections A (treatment to placebo, $p < 0.01$; placebo to treatment, $p < 0.01$) and C (treatment to placebo, $p = 0.03$; placebo to treatment, $p < 0.01$) were bidirectionally influenced by starting sequence. Scores in sections B (placebo to treatment, $p < 0.01$) and D (placebo to treatment, $p = 0.03$) were significantly negatively affected by starting in the placebo group and then receiving the treatment. After we controlled for sequence effects, we observed a significant improvement in gastrointestinal severity scores for gastric function (section A: treatment, $p < 0.01$; placebo, $p = 0.02$), small intestine pain (section C: treatment, $p = 0.02$; placebo, $p = 0.01$), and colon pain (section D: treatment, $p < 0.01$; placebo, $p = 0.03$) in both treatment and placebo groups (Table 3). There was no significant improvement in perceived gastrointestinal inflammation (section B) over the course of the intervention. In addition, there were no significant differences between the final time points of the treatment and placebo periods.

### Comprehensive metabolic profiles

Fourteen different analytes in heparinized whole blood were measured to determine effects of treatment consumption on liver and kidney function as well as other parameters of metabolic regulation (Table 2). After statistical tests were performed for sequence effects, a small treatment to placebo effect was noted for creatinine ($p = 0.05$). After we controlled for sequence effects, we observed that AST levels were lower

**Table 1.** Participant Demographics and Baseline Gastrointestinal Assessment Score

| Demographic | Women ($n = 26$) | Men ($n = 10$) | Study average |
|---|---|---|---|
| Age (years) | 39.7 ± 12.2 | 39.0 ± 12.8 | 39.5 ± 12.2 |
| Weight (lb) | 152.5 ± 31.6 | 170.6 ± 20.1 | 157.5 ± 29.8 |
| Height (inches) | 65.3 ± 2.7 | 71.8 ± 3.1 | 67.1 ± 4.0 |
| Body mass index (kg/m$^2$) | 25.1 ± 3.9 | 23.3 ± 2.4 | 24.6 ± 3.6 |
| True baseline gastrointestinal score | | | |
| Section A: gastric function | 12.0 ± 6.6 | 9.6 ± 8.3 | 11.4 ± 6.9 |
| Section B: gastrointestinal inflammation | 11.5 ± 8.6 | 6.3 ± 6.8 | 10.1 ± 6.9 |
| Section C: small intestine pain | 25.1 ± 12.8 | 18.7 ± 21.2 | 23.5 ± 15.0 |
| Section D: colon pain | 15.0 ± 10.3 | 12.9 ± 21.9 | 14.4 ± 13.5 |

*Note*. Values are means ± standard deviations.

**Table 2.** Values for Tested Blood Analytes at the Beginning and End of Each Intervention Period

| | | Treatment | | Placebo | |
|---|---|---|---|---|---|
| Analyte | Reference range | Baseline | Intervention | Baseline | Intervention |
| Sodium (mmol/L) | 128–145 | 140.1 ± 3.15 | 140.5 ± 2.77 | 140.6 ± 2.30 | 140.1 ± 2.41 |
| Potassium (mmol/L) | 3.6–5.1 | 3.93 ± 0.29 | 3.95 ± 0.33 | 3.96 ± 0.45 | 3.86 ± 0.37 |
| Total carbon dioxide (mmol/L) | 18–33 | 23.97 ± 2.12 | 24.94 ± 2.06[a] | 24.33 ± 2.53 | 25.0 ± 1.39 |
| Chloride (mmol/L) | 98–108 | 105.06 ± 2.39 | 104.86 ± 2.51 | 104.47 ± 2.57 | 104.92 ± 1.94 |
| Glucose (mg/dL) | 73–118 | 90.20 ± 9.09 | 89.68 ± 9.45 | 87.85 ± 11.37 | 91.24 ± 10.41 |
| Calcium (mg/dL) | 8.0–10.3 | 9.37 ± 0.30 | 9.47 ± 0.29 | 9.44 ± 0.56 | 9.38 ± 0.26 |
| Blood urea nitrogen (mg/dL) | 7–22 | 13.03 ± 3.37 | 13.20 ± 3.84 | 13.56 ± 4.51 | 13.15 ± 3.26 |
| Creatinine (mg/dL) | 0.6–1.2 | 0.95 ± 0.19[c] | 0.90 ± 0.14 | 0.91 ± 0.19 | 0.92 ± 0.15 |
| Alkaline phosphatase (U/L) | 42–144 | 52.80 ± 15.99 | 53.82 ± 14.13[b] | 53.58 ± 13.95 | 52.34 ± 14.08[a,b] |
| Alanine aminotransferase (U/L) | 10–47 | 28.23 ± 13.29 | 29.0 ± 14.79 | 23.64 ± 9.38 | 24.72 ± 12.22 |
| Aspartate aminotransferase (U/L) | 11–38 | 33.66 ± 15.65 | 30.14 ± 7.95[a,b] | 29.67 ± 6.57 | 29.62 ± 6.79[b] |
| Total bilirubin (mg/dL) | 0.2–1.6 | 1.03 ± 0.39 | 1.01 ± 0.47 | 0.97 ± 0.45 | 1.01 ± 0.41 |
| Albumin (g/dL) | 3.3–5.5 | 3.86 ± 0.21 | 3.89 ± 0.23 | 3.90 ± 0.35 | 3.85 ± 0.23 |
| Total protein (g/dL) | 6.4–8.1 | 7.37 ± 0.34 | 7.38 ± 0.29 | 7.36 ± 0.51 | 7.33 ± 0.31 |

*Note*. Values are means ± standard deviations.
[a]Denotes a significant difference ($p < 0.05$) between the baseline and treatment group after adjusting for period effects.
[b]Denotes a significant difference ($p < 0.05$) between the treatment and placebo endpoints after adjusting for period effects.
[c]Denotes a difference between the 2 baselines. The letter is placed in the baseline column whose sequence is driving this difference (i.e., a letter after the treatment baseline value indicates sequence effects in the treatment to placebo cohorts).

**Table 3.** Changes in Gastrointestinal Symptom Severity Scores with Treatment and Placebo

| | Treatment | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Change from baseline (SEM) | Reduced symptom severity | Increased symptom severity | Change from baseline (SEM) | Reduced symptom severity | Increased symptom severity |
| Section A | −3.46 (1.05) | 45% | 0% | −2.31 (1.02) | 61% | 13% |
| Section B | −1.58 (1.15) | 32% | 5% | −4.37 (1.47) | 52% | 5% |
| Section C | −3.85 (1.85) | 32% | 12% | −6.70 (2.86) | 40% | 16% |
| Section D | −2.58 (1.05) | 30% | 0% | −4.03 (2.02) | 38% | 17% |

*Note*. Changes in symptom severity were determined by score changes that resulted in reclassification of symptoms for a given category (i.e., moderate to mild).

after treatment than before treatment ($p = 0.03$) and they were also significantly lower at the end of the treatment period compared to the end of the placebo period ($p = 0.04$). ALP levels were significantly higher at the end of the placebo period relative to the start of placebo consumption ($p = 0.05$); however, they were lower than at the endpoint of the treatment period ($p < 0.01$). $tCO_2$ increased after the treatment relative to the beginning of the period ($p = 0.02$) but was not significantly different from the placebo period endpoint measure. Finally, it is important to note that although some of the changes in metabolic parameters were statistically significant, the average values of the analytes were well within clinical reference ranges at all measurements.

## Discussion

The PHAGE study was a randomized, double-blind, placebo-controlled crossover trial that was designed to carefully assess the safety, tolerability, and utility of a bacteriophage intervention that has great potential for promoting intestinal health and reducing suffering from gastrointestinal distress. Bacteriophages offer a novel and selective means of modifying the gut microbiota and thereby influencing the intestinal environment. Although bacteriophages are ubiquitous in the environment and are consistently consumed by humans in small amounts, opinions concerning the risks of bacteriophage interventions have fluctuated greatly over the past century. There have been a limited number of studies exploring the safety and tolerability of intentional consumption of bacteriophages and these studies have primarily been conducted in non-Western populations. Our data suggest that the bacteriophages present in the commercial product, PreforPro, are safe for daily human consumption. We observed a small but statistically significant increase in $tCO_2$ after PreforPro treatment. Low levels of $tCO_2$ in the blood could be indicative of chronic diarrhea, and bicarbonate

solutions are often used for oral rehydration in patients with watery diarrhea (22). The observed increase may be associated with reduced diarrhea in our test population, although we did not specifically monitor this parameter. We also observed lower AST and ALP in samples collected after the treatment compared to after the placebo control. In a previous study in mice, ALT and ALP increased after exposure to bacterial lipopolysaccharides, which are a component of the cell membrane of Gram-negative bacteria (23). Circulating lipopolysaccharide (LPS) is associated with system inflammation and tissue damage leading to the development of metabolic disease like type 2 diabetes (5). Because the bacteriophages used specifically target proinflammatory, gram-negative *E. coli*, it is plausible that the treatment can reduce circulating endotoxin through modulation of the gut microbiota and intestinal barrier function. Unfortunately, measures of LPS in plasma are notoriously unreliable so we were unable to measure this outcome.

It is important to note that despite any statistical differences in the measured metabolic parameters, all measurements remained within clinically accepted ranges after 28 days of consumption, highlighting its safety in a human population. This is in agreement with a recent study of 15 healthy adults in Bangladesh, in which the investigators reported no adverse events observed by self-report or clinical examination and clinically normal laboratory tests for liver, kidney, and hematological function after administration of a 9-phage cocktail dose of $3 \times 10^9$ and $3 \times 10^7$ plaque-forming units (24).

To determine the tolerability of consumption in a target population, we specifically recruited a population that was healthy but suffered from gastrointestinal complaints. There were no reports of adverse events during the trial, and self-assessment of gastrointestinal symptoms suggested improvements in most parameters measured. Interestingly, gastrointestinal symptom severity was reduced in most cases during both the treatment and placebo periods. In addition, there were bidirectional crossover effects, suggesting that participants perceived relief of gastrointestinal distress just from participating in the study. Regardless, the majority of individuals reduced or maintained baseline levels of gastrointestinal distress while on the treatment, suggesting that it was tolerable and did not exacerbate symptoms.

PreforPro is currently marketed as both a food and dietary ingredient and research in animal models demonstrates that when consumed simultaneously with probiotic bacteria, it stimulates their growth. The theoretical basis of this relationship is an alteration in biochemical cycles due to release of cell contents from phage targets that support the growth of other bacteria (25). Prebiotics are gaining widespread popularity as dietary supplements and are typically based on formulations of resistant fiber and oligosaccharides. However, typical prebiotics are often associated with increased flatulence, bloating, and other undesirable symptoms. Therefore, due to its high tolerability in a population of individuals with gastrointestinal distress, the treatment may prove to be a viable substitute for more traditional prebiotics.

One major strength of this study is its crossover design, in which each individual serves as his or her own control. This is advantageous given the interperson variability of the microbiota, particularly in target bacterial strains, as well as individual responses to a stimulus. An additional strength of this study design is the double-blinding, thus minimizing participant and researcher bias. Limitations of this study, like others, included variable compliance among study participants. In addition, lack of dietary assessment throughout the study may be seen as a weakness, as changes in diet are typical of consumers over a 10-week period and can promote or suppress gastrointestinal symptoms over short periods of time.

Daily tracking of stool consistency and frequency would have been desirable but likely would have placed an additional burden on participants, reducing compliance and increasing attrition. Finally, although the small sample size does not allow for direct assessment of subpopulations (e.g., ethnic or life-stage groups), it does provide precise intervention effects at the population level on designated outcomes of interest and offers an overall risk–benefit assessment of whether individuals should consume bacteriophages-containing products.

## Conclusions

Consumption of therapeutic doses of a mixture of 4 bacteriophages was both safe and tolerable in a target human population of healthy individuals reporting moderate GI distress. Our study suggests that bacteriophage may be used as a dietary supplement in healthy individuals with mild to moderate gastrointestinal distress without exacerbating symptoms. Future analyses of samples collected during this trial will focus on direct effects of phage consumption on the gut microbiota and intestinal and systemic inflammatory markers. However, larger and longer duration studies in populations with varying medical conditions are needed to fully elucidate their potential in human health.

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## Author disclosures

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