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A Novel Direct Butyrate Generator Reduces Belly Pain in a Randomized, Double-Blind, Placebo-Controlled Clinical Study

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Abstract: This study aimed to evaluate the effects of ButyraGen®, a direct butyrate generator, on digestive health in a real-world setting. A 6-week, randomized, double-blind, placebo-controlled, virtual clinical study was conducted starting with 596 participants. Participants were randomly assigned to either the 200 mg ButyraGen® active or placebo group. Questionnaires were administered electronically to assess gastrointestinal health outcomes. Statistically significant improvements were observed in belly pain for the active ButyraGen® group compared with the placebo group, particularly in women, and particularly in post-menopausal women. The active group showed a higher likelihood of achieving clinically significant improvements in digestive health measures. ButyraGen® supplementation demonstrated statistically significant benefits in rapidly improving gastrointestinal health and quality of life, especially in women and those with upper and lower GI symptoms.

Keywords: butyrate; ButyraGen®; digestive health; tributyrin; short-chain fatty acid; dietary supplement

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1. Introduction

Butyrate is a short-chain fatty acid (SCFA) which has been studied and touted for its role in gut health and other systemic benefits [1]. It is produced endogenously by gut bacteria when fermenting dietary fiber and can also be consumed exogenously through dietary supplementation. Butyrate supports gut health by reducing inflammation, strengthening the gut barrier, and promoting a balanced microbiome [1]. Acting as a signaling molecule, butyrate increases the production of tight junction proteins and mucin to enhance and strengthen the mucosal layer and provide protection against "leaky gut" and other digestive concerns, such as irritable bowel syndrome (IBS) [2,3]. The action of butyrate has been examined in animal models, and tributyrin, a food source of butyrate, by itself has been found beneficial in diseased individuals at extremely high doses [4].

ButyraGen® (NutriScience Innovations, LLC) is a patent-pending, novel dietary ingredient that directly generates butyrate. The novelty of ButyraGen® is that in contrast to other commonly available butyrate sources, ButyraGen® does not have an off-putting aroma and is in a powdered state. Its primary active component is tributyrin, a short-chain triglyceride, and its secondary active component is Sunfiber®, a partially hydrolyzed guar fiber. ButyraGen® dosing was pre-determined to be 200 mg/day from unpublished ex vivo human stool studies. We previously conducted a human randomized, placebo-controlled, cross-over pilot clinical study in healthy adults with no digestive issues to assess the tolerability and determine the mechanisms of action of ButyraGen® [5]. This 1-week placebo,

3-week active cross-over study demonstrated that ButyraGen® was safe and well tolerated. We observed significant decreases in stool acetate and propionate, two other SCFAs, and a non-significant decrease in stool butyrate. Butyrate in the blood was undetectable, possibly due to its transient nature or the low dose used in the supplement. However, secondary effects of butyrate generation and absorption were observed, including significant increases in triglycerides, which may be attributed to participants taking an active dose of ButyraGen (containing tributyrin) on the same day blood samples were collected, as tributyrin is a triglyceride-based supplement. There were also non-significant decreases in high-sensitivity C-reactive protein (hs-CRP) and blood glucose. These findings were indicative of downstream effects of butyrate generation, metabolism, and adsorption into the blood. A proposed mechanism of action involved butyrate generation in the small intestine by the action of pancreatic enzymes on tributyrin, followed by absorption into the portal vein and then activity in the liver. The reduction in stool butyrate may reflect increased utilization in the liver and other tissues [5].

Apart from this pilot study, we are not aware of any other randomized, controlled, blinded clinical study of tributyrin from food sources or at dietary supplement doses. The impetus behind the present study was to examine the effects of ButyraGen® consumption in a real-world setting. Butyrate may be helpful to healthy individuals with GI symptoms, at lower doses consistent with daily supplement use. The objective of the present study was to further investigate the role of ButyraGen® supplementation in supporting various aspects of digestive health through a 6-week, two-arm, randomized, double-blind, placebo-controlled clinical study. In the present study a series of validated questionnaires were used to assess upper and lower digestive health and the impact of digestive health on overall quality of life. This research study aims to address critical gaps in understanding the benefits of butyrate supplementation at practical doses, potentially offering a new avenue for improving gut health in healthy and at-risk populations.

2. Materials and Methods

Clinical Study Overview: This randomized, double-blind, placebo-controlled clinical study was conducted by Radicle Science Inc., Del Mar, CA, USA, with study number RADX-P-2407. This study adhered to the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6) and the Code of Federal Regulations of the Protection of Human Subjects (45 CFR, Part 46), including informed consent, which was obtained from all participants. This study evaluated the safety and effectiveness of ButyraGen® on self-reported GI-related health outcomes in approximately 600 adults aged 21 years and older that resided throughout the USA (Figure 1). This study was designed as a direct-to-consumer virtual trial with no in-person visits. Participants were randomly assigned to either the active or placebo group and consumed their assigned product daily for 6 weeks. Patient-reported assessments (questionnaires) were administered electronically via text message short message service (SMS) links or email. This study was approved by the Sterling Institutional Review Board (11730). The study overall protocol is registered at clinicaltrials.gov (study number RADX-P-2407) [6].

Recruitment and Compliance: Participants were recruited online through a variety of digital channels, including the social media platform Facebook and Radicle's own electronic mailing list. To qualify for the study, all participants expressed a desire to improve their digestive health, which was a core inclusion criterion. This study was conducted entirely virtually, with no in-person visit, to simulate real-world conditions. Therefore, diet and exercise were not controlled for. Compliance proved challenging due to the self-directed nature of the study and lack of in-person oversight. Participants were free to withdraw from the study at any time, and a portion did not complete all 6 weeks of the

study. The investigator had the authority to terminate a participant for medical or compliance reasons, but no one in this study was terminated by the investigator for medical or compliance reasons.

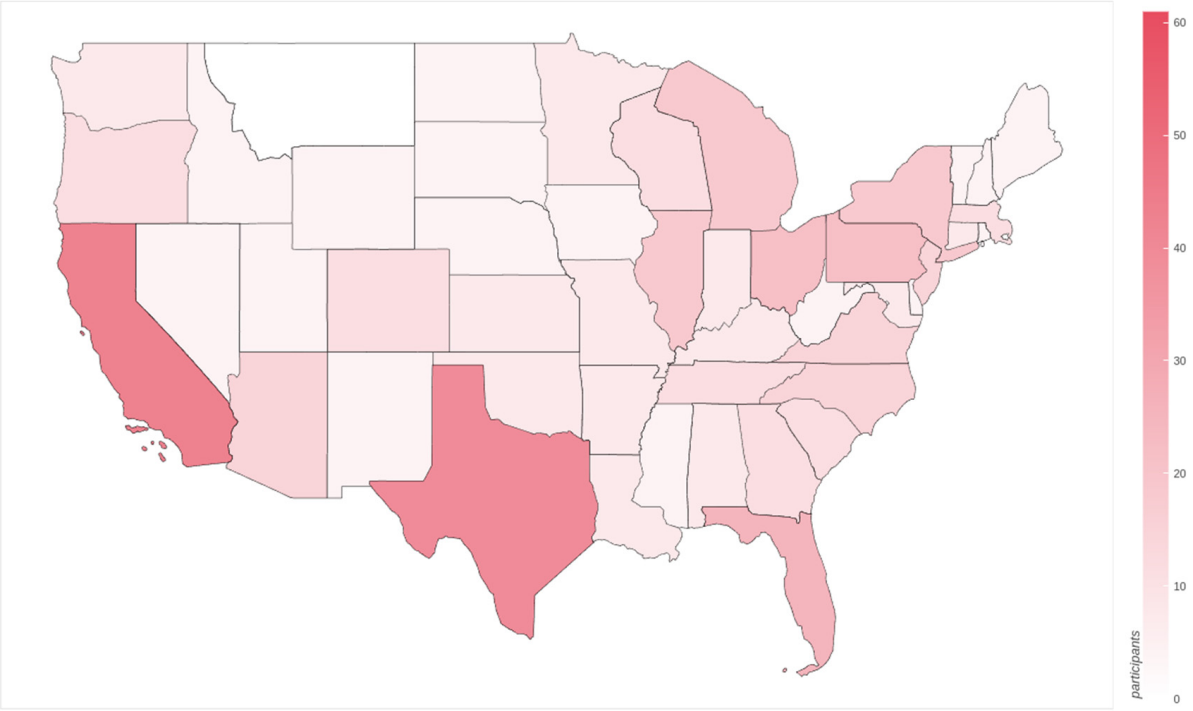


Figure 1. Population density of participants included in the study.

Inclusion and Exclusion Criteria: Inclusion criteria included adults (>21 years) with an expressed desire to improve their digestive health. Exclusion criteria included pregnancy; heavy drinking; diagnosed kidney, liver, or cardiovascular disease; chemotherapy or immunotherapy; and specific medications including antibiotics. The full inclusion and exclusion criteria were designed to ensure a study population representative of individuals with an interest in improving gut health while minimizing confounding variables that could interfere with study outcomes and can be found at clinicaltrials.gov (study number RADX-P-2407) [6].

Materials: The active and placebo products were manufactured and provided to Radicle Science by NutriScience Innovations. The active product contained ButyraGen®, a patent-pending complex of tributyrin, Sunfiber® (partially hydrolyzed guar fiber), acacia fiber, and other inactive constituents. Participants in the active group received a daily dose of 200 mg of ButyraGen®, which contained 50% tributyrin (providing 100 mg of tributyrin per day) and approximately 45% fiber (providing 90 mg of prebiotic fiber per day). The placebo product primarily consisted of maltodextrin, for its rapid digestion, meaning little to none reaches the colon to be fermented by gut bacteria into short-chain fatty acids like butyrate. Both the active and placebo powders were encapsulated in hydroxy propyl methyl cellulose capsules with identical inactive colorants used to ensure blinding. Each participant received a single bottle containing 45 capsules of their randomly assigned product (active or placebo), sufficient for the 6-week study. The active and placebo products were tested for potency, microbial contamination, heavy metals, pesticides, and residual solvents to ensure safety and quality before distribution.

Questionnaires: Participants were enrolled based on an inclusion/exclusion criterion questionnaire. Following enrollment, all participants provided demographic information and then completed a diet questionnaire along with a series of health questionnaires, which

included alcohol and cannabinoid use. The following health questionnaires (validated participant-reported assessments) were administered at baseline, weekly, and at the end of the study. The questionnaires used in this study included the following:

- Digestion-associated Quality of Life Questionnaire (DQLQ);
- PROMIS™ Belly Pain 5A*;
- PROMIS™ GI Gas and Bloating 13A*;
- PROMIS™ Constipation 9A*;
- PROMIS™ Diarrhea 6A*
- PROMIS™ Reflux 13A*;
- PROMIS™ Nausea and Vomiting 4A*.

* Validated tools used to assess GI issues. They are part of the Patient-Reported Outcomes Measurement Information System (PROMIS™), which was developed by the National Institutes of Health (NIH) [7].

Outcome Measurements: The primary GI collective outcome measure of the study was an assessment of gastrointestinal health, based on the collective responses to the following validated questionnaires:

- PROMIS™ Belly Pain 5A, which evaluated abdominal pain;
- PROMIS™ Reflux 13A, which assessed symptoms related to reflux;
- PROMIS™ Nausea and Vomiting 4A, which measured the severity of nausea and vomiting;
- PROMIS™ GI Gas and Bloating 13A, which evaluated gas and bloating;
- PROMIS™ Constipation 9A, which assessed constipation;
- PROMIS™ Diarrhea 6A, which measured the severity of diarrhea.

The secondary GI outcome measure was the following:

- GI-related Quality of Life (QOL) as assessed by the Digestion-associated QOL Questionnaire (DQLQ).

Statistical Analysis: Statistical analysis was performed on the dataset which was provided to the authors by Radicle Science. Participants included in the analysis completed the baseline questionnaire, reported taking at least 6 doses weekly (up to 7) in at least 5 of the 6 weekly questionnaires, and completed the final week-6 questionnaire. This modified per-protocol analysis was to account for the real-world situation with no in-person visits, where participants were included in the analysis even if they missed one dose per week and missed one of the 6 weekly questionnaires. The flow of participants through this process is shown in Figure 2 and Table 1 for the Belly Pain 5A questionnaire. Similar participant flows for the other health questionnaires are described in the Results Section.

Minimal Clinically Important Difference (MCID) analysis was used to analyze the results for meaningful clinical differences. MCID is a participant-centered concept that considers the magnitude of meaningful clinically relevant differences and how much value the participant places on the change [8]. MCID analysis examines the likelihood of meaningful improvements in participant questionnaires by the end of the study. An MCID was defined as an improvement of at least 0.5 standard deviations from baseline in the target health measure. A Poisson general linear model was used to fit questionnaire data to predict the occurrence of an MCID while controlling for covariates such as sex, age, and BMI across all data points, including both study arms and all participants. The *p*-values are determined from this model, not from raw MCID rates. The associated MCID risk ratio is computed to compare the likelihood of an improvement for the active and placebo study arms. A risk ratio greater than 1.0 indicates a higher probability of achieving clinically significant improvement in the active group versus the placebo group, with the 95% confidence interval (C.I.) representing the likely range of the true risk ratio.

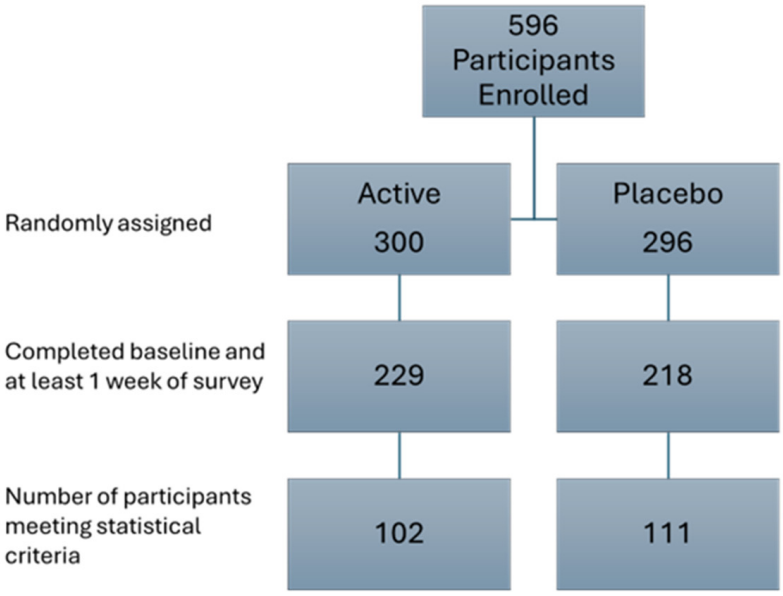


Figure 2. Participants meeting criteria through the study and analysis for the belly pain questionnaire (PROMIS™ Belly Pain 5A).

Table 1. Sex and age distribution for participants at baseline and week 6 for the belly pain questionnaire (PROMIS™ Belly Pain 5A).

Study Week	Age	ButyraGen		Placebo	
		Female	Male	Female	Male
Baseline	0–30	13	9	16	9
	30–40	65	31	53	34
	40–50	76	27	64	29
	50–60	34	21	46	11
	60+	16	8	21	13
Week 6	0–30	2	0	3	2
	30–40	19	8	13	12
	40–50	29	7	24	11
	50–60	14	9	20	7
	60+	8	6	13	6

The statistical analysis also considered key demographic subsets of the study participants, allowing for a deeper understanding of how specific population subgroups responded to the intervention. Since there were no significant outcomes for the primary (GI collective) and secondary (DQLQ) outcomes using intent-to-treat analysis, a modified per-protocol post hoc statistical analysis was performed on individual GI questionnaires within the GI collective as well as the DQLQ outcome.

The core analysis and development for this study were conducted by using Python version 3.12 [9], leveraging the following libraries:

- Statsmodels: Used to fit the Poisson general linear model [10].
- Pandas: Used for the manipulation and preprocessing of raw study data [11].
- Bokeh: Used to create visualizations and charts for the study [12].

We used a rigorous statistical approach to best reflect the clinical outcomes. This statistical framework provides meaningful insights into the effects of ButyraGen® supplementation on gastrointestinal health outcomes while accounting for real-world variability and consumer adherence.

3. Results

3.1. Study Demographics

A total of 596 participants were enrolled in the study. The study population analyzed here consisted of 32% males and 68% females, with a mean age of 44 years (range: 21–79). As calculated from Table 1, the percentage of male and female participants remained the same through week 6. Approximately 25% of the participants identified as racial minorities, and 36% reported having a prior gastrointestinal (GI) disorder diagnosis. Participants were geographically diverse, representing 47 of 50 US states.

3.2. Study Compliance

Thirty seven percent of the enrolled participants completed the modified per-protocol criteria, reflecting real-world compliance. Weekly questionnaire completion rates are shown in Figure 3. Similar completion rates were observed across both arms.

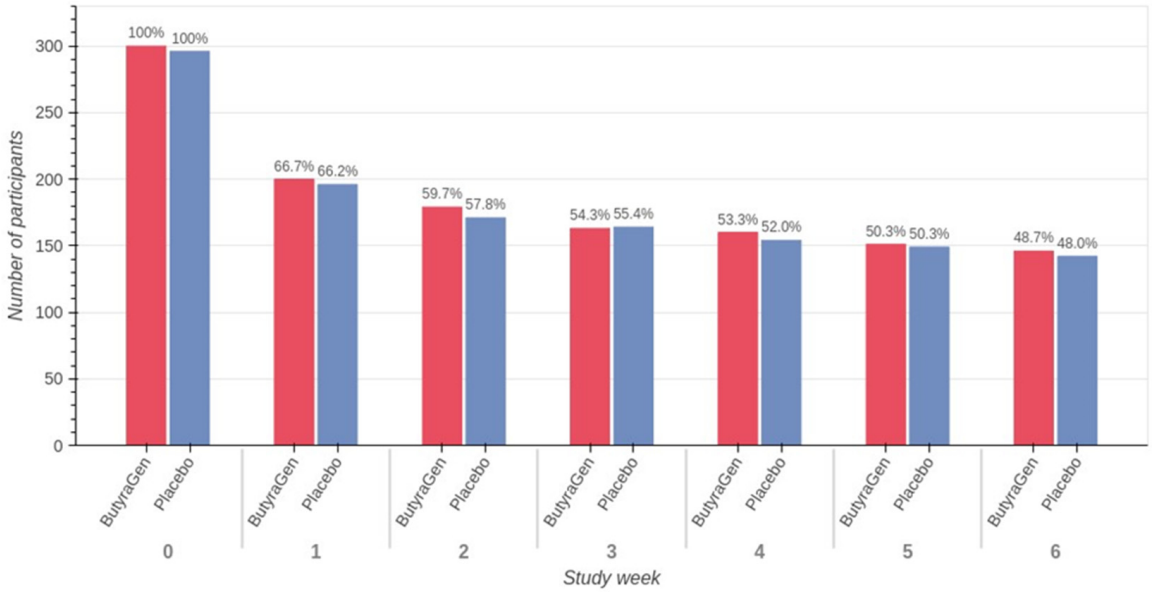


Figure 3. Weekly questionnaire completion rates by trial arm.

3.3. Tolerability

Both the active and placebo arm products were very tolerable and demonstrated similar tolerability. All side effects were mild or moderate across both trial arms; none were considered serious or required use of emergency healthcare services. A total of 15.7% of the active group (ButyraGen®) reported side effects, while 13.8% of the placebo group reported side effects, a non-statistically significant difference ($p = 0.559$, as calculated by Pearson's χ^2 test of independence). The entire population that completed baseline and at least one week (n = 447) were used to determine tolerability. The five top side effects were upset stomach/cramping, nausea, diarrhea, constipation, and headache, with the counts for active being almost identical to placebo in all cases, and both arms reported less than national averages of 40% for abdominal pain [13].

3.4. Belly Pain (PROMIS™ Belly Pain 5A)

Statistically significant improvements were observed in belly pain as measured by the PROMIS™ Belly Pain 5A questionnaire. Participants in the active group demonstrated a significantly higher likelihood (18%) of achieving an improvement compared with the placebo group ($p = 0.0064$, risk ratio = 1.29 (+/− 0.10)). These results are shown in Table 2.

Table 2. Belly pain results.

Questionnaire	PROMIS™ Belly Pain 5A
Total participants	213
Active participants	102
Placebo participants	111
MCID improvement *p*-value	0.0064
MCID risk ratio (+/− 95% C.I)	1.29 (+/− 0.10)
Difference in overall MCID (active–placebo)	18.0%

A temporal view (weekly) of the percent of the participants experiencing an improvement in belly pain (Figure 4) shows that even though the placebo participants experienced improvements, the active participants experienced a consistent improvement greater than the placebo group throughout the study, with significant differences compared with the placebo group observed in week 5 ($p < 0.05$) and week 6 ($p < 0.01$).

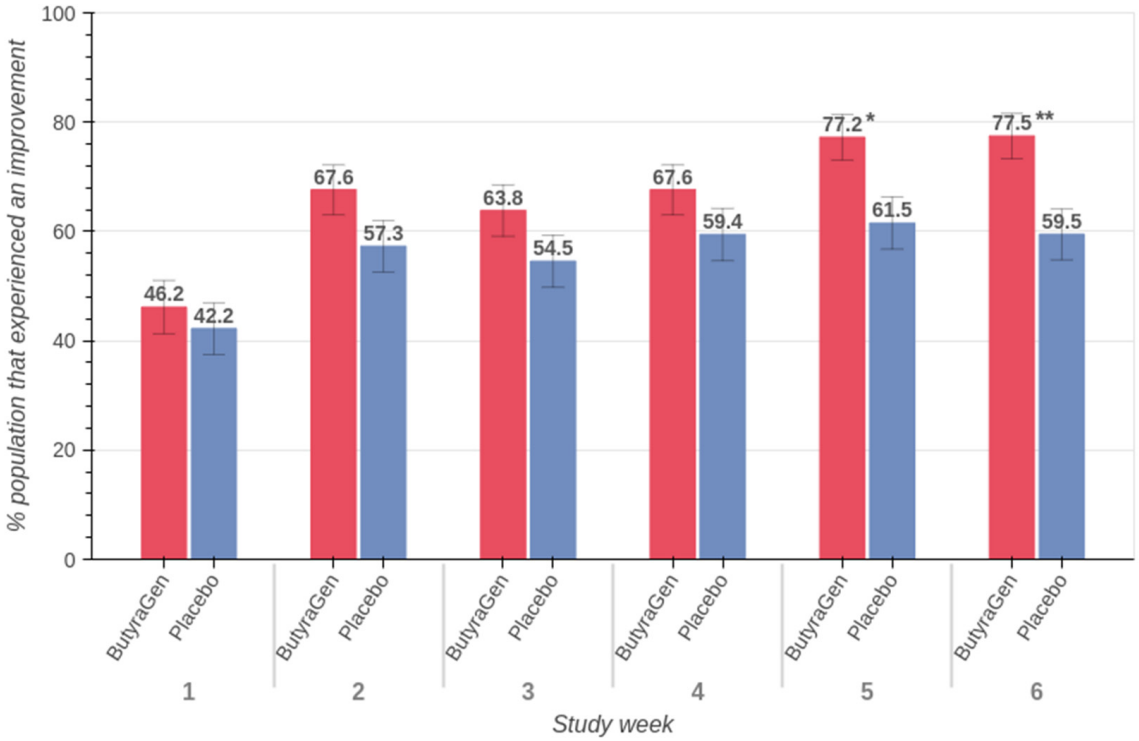


Figure 4. Temporal (week-to-week) changes in Belly Pain % participant improvement over the course of the 6-week study. Error bars represent Standard Error of the Mean (SEM). * $p < 0.05$ and ** $p < 0.01$.

Figure 5 illustrates the percentage of participants who completed weekly questionnaires assessing belly pain over the 6-week study period for both the ButyraGen® and placebo groups. The participant count remained steady throughout the study, with minimal variation between weeks. Both groups maintained high questionnaire completion rates, due to our analysis protocol which only included participants who completed five out of six weekly questionnaires and the week-6 questionnaire, ensuring robust data collection and study integrity.

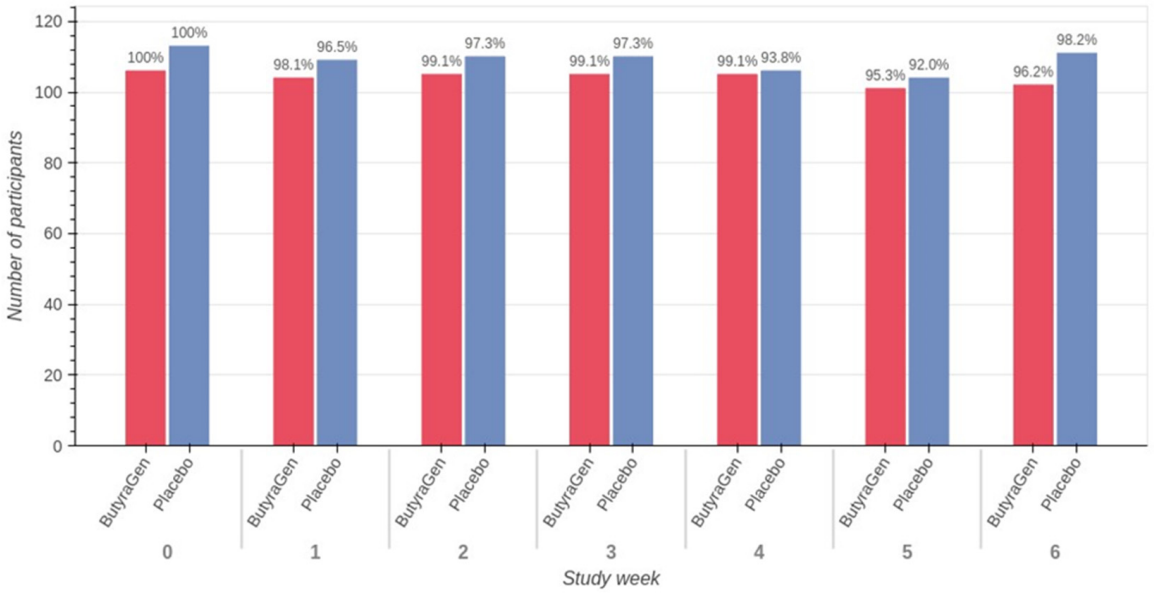


Figure 5. Weekly questionnaire completion rates for PROMIS™ Belly Pain 5A assessment.

Belly pain (abdominal pain or abdominal discomfort) encompasses a broad array of digestive issues between the chest and the groin [3]. The PROMIS™ Belly Pain 5A questionnaire is summarized in Table 3.

Table 3. PROMIS™ Belly Pain 5A questionnaire (Scale v1.0).

Question (In the Past 7 Days. . .)	1	2	3	4	5
How often did you have belly pain?	never *	one day	2–6 day	one/day	>1/day
At its worst, how would you rate your belly pain?	not bad at all	a little bad	somewhat bad	quite bad	very bad
How much did belly pain interfere with your day-to-day activities?	not at all	a little bit	somewhat	quite a bit	very much
How much did belly pain bother you?	not at all	a little bit	somewhat	quite a bit	very much
How often did you have discomfort in your belly?	never	rarely	sometimes	often	always

* If never go to last question.

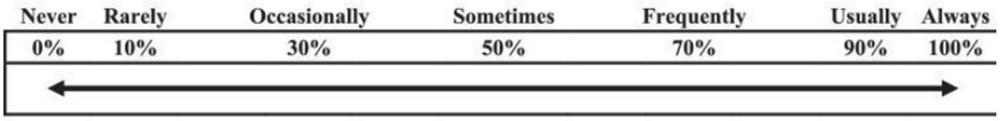
3.5. Upper and Lower GI

The post hoc analysis for the two upper GI questionnaires—nausea and vomiting (PROMIS™ Nausea and Vomiting 4A) and reflux (PROMIS™ Reflux 13A)—and the three lower GI questionnaires—gas and bloating (PROMIS™ GI Gas and Bloating 13A), constipation (PROMIS™ Constipation 9A), and diarrhea (PROMIS™ Diarrhea 6A)—did not show any significant improvements for active versus placebo groups overall or for specific population subsets with total participants > 100. The sole exception was for females who identified as post-menopausal, which is discussed in the section on women. On the other hand, the post hoc analysis of the DQLQ related to participants starting with (baseline) upper and lower GI issues did show significance in larger (>100 participants) populations, suggesting a possible improvement in both upper GI and lower GI for active versus placebo groups (Table 4).

Table 4. Participants showing significant improvement in their DQLQ starting with specific upper and lower GI conditions.

DQLQ Post Hoc Analysis	DQLQ	DQLQ
Specific population	Participants starting with normal/mild reflux	Participants starting with normal/mild constipation
Region	Upper GI	Lower GI
Total participants	157	106
Active participants	81	54
Placebo participants	76	52
MCID improvement *p*-value	0.0305	0.0440
MCID risk ratio (+/− 95% C.I)	1.39 (+/− 0.15)	1.45 (+/− 0.19)
Difference in overall MCID (active–placebo)	16.9%	18.8%

The DQLQ is presented in Figure 6.



Due to digestive events and experiences in the past 7 days,

	Never	Rarely	Occasionally	Sometimes	Frequently	Usually	Always
physical activities (running, walking, gardening, golfing, etc.) were unpleasant or avoided.	❍	❍	❍	❍	❍	❍	❍
my usual appetite changed.	❍	❍	❍	❍	❍	❍	❍
I was inconvenienced or physically uncomfortable.	❍	❍	❍	❍	❍	❍	❍
I avoided certain foods.	❍	❍	❍	❍	❍	❍	❍
I was self-conscious or bothered in public or around others.	❍	❍	❍	❍	❍	❍	❍
I used the restroom less than I wanted.	❍	❍	❍	❍	❍	❍	❍
I was distracted while doing various activities.	❍	❍	❍	❍	❍	❍	❍
social activities (spending time with friends or family, going out to eat, etc.) were unpleasant or avoided.	❍	❍	❍	❍	❍	❍	❍
consuming foods and beverages was less enjoyable.	❍	❍	❍	❍	❍	❍	❍

Figure 6. DQLQ.

Figures 7 and 8 illustrate the week-over-week % population which experienced improvements in the DQLQ for the specific populations detailed in Table 4. This temporal analysis aligns with the overall (baseline to week-6) analysis in Table 4. The figures show that participants in the active group consistently demonstrated greater improvements compared with the placebo group throughout the study. Notably, these differences were sta-

tistically significant in most week-over-week comparisons. Participants with normal/mild constipation and reflux showed statistically significant improvements over the placebo group for the DQLQ during weeks 1 and 2, demonstrating the fast action of ButyraGen®.

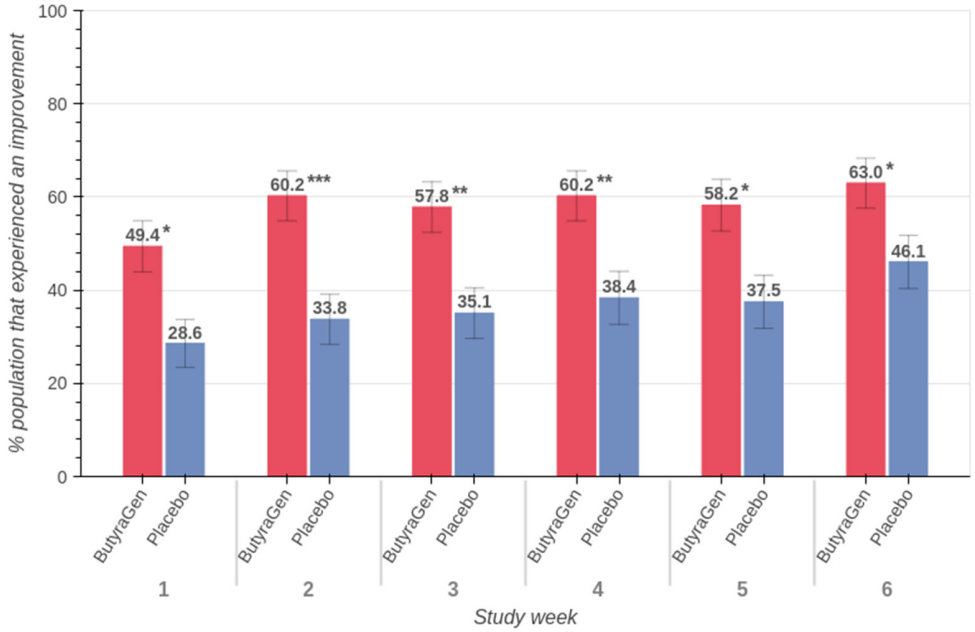


Figure 7. Temporal (week-to-week) changes in DQLQ participant % improvement starting with normal or mild baseline reflux over the course of the 6-week study. Error bars represent Standard Error of the Mean (SEM). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

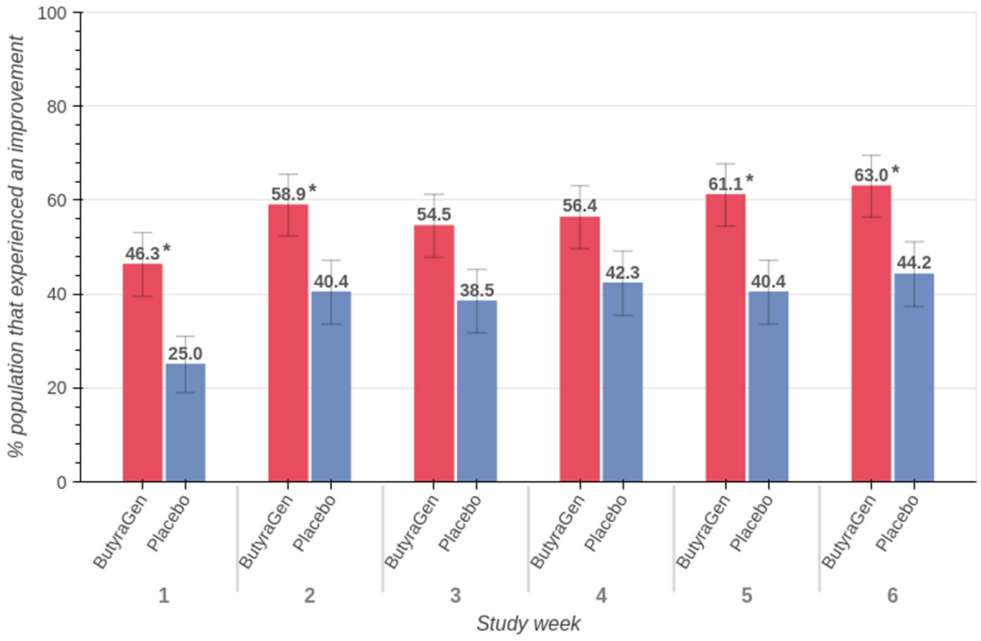


Figure 8. Temporal (week-to-week) changes in DQLQ participant % improvement starting with normal or mild baseline constipation over the course of the 6-week study. Error bars represent Standard Error of the Mean (SEM). * $p < 0.05$.

3.6. Women

The post hoc analysis of the female participants revealed significant improvements in belly pain and gas/bloating scores for the active group compared with the placebo. These findings were particularly pronounced in self-identified post-menopausal women.

These results are shown in Table 5. For belly pain (PROMIS™ Belly Pain 4A), the active group showed a significance difference in the percentage of participants experiencing an improvement greater than 20% ($p < 0.01$). Similarly, improvements in gas and bloating (PROMIS™ GI Gas and Bloating 13A) were observed among female participants, with 14% more participants experiencing improvement versus the placebo group ($p < 0.05$). Although the subgroup of post-menopausal women was <100 participants, the results are included here as this group represents a meaningful portion of the population and the results support a significant improvement in active versus placebo groups. A weekly analysis of the scores for female belly pain, gas/bloating, and belly pain for women self-reported as post-menopausal (Figures 9–11) indicates that the active group achieved a consistent % improvement versus placebo, with generally significant improvements by week 5 continuing through the completion of the study. Men did not see any significant improvement in their scores. These results align with the overall population findings but suggest a stronger effect in women.

Table 5. Post hoc analysis of female improvements in belly pain and gas and bloating.

Female-Specific Post Hoc Analysis	PROMIS™ Belly Pain 5A	PROMIS™ Belly Pain 5A	PROMIS™ GI Gas and Bloating 13A
Specific subset population	Women	Women self-identified as post-menopausal	Women who self-identified as experiencing GI distress
Total participants	145	55	125
Active participants	72	26	59
Placebo participants	73	29	66
MCID improvement *p*-value	0.0057	0.0230	0.0411
MCID risk ratio (+/− 95% C.I)	1.36 (+/− 0.11)	1.44 (+/− 0.16)	1.23 (+/− 0.10)
Difference in overall MCID (active–placebo)	20.3%	26.4%	14.9%

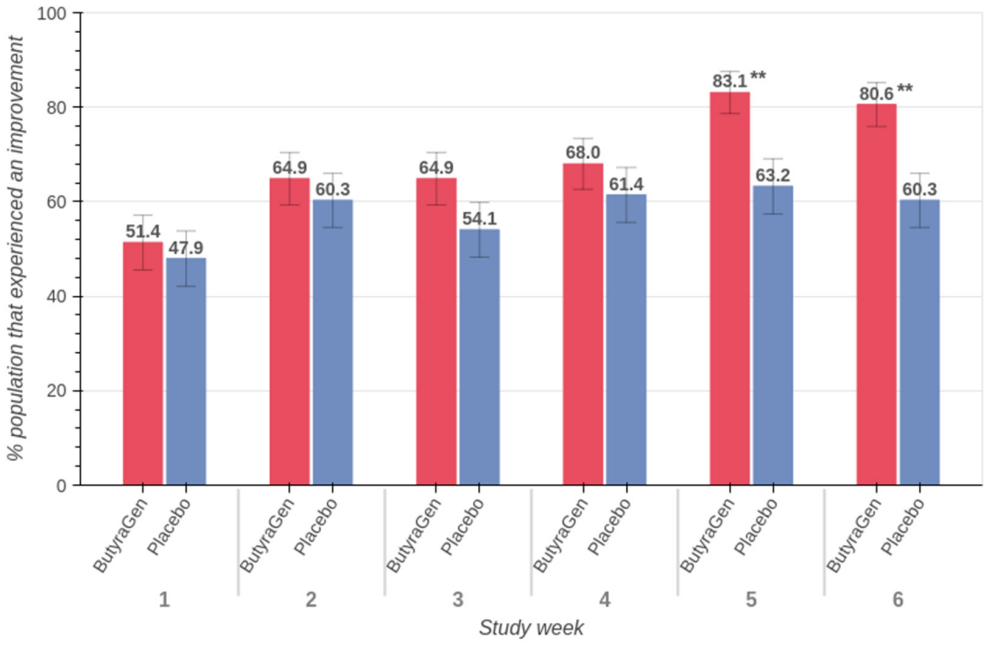


Figure 9. Temporal (week-to-week) changes in belly pain for females % participant improvement over the course of the 6-week study. Error bars represent Standard Error of the Mean (SEM). * $p < 0.05$ and ** $p < 0.01$.

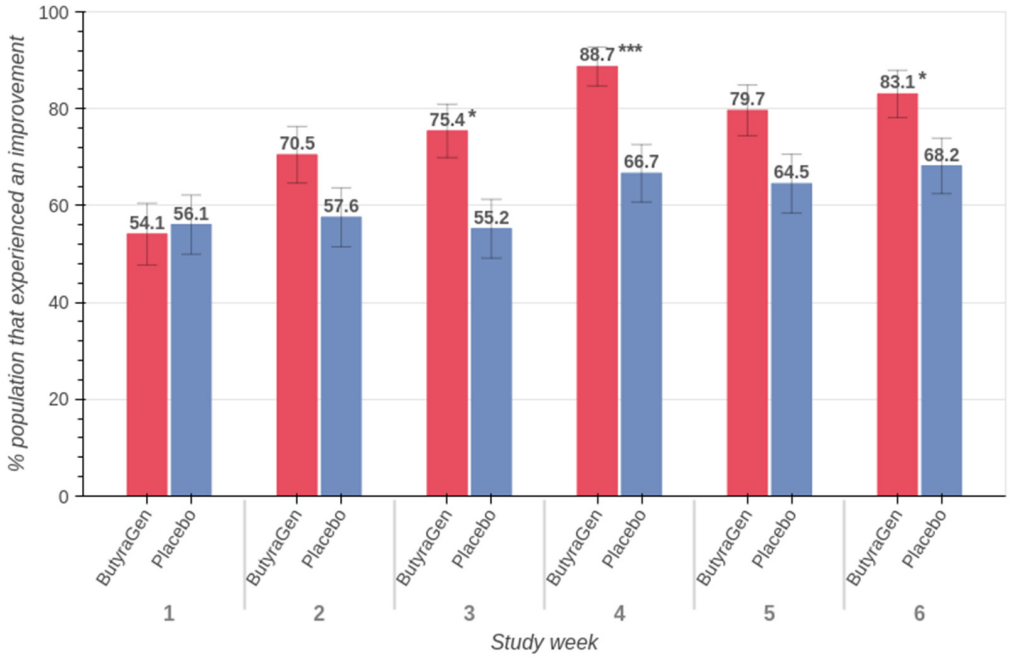


Figure 10. Temporal (week-to-week) changes in female gas and bloating with GI distress % participant improvement over the course of the 6-week study. Error bars represent Standard Error of the Mean (SEM). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

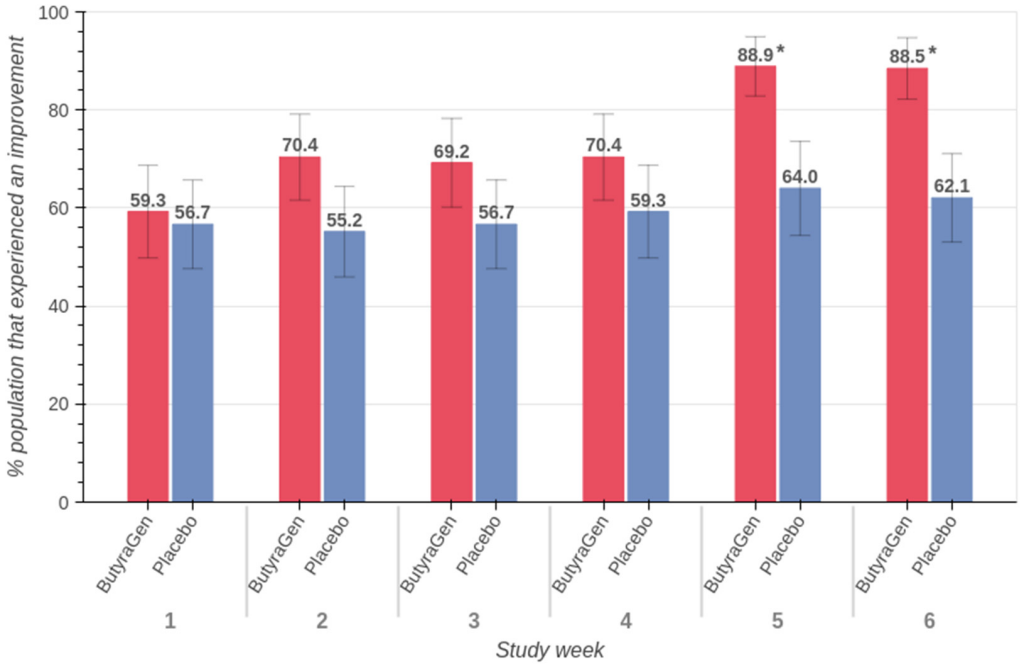


Figure 11. Temporal (week-to-week) changes in female Belly Pain % participant improvement for women with self-reported post-menopausal over the course of the 6-week study. Error bars represent Standard Error of the Mean (SEM). * $p < 0.05$.

3.7. Other Results

Other post hoc GI health statistical analyses were not significant, were marginally significant, or were significant for smaller subset populations and are not considered here.

4. Discussion

This study provides important insights into the potential benefits of ButyraGen® supplementation on gastrointestinal (GI) health, with significant findings that highlight its efficacy in specific populations and health measures. The results demonstrate that ButyraGen® supplementation led to meaningful clinical improvements in belly pain, particularly in women, and suggest potential applications for managing digestive discomfort and improving overall digestive quality of life.

4.1. Belly Pain Improvements and Clinical Relevance

Statistically significant improvements in belly pain were observed in the ButyraGen® group compared with the placebo group, as measured by the PROMIS™ Belly Pain 5A questionnaire. Notably, these improvements became significant during weeks 5 and 6, whereas our prior 3-week pilot study did not reveal significant improvements in digestive health [5]. This time-dependent response suggests that longer durations of supplementation may be necessary to achieve certain measurable benefits, potentially due to the cumulative effects of butyrate generation and its downstream impact on the gut. Butyrate is known for its role in supporting gut barrier integrity, reducing inflammation, modulating gut motility, and the moderation of abdominal pain [14–18]. These mechanisms are relevant to belly pain, which can be a symptom of conditions such as irritable bowel syndrome (IBS), leaky gut, and other inflammatory GI disorders. By promoting the production of tight junction proteins and enhancing mucosal defenses, butyrate may reduce the intestinal permeability often associated with abdominal discomfort [19].

The observed improvements in belly pain are clinically meaningful, as abdominal discomfort is a prevalent issue that presents within a wide range of digestive conditions, often not meeting diagnostic criteria for specific GI disorders, leading to challenges in management. By reducing belly pain, ButyraGen® supplementation may offer a practical, well-tolerated solution for individuals seeking relief from abdominal discomfort and associated digestive quality-of-life impairments.

4.2. Implications for GI Health and Quality of Life

In addition to belly pain, the post hoc analysis of the DQLQ demonstrated improvements in GI-related quality of life for participants with mild baseline GI symptoms. The DQLQ is designed to be sensitive to both physical and psychological changes related to GI health. This may explain the earlier significant improvements observed compared with the belly pain questionnaire, which measures the physical manifestation of discomfort. The DQLQ findings indicate that ButyraGen® may benefit individuals with normal-to-mild reflux or constipation, two common conditions that significantly impact digestive quality of life. The tributyrin and prebiotic fiber in ButyraGen® work synergistically to promote the production of short-chain fatty acids (SCFAs) in the upper and lower GI tracts, which may help alleviate digestive symptoms and support overall gut health. The ability to address both upper and lower GI symptoms through a single fast-acting intervention underscores the versatility of ButyraGen® as a dietary supplement.

The observed improvements in quality of life also suggest broader implications for ButyraGen® supplementation. By addressing GI symptoms and enhancing comfort, ButyraGen® may help individuals maintain regular daily activities, reduce stress associated with digestive discomfort, and improve overall well-being. These findings are particularly relevant given the increasing prevalence of GI disorders in the general population [20].

4.3. Potential Benefits in Women

The findings also highlight significant benefits in females, with further improvements observed in belly pain and gas/bloating scores. These benefits were particularly pronounced in post-menopausal women, suggesting a potential sex-specific or hormonal influence on the efficacy of ButyraGen®. Women, especially post-menopausal women, may experience unique changes in gut health due to hormonal shifts that affect the microbiome, gut motility, and inflammation levels. These hormonal shifts could be attributed to the estrobolome, a collection of gut microbiota involved in estrogen metabolism that plays a crucial role in regulating circulating estrogen levels via reactivation and absorption [21]. ButyraGen®, through its direct generation of butyrate, may help counteract these changes and the variability in hormonal shifts by promoting a healthy gut barrier, reducing inflammation, and supporting microbial diversity, independently of the state of the microbiome.

The larger improvement seen in women aligns with emerging evidence suggesting that biological sex and hormones play a significant role in shaping the gut microbiome and its response to various interventions [22]. Research on short-chain fatty acids, such as butyrate, have shown a moderation of gynecological disease progression due to hormonal normalization [23]. Women are more prone to experiencing digestive discomfort than men, with increased prevalence in post-menopausal women. This heightened susceptibility may be attributed to factors such as hormonal fluctuations, the unique complexity of the female gastrointestinal system, and increased psychological stress [24]. Notably, this finding highlights the importance of addressing the needs of post-menopausal women—a demographic often underrepresented in clinical research and for whom no studies to date have specifically examined the effects of butyrate on gastrointestinal issues [25]. These insights suggest that ButyraGen® could offer targeted support for this group.

4.4. Insights and Opportunities

This study was conducted entirely virtually with no in-clinic visits, representing real-world conditions and a diverse U.S. population. The virtual nature of the study prevented bias that can stem from proximity to testing facility locations. The use of validated tools such as PROMIS™ and the DQLQ helped ensure a robust and reliable set of questions. The large starting participant population was critical to obtaining a sufficient ending population in order to demonstrate statistical significance. The findings demonstrate statistically significant improvements in digestive health over the 6-week course and 200 mg ButyraGen® dose regimen. This study supported the safety and tolerability of ButyraGen® in a general population.

A virtually conducted clinical study inherently comes with certain limitations. Without in-clinic visits, collecting blood or stool samples or performing in-clinic tests was not possible, preventing the correlation of perceived digestive health with measurable digestive biomarkers. Additionally, ensuring participant compliance for remotely completing questionnaires is more challenging compared with an in-clinic setting, where investigators can provide direct oversight.

The delayed onset of some of the significant benefits, observed beginning in week 5, highlights intriguing questions about the time course of butyrate benefit. This finding prompts the question of whether extending the trial period could uncover additional health benefits. Additionally, the observed sex-specific differences raise important questions about the biological mechanisms driving these variations and how they might inform more personalized recommendations for different populations. These insights underscore the complexity of butyrate's impact and the potential for more targeted applications based

on individual or demographic characteristics, such as controlling for diet, exercise, and monitoring phases of the menstrual cycle.

5. Conclusions

This study demonstrates the ability of ButyraGen®, a direct butyrate-generating dietary supplement, to improve GI health and quality of life, with a fast-acting response. This improvement provides particular benefits for women, post-menopausal women, and those with mild GI symptoms. By addressing common digestive concerns, ButyraGen® represents a promising intervention for promoting gut health and enhancing overall well-being in diverse populations.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to legal restrictions.

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Abbreviations

The following abbreviations are used in this manuscript:

SCFA	Short-chain fatty acid
IBS	Irritable bowel syndrome
hs-CRP	High-sensitivity C-reactive protein
DQLQ	Digestion-associated Quality of Life Questionnaire
MCID	Minimal Clinically Important Difference
C.I.	Confidence interval
GI	Gastrointestinal

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