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ORIGINAL RESEARCH—CLINICAL

Safety and Tolerability of Microbial Inulinase Supplementation in Healthy Adults: A Randomized, Placebo-Controlled Trial

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BACKGROUND AND AIMS: Dietary fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) contribute to gastrointestinal (GI) symptoms in individuals with FODMAP sensitivity and irritable bowel syndrome. Oral enzyme supplementation is a strategy to reduce dietary FODMAP exposure and limit FODMAP-associated GI distress. This clinical trial investigated the safety of dietary supplementation with a food-grade, microbial inulinase known to hydrolyze fructan-type or inulin-type FODMAPs and related fructo-oligosaccharides in vitro. **METHODS:** A randomized, double-blind, placebo-controlled, parallel design trial was conducted in 60 healthy adult participants of both sexes. Following a 2-week run-in placebo phase, participants were randomized to consume inulinase or placebo capsules twice daily with meals for 4 weeks. The total daily dose of inulinase was 2000 inulinase activity units. Safety measures included blood clinical chemistry, hematology, lipid profile, high-sensitivity C-reactive protein, insulin, lactate, and uric acid. GI symptoms were recorded weekly using the 15-item Gastrointestinal Symptom Rating Scale. **RESULTS:** Fifty-eight participants completed the study. There were no clinically meaningful between-group differences in blood biomarkers. During the 4-week intervention period, 5 (16.7%) of 30 participants reported 5 adverse events in the inulinase group, and 8 (26.7%) of 30 participants reported 13 adverse events in the placebo group. No statistically significant between-group differences were observed in the change from baseline to 1, 2, 3, or 4 weeks of supplementation with respect to the 15-item Gastrointestinal Symptom Rating Scale overall or domain scores. **CONCLUSION:** Microbial inulinase supplementation demonstrated a favorable safety profile in healthy adults. Further investigation in a dose-ranging study in individuals with dietary FODMAP, fructan, or inulin sensitivity or irritable bowel syndrome is warranted. ClinicalTrials.gov: NCT05744700.

Keywords: Dietary Fiber; Enzyme; FODMAP; Food Sensitivity; Food Intolerance; Fructanase

Introduction

Food sensitivity, also known as food intolerance, describes a non-immunological, consistent, untoward food response typically arising from perturbed biochemical digestion of doses of certain foods that are broadly well-tolerated.[1] Dietary components associated with food sensitivity include lactose, gluten, histamine, fats, and fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs).[1,2] Clinical features of food sensitivity include gastrointestinal (GI) symptoms such as abdominal bloating, burping, flatulence, indigestion, diarrhea, and constipation. Food sensitivity is estimated to affect 15%–45% of the population,[3–6] consistent with a global survey showing that nearly 18% of 51,425 respondents experienced bloating at least once per week over the prior 3 months.[7] The prevalence of food sensitivity approaches 80% in individuals with functional GI disorders such as irritable bowel syndrome (IBS).[3,4,8–11]

A specific class of nondigestible FODMAPs called fructans (eg, inulins) are particularly associated with food sensitivity in both healthy individuals and individuals with IBS.[12–15] Fructans, inulin, and shorter chain fructo-oligosaccharides are polymers of fructose that naturally occur across many vegetables and fruits.[16] According to magnetic resonance imaging, oral inulin-type fructan administration is associated with increased colonic gas,[17,18] presumably a result of metabolism by resident gut microbes. One approach to limit the severity of FODMAP sensitivity is to reduce dietary intake of foods containing FODMAPs.[19–21] An unintended consequence of this strategy, though, can be reduction in dietary fiber intake and reduced intestinal abundance of certain beneficial, commensal bacteria. A meta-analysis of 9 clinical trials suggested that a low-FODMAP diet reduces fecal abundance of

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Abbreviations used in this paper: AE, adverse event; ANCOVA, analysis of covariance; FAS population, full analysis set population; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols; GI, gastrointestinal; GSRS, Gastrointestinal Symptom Rating Scale; hs-CRP, high-sensitivity C-reactive protein; IBS, irritable bowel syndrome; INU, inulinase activity unit; LS mean, least square mean; PP population, per protocol population; SAF population, safety population; TEAE, treatment-emergent adverse event.

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Bifidobacterium species in individuals with IBS.[22] Conversely, inulin-type fructan consumption is associated with increased fecal abundance of bifidobacteria.[23,24] Given that commensal bifidobacteria serve as biomarkers of healthy gut microbial metabolism and immunity,[25] and the established metabolic and GI health benefits of inulin and prebiotic fructo-oligosaccharides,[24,26–28] new approaches beyond FODMAP elimination diets are warranted to mitigate symptoms of FODMAP sensitivity.

An alternate dietary approach to FODMAP sensitivity is oral administration of enzymes. Microbial beta-galactosidase (ie, lactase) supplementation has repeatedly been shown to improve digestion of the FODMAP lactose and lessen GI symptoms following milk consumption.[29,30] Many FODMAP-targeting enzymes, though, are exclusively produced by commensal microbes in the human gut. This physiology lends itself well to the development of exogenous enzymes to supplement the repertoire of digestive enzymes secreted by the gut microbiota. The flagship example is microbial alpha-galactosidase (eg, the dietary supplement beano®), which has been shown in several clinical trials to reduce the severity of GI symptoms following consumption of galacto-oligosaccharides or foods like legumes that contain galactan-type FODMAPs.[31–35]

Since no human digestive enzyme can hydrolyze fructans, we set out to develop a new microbial enzyme to specifically address fructan-type FODMAP sensitivity. For this purpose, inulinase (ie, fructanase, fructan hydrolyase, beta-fructofuranosidase) is a logical choice of enzyme for its fructolytic activity and longstanding, safe use in food processing and fructose syrup manufacturing. In vitro GI digestion simulations have previously shown that a food-grade, microbial inulinase fermented from *Aspergillus tubingensis* effectively hydrolyzes fructans from several dietary substrates including inulin from chicory root, garlic, onion, and a mixed meal comprising a black bean patty, sautéed onions, and steamed Brussels sprouts.[36] This inulinase showed robust fructolytic activity at typical gastric pH and up to pH 6 and beyond,[36] suggesting that proton pump inhibitor use would likely not impact inulinase activity. Moreover, it was shown in vitro that fructan hydrolysis is pH-dependent, with greater hydrolysis at lower gastric pH.[36] This observation further suggests the utility of inulinase supplementation for FODMAP-sensitive individuals with reduced gastric acid output, as is sometimes observed with older age,[37] or individuals taking proton pump inhibitors.

Here we report on a randomized, placebo-controlled clinical trial to determine the safety and tolerability of twice daily inulinase supplementation for 4 weeks in healthy adults. Measures of safety included standard clinical chemistry and hematology. Blood lipids, high-sensitivity C-reactive protein (hs-CRP), insulin, lactate, and uric acid were also measured as they are potentially modulated by increased circulating fructose.[38–40] Adverse events (AEs) were monitored throughout the study, and tolerability was evaluated using the weekly Gastrointestinal Symptom Rating Scale (GSRS). Based on each of decades of safe human consumption of microbial enzymes obtained by fermentation and a favorable safety profile of this particular inulinase in a 90-day repeated-dose oral toxicity study in rats (unpublished data), we hypothesized that inulinase supplementation would be safe and well-tolerated in healthy adults.

Materials and Methods

Trial Protocol

This clinical trial (Protocol No. B03-22-01-T0037) was approved by Health Canada's Natural and Non-Prescription Health Products Directorate (Submission No. 258498; Ottawa, ON, Canada) and Sterling Institutional Review Board (IRB No. 10784-ABier; Atlanta, GA, USA). This single-site study was conducted between 28 April 2023 and 06 October 2023 at Apex Trials (Guelph, ON, Canada) in accordance with the protocol and consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, applicable International Council for Harmonisation Good Clinical Practice guidelines. All participants gave written informed consent before participation and were free to withdraw from the study at any time. The trial was registered at ClinicalTrials.gov (NCT05744700).

Participants

Participants were healthy adult females and males 20–60 years of age, with a body mass index 18.5–29.9 kg/m^2, who regularly consumed $\geq$2 meals daily. Detailed inclusion and exclusion criteria, as well as rationale to amend the trial protocol to expand the age range inclusion criterion, are provided in Supplementary Materials and Methods.

Trial Design

This study was a randomized, double-blind, placebo-controlled, 2-arm, parallel-group trial with a 2-week placebo run-in phase and a 4-week intervention period to assess the safety and tolerability of inulinase supplementation in healthy adults (Figure 1). A total of 60 participants were randomized to either the inulinase or placebo arm. The study included a total of 3 in-person visits: screening (visit 1), baseline (visit 2), and end of study (visit 3). The screening visit was followed by a run-in period of 14 days when participants consumed placebo capsules twice daily up until the day before the baseline visit. At the baseline visit on day 1, participants were instructed to consume one capsule of assigned study product twice daily with their 2 largest meals of the day. The end of study visit occurred on the day after 28 $\pm$ 3 days of study product consumption (day 29 $\pm$ 3).

Randomization and Masking

The run-in period was single-blinded to the participants. In a 1:1 ratio, participants were then randomly assigned to inulinase or placebo groups for 4 weeks based on a computer-generated algorithm (SAS® 9.4 PROC PLAN; SAS Institute Inc, Cary, NC, USA). Both the participants and the study staff remained blinded to which study product each participant received postrandomization. Assignment was known only to the designated, unblinded study staff also responsible for study

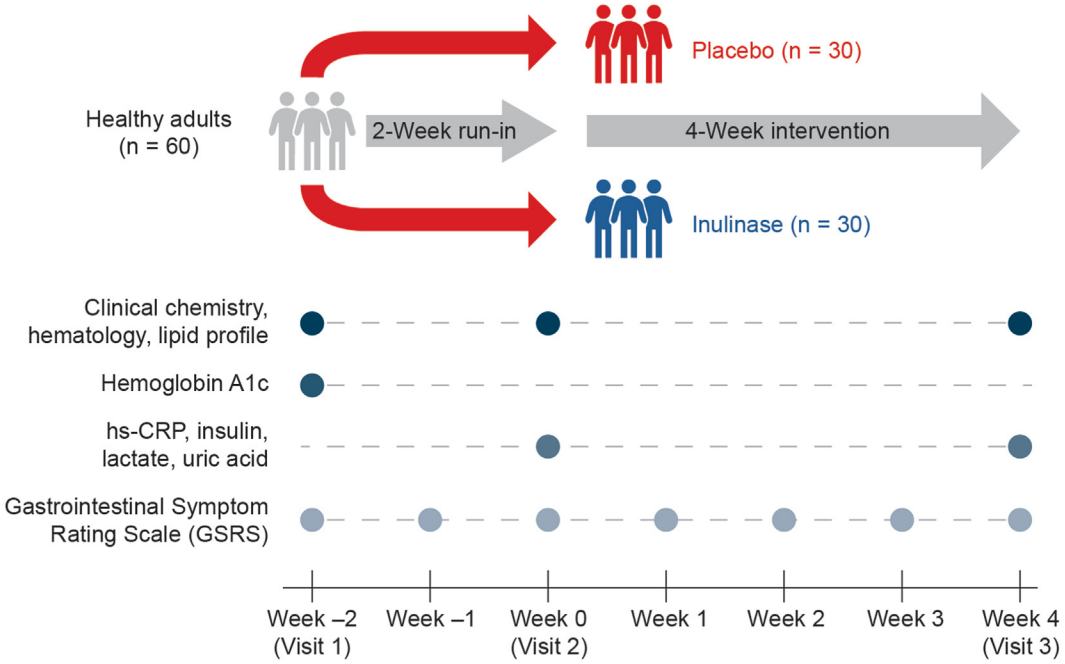

Figure 1. Summary schematic of the clinical trial design.

product labeling. Unblinding occurred following database lock and statistical analysis.

Study Products

OPTIZIOME® Inulinase (also sold as OPTIZIOME® Fructanase; BIO-CAT, Inc, Troy, VA, USA) is a non–genetically engineered, wild-type inulinase preparation comprising filtered enzyme concentrate obtained from *Aspergillus tubingensis* (reclassified from *Aspergillus niger* in 2022) and tapioca maltodextrin. This inulinase preparation is standardized by inulinase activity unit (INU), whereby 1 INU is defined as the amount of enzyme that liberates reducing sugars, such a fructose, from inulin at a rate of 1 μmol per minute at pH 4.5 °C and 40 °C.[1] For the clinical trial, 400 mg inulinase (Lot No. INP-HF09, BIO-CAT, Inc) was manufactured into size 0, opaque white, cellulose capsules (Arizona Custom Blends Manufacturing, LLC, Tempe, AZ, USA). Each inulinase capsule was formulated to contain 1000 INU. Placebo capsules were manufactured with 400 mg tapioca maltodextrin. Participants in the inulinase arm consumed an equivalent of 2000 INU per day (1000 INU per capsule). Compliance was defined as study product intake $\geq$90% during the run-in period and >80% and <120% during the intervention period (assessed by counting returned study products).

Rationale for Study Product Dosing

Participants in the inulinase arm consumed an equivalent of 2000 INU per day (1000 INU per capsule). The 1000 INU dose per meal was chosen as a high dose for first-in-human clinical safety and tolerability investigation based on the following: (i) in vitro efficacy on non-wheat dietary substrates,[36] (ii) the no-observed-adverse-effect-level informed by a 90-day repeated-dose oral toxicity study in rats (unpublished data), and (iii) usage rates in commercial dietary supplements. Participants were recommended to consume study product after 2 or 3 bites of meals to limit early transit to the duodenum and facilitate interaction with food in the stomach, as was previously shown to be effective by analysis of duodenal fluid aspirates after microbial protease administration with food.[41]

Outcomes

Laboratory assessments. Fasted blood samples were collected via arm venipuncture to assess serum clinical chemistry, whole blood hematology, serum lipids, whole blood hemoglobin A1c, serum hs-CRP, serum insulin, plasma lactate, and serum uric acid at the time points shown in Figure 1. All blood sample analyses were carried out at Dynacare® (Brampton, ON, Canada) using their standard methods.

AEs. All participants were asked to complete a daily diary to record AEs or changes in health. Clinic staff recorded all AEs observed, queried, or reported by participants.

GI symptoms. Participants completed the GSRS on a weekly basis to assess the effect of inulinase on GI symptoms. The 15 questions on the GSRS questionnaire are rated using a 7-point Likert scale, whereby higher ratings indicate greater symptom severity.[42] The GSRS overall score is determined by the score of 5 subscales: reflux (average score of 2 questions), diarrhea (average score of 3 questions), abdominal pain (average score of 3 questions), indigestion (average score of 4 questions), and constipation (average score of 3 questions). Each subscale score is the domain score. The average of all 5 domain scores is the GSRS overall score. The scores in visit 2 were treated as baseline, and change from baseline to week 1, week 2, week 3, and week 4 were calculated.

Vital signs and anthropometric measures. Blood pressure, heart rate, and weight were measured at all visits. Height was measured at screening.

Data Analysis

Three analysis sets were defined. The safety (SAF) population included all participants who received ≥1 dose of study product. The full analysis set (FAS) population included all participants who received ≥1 dose of study product and completed ≥1 postrandomization GSRS questionnaire and was used as the primary dataset for the GSRS analyses. Participants were excluded from the per protocol (PP) population for confounding protocol deviations, study product noncompliance, use of prohibited concomitant medications or supplements, or early discontinuation of study. The PP population was used to corroborate GSRS analyses from the FAS population.

Sample Size

A total of 60 participants were randomized into the inulinase or placebo groups in a 1:1 ratio, resulting in 30 participants in each group. This amount is standard and sufficient for safety studies and use of the GSRS questionnaire.[43] No sample size was calculated for this study.

Statistical Analysis

All calculations and analyses were performed using SAS® 9.4 or higher. Significance level was set at 0.05. For laboratory data, shift tables were generated to reflect the change from baseline to postrandomization or end of study. Shifts in these parameters were defined as normal to low, low to normal, high to normal, or normal to high, and assessed for clinical significance. Reference ranges are provided in Table A1. As a post hoc analysis, analysis of covariance (ANCOVA) was used to assess the change in laboratory parameters (responsible variable), with study product included as a fixed effect and baseline as a covariate. For GSRS overall and domain scores, ANCOVA was applied with the change from baseline as the responsible variable. Study product, week, and interaction between study product and week were included as fixed effects and baseline values as a covariate. Least square means were calculated together with 95% confidence interval and corresponding *P* value. Treatment-emergent AEs (TEAEs) were summarized by severity, relationship to study product, and categorized by AE term.

Table 1. Demographics and Characteristics of Randomized Participants at Screening

Parameter	Inulinase (n = 30)	Placebo (n = 30)	Total (n = 60)
Sex			
Female, n (%)	20 (66.7)	24 (80.0)	44 (73.3)
Male, n (%)	10 (33.3)	6 (20.0)	16 (26.7)
Ethnic origin			
White, n (%)	22 (73.3)	22 (73.3)	44 (73.3)
Other, n (%)	4 (13.3)	6 (20.0)	10 (16.7)
Not reported	4 (13.3)	2 (6.7)	6 (10.0)
Age, *y*			
Mean (SD)	37.2 (12.12)	35.7 (11.24)	36.5 (11.61)
Median	34.0	33.0	33.5
Range	20–60	21–56	20–60
Age group			
<40, n (%)	22 (73.3)	18 (60.0)	40 (66.7)
>40, n (%)	8 (26.7)	12 (40.0)	20 (33.3)
Body mass index, kg/m^2			
Mean (SD)	23.62 (2.523)	24.18 (2.796)	23.90 (2.656)
Median	23.75	24.10	23.90
Range	18.9–29.3	19.6–28.6	18.9–29.3
Systolic blood pressure, *mm Hg*			
Mean (SD)	110.3 (11.10)	108.5 (8.22)	109.4 (9.73)
Median	109.0	109.5	109.0
Range	92–135	92–123	92–135
Diastolic blood pressure, *mm Hg*			
Mean (SD)	71.0 (6.46)	71.4 (6.22)	71.2 (6.29)
Median	69.0	71.0	70.0
Range	60–85	61–84	60–85
Fasting plasma glucose, *mmol/L*			
Mean (SD)	4.73 (0.296)	4.71 (0.310)	4.72 (0.301)
Median	4.75	4.70	4.70
Range	4.0–5.2	4.1–5.4	4.0–5.4
Fasting plasma hemoglobin A1c, %			
Mean (SD)	5.38 (0.282)	5.29 (0.243)	5.34 (0.265)
Median	5.30	5.30	5.30
Range	4.9–5.9	4.8–5.8	4.8–5.9

Values represent the safety population at screening (visit 1), including all participants who received ≥1 dose of study product.
SD, standard deviation.

Results

Participants, Baseline Characteristics, and Compliance

A total of 156 individuals were screened for eligibility to randomize 60 participants. Demographics and baseline characteristics of the SAF population are presented in Table 1. A total of 60 generally healthy participants were enrolled with a median age of 33.5 years (range, 20–60 years) and median baseline body mass index of 23.9 kg/m^2 (range, 18.9–29.3 kg/m^2). The participant population self-identified as White (73.3%), Asian (13.3%), or Black or African American (3.3%), with 10% not reporting race. Two participants voluntarily withdrew from the study and were considered lost to follow-up without post-randomization GSRS or laboratory data collected. In the resulting FAS population of 58 participants, study product compliance for inulinase and placebo were 98.1% and 98.5%, respectively. The PP population consisted of 55 participants whereby 3 participants were removed from the FAS due to the following: (i) start of exclusionary medicine, (ii) missing GSRS responses, or (iii) <80% study product compliance. Baseline characteristics of the SAF population (Table 1) were similar to FAS and PP populations. The comprehensive participant flow chart is presented in Figure 2.

Blood Laboratory Assessments

The quantitative results of clinical chemistry, hematology, lipid profile, hs-CRP, insulin, lactate, and uric acid testing are presented in Table 2. All shifts in these parameters from baseline to end of study, apart from one instance of elevated hs-CRP in one participant in the placebo group, remained within clinically acceptable ranges (Tables A2–A5). Post hoc ANCOVA showed that out of the 37 blood parameters tested, 35 showed similar 4-week changes from baseline between inulinase and placebo groups (all $P \geq .05$). The 2 significant comparisons were a 5.9% reduction in creatinine and a 6.0% increase in estimated glomerular filtration rate in the placebo group, while inulinase showed no significant changes from baseline (all values remained within normal range). Four within-group changes from baseline specific to inulinase included a 3-fold increase in eosinophils ($P = .021$), <1% decreases in mean corpuscular hemoglobin

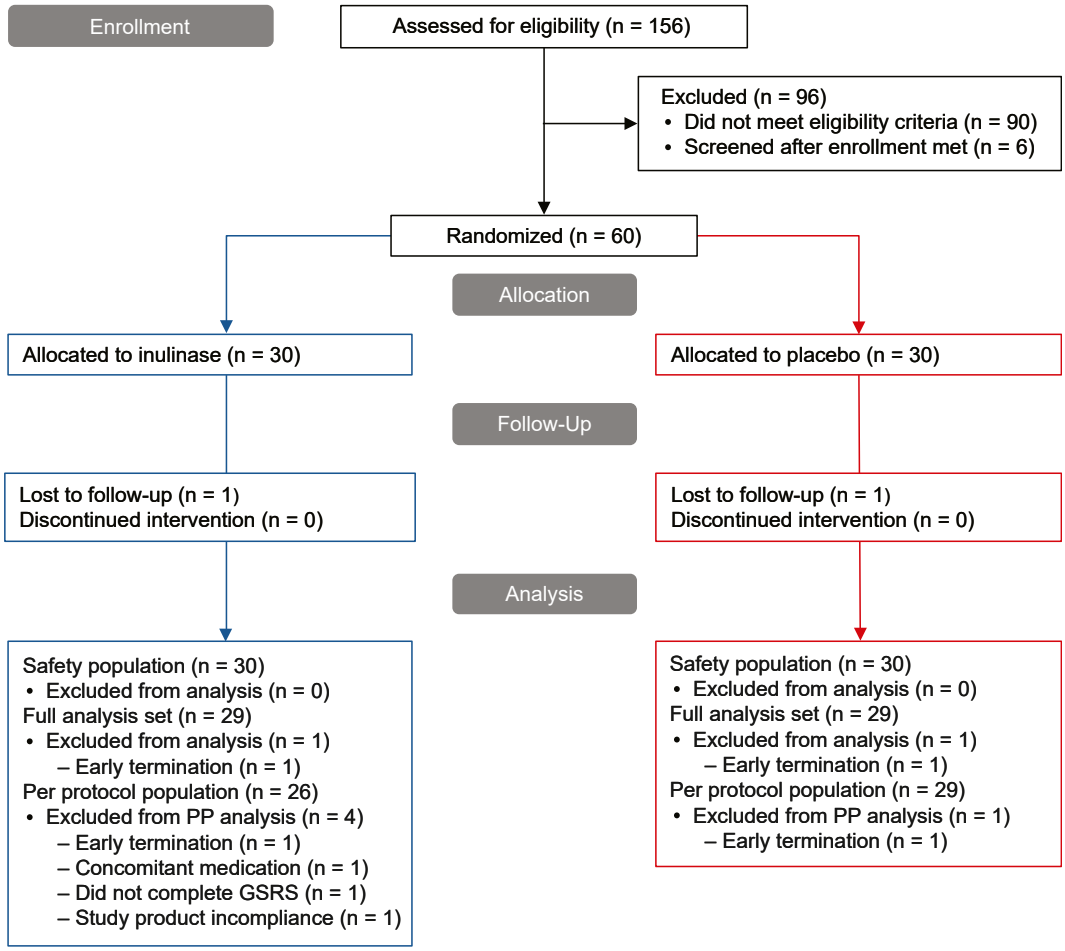

Figure 2. Participant enrollment flow chart and allocation to inulinase (2000 INU/d) or placebo. GSRS, Gastrointestinal Symptom Rating Scale; PP, per protocol.

Table 2. Blood Laboratory Assessments at Baseline and Week 4 of Dietary Supplementation

Parameter	Inulinase			Placebo		
	Baseline (n = 30)	Wk 4 (n = 29)	Change from baseline	Baseline (n = 30)	Wk 4 (n = 29)	Change from baseline
Clinical chemistry (serum)						
Alanine transferase, *U/L*	18.0 (9.50)	18.5 (10.78)	0.2 (5.38)	15.0 (5.45)	16.2 (5.88)	1.4 (3.44)
Albumin[a], *g/L*	45.6 (2.62)	45.3 (2.77)	−0.2 (1.70)	45.0 (2.85)	44.4 (2.70)	−0.5 (2.73)
Alkaline phosphatase, *U/L*	69.5 (22.88)	71.6 (23.88)	1.7 (8.90)	63.4 (20.32)	63.7 (20.05)	0.1 (8.70)
Aspartate transaminase, *U/L*	22.4 (9.62)	22.3 (8.76)	−0.3 (4.36)	19.3 (5.03)	19.6 (8.33)	0.3 (4.73)
Chloride[a], *mmol/L*	102.4 (1.99)	102.4 (2.63)	0.0 (1.85)	103.2 (2.20)	102.9 (1.75)	−0.3 (1.65)
Creatinine[a,b], *μmol/L*	75.2 (14.33)	75.1 (14.31)	−0.4 (4.80)[d]	78.4 (14.73)	73.8 (12.98)	−4.6 (7.65)[c,d]
Globulin[a], *g/L*	23.4 (3.48)	23.3 (3.40)	0.0 (2.05)	23.5 (3.53)	23.9 (3.67)	0.5 (2.28)
Glucose[a], *mmol/L*	4.91 (0.370)	4.96 (0.361)	0.06 (0.383)	4.83 (0.286)	4.80 (0.399)	−0.04 (0.366)
EGFR[a,b], *mL/min/1.73 m²*	97.4 (13.27)	97.2 (13.13)	0.3 (5.77)[d]	90.4 (15.59)	95.7 (15.40)	5.4 (8.65)[c,d]
Potassium[a], *mmol/L*	4.52 (0.357)	4.48 (0.391)	−0.06 (0.287)	4.51 (0.287)	4.45 (0.334)	−0.06 (0.390)
Protein[a], *g/L*	68.9 (3.47)	68.7 (3.80)	−0.2 (2.65)	68.4 (4.46)	68.3 (3.63)	0.0 (3.34)
Sodium[a], *mmol/L*	139.1 (1.76)	139.3 (1.76)	0.2 (1.50)	139.3 (2.04)	139.2 (1.71)	−0.2 (1.91)
Total bilirubin, *μmol/L*	9.8 (6.01)	10.7 (6.91)	0.9 (3.33)	9.7 (5.59)	9.7 (5.78)	−0.1 (3.47)
Urea, *mmol/L*	4.43 (1.387)	4.76 (1.778)	0.28 (0.840)	4.55 (1.527)	4.52 (1.501)	0.04 (0.813)
Hematology (whole blood)						
Basophils[a], *10^9/L*	0.016 (0.0224)	0.012 (0.0251)	−0.005 (0.0259)	0.008 (0.0177)	0.006 (0.0163)	−0.002 (0.0171)
Eosinophils, *10^9/L*	0.15 (0.159)	0.45 (0.946)	0.29 (0.938)[c]	0.12 (0.121)	0.11 (0.116)	−0.01 (0.058)
Erythrocytes[a], *10^{12}/L*	4.57 (0.304)	4.62 (0.335)	0.04 (0.194)	4.46 (0.480)	4.49 (0.436)	0.03 (0.207)
EDC[a], *%*	13.39 (0.678)	13.32 (0.780)	−0.09 (0.384)	13.53 (1.427)	13.46 (1.329)	−0.09 (0.490)
Hematocrit[a], *L/L*	0.412 (0.0258)	0.416 (0.0261)	0.002 (0.0166)	0.400 (0.0462)	0.403 (0.0425)	0.004 (0.0209)
Hemoglobin[a], *g/L*	139.7 (9.39)	139.6 (10.03)	−0.3 (4.89)	135.8 (16.66)	135.6 (15.05)	0.3 (5.44)
Leukocytes[a], *10^9/L*	5.50 (1.512)	5.58 (2.182)	0.14 (2.007)	5.19 (1.294)	5.17 (1.052)	0.08 (0.883)
Lymphocytes[a], *10^9/L*	1.60 (0.485)	1.63 (0.405)	0.03 (0.410)	1.65 (0.433)	1.61 (0.441)	−0.03 (0.307)
MCH[a], *pg*	30.6 (1.50)	30.3 (1.49)	−0.3 (0.71)[c]	30.7 (2.44)	30.3 (1.99)	−0.2 (0.83)
MCH concentration[a], *g/L*	338.4 (7.47)	335.4 (6.37)	−2.7 (7.61)[c]	339.9 (9.46)	336.8 (8.46)	−2.8 (8.67)
MCV, *fL*	90.5 (3.74)	90.2 (3.97)	−0.2 (0.91)	90.1 (5.01)	90.0 (5.06)	0.1 (0.92)
Mean platelet volume, *fL*	9.23 (1.101)	9.18 (1.145)	−0.07 (0.379)	9.07 (0.863)	9.01 (0.793)	−0.04 (0.334)
Monocytes, *10^9/L*	0.42 (0.137)	0.45 (0.172)	0.03 (0.116)	0.37 (0.102)	0.39 (0.098)	0.02 (0.098)
Neutrophils[a], *10^9/L*	3.19 (1.338)	2.95 (1.367)	−0.17 (1.149)	2.94 (1.091)	2.95 (0.895)	0.11 (0.857)
Platelets[a], *10^9/L*	247.5 (63.05)	244.0 (58.96)	−4.8 (30.16)	244.1 (45.83)	244.1 (46.55)	1.6 (26.34)
Lipids (serum)						
HDL, *mmol/L*	1.876 (0.3611)	1.828 (0.4729)	−0.058 (0.2553)	1.673 (0.4018)	1.641 (0.4229)	−0.045 (0.1575)
LDL[a], *mmol/L*	2.475 (0.6103)	2.493 (0.6558)	0.017 (0.3277)	2.448 (0.5724)	2.443 (0.5169)	0.014 (0.4025)
Total cholesterol, *mmol/L*	4.689 (0.7350)	4.693 (0.8431)	0.003 (0.4012)	4.444 (0.6860)	4.442 (0.7119)	0.004 (0.4718)
Triglycerides[a], *mmol/L*	0.733 (0.2728)	0.812 (0.3162)	0.099 (0.2551)[c]	0.705 (0.2163)	0.779 (0.2416)	0.071 (0.2123)
Other						
hs-CRP, *mg/L*	1.16 (1.284)	1.14 (1.156)	0.05 (0.877)	1.15 (1.212)	3.73 (11.657)	2.54 (11.635)
Insulin[a], *pmol/L*	46.9 (23.91)	47.6 (26.27)	1.4 (27.92)	44.6 (19.71)	48.2 (22.29)	3.7 (20.99)
Lactate, *mmol/L*	1.03 (0.231)	0.98 (0.374)	−0.06 (0.416)	0.82 (0.302)	0.92 (0.427)	0.10 (0.261)
Uric acid, *μmol/L*	272.4 (58.73)	278.3 (58.97)	3.2 (26.67)	260.5 (59.51)	251.3 (60.04)	−7.9 (34.33)

Values are means (standard deviation) from the safety population including all participants who received ≥1 dose of study product.
Least square means from the FAS population, including all participants who completed the study, were used for statistical comparisons.
EDC, erythrocyte distribution width; EGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.
[a]Significant effect of baseline values as a covariate.
[b]Significant fixed effect of study product.
[c]Significant within-group difference.
[d]Significant between-group difference.

(P = .015) and mean corpuscular hemoglobin concentration (P = .013), and a 13.5% increase in triglycerides (P = .025). These changes from baseline to end of study were not statistically different from changes observed in the placebo group, albeit the between-group comparison for eosinophils was nearly significant (P = .083).

Table 3. Summary of AEs During the Postrandomization Period by Study Product

	Inulinase (n = 30)		Placebo (n = 30)	
Event	Participants n (%)	Events	Participants n (%)	Events
TEAE[a]	5 (16.7)	5	8 (26.7)	13
Severity				
Mild	4 (13.3)	4	8 (26.7)	13
Moderate	1 (3.3)	1	0	0
Severe	0	0	0	0
Relationship to study product				
Not related	2 (6.7)	2	6 (20.0)	8
Suspected	3 (10.0)	3	3 (10.0)	5
Related	0	0	0	0
Leading to withdrawal	0	0	0	0
SAE	0	0	0	0
TEAEs by AE term				
Abdominal discomfort	0	0	1 (3.3)	1
Bloating	0	0	2 (6.7)	2
Constipation	1 (3.3)	1	1 (3.3)	1
Diarrhea	2 (6.7)	2	1 (3.3)	1
Dog bite	0	0	1 (3.3)	1
Elevated hs-CRP	0	0	1 (3.3)	1
Flatulence	1 (3.3)	1	0	0
Loose stools	1 (3.3)	1	1 (3.3)	2
Tonsilitis	0	0	1 (3.3)	1
Upper respiratory infection	0	0	1 (3.3)	1
Upset stomach	0	0	1 (3.3)	1
Vertigo	0	0	1 (3.3)	1

Values represent the safety population including all participants who received ≥1 dose of study product (n = 60).
SAE, serious adverse event.
[a]A treatment-emergent adverse event is defined as an adverse event that occurs after the first dose of study product postrandomization. Percentages are based on the total number of participants in each study product group.

AEs

During the 4-week intervention period, 13 participants experienced 18 TEAEs (Table 3). Five participants reported 5 TEAEs in the inulinase group and 8 participants reported 13 TEAEs in the placebo group. Most of the TEAEs reported across study product groups were diarrhea and loose stool and mild in severity. Only 1 TEAE was moderate in severity, which was an event of diarrhea reported by 1 participant in the inulinase group which lasted 6 days and resolved within 2 days after cessation of study product. This participant also experienced loose stool lasting 1 day during the placebo run-in period. No serious AEs or deaths were reported.

GI Symptoms

In the FAS population, there were no significant between-group differences observed in the changes from baseline to weeks 1, 2, 3, or 4 with respect to the GSRS overall score, any domain scores, or individual GSRS questions on abdominal discomfort, bloating, and burping (Table 4). Similar results were observed in the PP population.

Vital Signs and Anthropometrics

There were no significant between-group differences in vital signs and body measurements from baseline to end of study (Table A6).

Discussion

This study was a randomized, double-blind, placebo-controlled trial to determine the safety and tolerability of inulinase compared to a placebo in healthy adults across 4 weeks of twice daily supplementation. Clinical chemistry and hematology parameters did not demonstrate any consistent between-group differences in shifts from baseline to end of study, or any abnormal or clinically significant shifts. Five participants in the inulinase group had elevated eosinophils outside normal range at end of study; however, it was not high enough to be assessed as an AE or deemed clinically significant by the Principal Investigator. The rationale for a clinically significant eosinophil count was that moderate eosinophilia would be considered as $>1.5 \times 10^9$/L. By this criterion, only 1 of these 5 events would be considered clinically significant in the absence of any other symptoms. However, given the AE of diarrhea for this particular participant, the elevated eosinophils were not assessed as a separate event. An elevation in eosinophils can be caused by many medical conditions, including but not limited to allergies, infection, GI disorders, and asthma.[44] Two of the 5 participants reported mold and/or seasonal allergies and the other 2 participants did not report any AE or medical history. In the absence of other clinical symptoms, these eosinophil elevations were not deemed of concern. Moreover,

Table 4. GSRS Scores by Week and Study Product

GSRS parameter	Baseline	Wk 1	Wk 2	Wk 3	Wk 4	*P* values
GSRS overall score[a]						SP: .933
Inulinase	1.101 (0.1387)	1.151 (0.2117)	1.164 (0.2161)	1.137 (0.2145)	1.137 (0.2049)	W: .317
Placebo	1.268 (0.2586)	1.279 (0.2968)	1.309 (0.3325)	1.252 (0.2808)	1.269 (0.2611)	SP×W: .935
						B: .051
Abdominal pain (domain score)[a]						SP: .652
Inulinase	1.103 (0.2206)	1.130 (0.2768)	1.166 (0.3800)	1.107 (0.2871)	1.115 (0.2565)	W: .346
Placebo	1.379 (0.4064)	1.264 (0.3920)	1.241 (0.3322)	1.194 (0.2274)	1.310 (0.3666)	SP×W: .251
						B: <.001
Constipation (domain score)[a]						SP: .837
Inulinase	1.069 (0.1869)	1.166 (0.4109)	1.214 (0.5153)	1.167 (0.4210)	1.126 (0.3925)	W: .056
Placebo	1.195 (0.3276)	1.287 (0.4148)	1.345 (0.5464)	1.206 (0.3378)	1.218 (0.3246)	SP×W: .697
						B: .032
Diarrhea (domain score)[a]						SP: .432
Inulinase	1.092 (0.2339)	1.190 (0.3786)	1.155 (0.2944)	1.179 (0.3792)	1.150 (0.3637)	W: .883
Placebo	1.229 (0.3795)	1.276 (0.5569)	1.413 (0.8289)	1.356 (0.6041)	1.333 (0.5706)	SP×W: .684
						B: <.077
Indigestion (domain score)[a]						SP: .369
Inulinase	1.207 (0.3278)	1.214 (0.3171)	1.250 (0.3967)	1.214 (0.3832)	1.224 (0.3741)	W: .371
Placebo	1.483 (0.5129)	1.517 (0.6406)	1.526 (0.5318)	1.431 (0.5299)	1.414 (0.4079)	SP×W: .532
						B: <.001
Reflux (domain score)[a]						SP: .930
Inulinase	1.03 (0.129)	1.05 (0.157)	1.04 (0.131)	1.02 (0.094)	1.07 (0.175)	W: .321
Placebo	1.05 (0.205)	1.05 (0.205)	1.02 (0.093)	1.07 (0.221)	1.07 (0.221)	SP×W: .379
						B: <.001
Abdominal discomfort[b]						W1: .981
Inulinase	1.1 (0.26)	1.3 (0.71)	1.2 (0.57)	1.1 (0.42)	1.1 (0.44)	W2: .215
Placebo	1.3 (0.71)	1.2 (0.58)	1.3 (0.70)	1.1 (0.35)	1.2 (0.41)	W3: .518
						W4: .550
Bloating[b]						W1: .166
Inulinase	1.2 (0.49)	1.1 (0.31)	1.2 (0.50)	1.2 (0.48)	1.2 (0.47)	W2: .375
Placebo	1.7 (0.90)	1.7 (1.22)	1.8 (0.86)	1.7 (0.77)	1.5 (0.57)	W3: .503
						W4: .717
Burping[b]						W1: .717
Inulinase	1.1 (0.35)	1.1 (0.36)	1.1 (0.26)	1.1 (0.36)	1.1 (0.26)	W2: .782
Placebo	1.2 (0.41)	1.2 (0.38)	1.2 (0.38)	1.2 (0.38)	1.1 (0.31)	W3: .717
						W4: .656

Baseline represents the mean score during the 1 week leading up to randomization.
Values are means (standard deviation) from the FAS, population including all participants who completed the study (n = 29 per study group).
B, baseline; SP, study product; W, week.
[a]Changes from baseline for total GSRS, and GSRS, domain scores were analyzed using an ANCOVA, model, with the study product, week, and interaction between study product and week (SP×W) as a fixed effect, with the baseline as a covariate. Least square means of weeks 1, 2, 3, and 4 for each study product and difference in least square, means of weeks 1, 2, 3, and 4 between study products were calculated.
[b]For individual abdominal discomfort, bloating and burping scores, comparisons between study groups at weeks 1, 2, 3 and 4 were determined using Wilcoxon rank sum test and presented as W1, W2, W3, and W4 in the *P* value column. Within-group changes from baseline to weeks 1–4 were determined for significance using Signed Rank test (all $P > .05$).

neither elevated eosinophil counts nor eosinophilia were observed in a previous 90-day repeated-dose oral toxicity study of inulinase in rats (unpublished data).

Because the enzymatic activity of inulinase was expected to readily break down dietary fructans to fructose, circulating levels of fructo-metabolic byproducts, including hs-CRP, insulin, lactate, and uric acid were also assessed. These byproducts have been associated with gout, insulin resistance, inflammation, hyperuricemia, liver toxicity, and cardiovascular disease.[39,40] Additionally, serum lipid levels were analyzed as high fructose flux can lead to triglyceride accumulation.[38] There were no concerning increases in these parameters after 4 weeks of inulinase supplementation. Uric acid did appear to increase in the inulinase group and decrease in the placebo group; however, values remained within normal range.

Plasma fructose exposure aside, additional studies are needed to explore any possible effects of increased

intestinal exposure to fructose from oral inulinase co-administration with dietary fructans in individuals with IBS. Fructose malabsorption is associated with IBS and may be part of the etiology.[45,46] Nonetheless, several clinical trials have shown that fructan malabsorption is likely more problematic than fructose malabsorption in IBS.[15,47] Inulinase supplementation may also have the added benefit of reducing intestinal exposure to inulin-type fructans shown to be proinflammatory in mouse models.[48–50] In this regard, one limitation of the present study is that stool samples were not collected from participants. Future efficacy studies are warranted to investigate the effects of microbial inulinase supplementation on fecal inflammatory markers, short chain fatty acids, and metabolomic profiles.

Reports of AEs demonstrated no safety concerns for inulinase. Participants that consumed placebo reported twice the number of AEs than were reported by participants that consumed inulinase. One AE of diarrhea was reported as moderate in severity; however, this participant also had an AE of loose stool in the run-in period while taking placebo. All other AE terms reported by participants that consumed inulinase were mild in severity and were also reported by participants that consumed placebo.

GI symptoms at baseline were reflective of a healthy population, with GSRS overall and domain scores similar to those previously demonstrated in healthy adults.[42] There was no significant between-group difference observed in the GSRS overall score or any domain scores after 1, 2, 3, or 4 weeks of supplementation. It should be noted that the baseline values of mean GSRS scores were slightly higher in placebo group compared to inulinase. This may be attributed to the greater proportion of female participants in the placebo group, as females typically report higher incidences of bloating and other GI symptoms.[7] Most changes from baseline in GSRS scores were minimal, with the greatest change from baseline in scores of abdominal discomfort and diarrhea being less than 3 points out of a possible 7. Because a healthy population without diagnosed GI issues was recruited in this study, there was no expectation to appreciably reduce GI symptoms with inulinase supplementation. The GSRS questionnaire was used to assess the GI tolerability of inulinase in healthy adults, which was confirmed by the lack of difference observed between inulinase and placebo.

Conclusion

The results from the present clinical trial show that microbial inulinase is safe and well-tolerated in healthy adults. Further research is warranted to assess whether microbial inulinase supplementation can reduce fructan malabsorption, GI symptoms, and intestinal inflammation in individuals with FODMAP sensitivity and IBS, perhaps also in combination with other FODMAP-targeting enzymes such as alpha-galactosidase. Altogether, microbial inulinase supplementation is an attractive, candidate stop-gap approach to restoring some of the intestinal metagenomic carbohydrate-active enzyme functionality that has waned since the dawn of modern agriculture, processed foods, and low-fiber diets.

Supplementary Materials

Material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.gastha.2024.05.013.

References

1. Tuck CJ, Biesiekierski JR, Schmid-Grendelmeier P, et al. Food intolerances. Nutrients 2019;11:1684.
2. Patel PK, Tanpowpong P, Sriaroon P, et al. Nonallergic diseases associated with foods. J Allergy Clin Immunol Pract 2024;12:607–619.
3. Abraham P, Dhoble P, Desai D, et al. Self-reported food intolerances in an Indian population: need for individualization rather than a universal low-FODMAP diet. JGH Open 2023;7:772–776.
4. Lee HJ, Kim HJ, Kang EH, et al. Self-reported food intolerance in Korean patients with irritable bowel syndrome. J Neurogastroenterol Motil 2019;25:222–232.
5. Lomer MC. Review article: the aetiology, diagnosis, mechanisms and clinical evidence for food intolerance. Aliment Pharmacol Ther 2015;41:262–275.
6. Young E, Stoneham MD, Petruckevitch A, et al. A population study of food intolerance. Lancet 1994;343:1127–1130.
7. Ballou S, Singh P, Nee J, et al. Prevalence and associated factors of bloating: results from the Rome Foundation Global Epidemiology Study. Gastroenterology 2023;165:647–655.
8. Böhn L, Störsrud S, Törnblom H, et al. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. Am J Gastroenterol 2013;108:634–641.
9. Chey WD, Olden K, Carter E, et al. Utility of the Rome I and Rome II criteria for irritable bowel syndrome in U.S. women. Am J Gastroenterol 2002;97:2803–2811.
10. Monsbakken KW, Vandvik PO, Farup PG. Perceived food intolerance in subjects with irritable bowel syndrome – etiology, prevalence and consequences. Eur J Clin Nutr 2006;60:667–672.
11. Simrén M, Månsson A, Langkilde AM, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion 2001;63:108–115.
12. Bonnema AL, Kolberg LW, Thomas W, et al. Gastrointestinal tolerance of chicory inulin products. J Am Diet Assoc 2010;110:865–868.
13. Bruhwyler J, Carreer F, Demanet E, et al. Digestive tolerance of inulin-type fructans: a double-blind, placebo-controlled, cross-over, dose-ranging, randomized study in healthy volunteers. Int J Food Sci Nutr 2009;60:165–175.
14. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. Clin Gastroenterol Hepatol 2008;6:765–771.

15. Van den Houte K, Colomier E, Routhiaux K, et al. Efficacy and findings of a blinded randomized reintroduction phase for the low FODMAP diet in irritable bowel syndrome. Gastroenterology 2024;167:333–342.
16. Jovanovic-Malinovska R, Kuzmanova S, Winkelhausen E. Oligosaccharide profile in fruits and vegetables as sources of prebiotics and functional foods. Int J Food Prop 2014;17:949–965.
17. Gunn D, Abbas Z, Harris HC, et al. Psyllium reduces inulin-induced colonic gas production in IBS: MRI and in vitro fermentation studies. Gut 2022; 71:919–927.
18. Murray K, Wilkinson-Smith V, Hoad C, et al. Differential effects of FODMAPs (fermentable oligo-, di-, monosaccharides and polyols) on small and large intestinal contents in healthy subjects shown by MRI. Am J Gastroenterol 2014;109:110–119.
19. Bardacke JA, Yarrow L, Rosenkranz SK. The long-term effects of a low-fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet for irritable bowel syndrome management. Curr Dev Nutr 2023; 7:101997.
20. Black CJ, Staudacher HM, Ford AC. Efficacy of a low FODMAP diet in irritable bowel syndrome: systematic review and network meta-analysis. Gut 2022;71:1117–1126.
21. Dionne J, Ford AC, Yuan Y, et al. A systematic review and meta-analysis evaluating the efficacy of a gluten-free diet and a low FODMAPs diet in treating symptoms of irritable bowel syndrome. Am J Gastroenterol 2018; 113:1290–1300.
22. So D, Loughman A, Staudacher HM. Effects of a low FODMAP diet on the colonic microbiome in irritable bowel syndrome: a systematic review with meta-analysis. Am J Clin Nutr 2022;116:943–952.
23. Le Bastard Q, Chapelet G, Javaudin F, et al. The effects of inulin on gut microbial composition: a systematic review of evidence from human studies. Eur J Clin Microbiol Infect Dis 2020;39:403–413.
24. Nagy DU, Sándor-Bajusz KA, Bódy B, et al. Effect of chicory-derived inulin-type fructans on abundance of *Bifidobacterium* and on bowel function: a systematic review with meta-analyses. Crit Rev Food Sci Nutr 2023; 63:12018–12035.
25. O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol 2016;7:925.
26. Collado Yurrita L, San Mauro Martín I, Ciudad-Cabañas MJ, et al. Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled randomized clinical trials. Nutr Hosp 2014;30:244–252.
27. Hughes RL, Alvarado DA, Swanson KS, et al. The prebiotic potential of inulin-type fructans: a systematic review. Adv Nutr 2022;13:492–529.
28. Jackson PP, Wijeyesekera A, Williams CM, et al. Inulin-type fructans and 2'fucosyllactose alter both microbial composition and appear to alleviate stress-induced mood state in a working population compared to placebo (maltodextrin): the EFFICAD trial, a randomized, controlled trial. Am J Clin Nutr 2023;118:938–955.
29. DiPalma JA, Collins MS. Enzyme replacement for lactose malabsorption using a beta-D-galactosidase. J Clin Gastroenterol 1989;11:290–293.
30. Montalto M, Nucera G, Santoro L, et al. Effect of exogenous beta-galactosidase in patients with lactose malabsorption and intolerance: a crossover double-blind placebo-controlled study. Eur J Clin Nutr 2005; 59:489–493.
31. Di Nardo G, Oliva S, Ferrari F, et al. Efficacy and tolerability of α-galactosidase in treating gas-related symptoms in children: a randomized, double-blind, placebo controlled trial. BMC Gastroenterol 2013;13:142.
32. Di Stefano M, Miceli E, Gotti S, et al. The effect of oral alpha-galactosidase on intestinal gas production and gas-related symptoms. Dig Dis Sci 2007;52:78–83.
33. Ganiats TG, Norcross WA, Halverson AL, et al. Does Beano prevent gas? A double-blind crossover study of oral alpha-galactosidase to treat dietary oligosaccharide intolerance. J Fam Pract 1994;39:441–445.
34. Lettieri JT, Dain B. Effects of beano on the tolerability and pharmacodynamics of acarbose. Clin Ther 1998; 20:497–504.
35. Tuck CJ, Taylor KM, Gibson PR, et al. Increasing symptoms in irritable bowel symptoms with ingestion of galacto-oligosaccharides are mitigated by α-galactosidase treatment. Am J Gastroenterol 2018;113:124–134.
36. Guice JL, Hollins MD, Farmar JG, et al. Microbial inulinase promotes fructan hydrolysis under simulated gastric conditions. Front Nutr 2023;10:1129329.
37. Feldman M, Cryer B, McArthur KE, et al. Effects of aging and gastritis on gastric acid and pepsin secretion in humans: a prospective study. Gastroenterology 1996; 110:1043–1052.
38. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab 2005;2:5.
39. Jameel F, Phang M, Wood LG, et al. Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation. Lipids Health Dis 2014;13:195.
40. Silbernagel G, Machann J, Unmuth S, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. Br J Nutr 2011;106:79–86.
41. König J, Holster S, Bruins MJ, et al. Randomized clinical trial: effective gluten degradation by Aspergillus Niger-derived enzyme in a complex meal setting. Sci Rep 2017;7:13100.
42. Dimenäs E, Carlsson G, Glise H, et al. Relevance of norm values as part of the documentation of quality of life instruments for use in upper gastrointestinal disease. Scand J Gastroenterol Suppl 1996;221:8–13.
43. Kalman DS, Schwartz HI, Alvarez P, et al. A prospective, randomized, double-blind, placebo-controlled parallel-group dual site trial to evaluate the effects of a *Bacillus coagulans*-based product on functional intestinal gas symptoms. BMC Gastroenterol 2009;9:85.
44. Wechsler ME, Munitz A, Ackerman SJ, et al. Eosinophils in health and disease: a state-of-the-art review. Mayo Clin Proc 2021;96:2694–2707.
45. Benardout M, Le Gresley A, ElShaer A, et al. Fructose malabsorption: causes, diagnosis and treatment. Br J Nutr 2022;127:481–489.
46. Smiliotopoulos T, Zampelas A, Houliaras G, et al. Association of fructose consumption with prevalence of functional gastrointestinal disorders manifestations:

results from Hellenic National Nutrition and Health Survey (HNNHS). Br J Nutr 2023;130:1–12.
47. Sia T, Tanaka RO, Mousad A, et al. Fructose malabsorption and fructan malabsorption are associated in patients with irritable bowel syndrome. BMC Gastroenterol 2024;24:143.
48. Arifuzzaman M, Won TH, Li TT, et al. Inulin fibre promotes microbiota-derived bile acids and type 2 inflammation. Nature 2022;611:578–584.
49. Kamphuis JBJ, Reber L, Eutamène H, et al. Increased fermentable carbohydrate intake alters colonic mucus barrier function through glycation processes and increased mast cell counts. FASEB J 2022;36:e22297.
50. Singh P, Grabauskas G, Zhou SY, et al. High FODMAP diet causes barrier loss via lipopolysaccharide-mediated mast cell activation. JCI Insight 2021;6:e146529.

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Sean M. Garvey: Conceptualization; Investigation; Project administration; Visualization; Writing – original draft. Ashley LeMoire: Data curation; Formal analysis; Investigation; Visualization; Writing – original draft. Jun Wang: Data curation; Formal analysis; Validation; Writing – review & editing. Lois Lin: Data curation; Formal analysis; Investigation; Writing – review & editing. Bisma Sharif: Investigation; Project administration. Anthony Bier: Investigation. Robert C. Boyd: Investigation; Writing – review & editing. Joshua Baisley: Conceptualization; Investigation; Project administration. All authors had access to the study data and reviewed and approved the final manuscript.

Conflicts of Interest:
The authors disclose the following: Sean M. Garvey and Robert C. Boyd are employees of BIO-CAT, Inc. Ashley LeMoire, Jun Wang, Lois Lin, Bisma Sharif, Anthony Bier, and Joshua Baisley are employees of Nutrasource Pharmaceutical and Nutraceutical Services, Inc, the company that BIO-CAT, Inc contracted to conduct the study. BIO-CAT, Inc holds relevant patents U.S. Patent No. 5,445,957 and U.S. Patent No. 5,651,967.

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This clinical trial was approved by Health Canada's Natural and Non-Prescription Health Products Directorate (Submission No. 258498; Ottawa, ON, Canada) and Sterling Institutional Review Board (IRB No. 10784-ABier; Atlanta, GA, USA).

Data Transparency Statement:
The full trial protocol and datasets used in this study, including deidentified individual participant-level data, will be made available upon reasonable request from the corresponding author.

Reporting Guidelines:
CONSORT, Helsinki Declaration.