A Randomized, Double-blind, Placebo-controlled, Crossover Study to Investigate the Effect of an Arabinogalactan Product for 6 Weeks on the Gut Microbiome in Adults

Study Report
BIO-1906

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CONFIDENTIAL

STUDY REPORT SIGNATURE SHEET

Study Report BIO-1906

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By my signature below, I approve of this study report.

August 13th 2020

Sponsor Company: _____ _____
Signature Date

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1 ABBREVIATIONS AND DEFINITIONS

µg	microgram
AAHRPP	Association for the Accreditation of Human Research Participation Protection Programs
AE	adverse events
BMI	body mass index
BP	blood pressure
bpm	beats per minute
BSS	Bristol Stool scale
CFR	Code of Federal Regulations
dL	deciliter
eCRF	electronic case report forms
ET	Early Termination
FDA	Food and Drug Administration
fL	femtoliter
g	gram
GCP	Good Clinical Practices
GI	gastrointestinal
GRAS	Generally Recognized as Safe
h	hour
HIPAA	Health Insurance Portability and Accountability Act
IQR	interquartile range
IRB	Institutional Review Board
ITT	intent-to-treat
K/µL	kilo per microliter
kcal	kilocalorie
kg	kilogram
L	liter
m	meter
M/µL	million per microliter
Max	maximum
mg	miligram
Min	minimum
mm Hg	millimeter Mercury
mmol	millimole
mOsm	milliosmole
n	sample size
N/A	not applicable
NDC	non-digestible carbohydrate
pg	pictogram
PP	per protocol
SCFA	short-chain fatty acids
SD	standard deviation
SEM	standard error of the mean
SF-36	36-item Short Form Health Survey
U	International unit

2 SYNOPSIS

PROJECT TITLE	A Randomized, Double-blind, Placebo-controlled, Crossover Study to Investigate the Effect of an Arabinogalactan Product for 6 Weeks on the Gut Microbiome in Adults (ClinicalTrials.gov identifier: NCT04351841)
SPONSOR	Lonza, Inc.
SPONSOR LEAD	Aouatef Bellamine Ph.D.
RESEARCH OBJECTIVE	The objective of this study is to investigate the effect of ResistAid® on the gastrointestinal microbiota in healthy adults.
STUDY DESIGN	A randomized, double-blind, cross-over study with 3-week washout was conducted to assess the effects of consuming 15 g/day maltodextrin (Placebo) or ResistAid for 6 weeks on the fecal microbiota in healthy adults. Gut microbiome was measured prior to supplementation (Week 0) and after 3 and 6 weeks on the respective supplementations. Fecal short-chain fatty acids (SCFA) were assessed prior to the supplementation (baseline and at the end of washout) and after 6 weeks on the respective supplementations.
SUBJECTS	Healthy men and women (n=30, 16 females, 14 males), aged 21 to 59 years, with body mass index (BMI) 18.8 to 31.6 kg/m^2. Prior to any supplementation (at Week 0), subjects reported having 2-6 bowel movements/week.
MAIN OUTCOMES	The primary focus of this trial is to characterize the fecal microbiome including diversity and composition and short chain fatty acids (SCFA) after 6 weeks of study product consumption. Secondary outcomes included the following at baseline and after 3 and 6 weeks of study product consumption: • Plasma SCFA • Stool frequency: number of bowel movements recorded over the 3-day collection period • Stool consistency as measured by the 7-point Bristol Stool scale (BSS): 3-day average calculated as the sum of reported scores on the BSS divided by the number of bowel movements • Straining during bowel movements as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements • Discomfort during bowel movements as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements

	• Sensation of incomplete evacuation as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements • 8 domains of the 36-item Short Form Health Survey (SF-36): physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions
STUDY PRODUCT	Subjects consumed one serving/day of the following products for 6 weeks in a randomized, cross-over fashion: • Placebo: 15 g maltodextrin/ day • Active: 15 g ResistAid/ day Subjects were dispensed sachets containing the study products and were instructed to prepare a beverage by thoroughly mixing the placebo or active study product with water and the provided True Lemon flavoring mix each day prior to consumption. Subjects then consumed the entire self-prepared beverage, and rinsed the beverage container with some water to be sure the entire study product was consumed. Study product was consumed in the morning, with or without breakfast.
SUBJECTS & ANALYSIS POPULATIONS	The intent-to-treat (ITT) sample population consisted of all subjects randomized to the study (n=30), whereby two early termination subjects contributed baseline values only: one subject to the active group and a second subject to the placebo group. The per protocol (PP) population (n=21) excluded four early termination subjects, two subjects who failed to replicate their diet, two subjects who experienced cold/flu and consumed cold/flu medication during the supplementation period, and one subject who experienced unstable weight gain and loss (-6 to +4.5%) during the study period.
RESULTS SUMMARY	Compared to 15 g maltodextrin/ day, consumption of 15 g ResistAid/ day for 6 weeks by healthy adults resulted in a decrease in the ratio of fecal *Firmicutes* to *Bacteriodetes* driven by an increase in *Bacteriodetes* and a decrease in *Firmicutes*. Additionally, compared to 15 g maltodextrin/ day, 15 g ResistAid/ day for 6 weeks decreased alpha-diversity of fecal microbiome assessed by the Shannon Index and Simpson's Index, without affecting beta-diversity. PICRUSt analysis predicted five pathways, i.e., alpha-L-rhamnosidase, Chondroitin-sulfate-ABC endolyase, Chondroitin-sulfate-ABC exolyase, Succinate--CoA ligase, UDP-N-acetylglucosamine 2-epimerase, are significantly higher following 15 g ResistAid/ day compared to 15 g maltodextrin/ day. Fecal isovaleric acid, valeric acid, and hexanoic acid, but not acetic acid (marginal effect), butyric

	acid, isobutyric acid, and propionic acid were lower following 15 g ResistAid/ day compared to 15 g maltodextrin/ day. There were no significant differences in plasma SCFA between supplementations. A high proportion of subjects did not have bowel movement problems in regards to straining, discomfort, and incomplete evacuation. There were no statistically significant changes in number of bowel movements, stool consistency, straining, discomfort, and sensation of evacuation ratings, the proportion of subjects reporting moderate or severe issues or in the severity ratings for the individual gastrointestinal symptoms, and ratings for physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. Two adverse events were reported in one subject during the ResistAid supplementation (moderate bloating and mild decreased ease of bowel movement). No abnormal results for the chemistry profile, metabolic panel, and vitals were noted by the study physician. One subject lost 8.3% body weight during the placebo supplementation.

3 ETHICS

3.1 Conduct of Study and Confidentiality

This study was carried out in compliance with the protocol and in accordance with Good Clinical Practices (GCP) as described in the ICH Guidelines for GCP 1996, the applicable US Code of Federal Regulations (CFR) [i.e., 21 CFR including parts 50 and 56 concerning informed consent and Institutional Review Board (IRB) regulations], and the Declaration of Helsinki (2013 Version). Signed written informed consent for participation in the study was obtained from all subjects before protocol-specific procedures were carried out.

Subjects were informed of their right to withdraw from the study at any time. The study was explained verbally, as well as on the IRB-approved informed consent document. Each subject was given ample opportunity to inquire about details of the study and to read and understand the consent form before signing it. Consent was documented by the dated signature of the subject and each subject received a copy of the written informed consent document after signature. The informed consent document also included Health Insurance Portability and Accountability Act (HIPAA)-compliant wording, by which subjects authorized the use and disclosure of their Protected Health Information by the Investigator and by those persons who need that information for the purposes of this study. Subjects were also advised that the Sponsor, its employees or agents, the IRB, as well as representatives of the Food and Drug Administration (FDA), would have the right to audit and review pertinent medical records relating to this clinical trial as part of the informed consent.

Subjects' anonymity was maintained on electronic case report forms (eCRFs) and other documents by utilization of initials, number, or code, and not by using a subject's name. The Investigator kept a separate log showing codes, names, and addresses. All documents showing the subjects' identity were kept in strict confidence by the Investigator.

3.2 Institutional Review Board

The study protocol (Appendix SR1) was approved by IntegReview on August 2, 2019 prior to study commencement and subject recruitment. A protocol clarification memo was provided to the IRB on August 19, 2019 clarifying the exact flavoring agent used in the study and the timing of the first study product consumption (i.e., Day 0 instead of Day 1). A separate protocol clarification memo was provided to the IRB on October 29, 2019 clarifying the amount of blood drawn. IntegReview is fully accredited by the Association for the Accreditation of Human Research Participation Protection Programs (AAHRPP).

4 INTRODUCTION

4.1 Rationale

Dietary fiber is an important nutrient that supports gastrointestinal function, as well as the maintenance of blood glucose and cholesterol. Additionally, it is suggested that dietary fiber may provide other health benefits, such as maintenance of healthy weight through effects on satiety. The National Academy of Medicine established an adequate intake for fiber as 14

g/kcal, or 38 g and 25 g for men and women, respectively (IOM 2002). Currently, however, the majority of the U.S. population falls substantially below this level, with mean intakes of 18.9 g/day and 15.7 g/day in men and women age 20 and older, respectively (NHANES 2015-2016). Therefore, the average intake of fiber in the U.S. is ~40-50% below adequate intake levels.

Arabinogalactans, an isolated non-digestible carbohydrate (NDC), are hemicelluloses that are abundant in plants and, as such, have been part of the human diet for thousands of years (Dion 2016). Arabinogalactans are found in seeds, leaves, roots, and fruit of higher plants, such as cereals, beans, leeks, pear, corn, and wheat (Saeed 2011; Dion 2016). Specifically, arabinogalactans are proteoglycans that are composed primarily of D-galactose and L-arabinose linked together in a (1→3)-ß-D-galactopyranan backbone with (1→6)-linked side-chains (Dion 2016). Commercially, larch is a major source for arabinogalactans (Saeed 2011).

ResistAid®, an arabinogalactan product is isolated from larch (*Larix laricina*) using a patented water-based extraction process. ResistAid has been designated as Generally Recognized as Safe (GRAS) by the US FDA (2000) for multiple uses and has been used in numerous previous clinical studies in humans, with no significant safety issues observed at intakes of up to 30 g daily for up to 6 weeks (Robinson 2001). For example, Grube et al (2012) assessed the tolerability of 4.5 g/d ResistAid compared to a maltodextrin placebo over 12 weeks in healthy adults (n=101 and 98, respectively) and found no statistically significant or clinically-relevant differences between placebo and ResistAid. Robinson et al (2001) reported on the GI tolerance and microbiota effects of 15 g and 30 g of a different preparation of arabinogalactan and reported the product was well tolerated and resulted in significant increases in certain microbial populations considered to be beneficial (e.g., *Lactobacillus spp.)*

This study was designed to investigate the effect of daily consumption of 15 g of ResistAid on the gastrointestinal microbiota in healthy adults.

4.2 Objective

The objective of this study was to investigate the effect of ResistAid on the gastrointestinal microbiota in healthy adults.

4.3 Primary Outcome Variable

The primary focus of this trial was to characterize the fecal microbiome including diversity and composition, and short chain fatty acids (SCFA) by product after 6 weeks of study product consumption.

4.4 Secondary Outcome Variables

Secondary outcomes included the following for Weeks 3 and 6 of study product consumption:

- Plasma SCFA
- Stool frequency: number of bowel movements recorded over the 3-day collection period
- Stool consistency as measured by the 7-point Bristol Stool scale (BSS): 3-day average calculated as the sum of reported scores on the BSS divided by the number of bowel movements
- Straining during bowel movements as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements

- Discomfort during bowel movements as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements
- Sensation of incomplete evacuation as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements
- 8 domains of the 36-item Short Form Health Survey (SF-36): physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions

5 STUDY DESIGN & CONDUCT

The study was a randomized, crossover, single-center trial with one screening visit (Visit 1; Week -1) and 2 test periods [Test Period I (Visits 2, 3, and 4; Weeks 0, 3, and 6) and Test Period II (Visits 5, 6, and 7; Weeks 9, 12 and 15)] separated by a minimum 3-week washout period.

At Visit 1 (Week -1), subjects provided informed consent and completed assessments of medical history and prior, current medication/supplement use, and inclusion and exclusion criteria and a last menses query, where applicable. Additionally, height, body weight, and vital signs were measured and BMI was calculated. Fasting (12 ± 2 h) blood samples were collected for chemistry and hematology, and female subjects completed an in-clinic urine pregnancy test. Study and test day instructions were provided [(i.e., fasting compliance (12 ± 2 h, water only); maintenance of physical activity; and refraining from vigorous physical activity (i.e., sweat-inducing exercise, 24 h), and alcohol consumption (24 h) prior to and during Visit 2 (Week 0)]. Subjects were counseled to maintain habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha). Subjects were dispensed a Baseline Diet Diary with instructions to record intake 7 days prior to Visit 2 (Week 0). Subjects were also dispensed a gastrointestinal (GI) and Bowel Habits Diary and stool collection kit. Subjects were instructed to complete the GI and Bowel Habits Diary during the 3 days prior to Visit 2 (Week 0) and to collect fecal samples from one bowel movement during the 3 days prior to Visit 2 (Week 0).

At Visit 2 (Week 0), subjects arrived at the clinic fasted (12 ± 2 h, water only) to complete clinic visit procedures (concomitant medication/supplement use, assess inclusion/exclusion criteria, body weight and vital signs measurements, last menses query, where applicable). Adverse events (AE) were assessed and the GI Tolerance Questionnaire and SF-36 Questionnaire were administered. Subjects were queried about compliance with study and test day instructions. Fecal samples were collected and the GI and Bowel Habits Diary and Baseline Diet Diary were collected and reviewed. Fasting (12 ± 2 h) blood samples were collected and archived for possible later analysis of non-genetic indicators of physiology. Subjects were assigned to a randomization sequence. Subjects then prepared their first study product with guidance from study staff and the product was consumed in the clinic. Subjects were also dispensed study products according to their assigned randomization sequence for home consumption (1 serving per day in the morning, with or without breakfast). Subjects were dispensed a Study Product Diary to record study product intake. Subjects were dispensed a GI and Bowel Habits Diary and stool collection kit, and were instructed to complete the GI and Bowel Habits Diary during the 3 days immediately prior to Visit 3 (Week 3) and to collect fecal samples from one bowel movement during the same 3 days immediately prior to Visit 3 (Week 3). Subjects were

dispensed a copy of 5 days of their completed Baseline Diet Diary (reviewed at Visit 2, Week 0) that overlapped with the 3-day period of the GI and Bowel Habits Diary. Subjects were instructed to replicate the same food and beverage intake as closely as possible during the days prior to the collection of their fecal samples, which occurred during the 3 days immediately prior to Visit 3 (Week 3). Study instructions were also provided [(i.e., maintenance of physical activity; maintenance of habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha)].

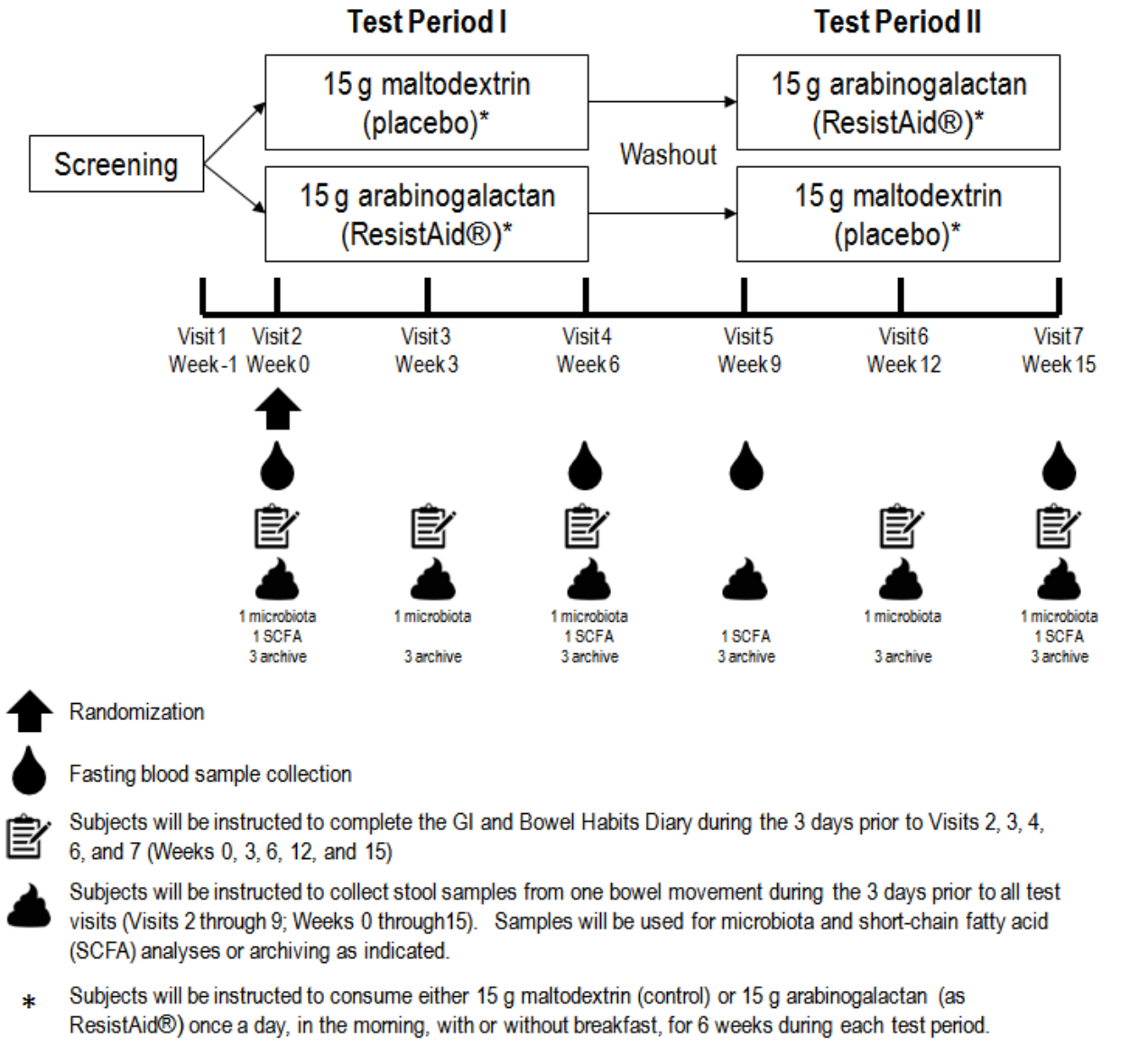
At Visit 3 (Week 3), subjects returned to the clinic for clinic visit procedures (i.e., concomitant medication/supplement use, assess inclusion/exclusion criteria, body weight and vital signs measurements, last menses query, where applicable). AEs were assessed and the GI Tolerance Questionnaire and SF-36 Questionnaire were administered. Subjects were queried about compliance with study instructions. The fecal samples were collected and the GI and Bowel Habits Diary were collected and reviewed. The Study Product Diary was collected/reviewed, any unused study products were collected, and compliance with study product consumption was assessed. Study product was re-dispensed back to subjects (with additional study product if necessary) for home consumption (one sachet per day in the morning, with or without breakfast) along with blank Study Product Diary to record study product intake. Additionally, subjects were dispensed 3-day Analysis Diet Records with instructions to record all food and beverages consumed during 3 days (2 weekdays and one weekend) following Visit 3 (Week 3) that did not coincide with the 5 diet replication days immediately prior to Visit 4 (Week 6). Subjects were dispensed a GI and Bowel Habits Diary and stool collection kit, and were instructed to complete the GI and Bowel Habits Diary during the 3 days immediately prior to Visit 4 (Week 6) and to collect fecal samples from one bowel movement during the same 3 days immediately prior to Visit 4 (Week 6). Subjects were dispensed a copy of 5 days of their completed Baseline Diet Diary (reviewed at Visit 2, Week 0) that overlapped with the 3-day period of the GI and Bowel Habits Diary. Subjects were instructed to replicate the same food and beverage intake as closely as possible during the days prior to the collection of their fecal samples, which occurred during the 3 days immediately prior to Visit 4 (Week 6). Study and test day instructions were provided [(i.e., fasting compliance (12 ± 2 h, water only); maintenance of physical activity; maintenance of habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha); and refraining from vigorous physical activity (i.e., sweat-inducing exercise, 24 h) and alcohol consumption (24 h) prior to and during Visit 4 (Week 6)].

At Visit 4 (Week 6), subjects arrived at the clinic fasted (12 ± 2 h, water only) to undergo clinic visit procedures (concomitant medication/supplement use, assess inclusion/exclusion criteria, body weight, and vital signs measurements, last menses query, where applicable). AEs were assessed and the GI Tolerance Questionnaire and SF-36 Questionnaire were administered. Subjects were queried about compliance with study instructions. The fecal samples were collected and GI and Bowel Habits Diary and the 3-day Analysis Diet Record were collected and reviewed. The Study Product Diary was collected/reviewed, any unused study products were collected, and compliance with study product consumption was assessed. Fasting (12 ± 2 h) blood samples were collected for chemistry and hematology and archived for possible later analysis of non-genetic indicators of physiology. Subjects were dispensed a stool collection kit and were instructed to collect fecal samples from one bowel movement during the 3 days immediately prior to Visit 5 (Week 9). Subjects were also dispensed a copy of 5 days of their completed Baseline Diet Diary (reviewed at Visit 2, Week 0) that overlapped with the 3-day

period of the fecal sample collection. Subjects were instructed to replicate the same food and beverage intake as closely as possible during the days prior to the collection of their fecal samples, which occurred during the 3 days immediately prior to Visit 5 (Week 9). Study and test day instructions were provided [(i.e., fasting compliance (12 ± 2 h, water only); maintenance of physical activity; maintenance of habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha); and refraining from vigorous physical activity (i.e., sweat-inducing exercise, 24 h) and alcohol consumption (24 h) prior to and during Visit 5 (Week 9)]. Subjects were instructed to begin the 3-week washout period and return to the clinic to begin Test Period II at Visit 5 (Week 9).

At Visit 5 (Week 9), subjects returned to the clinic fasted (12 ± 2 h, water only), crossed over to the other study product in their test sequence, and repeated the procedures from Visits 2 (Week 0) with the exclusion of the randomization procedure. At Visit 6 (Week 12), subjects repeated the procedures from Visit 3 (Week 3) and at Visit 7 (Week 15), subjects repeated relevant in-clinic procedures from Visit 4 (Week 6) and concluded the trial.

Figure 1. Study design



Please refer to the protocol for more details (Appendix SR1).

5.1 Flow Chart

	Screen	Test Period 1			Test Period II		
Visit[1]	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Weeks	**-1**	**0**	**3**	**6**	**9**	**12**	**15**
Informed Consent/HIPAA[2]	X						
Medical History	X						
Clinic Visit[3]	X	X	X	X	X	X	X
In-clinic Urine Pregnancy Test[4]	X						

	Screen	Test Period 1			Test Period II		
Visit[1]	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Weeks	**-1**	**0**	**3**	**6**	**9**	**12**	**15**
Chemistry Profile[5]	X			X			X
Hematology Profile[6]	X			X			X
Randomization		X					
Study/Test Day Instruction Review/Query[7]	X	X	X	X	X	X	X
Dispense Baseline Diet Diary for Habitual Diet[8]	X						
Collect and Review Baseline Diet Diary[8]		X					
Dispense Copy of Baseline Diet Diary for Replication during Stool Collection Period[9]		X	X	X	X	X	
Dispense 3-day Analysis Diet Record[10]			X			X	
Collect/Review 3-day Analysis Diet Record[10]				X			X
In-clinic Study Product Consumption		X			X		
Dispense Study Product		X	X		X	X	
Dispense Study Product Diary		X	X		X	X	
Collect Unused Study Product and Collect/Review Study Product Diary[11]			X	X		X	X
Dispense Stool Collection Kit[12]	X	X	X	X	X	X	
Stool Collection (for archiving)[13]		X	X	X	X	X	X
Stool Microbiome[14]		X	X	X		X	X
Stool Short-chain Fatty Acids[15]		X		X	X		X
Dispense GI and Bowel Habit Diary[16]	X	X	X		X	X	
Collect/Review GI and Bowel Habit Diary		X	X	X		X	X
Fasting blood collection (for archiving)[17]		X		X	X		X
GI Tolerance Questionnaire[18]		X	X	X	X	X	X
SF-36 Questionnaire		X	X	X	X	X	X
AE Assessment		X	X	X	X	X	X

Footnotes:

[1]A window of +1 week was allowed for Visits 2 and 5, anchored to Visits 1 and 4, respectively. A window of +3 d was allowed for Visits 3 and 4, anchored to Visit 2, and for Visits 6 and 7, anchored to Visit 5.

[2]HIPAA = Health Insurance Portability and Accountability Act authorization for disclosure of protected health information. Signed document authorized the use and disclosure of the subject's Protected Health Information by the Investigator and by those persons who need that information for the purposes of the study.

[3]Clinic visit procedures included measurement of height (Visit 1 only), vital signs, and body weight, calculation of BMI (visit 1 only), evaluation of inclusion and exclusion criteria, concomitant medication/supplement use, and last menses query (where applicable).

[4]Urine pregnancy tests were completed for all women.

[5]Fasting (12 ± 2 h) chemistry profile.

[6]Fasting (12 ± 2 h) hematology panel.

[7]Study instructions were provided [(i.e., maintenance of physical activity; maintenance of habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha)]. Additionally, subjects were instructed to fast (12 ± 2 h, water only) and refrained from vigorous physical activity (i.e., sweat-inducing exercise, 24 h), use of tobacco products (1 h) and alcohol consumption (24 h) prior to and during Visits 1, 2, 4, 5, and 7 (Weeks -1, 0, 6, 9, and 15).

[8]Food and beverage intake for 7 days were assessed by the Baseline Diet Diary after screening [between Week -1 and 0) whereby 3 days (2 weekdays and one weekend)] were analyzed for habitual nutrient intakes.

[9] Subjects were dispensed a copy of 5 days of their completed Baseline Diet Diary (reviewed at Visit 2, Week 0) that overlapped with the 3-day period of the GI and Bowel Habits Diary. Subjects were instructed to replicate the same food and beverage intake as closely as possible during the days prior to the collection of their fecal samples, which occurred during the 3 days immediately prior to Visit 3 (Week 3).

[10]Subjects were instructed to record all food and beverages consumed during 3 days (2 weekdays and one weekend) following Visits 3 and 6 (Weeks 3 and 12) that did not coincide with the 5 replication days immediately prior to Visits 4 and 7 (Weeks 6 and 15). These were analyzed for nutrient intakes during the supplementation period.

[11]Counting of unused study products was used to assess compliance.

[12]Stool collection kit was dispensed for the collection of fecal samples. All collected fecal samples were stored in a freezer. The samples were transported in a cooler and brought to the study site at the next scheduled visit.

[13]Collection and archiving of three cryotube fecal samples at all tests visits.

[14]16s RNA sequencing was performed to assess microbial diversity and composition in fecal samples (1 tube per visit; OMNIgene method).

[15]Collection of 1 cryotube for short-chain fatty acid analysis per visit.

[16]GI and Bowel Habits Diary was collected during the 3 days prior to each test visit (Visits 2 through 7; Weeks 0 through 15).

[17]Collection of samples for possible future analyses of markers of inflammation and intestinal integrity in blood (e.g., high sensitivity C-Reactive Protein, zonulin, lipopolysaccharide binding protein).

[18] Subjects were asked a series of questions regarding the presence and severity of GI symptoms occurring during the past 3 weeks, in which each response is ranked on a 4-point scale ranging from none to severe. Individual components included the severity of GI symptoms including: gas/flatulence, nausea, vomiting, abdominal cramping, abdominal distention/bloating, borborygmus/stomach rumbling, burping, and/or reflux (heartburn).

6 SUBJECTS

6.1 Selection of Subjects

Subjects were men and women, 18 to 60 years of age (inclusive), each with a BMI 18-32.0 kg/m^2 (inclusive) and with self-reported regular bowel movement at Visit 1 (Week -1). Each subject had to meet all of the inclusion criteria and none of the exclusion criteria listed below and in the protocol (Appendix SR1) at the screening visit in order to participate in this study.

6.1.1 Inclusion Criteria

Subjects were included in the study when they met any of the following inclusion criteria, unless waivers were provided (see Section 10):

1. Male or female, 18-60 years of age, inclusive at Visit 1 (Week -1).
2. BMI of 18.0 to 32.0 kg/m^2, inclusive, at Visit 1 (Week -1).
3. Self-reported regular bowel movement at Visit 1 (Week -1).
4. Non-user of all tobacco and smoking products (including, but not limited to cigarettes, cigars, chewing tobacco, e-cigarettes) and nicotine products (e.g., nicotine patches, nicotine gums) and has no plans to change smoking habits during the study period.
5. Non-user of any marijuana or hemp products and has no plans to use marijuana or hemp products during the study period.
6. Willing to maintain physical activity patterns, body weight, and habitual diet throughout the trial, except for exclusion of fermented foods that do or might contain live probiotics (e.g., yogurt, kombucha) and inclusion of study products.
7. Willing to abstain from alcohol consumption and avoid vigorous physical activity for 24 h prior to and during Visits 1, 2, 4, 5, and 7 (Weeks -1, 0, 6, 9, and 15).
8. Willing to refrain from exclusionary medications, supplements, and products throughout the study.
9. Willing and able to comply with the visit schedule and fecal sample collection/processing/storage requirements during the study period.
10. No health conditions that would prevent him/her from fulfilling the study requirements as judged by the Clinical Investigator on the basis of medical history and routine laboratory test results.
11. Understands the study procedures and signs forms providing informed consent to participate in the study and authorizes the release of relevant protected health information to the Clinical Investigator.

6.1.2 Exclusion Criteria

Subjects were to be excluded from study participation when they met any of the following exclusion criteria, unless waivers were provided (see Section 10):

1. Abnormal laboratory test results of clinical significance at Visit 1 (Week -1), at the discretion of the Clinical Investigator. One re-test will be allowed on a separate day prior to Visit 2 (Week 0), for subjects with abnormal laboratory test results.
2. Clinically important GI condition that would potentially interfere with the evaluation of the study product (e.g., inflammatory bowel disease, irritable bowel syndrome, gastric reflux, indigestion, dyspepsia, Crohn's disease, celiac disease, history of surgery for weight loss, gastroparesis, and clinically significant lactose and gluten intolerance or allergies).
3. Recent (within 2 weeks of Visit 1; Week -1) history of an episode of acute GI illness such as nausea/vomiting or diarrhea (defined as ≥3 loose or liquid stools/day).

4. Self-reported history (within 6 weeks of Visit 1; Week -1) of constipation (defined as <3 bowel movements per week).
5. History or presence of uncontrolled and/or clinically important pulmonary (including uncontrolled asthma), cardiac (including, but not limited to, atherosclerotic disease, history of myocardial infarction, peripheral arterial disease, stroke), hepatic, renal, endocrine, hematologic, immunologic, neurologic (such as Alzheimer's or Parkinson's disease), psychiatric (including depression and/or anxiety disorders) or biliary disorders.
6. Uncontrolled hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) as defined by the blood pressure measured at Visit 1 (Week -1). One re-test will be allowed on a separate day prior to Visit 2 (Week 0), for subjects whose blood pressure exceeds either of these cut points at Visit 1 (Week -1), in the judgment of the Clinical Investigator.
7. Known allergy intolerances or sensitivity to any of the ingredients in the study product.
8. Extreme dietary habits (e.g., Atkins diet/ketogenic diet, very high protein, very high fiber, vegetarian), in the opinion of the Clinical Investigator.
9. History or presence of cancer in the prior 2 years, except for non-melanoma skin cancer.
10. Major trauma or any other surgical event within 3 months of Visit 1 (Week -1).
11. Signs or symptoms of an active infection of clinical relevance within 5 days of Visit 1 (Week -1). The visit may be rescheduled such that all signs and symptoms have resolved (at the discretion of the Clinical Investigator) at least 5 days prior to Visit 1 (Week -1). If an infection occurs during the study period, test visits will be rescheduled until all signs and symptoms have resolved (at the discretion of the Clinical Investigator) at least 5 days prior to study visits.
12. Weight loss or gain >4.5 kg in the 3 months prior to Visit 1 (Week -1).
13. Currently or planning to be on a weight loss regimen during the duration of the study.
14. Antibiotic use within 2 months of Visit 1 (Week -1).
15. Use of steroids within 1 month of Visit 1 (Week -1).
16. Chronic use (i.e., daily on a regular basis) of anti-inflammatory medications (e.g., NSAIDS) within 1 month of Visit 1 (Week -1).
17. Use of medications (over-the-counter or prescription) and/or dietary supplements, known to influence GI function, including but not limited to prebiotics or probiotics, laxatives, enemas, fiber supplements and/or suppositories, anti-diarrheal agents, and/or anti-spasmodic within 2 weeks of Visit 1 (Week -1).
18. Bismuth subsalicylate (e.g., Pepto Bismol) and antacids (e.g., Tums) ≤2 times/ week starting from 2 weeks prior to Visit 1 (Week -1), with the exception of 7 days prior to the stool collection period, during which consumption of these products are not allowed.
19. Consumption of fermented foods or beverages that do or might contain live probiotics within 2 weeks of Visit 1 (Week -1).
20. Participated in colonoscopy or colonoscopy preparation within 3 months prior to Visit 1 (Week -1).

21. Exposure to any non-registered drug product within 4 weeks prior to Visit 1 (Week -1).
22. Recent history of (within 12 months of screening; Visit 1; Week -1) or strong potential for alcohol or substance abuse. Alcohol abuse is defined as >14 drinks per week (1 drink = 12 oz beer, 5 oz wine, or 1½ oz distilled spirits).
23. Had a condition the Clinical Investigator believed would interfere with his/her ability to provide informed consent, comply with the study protocol, which might confound the interpretation of the study results, or put the subject at undue risk.

6.2 Early Termination Procedures

If a subject decided to withdraw, the subject was designated as an Early Termination (ET). When a subject withdrew from the study, all efforts were made to complete and report observations as thoroughly as possible and the primary reason for the withdrawal was documented.

7 STUDY PRODUCTS

7.1 Study Foods

Placebo: 15 g maltodextrin/ day
Active: 15 g ResistAid/ day

Subjects were instructed to consume one serving daily for 6 weeks. Subjects were dispensed sachets containing the study product and were instructed to prepare a beverage by thoroughly mixing the study products with water and True Lemon flavoring each day prior to consumption. Subjects were instructed to consume the entire beverage, and rinse the beverage container with some water to be sure the entire study product was consumed. Study products were consumed in the morning, with or without breakfast.

8 MEASUREMENTS & METHODS

8.1 Clinical Assessments

Clinic visit procedures included measurement of height (Visit 1; Week -1 only), body weight (Visit 1 through 7; Weeks -1 through 15); BMI calculation (Visit 1; Week -1 only); and review of inclusion/exclusion criteria [for eligibility at Visit 1 (Week -1)] or major changes in health, diet, or lifestyle (at subsequent visits), last menses query (where applicable) and concomitant medication/supplement use.

Standardized vital signs measurements were assessed at each clinic visit and included resting blood pressure and heart rate measured using an automated blood pressure measurement device. Blood pressure was obtained after the subject had been sitting for at least 5 minutes. Systolic and diastolic pressures were measured once using an appropriate sized cuff (bladder within the cuff must encircle ≥80% of the arm). When necessary, clinic staff took a second blood pressure and heart rate and the second measurement was recorded in the electronic case report form (eCRF).

8.2 Laboratory Measurements

The procedures for all clinical laboratory measurements were outlined in a laboratory instruction document. Laboratory parameters that were missing were entered in the eCRF as "not done."

The following were performed at Visits 1, 4, and 7 (Weeks -1, 6, and 15) as a part of the fasting (12 ± 2 h) blood chemistry profile: Albumin, albumin/globulin ratio, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, anion gap, total bilirubin, calcium, carbon dioxide, chloride, creatinine, blood urea nitrogen, blood urea nitrogen/creatinine ratio, potassium, sodium, total protein, osmolality, globulin, glucose, and glomerular filtration rate.

The following were performed at Visits 1, 4, and 7 (Weeks -1, 6, and 15) as a part of the fasting (12 ± 2 h) blood hematology: White blood cell count, red blood cell count, hemoglobin concentration, hematocrit (as volume percent), mean corpuscular volume, mean corpuscular hemoglobin concentration, neutrophils, lymphocytes, monocytes, eosinophils, basophils, granulocytes, and platelet count.

Fecal samples for microbiome analyses were collected at Visits 2, 3, 4, 6, and 7 (Weeks 0, 3, 6, 12, and 15). No additional samples were stored as a backup.

Fecal samples for short-chain fatty acid analyses were collected at Visits 2, 4, 5, and 7 (Weeks 0, 6, 9, and 15). No additional samples were stored as a backup.

Fecal samples were collected and archived for possible later analysis of non-genetic indicators of physiology at Visits 2, 3, 4, 5, 6, and 7 (Weeks 0, 3, 6, 9, 12, and 15).

Fasting blood samples were also collected and archived for possible later analysis of non-genetic indicators of physiology at Visits 2, 4, 5, and 7 (Weeks 0, 6, 9, and 15).

An in-clinic urine pregnancy test was performed on all women at Visit 1 (Week -1).

8.2.1 Test Day Instructions/Query

Prior to Visits 2, 4, 5, and 7 (Weeks 0, 6, 9, and 15), subjects received the following test day instructions: fasting compliance (12 ± 2 h, water only); maintenance of physical activity; maintenance of habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha); and refraining from vigorous physical activity (i.e., sweat-inducing exercise, 24 h) and alcohol consumption (24 h) prior to and during Visits 2, 4, 5, and 7 (Weeks 0, 6, 9, and 15).

Prior to Visits 3 and 6 (Weeks 3 and 12), subjects received the following test day instructions: maintenance of physical activity; maintenance of habitual diet as much as possible with the exception of excluding fermented foods or beverages that do or might contain live probiotics (e.g., yogurt, kombucha).

A query regarding compliance with these instructions was performed at each test visit.

8.2.2 Baseline and 3-day Analysis Diet Records

8.2.2.1 Baseline Diet Diary

A Baseline Diet Diary was dispensed at Visit 1 (Week -1). Subjects were asked to record all food and beverages consumed 7 days (Weeks -1 to 0) prior to Visit 2 (Week 0). Three (2 weekdays and one weekend day) of the 7 recorded days were analyzed for selected nutrient

intakes, using the Food Processor® Nutrition Analysis & Fitness Software (version 10.4, Salem, OR). Subjects were dispensed a copy of 5 days of their completed Baseline Diet Diary (reviewed at Visit 2, Week 0) that overlapped with the 3-day period of the GI and Bowel Habits Diary. Subjects were instructed to replicate the same food and beverage intake as closely as possible during the days prior to the collection of their fecal samples, which occurred during the 3 days immediately prior to each subsequent test visit (Visits 3 through 7; Weeks 3 through 15).

8.2.2.2 3-day Analysis Diet Record

3-day Diet Records were dispensed at Visits 3 and 6 (Weeks 3 and 12). Subjects were instructed to record all food and beverages consumed during 3 days (2 weekdays and one weekend) following Visits 3 and 6 (Weeks 3 and 12) that did not coincide with the 5 diet replication days immediately prior to Visits 4 and 7 (Weeks 6 and 15). These were analyzed for nutrient intakes during the supplementation period, using the Food Processor® Nutrition Analysis & Fitness Software.

8.2.3 Study Product Diary

Subjects were dispensed a Study Product Diary at Visits 2, 3, 5, and 6 (Weeks 0, 3, 9, and 12). Subjects were instructed to record study product intake each day during each test period. Study Product Diary was collected and reviewed at Visits 3, 4, 6, and 7 (Weeks 3, 6, 12, and 15).

8.2.4 Gastrointestinal and Bowel Habit Diary

Subjects completed a GI and Bowel Habits Diary during the 3-day periods immediately prior to Visits 2, 3, 4, 6, and 7 (Weeks 0, 3, 6, 12, and 15). The diary provided information on stool frequency and consistency, straining and discomfort during bowel movement, and any sensation of incomplete evacuation.

8.2.5 Fecal Sample Collection and Analysis

Fecal samples were collected using Omnigene®-GUT DNA Genotek tube per manufacturer's instructions (for gut microbiome analyses) or cryotube (for short-chain fatty acid and archiving) and all samples were frozen immediately. Subjects were instructed to collect fecal samples from one bowel movement during the 3-day periods immediately prior to all test visits as follows:

- One sample collection using an Omnigene®-GUT DNA Genotek tube prior to Visits 2, 3, 4, 6, and 7 (Weeks 0, 3, 6, 12, and 15),
- One sample into a cryotube for short-chain fatty acid analysis prior to Visits 2, 4, 5, and 7 (Weeks 0, 6, 9, and 15), and
- Three samples into three cryotubes to be frozen immediately for archiving prior to each test visit.

Fecal samples were collected by subjects using the provided stool collection kit as instructed by study staff. The time and date the fecal sample was collected were recorded in the eCRF.

Microbiome in fecal samples was analyzed using a 16S rRNA sequencing method, performed by the University of Wisconsin Biotechnology Center at Madison.

Fecal short-chain fatty acids (SCFA; acetic, propionic, butyric, isobutyric, isovaleric, valeric, and hexanoic acids) were analyzed by Creative Proteomics (Shirley, NY) using a gas chromatography – mass spectrometry method. Briefly, approximately 1 g of each fecal sample was weighed into a 15 mL centrifugation tube and homogenized in 5 mL water by overnight shaking. The samples were centrifuged at 358 g and an aliquot of fecal water was used for derivatization. The aliquot of fecal water was diluted in water containing labeled internal standards for each chain length. Free SCFA were derivatized using methyl chloroformate in 1-propanol yielding propyl esters before subsequent liquid-liquid extraction into hexane and analysis on a Supelco SLB-5ms (30 m x 0.25 mm, 1.0 µm) column and detection using GC-EI-MS in SIM-mode. Quantification was performed against an external standard curve of seven points for each analyte. Quality and quantification parameters are as follows:

Analyte	Repeatability (CV%) n=3	Intermediate precision % n=15	Limit of quantification (µg/g)
Acetic acid	1.2	2.0	1.56
Propionic acid	2.7	5.1	1.56
Butyric acid	1.6	3.5	1.56
Isobutyric acid	1.7	5.4	1.56
Valeric acid	1.0	2.7	1.56
Isovaleric acid	1.3	2.5	1.56
Hexanoic acid	3.3	18.1	1.50

Plasma SCFA was analyzed by Pine Lake Laboratories using a gas chromatography – mass spectrometry method. Briefly, 100 mg of plasma was diluted with 8 mL water. The samples were centrifuged and the liquid layer was transferred into a separate tube. 1 mL of each extracted liquid layer was mixed with 950 µL methanol and 50 µL internal standard. An aliquot of this mixture was transferred into an appropriate vial for analysis on a Supelco Nukol (30 m x 0.25mm, 0.25 µm) column. For more details on the method, please refer to Appendix SR7.

8.2.6 GI Tolerance Questionnaire

At Visits 2 through 7 (Weeks 0 through 15), subjects were asked a series of questions regarding the presence and severity of GI symptoms occurring in the previous 3 weeks, in which each response was ranked on a 4-point scale ranging from none to severe. Individual components included the severity of GI symptoms including: gas/flatulence, nausea, vomiting, abdominal cramping, abdominal distention/bloating, borborygmus/stomach rumbling, burping, and/or reflux (heartburn).

8.2.7 SF-36 Questionnaire

At Visits 2 through 7 (Weeks 0 through 15), subjects completed the SF-36 Questionnaire. The SF-36 Questionnaire assessed eight health concepts: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. It also included a single item that provides an indication of perceived change in health.

8.2.8 AE Assessment

Open-ended questions for AE assessment were performed at Visits 2 through 7 (Weeks 0 through 15).

8.3 Compliance

Compliance was monitored throughout the study by the assessment of amount of unused study products collected at Visits 3, 4, 6, and 7 (Weeks 3, 6, 12, and 15). Final compliance was based on total study product consumed by the end of each test period [i.e., Visits 4 and 7 (Weeks 6 and 15)] and was recorded as a percent of scheduled intakes of study products. Non-compliance was defined as consumption of <80% or >120% of the scheduled intake (based on amount of unused study product collected back).

9 STATISTICAL METHODS

9.1 Randomization

A randomization sequence was prepared by a Biofortis statistician and uploaded onto Medrio eCRF platform (Medrio Inc, San Francisco, CA). When a participant was determined to be eligible for the study, a randomization number was assigned to the participant through the randomization module of the Medrio platform. The randomization number was recorded in the participant's source documentation.

9.2 Sample Size

No formal sample size calculation was conducted and the sample size selected was based on published studies on arabinogalactan and GI outcomes (Grube 2012, Robinson 2001 and 2013). A total of 30 subjects were randomized with the goal of completing 25 subjects. No subjects were replaced in the event of early terminations.

For a continuous outcome measure, there was approximately 70% power to detect a 0.50 effect size assuming negligible carry-over effect, a 0.05 significance level, and a correlation between paired measures of 0.5 with 25 subjects completing (~12 randomized to each sequence). In the event of no attrition, power would increase to approximately 78% under the same design parameters.

9.3 OUTCOMES

9.3.1 Primary Outcome

The primary focus of this trial was to characterize the fecal microbiome including diversity and composition and short chain fatty acids after 6 weeks of study product consumption.

9.3.2 Secondary Outcomes

Secondary outcomes included the following after 3 and 6 weeks of study product consumption:

- SCFA plasma levels
- Stool frequency: number of bowel movements recorded over the 3-day collection period

- Stool consistency as measured by the 7-point BSS: 3-day average calculated as the sum of reported scores on the BSS divided by the number of bowel movements
- Straining during bowel movements as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements
- Discomfort during bowel movements as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements
- Sensation of incomplete evacuation as measured by a 4-point Likert scale: 3-day average calculated as the sum of reported scores divided by the number of bowel movements
- 8 domains of the SF-36: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions

9.3.3 Additional Measures

A 3-day diet record was collected with instructions to complete intake during the 3 days (2 weekdays and one weekend) following Visits 3 and 6 (Weeks 3 and 12) that did not coincide with the 5 replication days immediately prior to Visits 4 and 7 (Weeks 6 and 15). Records were analyzed for dietary composition using the Food Processor Nutrition Analysis and Fitness Software. Specific nutrients/outcomes of interest include: total energy intake (kcal/day) and total dietary fiber (g/day). Additionally, the following were calculated:

- Carbohydrate (% of total energy/day) = [(Total Carbohydrate g/day)*(4 kcal/g)]/Total Energy intake*100
- Protein (% of total energy/day) = [(Total Protein g/day) X (4 kcal/g)]/ Total Energy intake*100
- Total fat (% of total energy/day) = [(Total Fat in g/day) X (9 kcal/g)]/ Total Energy intake*100

9.4 Statistical Analysis

9.4.1 Demographics

Subject demographic and anthropometric measurements were summarized and presented with descriptive statistics for each analysis population. Continuous variables were presented with the number of participants, mean, standard deviation, standard error of the mean, median, interquartile range limits (25th and 75th percentiles), and range limits (minimum and maximum values). Categorical variables were summarized as counts and percentages.

9.4.2 Outcomes

All statistical analyses that were performed by personnel from Biofortis were conducted using SAS for Windows (version 9.4, Cary, NC) and/or R (version 3.6.0, R Core Team 2019). All P-values are two-sided and considered significant at the <0.05 level; values ≥ 0.05 but <0.10 were considered marginally significant unless otherwise stated. Analyses were performed based on observed data, and missing values were not imputed unless otherwise stated.

Specific statistical analysis procedures are provided in the results section (Section 11) for each outcomes assessed.

10 ANALYSIS POPULATION

A summary of the subject disposition is provided in **Figure 3**. A total of 45 subjects were screened for participation and 30 subjects (16 females, 14 males) were enrolled.

All subjects enrolled in the study fulfilled the required inclusion and exclusion criteria with the exception of five subjects:

- One subject (#006) was on an exclusionary medication (calcium supplement) at Visit 1. With the exception of consent and medical history/medication review, all study procedures were then completed following a 14-day washout as per protocol.
- Four subjects (#012, #022, #028, and #038) ate fermented food prior to Visit 2 (sour dough/sour dough bread) which was determined based on subject's 3-day diet records. Subjects were instructed to refrain from consuming any sour dough or sour dough bread throughout the study.

All five subjects were approved to enroll in the study.

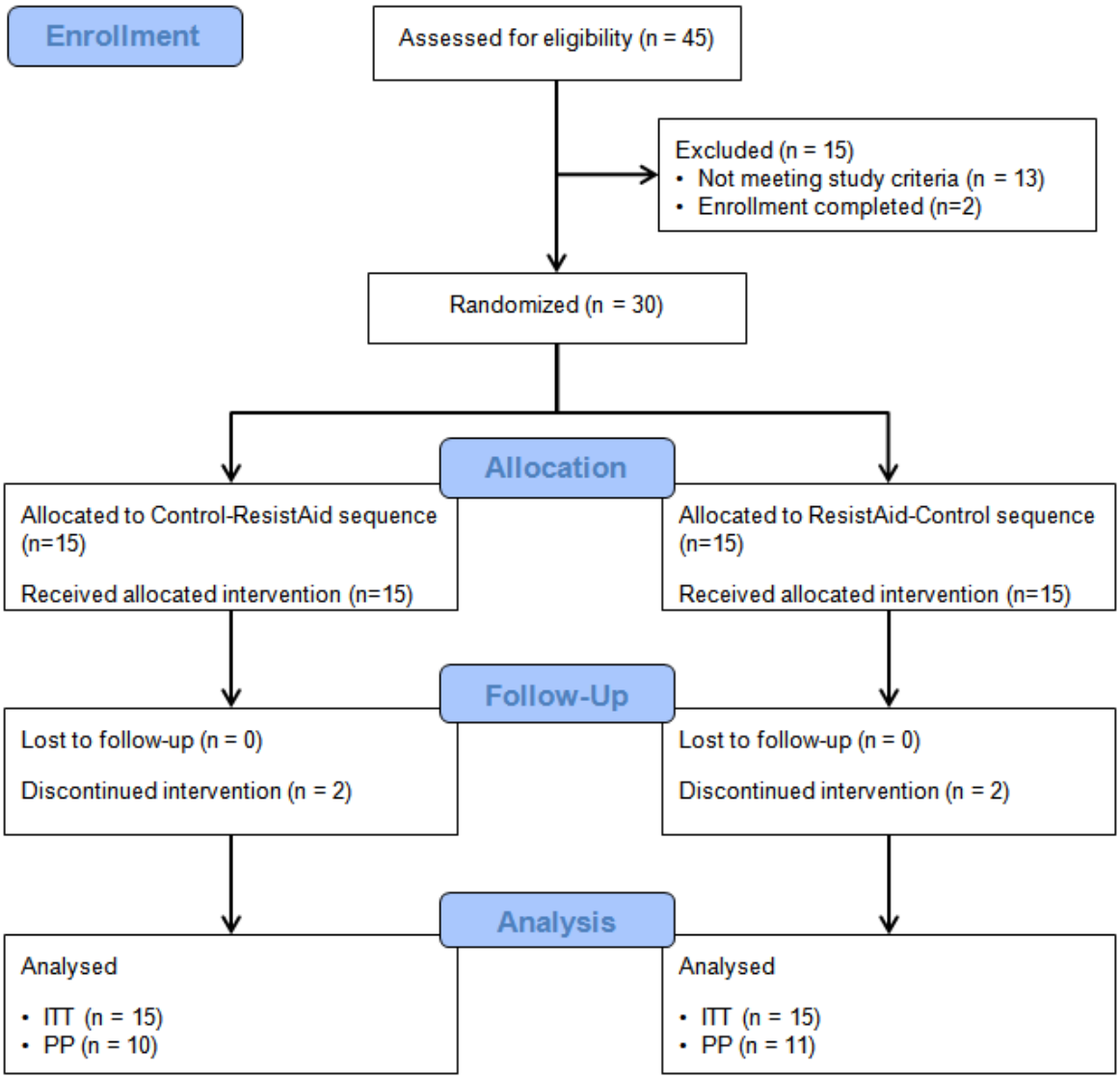
Nine subjects were excluded from the PP populations due to the following reasons:

- Early termination: 4 subjects (Subject #028, #022, #012, #023)
- Illness during intervention: 1 subject (Subject #005 reported having the flu/cold during the study)
- Intake of exclusionary medication: 1 subject (Subject #035 took exclusionary medication due to a cold/flu during the study)
- Failure to replicate diet: 2 subjects (Subject #015 and #032)
- Weight change: 1 subject (Subject #029)

One subject (Subject #22) only contributed baseline data due to termination prior to Visit 3. Another subject (Subject #28) contributed baseline and Week 3 data for the ResistAid supplementation prior to withdrawing from the study. Finally, Subject #12 contributed baseline and Week 3 data for the placebo supplementation prior to withdrawing from the study.

Please refer to Appendix SR3 for more details.

Figure 3. Subject Disposition



Abbreviation: ET, early termination; ITT, intent-to-treat; PP, per protocol; n, sample size

11 OUTCOME RESULTS

11.1 Subject Characteristics

Selected demographics and baseline characteristics for the ITT and PP sample populations are listed in **Table B** and are also available in Appendix SR4.

Table B. Baseline Characteristics of the ITT and PP Populations

Characteristic		ITT (n=30)	PP (n=21)
Gender	Female	16 (53.3%)	10 (47.6%)
	Male	14 (46.7%)	11 (52.4%)
Age (years)	Mean (SD)	41.10 (9.46)	41.86 (9.05)

Characteristic		ITT (n=30)	PP (n=21)
	Median (Min, Max)	40.00 (21.00, 59.00)	40.00 (27.00, 59.00)
	IQR	36.00, 50.00	37.00, 53.00
Ethnicity	Hispanic/Latino	4 (13.3%)	3 (14.3%)
	Not Hispanic/Latino	26 (86.7%)	18 (85.7%)
Race	American Indian Or Alaskan Native	1 (3.3%)	1 (4.8%)
	Asian	1 (3.3%)	0 (0.0%)
	Black/African American	3 (10.0%)	1 (4.8%)
	Other	1 (3.3%)	1 (4.8%)
	White	24 (80.0%)	18 (85.7%)
BMI (kg/m^2)[1]	Mean (SD)	26.43 (3.43)	26.56 (2.79)
	Median (Min, Max)	26.35 (18.80, 31.60)	26.30 (22.00, 31.40)
	IQR	24.00, 28.90	24.10, 28.80
Systolic BP (mm Hg)[2]	Mean (SD)	113.87 (13.20)	116.86 (11.56)
	Median (Min, Max)	113.00 (85.00, 139.00)	116.00 (98.00, 139.00)
	IQR	106.00, 123.00	107.00, 125.00
Diastolic BP (mm Hg)[2]	Mean (SD)	72.07 (10.68)	74.71 (8.93)
	Median (Min, Max)	71.50 (50.00, 93.00)	75.00 (59.00, 92.00)
	IQR	64.00, 79.00	69.00, 80.00

[1]BMI was calculated at Week 0
[2]Included blood pressure obtained at Week 0 and Week 9
Abbreviations: BMI, body mass index; BP, blood pressure; kg, kilogram; m, meter; Max, maximum; Min, minimum; mm Hg, millimeter Mercury; n, sample size; SD, standard deviation; IQR, interquartile range

11.2 Diet Intake

Intakes of selected dietary components obtained from the 3-day Diet Records are listed in **Table B1 and B2** and are also available in Appendix SR4.

Table B1. Habitual Diet Intake for the ITT Population

Dietary Component		All (Week 0) n=30	Placebo (Week 6) n=27	ResistAid (Week 6) n=25
Total energy intake (kcal)	Mean (SD)	1858.0 (683.9)	1858.0 (594.0)	1723.2 (528.2)
	SEM	124.9	114.3	105.6
	Median (range)	1716.9 (780.4 4058.3)	1870.8 (928.6, 3243.0)	1721.9 (744.4, 2800.0)
	IQR	1453.7, 2096.2	1362.8, 2149.5	1396.4, 2040.9
Carbohydrate (% total kcal)	Mean (SD)	39.69 (12.91)	37.54 (9.82)	39.77 (10.32)
	SEM	2.36	1.89	2.06
	Median (range)	39.06 (16.33, 63.49)	36.39 (14.24, 58.06)	39.45 (13.13, 55.80)
	IQR	28.50, 48.24	31.09, 44.59	33.07, 47.82
Total dietary fiber (g)	Mean (SD)	15.10 (7.78)	15.01 (7.24)	14.41 (6.10)
	SEM	1.42	1.39	1.22
	Median (range)	13.68 (5.53, 46.24)	13.99 (2.38, 32.11)	12.68 (4.39, 30.47)
	IQR	10.41, 18.32	9.52, 17.44	10.03, 17.61
Protein (% total kcal)	Mean (SD)	19.15 (5.67)	20.25 (6.14)	19.30 (4.47)
	SEM	1.03	1.18	0.89
	Median (range)	17.90 (10.78, 34.27)	19.56 (10.77, 36.85)	19.74 (11.84, 28.66)
	IQR	15.42, 23.38	15.58, 23.63	15.67, 22.11
Fat (% total kcal)	Mean (SD)	40.72 (7.70)	41.59 (7.77)	40.89 (7.28)
	SEM	1.41	1.50	1.46
	Median (range)	40.16 (26.67, 57.73)	42.40 (24.67, 64.96)	41.17 (30.80, 62.96)
	IQR	37.10, 45.32	36.22, 45.84	35.34, 42.95

Abbreviations: g, gram; IQR, interquartile range; kcal, kilocalorie; n, sample size; SD, standard deviation; SEM, standard error of the mean

Table B2. Habitual Diet Intake for the PP Population

Dietary Component		All (Week 0) n=30	Placebo (Week 6) n=27	ResistAid (Week 6) n=25
Total energy intake (kcal)	Mean (SD)	2005.9 (722.0)	1990.2 (597.8)	1796.4 (491.2)
	SEM	157.5	130.5	107.2
	Median (range)	1875.3 (1137.1, 4058.3)	1988.2 (928.6, 3243.0)	1749.3 (917.5, 2800.0)
	IQR	1559.2, 2150.7	1746.6, 2296.3	1506.9, 2151.0
Carbohydrate (% total kcal)	Mean (SD)	38.05 (13.93)	36.79 (10.22)	39.29 (10.62)
	SEM	3.04	2.23	2.32
	Median (range)	39.07 (16.33, 62.67)	36.23 (14.24, 58.06)	38.74 (13.13, 55.80)
	IQR	26.40, 47.95	30.68, 44.59	32.81, 47.82
Total dietary fiber (g)	Mean (SD)	15.49 (8.69)	15.55 (6.62)	15.08 (6.21)
	SEM	1.90	1.44	1.36
	Median (range)	12.38 (5.53, 46.24)	16.29 (2.38, 29.59)	15.03 (6.49, 30.47)
	IQR	10.47, 17.40	12.69, 17.44	10.03, 17.79
Protein (% total kcal)	Mean (SD)	19.74 (5.96)	20.08 (5.98)	19.38 (4.62)
	SEM	1.30	1.31	1.01
	Median (range)	18.15 (10.78, 34.27)	19.56 (10.77, 36.85)	19.40 (11.84, 28.66)
	IQR	16.28, 23.97	16.71, 22.94	15.67, 22.11
Fat (% total kcal)	Mean (SD)	41.38 (8.65)	42.23 (8.20)	41.10 (7.83)
	SEM	1.89	1.79	1.71
	Median (range)	41.15 (26.67, 57.73)	42.45 (24.67, 64.96)	41.17 (30.80, 62.96)
	IQR	36.18, 47.54	36.92, 46.27	34.58, 42.95

Abbreviations: g, gram; IQR, interquartile range; kcal, kilocalorie; n, sample size; SD, standard deviation; SEM, standard error of the mean

11.3 Compliance

For the ITT population, compliance for the ResistAid supplementation ranged from 37.5% to 133.3% with an average of 99.2%. The low (37.5%) compliance rate is due to an early termination subject that withdrew prior to Week 3. Meanwhile, compliance for the placebo supplementation ranged from 85.8% to 109.1% with an average of 99.7%.

For the PP population, compliance for the ResistAid supplementation ranged from 95.2% to 104.8% with an average of 100.5%. Meanwhile, compliance for the placebo supplementation ranged from 85.8% to 107% with an average of 99.3%.

11.4 Primary Outcomes

11.4.1 Fecal Bacterial Phylotypes

11.4.1.1 Statistical Analysis Approach

The following specific bacterial phylotypes were measured: *Firmicutes, Bacteroidetes, Bifidobacterium*, and *Lactobacillus*. Additionally, the ratio of *Firmicutes* to *Bacteroidetes* was analyzed. Using graphical methods and 1.5*interquartile rule, 1 subject was identified as an outlier at the 3-week time point for the *Firmicutes* to *Bacteroidetes* ratio and data from this subject was removed from the *Firmicutes* to *Bacteroidetes* ratio analysis. A linear mixed model was used to estimate the difference in the specific bacterial phylotypes at each of the 3-week and 6-week time point. For each, a univariate fixed effect model for supplementation/intervention effect as well as a final adjusted multivariable model was estimated. The multivariable model considered terms for period, sequence, baseline measure, age and BMI. Final model was selected with the backwards elimination approach. All models included a random intercept for subject nested within sequence.

Of note, *Lactobacillus* was not detected (i.e., relative abundance = 0) in over 95% of samples; therefore, it was not modeled.

11.4.1.2 Results

The unadjusted relative abundance for specific bacterial phylotypes for the ITT and PP populations are shown in **Tables C1 and C2**. The ratio of *Firmicutes* to *Bacteriodetes* was significantly different ($P<0.05$) following the ResistAid supplementation compared to placebo in the ITT and PP populations whereby the ratio was lower following the ResistAid supplementation compared to placebo. In both populations, *Bacteriodetes* was greater following the ResistAid supplementation compared to placebo and *Firmicutes* was lower following the ResistAid supplementation compared to placebo. Finally, *Bifidobacterim* was marginally different ($P=0.084$) following the ResistAid supplementation compared to placebo in the ITT population only whereby the relative abundance was greater following the ResistAid supplementation compared to placebo. There were no other statistically or marginally significant observations.

Refer to Appendix SR4 Tables T9 and T10 for more details.

The unadjusted relative abundances of the selected bacterial phylotypes at the phylum level are shown in **Figure 3a**, with a magnified area for lower abundant phyla shown in **Figure 3b**. Additional graphs are available in Appendix SR5.

Table C1. Relative Abundance of Selected Bacterial Phylotypes for the ITT Population

Bacterial Phylotypes[1]	Week	All n=30	Placebo n=27-28	ResistAid n=26-27	Univariate model P-value	Adjusted model P-value[3]
Bacteroidetes	0	0.4133 (0.2325, 0.6726)				
	3		0.4390 (0.0150, 0.6706)	0.5090 (0.3366, 0.6865)	0.007	0.007
	6		0.4347 (0.1236, 0.6219)	0.4820 (0.1916, 0.7684)	0.042	0.043+
Firmicutes	0	0.5471 (0.2706, 0.6880)				
	3		0.4884 (0.3151, 0.9271)	0.4448 (0.2605, 0.5754)	0.006	0.007
	6		0.5267 (0.3342, 0.8335)	0.4466 (0.2005, 0.7285)	0.010	0.010+
Firmicutes to Bacteroidetes	0	1.3471 (0.4482, 2.9489)				
	3		1.0954 (0.4699, 61.787)	0.8768 (0.3795, 1.7097)	0.017	0.007
	6		1.2268 (0.5415, 6.7438)	0.9566 (0.2610, 3.7477)	0.024	0.025
Bifidobacterium	0	0.0046 (0.0000, 0.0400)				
	3		0.0034 (0.0000, 0.0515)	0.0074 (0.0000, 0.0488)	0.077	0.084†
	6		0.0055 (0.0000, 0.0360)	0.0064 (0.0000, 0.1133)	0.370	0.371
Lactobacillus	0	0.0000 (0.0000, 0.0033)				
	3		0.0000 (0.0000, 0.0014)	0.0000 (0.0000, 0.0011)	N/A[2]	N/A
	6		0.0000 (0.0000, 0.0011)	0.0000 (0.0000, 0.0000)	N/A	N/A

[1]Data are unadjusted median (range). Median (range) is presented because the mean is skewed due to distribution of the data.
[2]*Lactobacillus* was not detected in over 95% of samples; therefore, it was not modeled.

[3]Adjusted for the respective baseline bacterial phylotype relative abundance
+Adjusted model also contained a term for baseline BMI
†Adjusted model also contained a term for randomized sequence
Abbreviations: n, sample size; N/A, not applicable

Table C2. Relative Abundance of Selected Bacterial Phylotypes for the PP Population

Bacterial Phylotypes[1]	Week	All n=21	Placebo n=21	ResistAid n=21	Univariate model P-value	Adjusted model P-value[3]
Bacteroidetes	0	0.4047 (0.2325, 0.6696)				
	3		0.4282 (0.0150, 0.6706)	0.5064 (0.3366, 0.6865)	0.012	0.012
	6		0.4370 (0.1236, 0.6219)	0.5204 (0.1916, 0.7684)	0.014	0.008+
Firmicutes	0	0.5395 (0.2706, 0.6880)				
	3		0.4841 (0.3151, 0.9271)	0.4549 (0.2605, 0.5754)	0.014	0.010
	6		0.5220 (0.3342, 0.8335)	0.4376 (0.2005, 0.7285)	0.003	0.003+
Firmicutes to Bacteroidetes	0	1.4000 (0.4482, 2.9489)				
	3		1.0952 (0.4699, 61.787)	0.8768 (0.3795, 1.7097)	0.039	0.014
	6		1.2000 (0.5415, 6.7438)	0.8529 (0.2610, 3.7477)	0.008	0.008
Bifidobacterium	0	0.0068 (0.0000, 0.0400)				
	3		0.0036 (0.0000, 0.0515)	0.0052 (0.0000, 0.0386)	0.174	0.174
	6		0.0055 (0.0000, 0.0304)	0.0066 (0.0000, 0.1133)	0.494	0.494
Lactobacillus	0	0.0000 (0.0000, 0.0033)				

Bacterial Phylotypes[1]	Week	All n=21	Placebo n=21	ResistAid n=21	Univariate model P-value	Adjusted model P-value[3]
	3		0.0000 (0.0000, 0.0014)	0.0000 (0.0000, 0.0011)	N/A[2]	N/A
	6		0.0000 (0.0000, 0.0000)	0.0000 (0.0000, 0.0000)	N/A	N/A

[1]Data are unadjusted median (range). Median (range) is presented because the mean is skewed due to distribution of the data.
[2]*Lactobacillus* was not detected in over 95% of samples; therefore, it was not modeled.
[3]Adjusted for the respective baseline bacterial phylotype relative abundance
+Adjusted model also contained a term for baseline BMI
Abbreviations: n, sample size; N/A, not applicable

Figure 3a. Relative Abundance of Selected Bacterial Phylotypes at the Phylum Level for the ITT Population

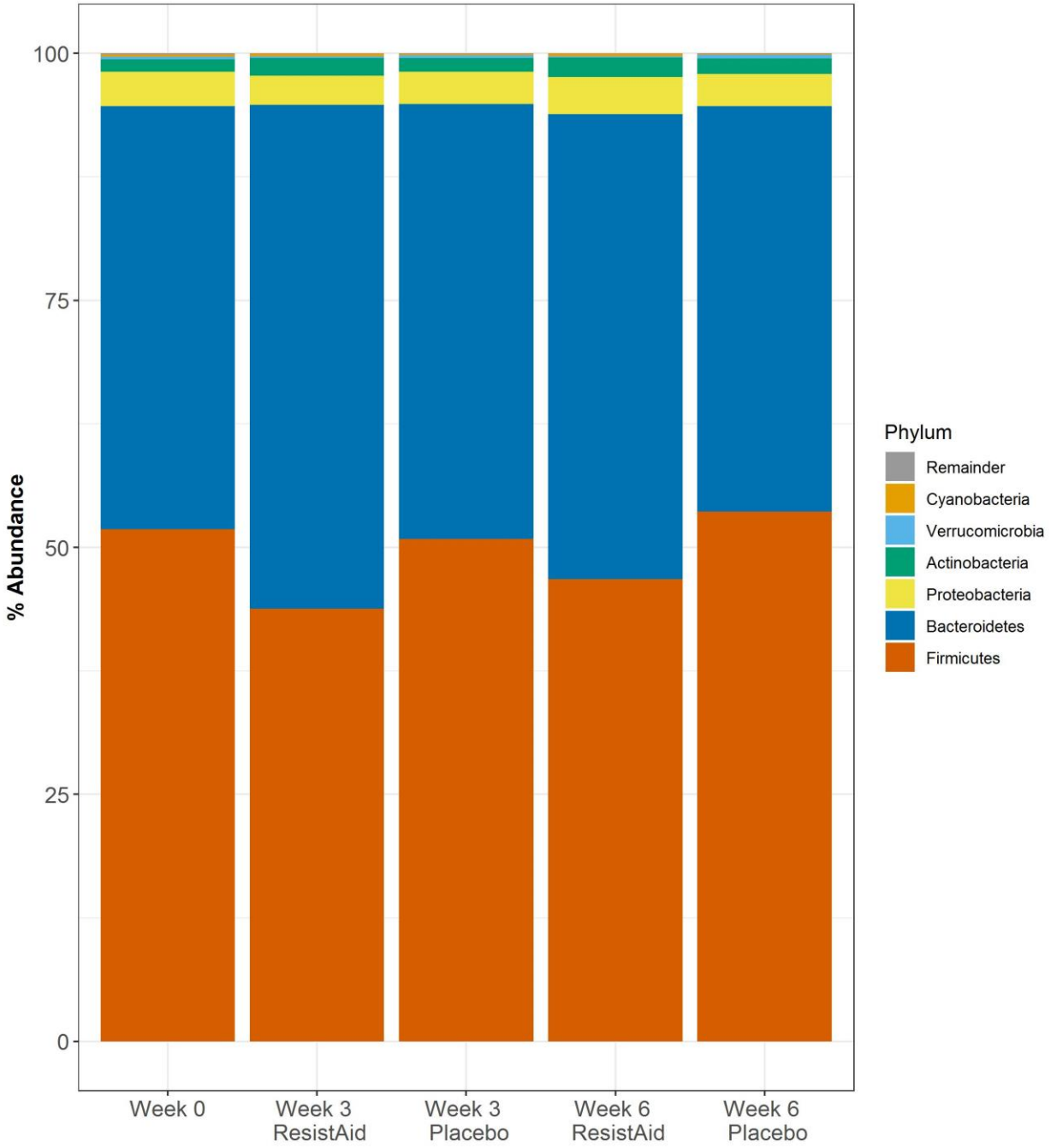
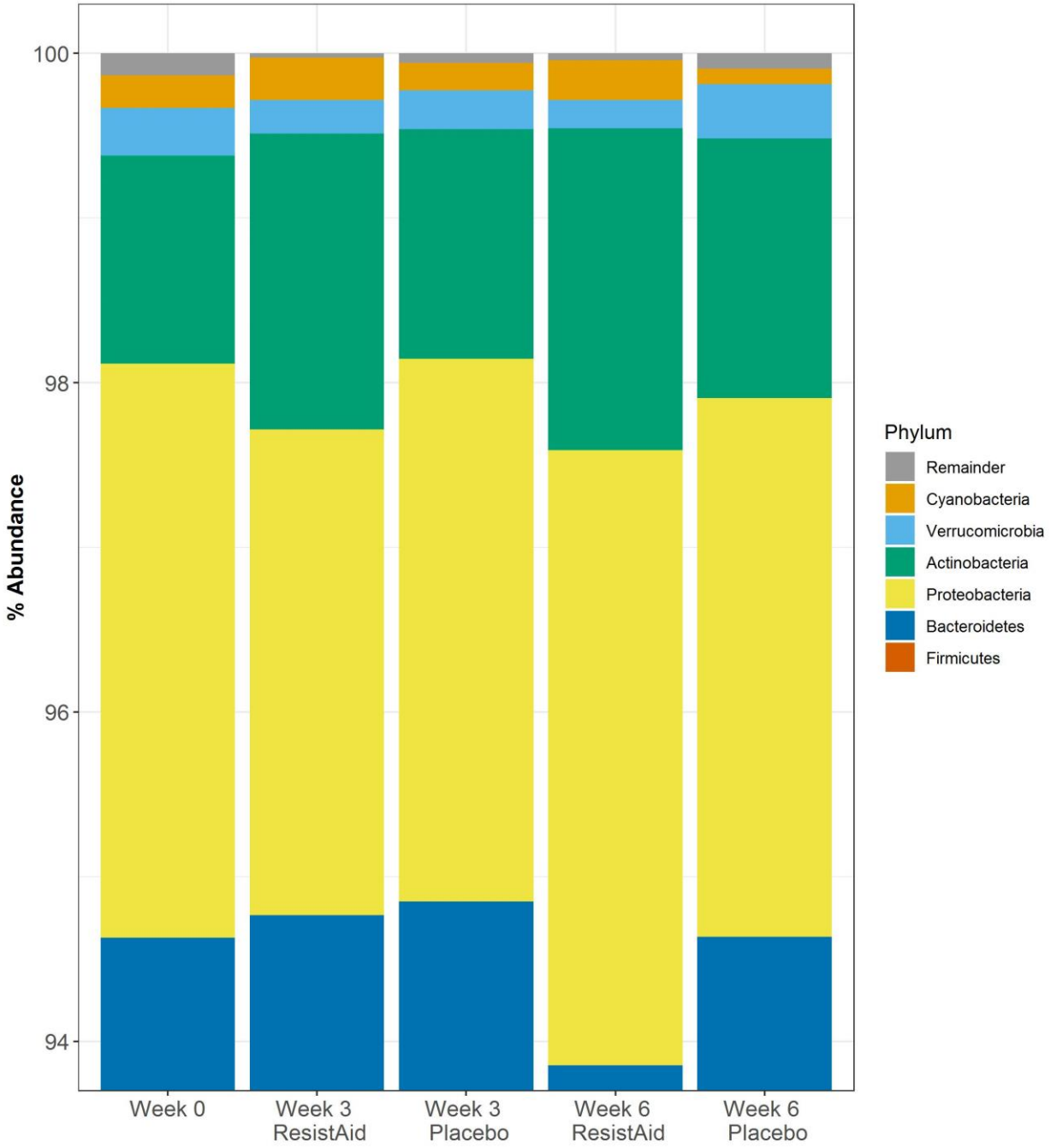


Figure 3b. Magnified Area for Selected Bacterial Phylotypes with Lower Abundance at the Phylum Level for the ITT Population



11.4.2 Fecal Microbiome Alpha- and Beta-Diversity

11.4.2.1 Statistical Analysis Approach

Alpha- and beta-diversity analyses were performed by UW Biotechnology Center.

Alpha-diversity metrics describe the diversity within each sample unit (i.e., each subject at each visit) and are presented as the Chao1, Shannon Index, and Simpson's Index. Chao1 uses presence or absence of species to calculate diversity (number of species present in a sample), Shannon assumes all species are represented in a sample and that they are randomly sampled. Rare and lower abundant species are also taken into consideration. Simpson uses similar concept but gives more weight to common or dominant species. In this case, a few rare species with only a few representatives will not affect the diversity. In both Shannon and Simpson analyses, higher value indicates higher alpha diversity in the samples.

The alpha-diversity indices, Chao1, Shannon, and Simpson, were modeled with a linear mixed model. For each, a univariate fixed effect model for supplementation/intervention effect as well as a final adjusted multivariable model was estimated. The multivariable model considered terms for period, sequence, baseline measure, age and BMI. Final model was selected with the backwards elimination approach. All models included a random intercept for subject nested within sequence.

Beta-diversity metrics describe the difference or similarity between collections of sample units (e.g., Week 0 and Weeks 3 and 6 of each supplementation; within and between supplementations) and are expressed as non-phylogenetic beta diversity (Bray-Curtis dissimilarity index) which considers abundance distribution of bacteria.

11.4.2.2 Results

The total number of OTU and frequency analyzed in the 16S rRNA sequencing was 2561 and 1065519, respectively. In the genus and species level, 238 and 461 OTU was detected and analyzed in the fecal samples, respectively. Shannon Index and the Simpson's Index were significantly different ($P<0.05$) following the ResistAid supplementation compared to placebo at Weeks 3 and 6 in the ITT and PP populations whereby the indices were lower ($P<0.05$) at Weeks 3 and 6 following the ResistAid supplementation compared to placebo for both populations (**Figure 4**) when adjusting for the respective baseline alpha diversity measure. Chao1 was marginally different ($P=0.082$) at Week 3 following the ResistAid supplementation compared to placebo in the ITT population only whereby Chao1 was lower following the ResistAid supplementation compared to placebo (**Figure 4**). While the PP population did not retain statistical significance at the 0.05 level, the directionality of the effect was consistent with that of the ITT population. There were no other statistically or marginally significant observations.

All sample units are similar as indicated by $P>0.1$ for the Bray-Curtis dissimilarity indices. Principal coordinates analysis plot for Bray-Curtis is shown in **Figure 5.**

Refer to Appendix SR4 Table T11 for more details.

Figure 4. Alpha-Diversity Expressed as Chao1, Shannon, and Simpson's Indices for ITT Population

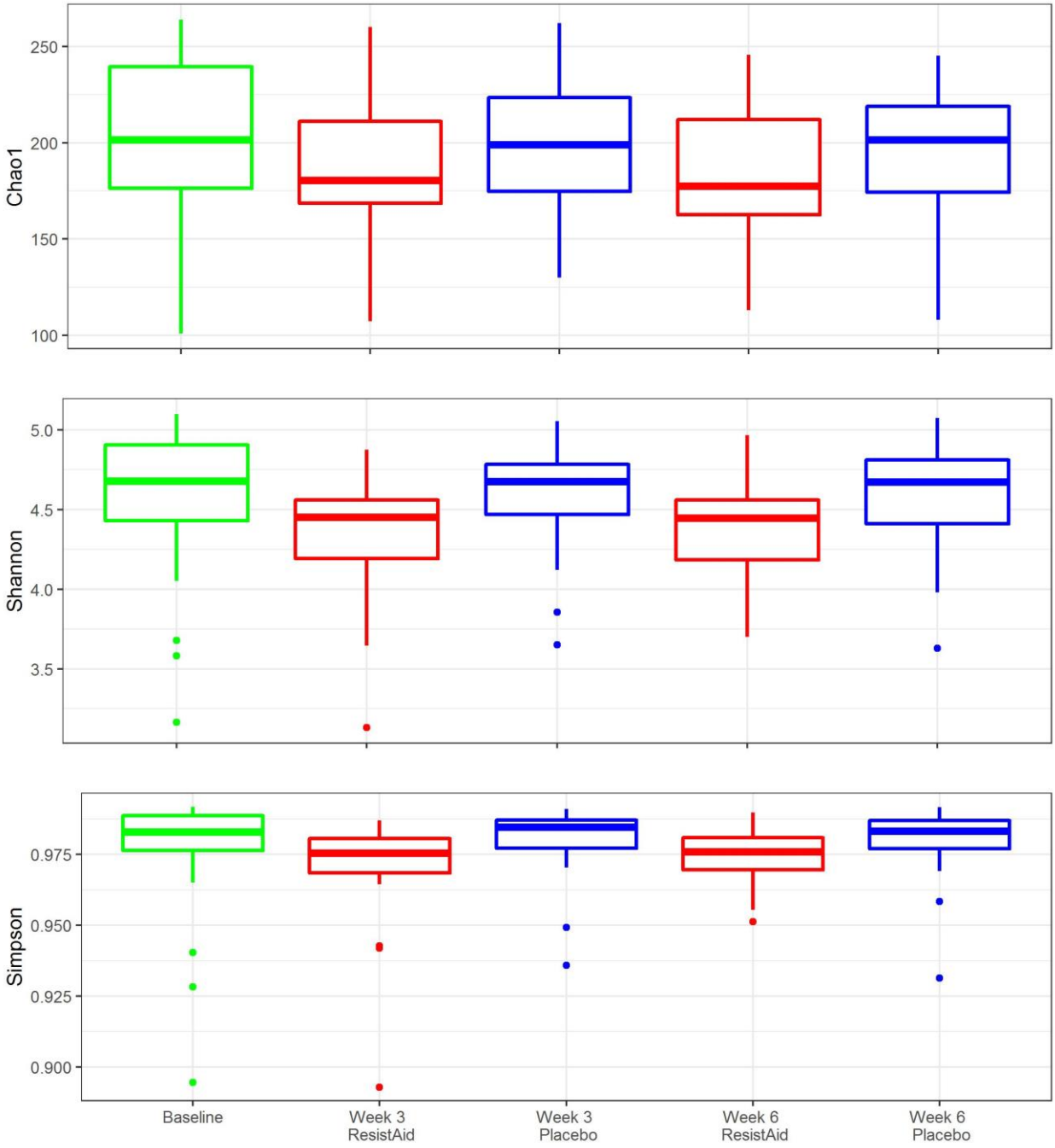
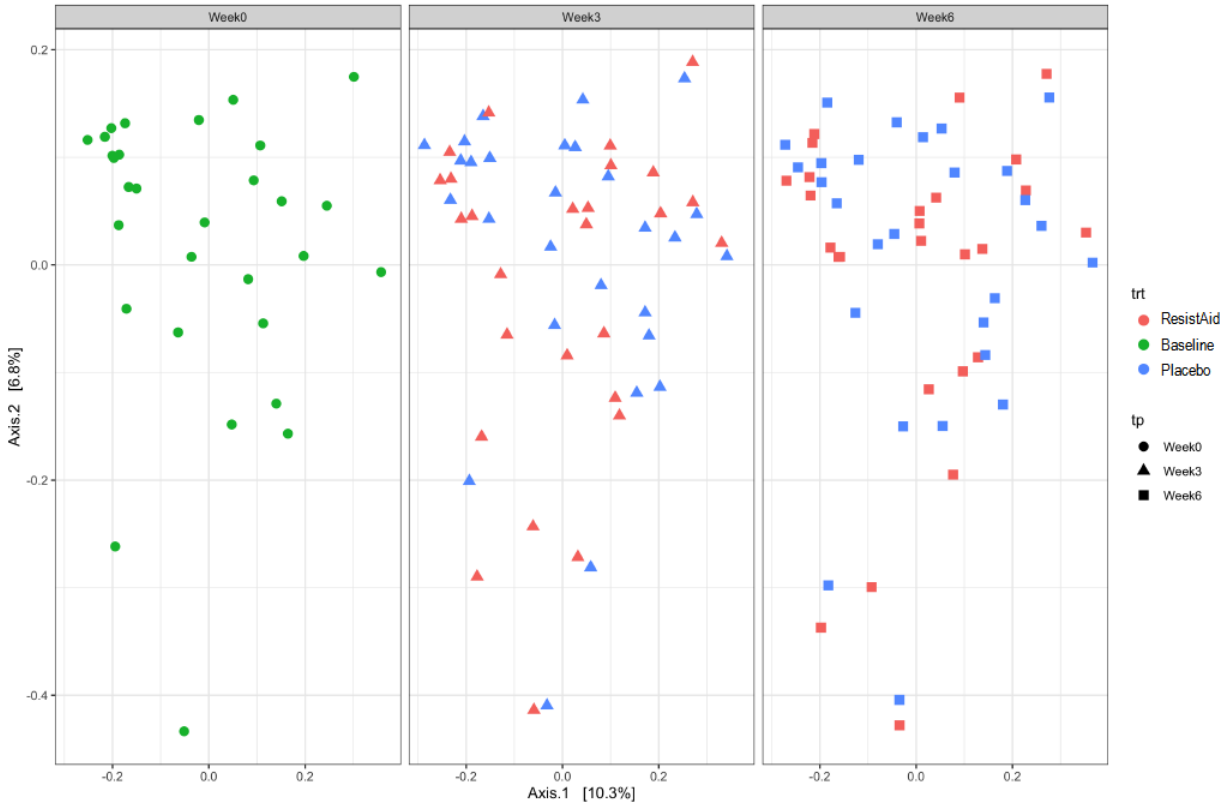


Figure 5. Bray-Curtis Principal Coordinates Analysis Plot for ITT population



11.4.3 Fecal SCFA

11.4.3.1 Statistical Analysis Approach

A linear mixed model was used to estimate the difference in SCFAs at the 6-week time point. For each, a univariate fixed effect model for supplementation/intervention effect as well as a final adjusted multivariable model was estimated. The multivariable model considered terms for period, sequence, baseline measure, age and BMI. Final model was selected with the backwards elimination approach. All models included a random intercept for subject nested within sequence. A log transformation was used for isobutyric acid, butyric acid, isovaleric acid, valeric acid, and hexanoic acid.

11.4.3.2 Results

Unadjusted data for fecal SCFA for the ITT and PP populations are shown in **Tables D1 and D2**, respectively. Fecal isovaleric acid, valeric acid, and hexanoic acid were significantly different ($P<0.05$) following the ResistAid supplementation compared to placebo at Week 6 in the ITT and PP populations whereby concentrations of these fatty acids were lower ($P<0.05$) following the ResistAid supplementation compared to placebo for both populations. Acetic acid was marginally lower in the ITT with ResistAid supplementation ($p=0.088$) when adjusted for the baseline measure. Isobutyric acid was significantly different ($P<0.05$) following the ResistAid supplementation compared to placebo at Week 6 in the PP population only whereby isobutyric acid concentration was lower ($P<0.05$) following the ResistAid supplementation compared to

placebo. There were no other statistically or marginally significant observations. Refer to Appendix SR4 Tables T12 and T13 for more details.

Table D1. Fecal SCFA for ITT Population

Fecal SCFA[1]		Placebo n=27-28	ResistAid n=26-29	Unadjusted model P-value	Adjusted model P-value[3]
Acetic acid (µg/g)	Baseline[2]	3449.71 (1127.79, 5504.21)	3129.35 (1118.19, 8190.20)		
	Week 6	3211.72 (906.83, 8493.28)	2740.17 (899.82, 6188.77)	0.205	0.088
Butyric acid (µg/g)	Baseline	860.31 (215.63, 2224.00)	739.95 (302.01, 3169.64)		
	Week 6	830.48 (268.05, 2080.77)	749.30 (141.58, 2081.72)	0.194	0.179
Hexanoic acid (µg/g)	Baseline	25.91 (2.61, 500.35)	23.00 (1.88, 677.19)		
	Week 6	33.45 (1.82, 999.27)	15.95 (2.45, 245.62)	0.27	0.023
Isobutyric acid (µg/g)	Baseline	130.58 (40.54, 345.04)	142.02 (40.39, 328.70)		
	Week 6	113.53 (66.26, 325.95)	105.17 (37.76, 424.81)	0.102	0.112
Isovaleric acid (µg/g)	Baseline	113.27 (25.04, 278.86)	139.72 (16.37, 279.36)		
	Week 6	112.51 (59.60, 335.40)	83.28 (34.02, 428.41)	0.055	0.044
Propionic acid (µg/g)	Baseline	938.64 (276.92, 2239.87)	856.43 (361.10, 2088.83)		
	Week 6	841.98 (407.90, 1734.40)	918.95 (337.03, 1768.10)	0.745	0.745+
Valeric acid (µg/g)	Baseline	188.09 (28.81, 399.66)	187.52 (16.97, 650.16)		
	Week 6	191.45 (30.13, 452.37)	135.61 (31.28, 419.28)	0.014	0.013

[1]Data are unadjusted median (range). Median (range) is presented because the mean is skewed due to distribution of the data.
[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[3]Adjusted for the respective baseline SCFA, BMI, and/or randomized sequence
+ Final adjusted model only retained product and is equivalent to univariate model
Abbreviations: g, gram; µg, microgram; n, sample size; SCFA, short-chain fatty acid

Table D2. Fecal SCFA for PP Population

Fecal SCFA[1]		Placebo n=21	ResistAid n=21	Unadjusted model P-value	Adjusted model P-value[3]
Acetic acid (µg/g)	Baseline[2]	3451.09 (1127.79, 5504.21)	3355.30 (1118.19, 8190.20)		
	Week 6	3023.63 (990.08, 8493.28)	2627.63 (899.82, 6188.77)	0.333	0.204
Butyric acid (µg/g)	Baseline	989.71 (248.89, 2224.00)	811.76 (302.01, 3169.64)		
	Week 6	830.48 (268.05, 1967.70)	671.73 (141.58, 2067.62)	0.068	0.113
Hexanoic acid (µg/g)	Baseline	21.45 (2.61, 500.35)	23.00 (1.88, 677.19)		
	Week 6	46.89 (1.82, 999.27)	19.36 (2.45, 234.25)	0.027	0.048
Isobutyric acid (µg/g)	Baseline	159.96 (40.54, 345.04)	144.09 (40.39, 328.70)		
	Week 6	127.86 (66.26, 325.95)	103.97 (37.76, 199.90)	0.024	0.024
Isovaleric acid (µg/g)	Baseline	147.63 (25.04, 278.86)	140.86 (16.37, 279.36)		
	Week 6	113.50 (59.60, 335.40)	80.50 (34.02, 206.12)	0.017	0.015
Propionic acid (µg/g)	Baseline	963.16 (291.10, 2239.87)	934.67 (361.10, 2088.83)		
	Week 6	841.98 (407.90, 1734.40)	874.62 (337.03, 1547.29)	0.312	0.312
Valeric acid (µg/g)	Baseline	226.40 (28.81, 399.66)	187.52 (16.97, 650.16)		
	Week 6	198.77 (30.13, 452.37)	128.86 (31.28, 225.01)	0.002	0.002

[1]Data are unadjusted median (range). Median (range) is presented because the mean is skewed due to distribution of the data.
[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[3]Adjusted for the respective baseline SCFA, BMI, and/or randomized sequence
Abbreviations: g, gram; µg, microgram; n, sample size; SCFA, short-chain fatty acid

11.5 Secondary Outcomes

11.5.1 Bowel Habits Diary

11.5.1.1 Statistical Analysis Approach

A high proportion of subjects did not have problems in regards to straining, discomfort, and incomplete evacuation. Therefore, outcomes were dichotomized to on average no issue (score = 0) and at least some mild to severe symptom reported (score > 0).

The number of bowel movements over the 3-day period was assumed to follow a Poisson distribution. A generalized linear mixed model following a Poisson distribution with a log link was used to model the number of bowel movements at each of 3-week and 6-week time points. Maximum likelihood with adaptive quadrature was used to estimate model parameters. Stool consistency was modeled with a linear mixed model at each of 3-week and 6-week time points. No data transformation was indicated. Due to the high proportion of subjects without an issue for straining, discomfort, and incomplete evacuation, outcomes were modeled categorically with a generalized linear mixed model following a binary distribution and a logit link; maximum likelihood with adaptive quadrature was used to estimate model parameters. For each outcome, univariate fixed effect models for supplementation effect as well as a final adjusted multivariable model was estimated. The multivariable model considered terms for period, sequence, baseline measure, age, and BMI. Final model was selected with the backwards elimination approach. All models included a random intercept for subject nested within sequence.

11.5.1.2 Results

There were no statistically significant differences (P>0.05) in number of bowel movements, stool consistency, straining, discomfort, and sensation of evacuation ratings between supplementations (**Tables E1 and E2).** Dichotomized data for straining, discomfort, and incomplete evacuation are shown in **Tables E3 and E4**. Refer to Appendix SR4 Tables T2 and T3 for more details.

Table E1. Bowel Habits Diary Outcomes for the ITT Population

Parameters[1]		All n=30	Placebo n=27-28	ResistAid n=26-27	Adjusted model P-value[2]
Number of bowel movements	Week 0	3.43 (1.28)			
	Week 3		3.86 (1.69)	4.15 (1.92)	0.587
	Week 6		3.70 (1.54)	3.81 (1.60)	0.859
Stool consistency	Week 0	3.53 (1.07)			
	Week 3		3.76 (1.16)	3.56 (1.03)	0.459
	Week 6		3.59 (1.15)	3.69 (0.96)	0.813+
Straining during bowel movement	Week 0	0.42 (0.49)			

Parameters[1]		All n=30	Placebo n=27-28	ResistAid n=26-27	Adjusted model P-value[2]
	Week 3		0.31 (0.47)	0.43 (0.60)	N/A†
	Week 6		0.52 (0.53)	0.40 (0.51)	N/A†
Discomfort during bowel movement	Week 0	0.23 (0.32)			
	Week 3		0.15 (0.29)	0.19 (0.36)	N/A†
	Week 6		0.33 (0.48)	0.14 (0.27)	N/A†
Sensation of incomplete evacuation	Week 0	0.29 (0.54)			
	Week 3		0.24 (0.50)	0.28 (0.43)	N/A†
	Week 6		0.31 (0.44)	0.30 (0.50)	N/A†

[1]Data are unadjusted mean (standard deviation).
[2]Backwards selected models considered baseline value, age, and BMI as well as design effects period and randomized sequence
+ Backwards selected model equivalent to the univariate mixed effects model
† Not modelled continuously
Abbreviations: n, sample size; N/A, not applicable

Table E2. Bowel Habits Diary Outcomes for the PP Population

Parameters[1]		All n=21	Placebo n=21	ResistAid n=21	Adjusted model P-value[2]
Number of bowel movements	Week 0	3.52 (1.40)			
	Week 3		4.05 (1.77)	4.05 (1.88)	1.000
	Week 6		3.81 (1.66)	3.81 (1.40)	1.000
Stool consistency	Week 0	3.40 (0.81)			
	Week 3		3.75 (1.05)	3.56 (1.06)	0.526
	Week 6		3.65 (1.04)	3.84 (0.94)	0.336+
Straining during bowel movement	Week 0	0.45 (0.50)			
	Week 3		0.35 (0.52)	0.43 (0.65)	N/A†
	Week 6		0.47 (0.51)	0.40 (0.52)	N/A†

Parameters[1]		All n=21	Placebo n=21	ResistAid n=21	Adjusted model P-value[2]
Discomfort during bowel movement	Week 0	0.21 (0.27)			
	Week 3		0.20 (0.32)	0.16 (0.32)	N/A†
	Week 6		0.32 (0.48)	0.17 (0.30)	N/A†
Sensation of incomplete evacuation	Week 0	0.13 (0.24)			
	Week 3		0.19 (0.49)	0.22 (0.41)	N/A†
	Week 6		0.23 (0.34)	0.30 (0.53)	N/A†

[1]Data are unadjusted mean (standard deviation).
[2]Backwards selected models considered baseline value, age, and BMI as well as design effects period and randomized sequence
† Not modelled continuously
Abbreviations: n, sample size; N/A, not applicable

Table E3. Bowel Habits Diary Dichotomized Outcomes for the ITT Population

Parameters[1]		All	Placebo	ResistAid	Adjusted model p-value[2]
Straining during bowel movement	Week 0	16 (53.33)			
	Week 3		12 (42.86)	12 (44.44)	0.946
	Week 6		18 (66.67)	15 (57.69)	0.345
Discomfort during bowel movement	Week 0	13 (43.33)			
	Week 3		8 (28.57)	8 (29.63)	0.942
	Week 6		12 (44.44)	7 (26.92)	0.164
Sensation of incomplete evacuation	Week 0	11 (36.67)			
	Week 3		8 (28.57)	11 (40.74)	0.207
	Week 6		11 (40.74)	10 (38.46)	0.773

[1]Data are unadjusted proportion i.e., n (%) reporting presence of mild-to-severe symptoms.
[2]Backwards selected model equivalent to the univariate mixed effects model
Abbreviations: n, sample size

Table E4. Bowel Habits Diary Dichotomized Outcomes for the PP Population

Parameters[1]		All	Placebo	ResistAid	Adjusted model p-value[2]
Straining during bowel movement	Week 0	12 (57.14)			
	Week 3		9 (42.86)	9 (42.86)	1.000
	Week 6		14 (66.67)	12 (57.14)	0.433
Discomfort during bowel movement	Week 0	9 (42.86)			
	Week 3		8 (38.10)	6 (28.57)	0.433
	Week 6		9 (42.86)	7 (33.33)	0.344
Sensation of incomplete evacuation	Week 0	6 (28.57)			
	Week 3		4 (19.05)	7 (33.33)	0.207
	Week 6		8 (38.10)	7 (33.33)	0.663

[1]Data are unadjusted proportion i.e., n (%) reporting presence of mild-to-severe symptoms.
[2]Backwards selected model equivalent to the univariate mixed effects model
Abbreviations: n, sample size

11.5.2 GI Tolerance Questionnaire

11.5.2.1 Statistical Analysis Approach

The proportion with at least one moderate-to-severe symptom was compared between supplementation groups with the Fisher's exact test, unadjusted for the crossover design. If no subject experienced an issue, the test was not performed.

11.5.2.2 Results

There were no statistically significant differences (P>0.05) in the proportion of subjects reporting moderate or severe issues (**Table F1 and F2**) or in the severity ratings for the individual GI Tolerance Questionnaire components between supplementations (**Tables F3 and F4**). Refer to Appendix SR4 Table T6 for more details.

Table F1. GI Tolerance Questionnaire Dichotomized Scores for ITT Population

		Placebo	ResistAid	P value[4]
Proportion of subjects who reported moderate or severe issues[1]	Baseline[3]	4 (14.3)	5 (17.2)	1.000
	Week 3	4 (14.3)	10 (37.0)	0.068
	Week 6	3 (11.1)	8 (30.8)	0.099

		Placebo	ResistAid	P value[4]
Proportion of subjects who developed moderate or severe issues during the study[2]	Baseline	N/A	N/A	N/A
	Week 3	3 (10.7)	7 (25.9)	0.177
	Week 6	2 (7.4)	5 (19.2)	0.250

[1]Subjects who reported at least 1 ≥moderate issue. Data are unadjusted proportion n (%).
[2]Subjects who reported at least 1 ≥moderate issue that was not previously reported at baseline (i.e., at least 1 ≥moderate issue developed during the study). Data are unadjusted proportion n (%).
[3]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[4]Fisher's exact test
Abbreviations: n, sample size; N/A, not applicable

Table F2. GI Tolerance Questionnaire Dichotomized Scores for PP Population

		Placebo	ResistAid	P value[4]
Proportion of subjects who reported moderate or severe issues[1]	Baseline[3]	3 (14.3)	3 (14.3)	1.000
	Week 3	2 (9.5)	7 (33.3)	0.130
	Week 6	2 (9.5)	6 (28.6)	0.238
Proportion of subjects who developed moderate or severe issues during the study[2]	Baseline	N/A	N/A	N/A
	Week 3	1 (4.8)	5 (23.8)	0.184
	Week 6	1 (4.8)	4 (19.0)	0.343

[1]Subjects who reported at least 1 ≥moderate issue. Data are unadjusted proportion n (%).
[2]Subjects who reported at least 1 ≥moderate issue that was not previously reported at baseline (i.e., at least 1 ≥moderate issue developed during the study). Data are unadjusted proportion n (%).
[3]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[4]Fisher's exact test
Abbreviations: n, sample size; N/A, not applicable

Table F3. GI Tolerance Questionnaire Dichotomized Individual Severity Scores for ITT Population

Component[1]		Severity category	Placebo	ResistAid	P value[3]
Abdominal cramping	Baseline[2]	< Moderate	28 (100.0)	29 (100.0)	N/A[4]
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	27 (96.4)	27 (100.0)	1.000
		≥ Moderate	1 (3.6)	0 (0.0)	
	Week 6	< Moderate	27 (100.0)	26 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	

Component[1]		Severity category	Placebo	ResistAid	P value[3]
Abdominal distention/bloating	Baseline	< Moderate	27 (96.4)	28 (96.6)	1.000
		≥ Moderate	1 (3.6)	1 (3.4)	
	Week 3	< Moderate	27 (96.4)	23 (85.2)	0.193
		≥ Moderate	1 (3.6)	4 (14.8)	
	Week 6	< Moderate	26 (96.3)	23 (88.5)	0.351
		≥ Moderate	1 (3.7)	3 (11.5)	
Burping	Baseline	< Moderate	27 (96.4)	28 (96.6)	1.000
		≥ Moderate	1 (3.6)	1 (3.4)	
	Week 3	< Moderate	26 (92.9)	24 (88.9)	0.669
		≥ Moderate	2 (7.1)	3 (11.1)	
	Week 6	< Moderate	26 (96.3)	26 (100.0)	1.000
		≥ Moderate	1 (3.7)	0 (0.0)	
Borborygmus/stomach rumbling	Baseline	< Moderate	28 (100.0)	28 (96.6)	1.000
		≥ Moderate	0 (0.0)	1 (3.4)	
	Week 3	< Moderate	27 (96.4)	26 (96.3)	1.000
		≥ Moderate	1 (3.6)	1 (3.7)	
	Week 6	< Moderate	26 (96.3)	25 (96.2)	1.000
		≥ Moderate	1 (3.7)	1 (3.8)	
Gas/flatulence	Baseline	< Moderate	26 (92.9)	24 (82.8)	0.423
		≥ Moderate	2 (7.1)	5 (17.2)	
	Week 3	< Moderate	27 (96.4)	21 (77.8)	0.051
		≥ Moderate	1 (3.6)	6 (22.2)	
	Week 6	< Moderate	24 (88.9)	19 (73.1)	0.175
		≥ Moderate	3 (11.1)	7 (26.9)	
Nausea	Baseline	< Moderate	28 (100.0)	29 (100.0)	N/A

Component[1]		Severity category	Placebo	ResistAid	P value[3]
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	28 (100.0)	27 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	27 (100.0)	26 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
Reflux	Baseline	< Moderate	28 (100.0)	29 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	28 (100.0)	27 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	27 (100.0)	25 (96.2)	0.491
		≥ Moderate	0 (0.0)	1 (3.8)	
Vomiting	Baseline	< Moderate	28 (100.0)	29 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	28 (100.0)	27 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	27 (100.0)	26 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	

[1]Data are unadjusted proportion i.e., n (%) reporting < moderate or ≥ moderate symptoms.
[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[3]Fisher's exact test
[4]Fisher's exact test was not performed because no subject reported ≥ moderate symptoms
Abbreviations: n, sample size; N/A, not applicable

Table F4. GI Tolerance Questionnaire Dichotomized Individual Severity Scores for PP Population

Component[1]	Week	Severity category	Placebo	ResistAid	P value[3]
Abdominal cramping	Baseline[2]	< Moderate	21 (100.0)	21 (100.0)	N/A[4]
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	21 (100.0)	21 (100.0)	N/A

Component[1]	Week	Severity category	Placebo	ResistAid	P value[3]
		≥ Moderate	0 (0.0)	0 (0.0)	
Abdominal distention/bloating	Baseline	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	21 (100.0)	19 (90.5)	0.488
		≥ Moderate	0 (0.0)	2 (9.5)	
	Week 6	< Moderate	21 (100.0)	20 (95.2)	1.000
		≥ Moderate	0 (0.0)	1 (4.8)	
Burping	Baseline	< Moderate	20 (95.2)	20 (95.2)	1.000
		≥ Moderate	1 (4.8)	1 (4.8)	
	Week 3	< Moderate	20 (95.2)	19 (90.5)	1.000
		≥ Moderate	1 (4.8)	2 (9.5)	
	Week 6	< Moderate	20 (95.2)	21 (100.0)	1.000
		≥ Moderate	1 (4.8)	0 (0.0)	
Borborygmus/stomach rumbling	Baseline	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	20 (95.2)	20 (95.2)	1.000
		≥ Moderate	1 (4.8)	1 (4.8)	
	Week 6	< Moderate	20 (95.2)	20 (95.2)	1.000
		≥ Moderate	1 (4.8)	1 (4.8)	
Gas/flatulence	Baseline	< Moderate	19 (90.5)	18 (85.7)	1.000
		≥ Moderate	2 (9.5)	3 (14.3)	
	Week 3	< Moderate	21 (100.0)	17 (81.0)	0.107
		≥ Moderate	0 (0.0)	4 (19.0)	
	Week 6	< Moderate	19 (90.5)	15 (71.4)	0.238
		≥ Moderate	2 (9.5)	6 (28.6)	

Component[1]	Week	Severity category	Placebo	ResistAid	P value[3]
Nausea	Baseline	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
Reflux	Baseline	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	21 (100.0)	20 (95.2)	1.000
		≥ Moderate	0 (0.0)	1 (4.8)	
Vomiting	Baseline	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 3	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	
	Week 6	< Moderate	21 (100.0)	21 (100.0)	N/A
		≥ Moderate	0 (0.0)	0 (0.0)	

[1]Data are unadjusted proportion i.e., n (%) reporting < moderate or ≥ moderate symptoms.
[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[3]Fisher's exact test
[4]Fisher's exact test was not performed because no subject reported ≥ moderate symptoms
Abbreviations: n, sample size; N/A, not applicable

11.5.3 SF-36

11.5.3.1 Statistical Analysis Approach

All items are scored so that a high score indicates a better health state with 0 as the lowest possible score and 100 as the highest possible score. The following details the analyses performed for each of the 8 domain scores. Due to the distributions of the domains, a model was only fit for emotional well-being (rank transformation), energy/fatigue (rank transformation), and general health (no transformation). For each of the specified domains, a univariate fixed effects model as well as a final adjusted multivariable model was estimated. The multivariable model considered terms for period, sequence, baseline measure, and age; final model was selected with

backwards elimination approach. All models included a term for a random intercept for subject nested within sequence.

For the other domains (pain, physical functioning, role limitations due to physical health, role limitations due to emotional problems, and social functioning), there appeared to be a ceiling effect. For pain, approximately 76% and 72% had a score of 100 for Week 3 and Week 6, respectively. For all others, the proportion of subjects with a score of 100 exceeded 92% for both measured time points.

11.5.3.2 Results

There were no statistically significant differences (P>0.05) in the scores for all 8 domains, e.g., emotional well-being, energy/fatigue, and general health, between supplementations (**Tables G1 and G2**). Refer to Appendix SR4 Table T4 and T5 for more details.

Table G1. SF-36 Scores for ITT Population

Domain[1]		Placebo n=27-28	ResistAid n=26-29	Adjusted model P-value
Emotional well-being	Week 0	87.43 (10.31)	87.45 (10.94)	
	Week 3	85.57 (14.82)	87.11 (11.33)	0.936
	Week 6	88.00 (8.45)	90.15 (8.33)	0.238
Energy/fatigue	Week 0	78.04 (16.96)	75.69 (16.08)	
	Week 3	75.00 (16.61)	77.96 (15.46)	0.228
	Week 6	78.52 (16.34)	77.31 (16.81)	0.808
General health	Week 0	88.93 (11.41)	86.55 (11.43)	
	Week 3	87.68 (13.23)	87.59 (11.21)	0.425
	Week 6	87.96 (10.94)	88.27 (10.95)	0.360
Pain	Week 0	93.21 (8.92)	96.81 (6.16)	
	Week 3	96.70 (6.94)	97.41 (4.47)	N/A[2]
	Week 6	96.20 (6.55)	95.19 (9.62)	N/A
Physical functioning	Week 0	99.82 (0.94)	97.24 (10.40)	
	Week 3	99.82 (0.94)	99.44 (2.12)	N/A
	Week 6	99.81 (0.96)	99.81 (0.98)	N/A

Domain[1]		Placebo n=27-28	ResistAid n=26-29	Adjusted model P-value
Role limitations due to emotional problems	Week 0	100.00 (0.00)	98.85 (6.19)	
	Week 3	96.43 (18.90)	96.30 (19.25)	N/A
	Week 6	96.30 (14.12)	100.00 (0.00)	N/A
Role limitations due to physical health	Week 0	100.00 (0.00)	99.14 (4.64)	
	Week 3	98.21 (9.45)	100.00 (0.00)	N/A
	Week 6	97.22 (10.59)	98.08 (6.79)	N/A
Social functioning	Week 0	99.11 (4.72)	96.98 (14.04)	
	Week 3	96.88 (10.55)	96.76 (10.74)	N/A
	Week 6	97.69 (6.97)	99.04 (4.90)	N/A

[1]Data are unadjusted means (standard deviation).
[2]These domains were not modelled due to the uneven data distribution
Abbreviations: n, sample size; N/A, not applicable

Table G2. SF-36 Scores for PP Population

Domain[1]		Placebo n=21	ResistAid n=21	Adjusted model P-value
Emotional well-being	Week 0	89.33 (8.97)	88.76 (9.26)	
	Week 3	89.71 (7.65)	87.81 (12.03)	0.862
	Week 6	88.95 (8.57)	90.29 (8.82)	0.263
Energy/fatigue	Week 0	80.95 (18.00)	77.62 (15.94)	
	Week 3	79.29 (14.69)	79.52 (16.19)	0.553
	Week 6	79.76 (16.54)	78.57 (15.74)	0.953
General health	Week 0	90.00 (10.49)	89.29 (10.64)	
	Week 3	90.00 (10.37)	88.33 (11.55)	0.528
	Week 6	89.05 (10.56)	89.29 (10.16)	0.614

Domain[1]		Placebo n=21	ResistAid n=21	Adjusted model P-value
Pain	Week 0	94.05 (8.57)	97.62 (5.39)	
	Week 3	97.14 (6.44)	97.62 (4.36)	N/A[2]
	Week 6	96.55 (6.91)	95.48 (9.96)	N/A
Physical functioning	Week 0	99.76 (1.09)	96.90 (12.09)	
	Week 3	99.76 (1.09)	99.29 (2.39)	N/A
	Week 6	99.76 (1.09)	99.76 (1.09)	N/A
Role limitations due to emotional problems	Week 0	100.00 (0.00)	98.41 (7.27)	
	Week 3	100.00 (0.00)	95.24 (21.82)	N/A
	Week 6	96.83 (14.55)	100.00 (0.00)	N/A
Role limitations due to physical health	Week 0	100.00 (0.00)	100.00 (0.00)	
	Week 3	100.00 (0.00)	100.00 (0.00)	N/A
	Week 6	100.00 (0.00)	98.81 (5.46)	N/A
Social functioning	Week 0	100.00 (0.00)	99.40 (2.73)	
	Week 3	99.40 (2.73)	96.43 (11.95)	N/A
	Week 6	99.40 (2.73)	98.81 (5.46)	N/A

[1]Data are unadjusted means (standard deviation).
[2]These domains were not modelled due to the uneven data distribution
Abbreviations: n, sample size; N/A, not applicable

11.5.4 Plasma SCFA

11.5.4.1 Statistical Analysis Approach

The measured plasma SCFAs included: acetic, propionic, and butyric acids. Propionic and butyric acid had samples that were below the LOD (<1.00). In the event that a particular sample was <1.00, it was replaced with 1/2*LOD (0.500). A linear mixed model was used to estimate the difference in acetic acid and propionic acid at the 6-week time point. For each, a univariate fixed effect model for intervention effect as well as a final adjusted multivariable model was estimated. The multivariable model considered terms for period, sequence, baseline measure, age and BMI. Final model was selected with the backwards elimination approach. All models included a random intercept for subject nested within sequence

The presence of butyric acid was modeled with a generalized linear mixed model following a binomial distribution with a logit link. Due to the number of events, a model considering product and the presence of butyric acid at baseline as fixed effects was fit in order to have estimable effects; a random intercept for subject nested within sequence was included.

11.5.4.2 Results

There were no significant or marginally significant differences between interventions for plasma acetic acid, butyric acid, and propionic acid (**Tables H1 and H2**). Refer to Appendix SR4 Tables T14 and T15 for more details.

Table H1. Plasma SCFA for ITT Population

Plasma SCFA[1]		Placebo n=27-28	ResistAid n=26-29	Unadjusted model P-value	Adjusted model P-value[3]
Acetic acid (µg/mL)	Baseline[2]	68.80 (35.52, 97.43)	65.86 (50.16, 85.61)		
	Week 6	84.47 (65.07, 102.44)	83.96 (68.87, 120.01)	0.947	0.822
Butyric acid (µg/mL)	Baseline	0.50 (0.50, 2.36)	0.50 (0.50, 1.48)		
	Week 6	0.50 (0.50, 2.64)	0.50 (0.50, 2.33)	0.939*	0.993*
Propionic acid (µg/mL)	Baseline	0.50 (0.50, 2.26)	0.50 (0.50, 2.85)	0.790	0.710
	Week 6	4.52 (1.07, 6.44)	4.88 (0.50, 6.40)		

[1]Data are unadjusted median (range). Median (range) is presented because the mean is skewed due to distribution of the data.
[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[3]Adjusted for the respective baseline SCFA, BMI, and/or randomized sequence.
*Obtained from generalized linear mixed models on odds of having detectable butyric acid
Abbreviations: g, gram; mL, milliliter; n, sample size; SCFA, short-chain fatty acid

Table H2. Plasma SCFA for PP Population

Plasma SCFA[1]		Placebo n=21	ResistAid n=21	Unadjusted model P-value	Adjusted model P-value[3]
Acetic acid (µg/mL)	Baseline[2]	67.07 (35.52, 97.43)	66.51 (50.16, 85.61)		
	Week 6	82.28 (65.07, 102.44)	86.20 (69.96, 120.01)	0.538	0.550
Butyric acid (µg/mL)	Baseline	0.50 (0.50, 2.30)	0.50 (0.50, 1.48)		
	Week 6	0.50 (0.50, 2.21)	0.50 (0.50, 2.33)	0.592*	0.665*
	Baseline	0.50 (0.50, 2.26)	0.50 (0.50, 2.85)	0.558	0.359

Plasma SCFA[1]		Placebo n=21	ResistAid n=21	Unadjusted model P-value	Adjusted model P-value[3]
Propionic acid (µg/mL)	Week 6	3.93 (1.07, 6.44)	4.55 (0.50, 6.40)		

[1]Data are unadjusted median (range). Median (range) is presented because the mean is skewed due to distribution of the data.
[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
[3]Adjusted for the respective baseline SCFA, BMI, and/or randomized sequence.
*Obtained from generalized linear mixed models on odds of having detectable butyric acid
Abbreviations: g, gram; mL, milliliter ; n, sample size; SCFA, short-chain fatty acid

11.6 Post Hoc Microbiome Analysis

The PICRUSt bioinformatics software package was used to predict functional abundances using 16s rRNA marker gene. Note, this package can be used to generate hypotheses but should be interpreted cautiously. Predictions are limited to the gene contents of existing reference genomes. The ANOVA test was used to evaluate if at least 1 treatment group/time point combination was significantly different (**Appendix SR6**). The top 5 significantly different predicted functions were further explored and included: Alpha-L-rhamnosidase ($p<0.001$), Chondroitin-sulfate-ABC endolyase ($p<0.001$), Chondroitin-sulfate-ABC exolyase ($p<0.001$), Succinate--CoA ligase ($p<0.001$), UDP-N-acetylglucosamine 2-epimerase ($p<0.001$). For each, the pairwise comparisons were evaluated the ResistAid supplementation was higher than the placebo supplementation at each time point and the ResistAid supplementation was higher at each time point compared to baseline (**Figures 6-10**).

Additionally, the DeSeq2 package was used to compare the differential abundance for taxa by treatment group and time points. DeSeq2 only allows for pairwise comparisons. Significant taxa differences between placebo and ResistAid supplementation are shown below (**Table I**).

Table I. Summary of significant differences between supplementations in taxa abundance

Time point	Taxa	Adjusted p-value	Direction of change
Week 3	D_5__.Eubacterium..ruminantium.group	0.001	Upregulated with ResistAid supplementation
	D_5__Howardella	0.005	Upregulated with ResistAid supplementation
	D_5__GCA.900066225	<0.001	Upregulated with ResistAid supplementation
	D_5__DTU089	<0.001	Upregulated with ResistAid supplementation
	D_5__CAG.352	0.001	Upregulated with placebo supplementation
	D_5__Victivallis	0.003	Upregulated with placebo supplementation
Week 6	D_5__Eisenbergiella	<0.001	Upregulated with ResistAid supplementation
	D_5__DTU089	<0.001	Upregulated with ResistAid supplementation

Figure 6. Proportion of sequences for alpha-L-rhamnosidase

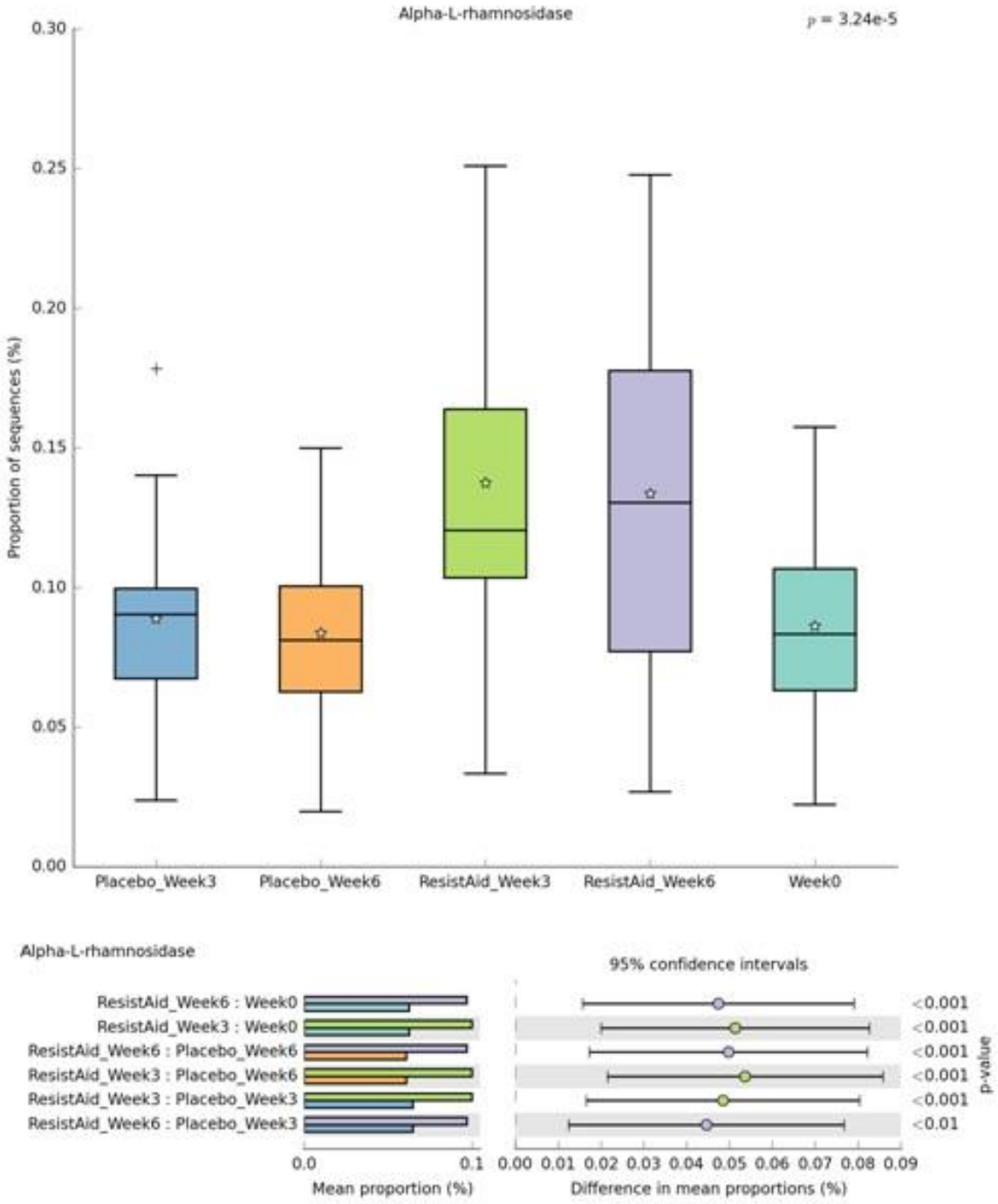


Figure 7. Proportion of sequences for Chondroitin-sulfate-ABC endolyase

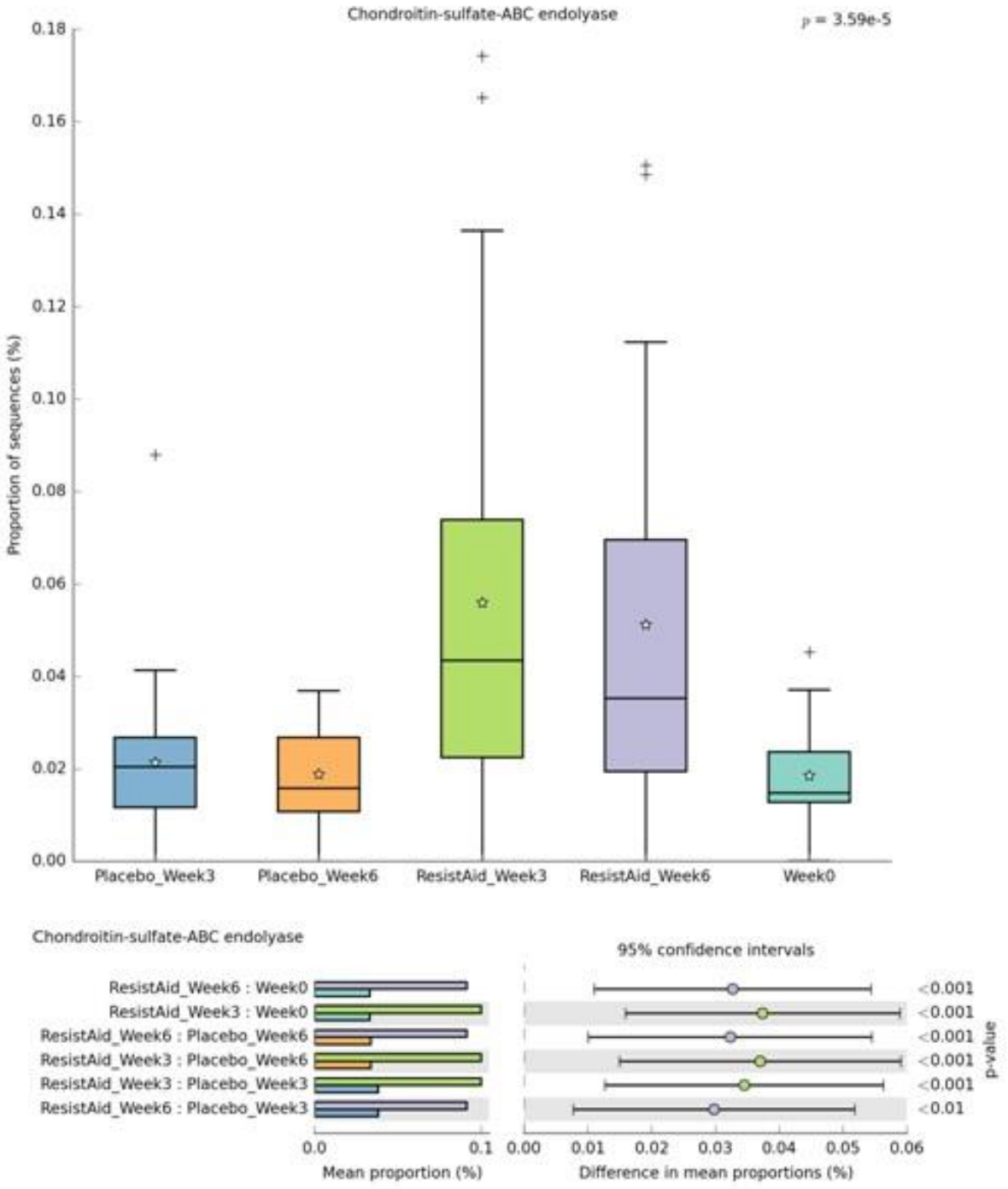


Figure 8. Proportion of sequences for Chondroitin-sulfate-ABC exolyase

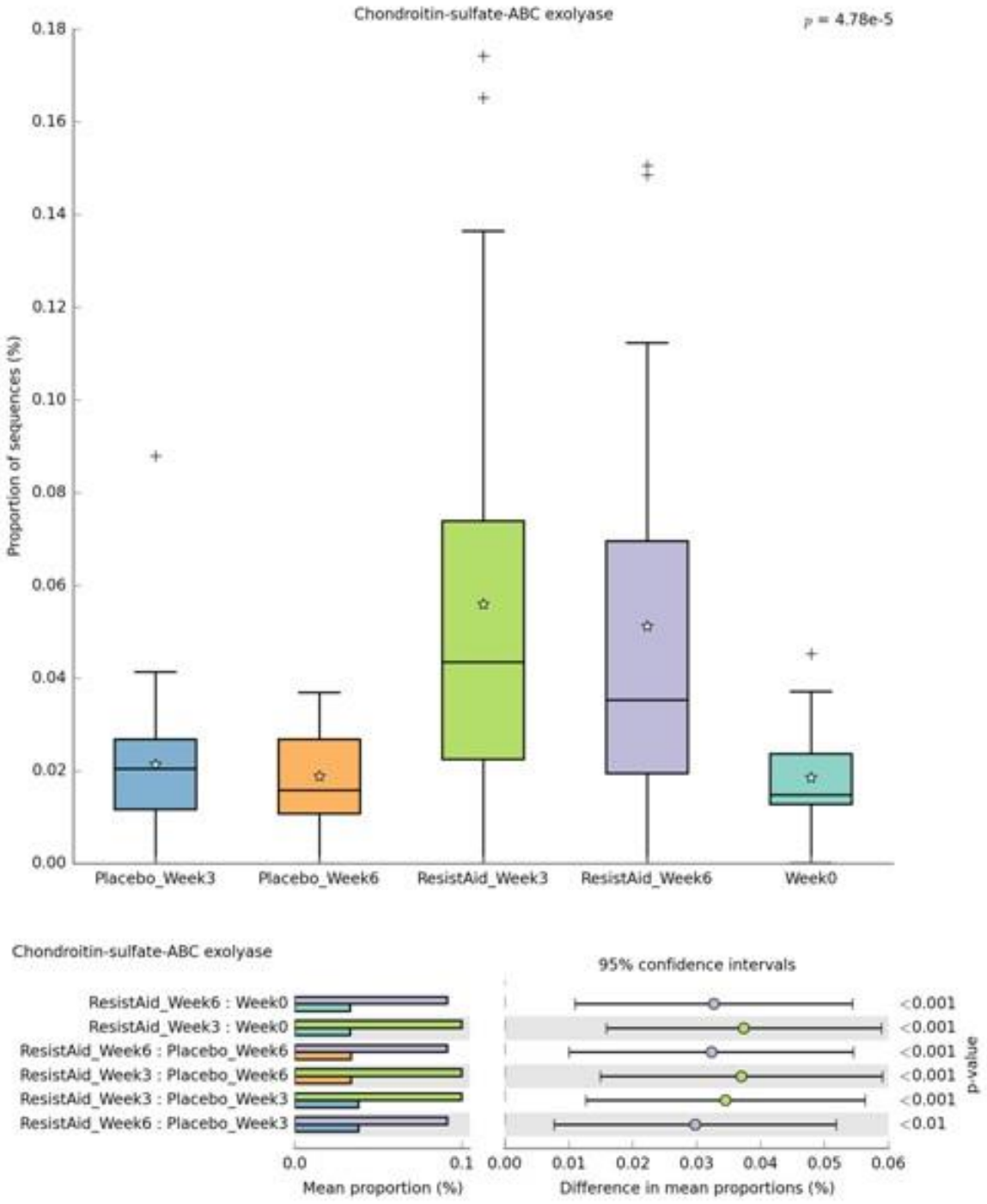


Figure 9. Proportion of sequences for Succinate--CoA ligase

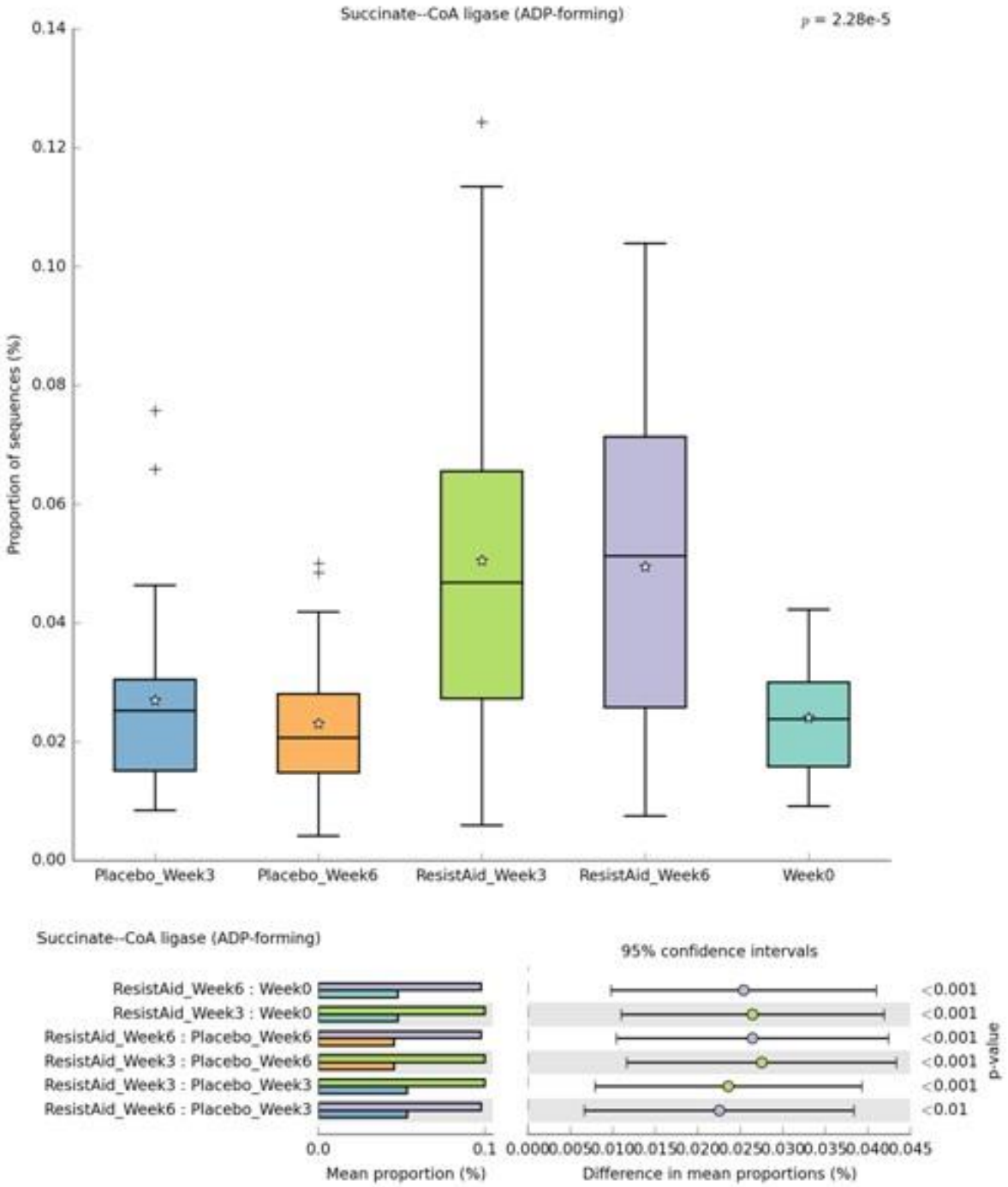
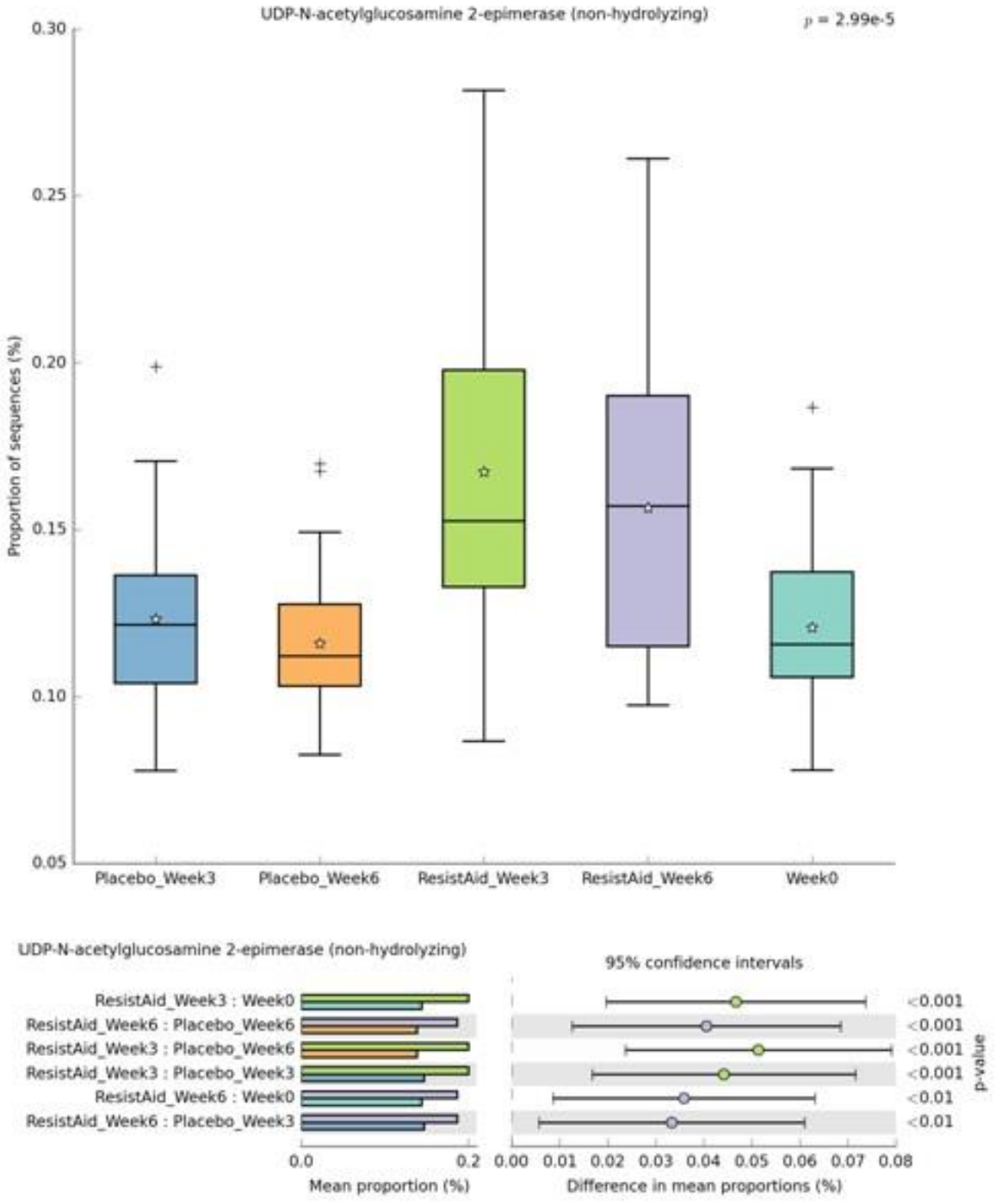


Figure 10. UDP-N-acetylglucosamine 2-epimerase



12 SAFETY

12.1 Adverse Events

Twenty mild or moderate adverse events were reported by 11 subjects during the study and no serious adverse events were reported. Of these, two adverse events were judged by the Clinical Investigator to be definitely or probably related to the study product and both were reported by one subject (#005). Subject #005 reported moderate bloating and mild decreased ease of bowel movement passage two days after start of consumption of the ResistAid product. Details for all the adverse events can be found in Appendix SR3.

12.2 Body Weight, Vitals, Chemistry Profile, and Hematology Panel

Descriptive statistics for body weight, vitals (blood pressure and heart rate), chemistry profile, and hematology panel for the ITT and PP populations are shown in **Tables J1, J2, J3, J4, and J5** below. No abnormal results for the chemistry profile, metabolic panel, and vitals were noted by the study physician. One subject (#029) lost 8.3% body weight during the placebo supplementation and another (Subject #025) lost 9.1% during the washout period.

Table J1. Body Weight and Vitals for the ITT and PP Populations

Parameter[1]		ITT		PP	
		Placebo	ResistAid	Placebo	ResistAid
		n=27-28	n=26-29	n=21	n=21
Body Weight (kg)	Baseline[2]	77.29 (16.09)	79.26 (16.59)	79.16 (14.86)	79.30 (15.14)
	Week 3	77.12 (16.48)	78.45 (16.38)	78.96 (14.94)	79.34 (14.98)
	Week 6	78.21 (16.16)	78.05 (16.16)	79.39 (14.98)	79.30 (14.71)
Systolic Blood Pressure (mm Hg)	Baseline	116.07 (15.68)	119.93 (13.79)	118.90 (13.90)	120.95 (12.90)
	Week 3	115.18 (13.36)	116.07 (11.48)	117.43 (11.46)	117.33 (10.18)
	Week 6	118.19 (12.45)	117.96 (11.84)	120.62 (10.84)	118.52 (9.29)
Diastolic Blood Pressure (mm Hg)	Baseline	73.04 (13.23)	74.59 (10.99)	75.43 (11.35)	76.62 (10.71)
	Week 3	73.64 (11.49)	74.19 (9.92)	75.90 (9.98)	75.43 (8.58)
	Week 6	74.67 (9.04)	72.92 (12.06)	76.00 (7.40)	73.24 (7.90)
Heart Rate (bpm)	Baseline	67.68 (9.68)	63.79 (7.56)	66.38 (8.72)	63.95 (7.68)
	Week 3	67.14 (8.58)	68.22 (10.81)	66.48 (8.01)	67.86 (10.08)
	Week 6	67.74 (9.17)	66.81 (8.34)	66.67 (8.56)	65.90 (7.76)

[1]Data are unadjusted mean (standard deviation).

[2]Baseline refers to data collected prior to the start of any supplementation (i.e., prior to Week 0) and following washout and prior subjects crossing over to the next supplementation.
Abbreviations: bpm, beats per minute; ITT, Intent-to-treat; kg, kilogram; mm Hg, millimeter Mercury; n, sample size; PP, per protocol

Table J2. Chemistry Profile for the ITT Population

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Glucose (mg/dL)	Week 0	91.57 (6.06)		
	Week 6		92.85 (9.18)	92.73 (6.34)
Sodium (mmol/L)	Week 0	141.03 (1.54)		
	Week 6		140.19 (1.49)	140.81 (1.60)
Potassium (mmol/L)	Week 0	4.64 (0.45)		
	Week 6		4.46 (0.42)	4.57 (0.52)
Chloride (mmol/L)	Week 0	106.83 (2.00)		
	Week 6		106.70 (2.52)	107.42 (1.68)
Carbon Dioxide (mmol/L)	Week 0	28.17 (1.78)		
	Week 6		28.78 (1.87)	28.62 (1.36)
Blood Urea Nitrogen (mg/dL)	Week 0	13.97 (3.25)		
	Week 6		13.30 (3.14)	13.42 (3.86)
Creatinine (mg/dL)	Week 0	0.92 (0.15)		
	Week 6		0.93 (0.15)	0.94 (0.17)
Total Calcium (mg/dL)	Week 0	8.99 (0.37)		
	Week 6		9.07 (0.39)	9.10 (0.36)
Alkaline Phosphatase (U/L)	Week 0	65.57 (14.41)		
	Week 6		64.96 (11.83)	65.19 (13.80)

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Alanine Aminotransferase (U/L)	Week 0	25.33 (7.79)		
	Week 6		26.26 (8.03)	25.96 (6.51)
Aspartate Aminotransferase (U/L)	Week 0	18.47 (4.59)		
	Week 6		18.33 (5.87)	16.96 (3.92)
Total Bilirubin (mg/dL)	Week 0	0.61 (0.28)		
	Week 6		0.70 (0.36)	0.60 (0.35)
Total Protein (g/dL)	Week 0	7.45 (0.49)		
	Week 6		7.30 (0.48)	7.30 (0.42)
Albumin (g/dL)	Week 0	3.88 (0.24)		
	Week 6		3.92 (0.27)	3.92 (0.27)
Globulin (g/dL)	Week 0	3.57 (0.38)		
	Week 6		3.38 (0.39)	3.37 (0.37)
Albumin / Globulin Ratio	Week 0	1.09 (0.13)		
	Week 6		1.18 (0.16)	1.18 (0.17)
Anion Gap (mmol/L)	Week 0	6.03 (1.56)		
	Week 6		4.70 (2.25)	4.77 (1.39)
Blood Urea Nitrogen / Creatinine Ratio	Week 0	15.38 (3.37)		
	Week 6		14.69 (4.70)	14.75 (5.15)
Calculated Osmolality (mOsm/kg)	Week 0	292.10 (3.48)		
	Week 6		290.22 (3.23)	291.58 (3.61)

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Glomerular Filtration Rate for African Americans (mL/minute/1.73 m^2)	Week 0	104.73 (18.81)		
	Week 6		102.89 (17.45)	104.38 (20.58)
Glomerular Filtration Rate for Non-African Americans (mL/minute/1.73 m^2)	Week 0	90.70 (16.17)		
	Week 6		89.19 (14.93)	90.46 (17.77)

[1]Data are unadjusted mean (standard deviation).
Abbreviations: dL, deciliter; g, gram; ITT, Intent-to-treat; L, liter; m, meter; mg, milligram; mmol, millimole; mOsm, milliosmole; n, sample size; PP, per protocol; U, International unit

Table J3. Chemistry Profile for the PP Population

Parameter[1]		All n=21	Placebo n=21	ResistAid n=21
Glucose (mg/dL)	Week 0	92.76 (5.89)		
	Week 6		94.05 (8.66)	93.29 (6.75)
Sodium (mmol/L)	Week 0	140.90 (1.58)		
	Week 6		140.24 (1.37)	140.86 (1.71)
Potassium (mmol/L)	Week 0	4.62 (0.49)		
	Week 6		4.44 (0.37)	4.59 (0.56)
Chloride (mmol/L)	Week 0	106.62 (1.91)		
	Week 6		106.90 (2.57)	107.62 (1.72)
Carbon Dioxide (mmol/L)	Week 0	28.14 (1.68)		
	Week 6		28.67 (1.83)	28.57 (1.40)
Blood Urea Nitrogen (mg/dL)	Week 0	14.19 (3.49)		
	Week 6		13.81 (2.84)	13.76 (3.94)

Parameter[1]		All n=21	Placebo n=21	ResistAid n=21
Creatinine (mg/dL)	Week 0	0.92 (0.16)		
	Week 6		0.93 (0.16)	0.95 (0.17)
Total Calcium (mg/dL)	Week 0	9.05 (0.37)		
	Week 6		9.00 (0.36)	9.13 (0.38)
Alkaline Phosphatase (U/L)	Week 0	65.76 (14.76)		
	Week 6		65.14 (12.85)	65.76 (14.90)
Alanine Aminotransferase (U/L)	Week 0	26.19 (8.60)		
	Week 6		27.24 (7.77)	26.19 (7.09)
Aspartate Aminotransferase (U/L)	Week 0	18.57 (4.20)		
	Week 6		19.19 (5.17)	17.14 (3.77)
Total Bilirubin (mg/dL)	Week 0	0.61 (0.28)		
	Week 6		0.68 (0.33)	0.60 (0.33)
Total Protein (g/dL)	Week 0	7.44 (0.47)		
	Week 6		7.25 (0.44)	7.30 (0.43)
Albumin (g/dL)	Week 0	3.91 (0.22)		
	Week 6		3.91 (0.28)	3.96 (0.25)
Globulin (g/dL)	Week 0	3.53 (0.40)		
	Week 6		3.33 (0.38)	3.34 (0.40)
Albumin / Globulin Ratio	Week 0	1.11 (0.14)		
	Week 6		1.20 (0.17)	1.20 (0.18)

Parameter[1]		All n=21	Placebo n=21	ResistAid n=21
Anion Gap (mmol/L)	Week 0	6.14 (1.53)		
	Week 6		4.67 (2.01)	4.67 (1.43)
Blood Urea Nitrogen /Creatinine Ratio	Week 0	15.62 (3.75)		
	Week 6		15.49 (4.86)	15.00 (5.62)
Calculated Osmolality (mOsm/kg)	Week 0	292.00 (3.62)		
	Week 6		290.52 (3.01)	291.86 (3.86)
Glomerular Filtration Rate for African Americans (mL/minute/1.73 m^2)	Week 0	105.33 (20.04)		
	Week 6		104.05 (17.73)	102.00 (20.21)
Glomerular Filtration Rate for Non-African Americans (mL/minute/1.73 m^2)	Week 0	91.24 (17.27)		
	Week 6		90.10 (15.26)	88.38 (17.36)

[1]Data are unadjusted mean (standard deviation).
Abbreviations: dL, deciliter; g, gram; ITT, Intent-to-treat; L, liter; m, meter; mg, milligram; mmol, millimole; mOsm, milliosmole; n, sample size; PP, per protocol; U, International unit

Table J4. Hematology Panel for the ITT Population

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Red Blood Cell (M/µL)	Week 0	4.72 (0.41)		
	Week 6		4.73 (0.46)	4.72 (0.43)
Red Blood Cell Distribution Width (%)	Week 0	12.74 (0.67)		
	Week 6		12.56 (0.64)	12.63 (0.64)
Hematocrit (%)	Week 0	43.32 (2.86)		
	Week 6		42.98 (3.19)	43.05 (3.14)

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Hemoglobin (g/dL)	Week 0	14.28 (1.12)		
	Week 6		14.19 (1.08)	14.20 (1.21)
Mean Corpuscular Hemoglobin (pg)	Week 0	30.28 (1.41)		
	Week 6		30.06 (1.46)	30.12 (1.32)
Mean Corpuscular Hemoglobin Concentration (g/dL)	Week 0	32.95 (1.02)		
	Week 6		33.02 (1.05)	32.96 (1.04)
Mean Corpuscular Volume (fL)	Week 0	91.89 (3.51)		
	Week 6		91.07 (3.39)	91.42 (3.45)
Platelet (K/µL)	Week 0	261.40 (60.15)		
	Week 6		257.41 (66.55)	259.62 (72.81)
White Blood Cell (K/µL)	Week 0	6.44 (1.29)		
	Week 6		5.92 (1.07)	5.94 (1.15)
Basophil (%)	Week 0	0.67 (0.28)		
	Week 6		0.70 (0.24)	0.73 (0.31)
Basophil Absolute (K/µL)	Week 0	0.04 (0.02)		
	Week 6		0.04 (0.02)	0.04 (0.02)
Eosinophil (%)	Week 0	2.58 (1.37)		
	Week 6		2.47 (1.56)	3.57 (3.50)
Eosinophil Absolute (K/µL)	Week 0	0.16 (0.09)		
	Week 6		0.14 (0.09)	0.22 (0.26)

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Lymphocyte (%)	Week 0	32.59 (9.34)		
	Week 6		34.01 (8.74)	33.43 (10.03)
Lymphocyte Absolute (K/µL)	Week 0	2.04 (0.50)		
	Week 6		1.98 (0.48)	1.93 (0.50)
Monocyte (%)	Week 0	8.35 (1.84)		
	Week 6		8.33 (1.92)	8.40 (2.07)
Monocyte Absolute (K/µL)	Week 0	0.53 (0.14)		
	Week 6		0.49 (0.11)	0.49 (0.11)
Neutrophil (%)	Week 0	55.56 (9.86)		
	Week 6		54.24 (9.57)	53.64 (9.59)
Neutrophil Absolute (K/µL)	Week 0	3.64 (1.09)		
	Week 6		3.24 (0.91)	3.23 (0.98)
Immature Granulocyte (%)	Week 0	0.26 (0.12)		
	Week 6		0.24 (0.13)	0.23 (0.14)
Immature Granulocyte Absolute (K/µL)	Week 0	0.02 (0.01)		
	Week 6		0.01 (0.01)	0.01 (0.01)

[1]Data are unadjusted mean (standard deviation).
Abbreviations: ITT, Intent-to-treat; n, sample size; PP, per protocol

Table J5. Hematology Panel for the PP Population

Parameter[1]		All n=30	Placebo n=27-28	ResistAid n=26-29
Red Blood Cell (M/µL)	Week 0	4.77 (0.44)		
	Week 6		4.73 (0.46)	4.74 (0.42)
Red Blood Cell Distribution Width (%)	Week 0	12.56 (0.55)		
	Week 6		12.41 (0.50)	12.47 (0.54)
Hematocrit (%)	Week 0	43.91 (2.88)		
	Week 6		43.11 (3.19)	43.24 (3.10)
Hemoglobin (g/dL)	Week 0	14.57 (1.07)		
	Week 6		14.27 (1.10)	14.32 (1.16)
Mean Corpuscular Hemoglobin (pg)	Week 0	30.58 (1.36)		
	Week 6		30.23 (1.41)	30.29 (1.12)
Mean Corpuscular Hemoglobin Concentration (g/dL)	Week 0	33.16 (1.06)		
	Week 6		33.12 (1.14)	33.12 (1.00)
Mean Corpuscular Volume (fL)	Week 0	92.23 (3.62)		
	Week 6		91.31 (3.32)	91.50 (3.33)
Platelet (K/µL)	Week 0	260.76 (68.37)		
	Week 6		257.00 (72.21)	262.00 (80.41)
White Blood Cell (K/µL)	Week 0	6.67 (1.38)		
	Week 6		6.22 (0.98)	6.04 (1.15)
Basophil (%)	Week 0	0.68 (0.28)		

Parameter[1]		All	Placebo	ResistAid
		n=30	n=27-28	n=26-29
	Week 6		0.71 (0.26)	0.77 (0.32)
Basophil Absolute (K/µL)	Week 0	0.04 (0.02)		
	Week 6		0.04 (0.02)	0.05 (0.02)
Eosinophil (%)	Week 0	2.58 (1.30)		
	Week 6		2.60 (1.64)	3.93 (3.80)
Eosinophil Absolute (K/µL)	Week 0	0.17 (0.08)		
	Week 6		0.16 (0.10)	0.25 (0.28)
Lymphocyte (%)	Week 0	32.00 (10.29)		
	Week 6		33.07 (8.64)	32.99 (9.86)
Lymphocyte Absolute (K/µL)	Week 0	2.08 (0.58)		
	Week 6		2.04 (0.50)	1.95 (0.55)
Monocyte (%)	Week 0	8.34 (1.83)		
	Week 6		8.08 (1.60)	8.38 (1.90)
Monocyte Absolute (K/µL)	Week 0	0.56 (0.15)		
	Week 6		0.50 (0.12)	0.50 (0.11)
Neutrophil (%)	Week 0	56.15 (10.53)		
	Week 6		55.29 (8.91)	53.70 (8.68)
Neutrophil Absolute (K/µL)	Week 0	3.81 (1.14)		
	Week 6		3.46 (0.83)	3.28 (0.93)
Immature Granulocyte (%)	Week 0	0.25 (0.12)		

Parameter[1]		All	Placebo	ResistAid
		n=30	n=27-28	n=26-29
	Week 6		0.26 (0.12)	0.23 (0.14)
Immature Granulocyte Absolute (K/µL)	Week 0	0.02 (0.01)		
	Week 6		0.02 (0.01)	0.01 (0.01)

[1]Data are unadjusted mean (standard deviation).

Abbreviations: fL, femtoliter; ITT, Intent-to-treat; K/µL, kilo per microliter; M/µL, Million microliter; n, sample size; pg, pictogram; PP, per protocol

13 CONCLUSION

Compared to 15 g maltodextrin/ day, consumption of 15 g ResistAid/ day for 6 weeks by healthy adults resulted in a decrease in the ratio of fecal *Firmicutes* to *Bacteriodetes* driven by an increase in *Bacteriodetes* and a decrease in *Firmicutes*. Additionally, compared to 15 g maltodextrin/ day, 15 g ResistAid/ day for 6 weeks decreased alpha-diversity of fecal microbiome assessed by the Shannon Index and Simpson's Index, without affecting beta-diversity. PICRUSt analysis predicted five pathways, i.e., alpha-L-rhamnosidase, Chondroitin-sulfate-ABC endolyase, Chondroitin-sulfate-ABC exolyase, Succinate--CoA ligase, UDP-N-acetylglucosamine 2-epimerase, are significantly higher following 15 g ResistAid/ day compared to 15 g maltodextrin/ day. Fecal isovaleric acid, valeric acid, and hexanoic acid, but not acetic acid (marginal effect), butyric acid, isobutyric acid, and propionic acid were lower following 15 g ResistAid/ day compared to 15 g maltodextrin/ day. Plasma acetic acid, butyric acid, and propionic acid were not significantly different between the supplementations. A high proportion of subjects did not have bowel movement problems in regards to straining, discomfort, and incomplete evacuation. There were no statistically significant changes in number of bowel movements, stool consistency, straining, discomfort, and sensation of evacuation ratings, the proportion of subjects reporting moderate or severe issues or in the severity ratings for the individual gastrointestinal symptoms, and ratings for physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions.

Two adverse events were reported in one subject during the ResistAid supplementation (moderate bloating and mild decreased ease of bowel movement). No abnormal results for the chemistry profile, metabolic panel, and vitals were noted by the study physician. One subject lost 8.3% body weight during the placebo supplementation.

14 LIMITATIONS

Participants in the trial were chiefly Caucasian which may limit generalizability; however, their low average habitual dietary fiber intake is typical across all socioeconomic demographics in the Western countries. Subjects were healthy adults and may already have a healthy microbiome

which may explain the lack of changes in certain bacterial strains previously reported (Lactobacillus ssp.). Additionally, some SCFAs were not detectable or were at very low concentrations, thus contributing to a lack of measurable change. The 16S rRNA sequencing used to measure the gut microbiome composition and predicted functions did not provide detailed arabinogalactan-induced changes in bacterial species and strains. Further analysis is required. It is also important to note that our speculated changes in the metabolic pathways need to be confirmed by additional measurements using a metabolomics approach potentially in subsequent trials.

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