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ORIGINAL RESEARCH

A Novel Butyrate Generator Helps Modulate the Gut-Brain Axis: A Randomized Double-Blind Placebo-Controlled Clinical Study

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Purpose: ButyraGen® (Registered Trademark of NutriScience Innovations, LLC), generates butyrate, a short-chain fatty acid, in the small intestine without the drawbacks of conventional formulations. Butyrate has roles in metabolism, immunity, and inflammation, and has emerging relevance in gut-brain axis signaling and psychological health. This randomized, double-blind, placebo-controlled trial evaluated ButyraGen's effects on mood and well-being.

Patients and Methods: A starting cohort of 596 adults were randomized and double-blinded to receive 200 mg/day of ButyraGen or placebo for six weeks in a fully virtual study. Validated questionnaires were administered to assess changes in anxiety, depression, and functional outcomes. Analyses followed an Intent-to-Treat protocol, with Minimal Clinically Important Difference (MCID) used to identify meaningful improvements through post-hoc analyses.

Results: ButyraGen significantly reduced self-reported anxiety in men, who were over twice as likely to achieve clinically meaningful improvement versus placebo. Across all participants, improvements in feelings of fear and unease were observed. No overall benefit for self-reported depression was seen, but participants without gastrointestinal disease had a twofold greater likelihood of improvement compared to placebo. Further analysis showed improvements in hopelessness and helplessness among younger adults and men. Functional improvements were also seen for reduced distraction and improved social engagement.

Conclusion: These results suggest that ButyraGen may supports both psychological and physiological well-being, particularly in men, younger individuals, and those without gastrointestinal disease. Benefits began to appear within weeks two and three and were sustained through six weeks, highlighting its potential as a fast-acting intervention for both gut and brain health, indicative of the gut-brain axis connection.

Keywords: ButyraGen, cognitive, digestion, butyrate, mood, psychological well-being

Introduction

Butyrate, a short-chain fatty acid (SCFA), is well known for its systemic benefits, including its role in metabolic regulation, immune function, and inflammation control.[1] However, its effects on the gut-brain axis remain less explored, with emerging research beginning to uncover its influence on brain health and neurological function.[2,3] The gut-brain axis facilitates bidirectional communication between the enteric nervous system (ENS) within the gastrointestinal (GI) tract, and the central nervous system (CNS). The ENS, which regulates basic gut functions, utilizes neurotransmitters and signaling molecules similar to those found in the brain. A key neural pathway for this communication is the vagus nerve, which modulates the hypothalamic-pituitary-adrenal (HPA) axis, a central regulator of the body's adaptive stress response.[3]

Communication between the gut and brain also occurs through biochemical signaling pathways involving microbial metabolites, hormones, and neurotransmitters that can be synthesized or modulated by the gut microbiota or introduced through diet and supplementation. Short chain fatty acids, including butyrate, or butyric acid, play a crucial role in this

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regard, as they contribute to intestinal barrier integrity, mucus production, and inflammation regulation, key factors in maintaining gut and brain health.

Butyrate is primarily produced endogenously by gut bacteria during the fermentation of dietary fiber but can also be supplemented exogenously. Butyrate interacts with various receptors attached to many cell types throughout the body. Although the mechanisms by which butyrate regulates biochemical activity in these cells is just beginning to be investigated and understood, the widespread distribution of butyrate receptors underscores its broad physiological importance.

There is mounting evidence of a relationship between butyrate and the brain and nervous system.[1,3–5] While the precise pathways remain to be fully elucidated, free fatty acid receptors (FFARs), which are sensitive to SCFAs, have been found on many nerve and brain cells, suggesting direct interactions. Beyond direct signaling, butyrate acts as a histone deacetylase (HDAC) inhibitor, and studies have shown that HDAC inhibition in brain cells upregulates genes involved in oxidative stress resistance and neurotrophic factor production. Butyrate has also been shown to positively enhance development and homeostasis of microglia, the immune cells specific to the brain and nervous system, which are essential for regulating neuroinflammation and supporting cognitive function.[6] Higher levels of butyrate have been associated with increased neurogenesis and may offer neuroprotective benefits in neurodegenerative diseases such as Alzheimer's, Parkinson's, and multiple sclerosis.[7–10] Through its roles in neuroprotection, inflammation reduction, and enhancement of neuroplasticity, butyrate holds considerable promise for supporting brain health but has yet to be studied in a human clinical setting.

ButyraGen is a novel, complex dietary ingredient that directly generates butyrate. Its primary active component is tributyrin, a short chain triglyceride, with additional active components including Sunfiber® (Registered Trademark of Taiyo International, Inc)., a partially hydrolyzed guar fiber, and other fermentable fibers. 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. ButyraGen dosing was pre-determined to be 200 mg/day from ex vivo human stool data.

We previously conducted a randomized, placebo-controlled, cross-over pilot study in healthy adults with no digestive issues to assess the tolerability and determine mechanisms of action of ButyraGen.[11] This 1-week placebo, 3-week active cross over study, demonstrated that ButyraGen was safe and well tolerated. While butyrate in the blood was undetectable, likely due to its transient nature or the low supplement dose, secondary effects indicated downstream absorption and metabolic activity, including significant increases in triglycerides, attributable to the tributyrin component taken on the same day of blood sampling. Non-significant decreases in hs-CRP and blood glucose were also observed, suggesting beneficial metabolic effects. A proposed mechanism of action involves 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, allowing it to have a systematic effect. The dietary prebiotic fiber components are fermentable in the large intestine.

Based on the proposed mechanism of action, a large cohort, real world setting, randomized double-blind placebo-controlled clinical study was conducted. ButyraGen® was found to significantly reduce belly pain, particularly in women, and even more so in post-menopausal women. We additionally found that 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.[12]

During this clinical study, we also collected self-reported data related to mood and emotional well-being, specifically focusing on symptoms commonly associated with anxiety and depression, which we analyze and present here for the first time. These data were assessed using validated questionnaires designed to capture subclinical variations in psychological states, specifically focusing on symptoms commonly associated with anxiety and depression, rather than to serve as diagnostic tools. Our aim here was to examine the effects of ButyraGen consumption on the gut-brain axis in a real-world setting at a practical supplement dose. Specifically, we investigated ButyraGen's role in supporting various aspects of the gut-brain axis, such as self-reported non-clinical anxiety and depression through a 6-week, 2-arm, randomized, double-blind, placebo-controlled clinical trial. Validated questionnaires were used to assess stress and brain health and their impact on overall digestive quality of life. This research aims to address critical gaps in understanding the effects of butyrate supplementation at practical doses on the gut-brain axis, potentially offering a new avenue for improving brain and gut health in healthy and at-risk populations.

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. All participants provided electronic informed consent via a secure web portal, confirming their understanding of study procedures, risks, and rights, with the option to ask questions, receive a copy of the form, and withdraw at any time without penalty. This study evaluated the safety and effectiveness of ButyraGen on self-reported health outcomes starting with the recruitment of a starting cohort of approximately 600 adults aged 21 years and older that resided throughout the USA. This study was designed as a direct-to-consumer, virtual trial with no in-person visits. Participants were stratified by assigned sex at birth and self-reported GI health score, blinded and randomly assigned using block randomization to either the active or placebo group and instructed to consume their alpha-numeric coded assigned product daily for 6 weeks. The investigators were also blinded to the participants assigned study products. Patient-reported assessments (questionnaires) were administered electronically via text message short message service (SMS) links or email. Participants verified their study product via an alpha-numeric code to confirm they received the correct product. Weekly email check-ins captured self-reported supplement use (days taken, capsule count) and side effects. Automated 24-hour follow-up email reminders were sent for missed assessments. 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 / NCT06376695.[13]

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. This study was conducted entirely virtually, with no in-person visit, to simulate real-world conditions. Therefore, diet and exercise were not controlled for and are limitations to the study design. The self-directed format and absence of in-person oversight mirrored real-world use, enhancing ecological validity but potentially contributing to variability in compliance. Participants were required to answer all health-related items within the questionnaires. Reminders were sent at the 24hr mark, if the questionnaire was not completed it was not included in the statistical analyses. Self-reported weekly checks on days product taken and capsule count were conducted to ensure compliance.

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.

Inclusion and Exclusion Criteria

Inclusion criteria included adults (>21 years) residing in the US who expressed a desire to improve their digestive health and had the potential to improve in their GI health score by at least 30%. Exclusion criteria included pregnancy; heavy drinking; diagnosed kidney, liver, or cardiovascular disease; 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.[13]

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, chosen for its

rapid digestion, meaning little to none reached the colon to be fermented by gut bacteria into SCFAs like butyrate, and silicon dioxide a processing aid. Both the active and placebo powders were encapsulated in hydroxy propyl methyl cellulose capsules with identical features, using inactive colorants 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, moisture 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. Health questionnaires (validated participant-reported assessments) were administered at baseline, weekly, and at the end of the study. The questionnaires used in this analysis included the following:

- PROMIS Anxiety 4A questionnaire* [Anxiety is self-reported and not medically diagnosed from this questionnaire]
- PROMIS Depression 4A questionnaire* [Depression is self-reported and not medically diagnosed from this questionnaire]
- Digestion-associated quality of life Questionnaire (DQLQ) [This questionnaire includes both digestive and anxiety questions which are helpful in associating gut with brain]

*The PROMIS Gastrointestinal Anxiety and Depression Scales are validated tools used to assess anxiety and depression in adults. They are part of the Patient-Reported Outcomes Measurement Information System (PROMIS^{TM}), which was developed by the National Institutes of Health (NIH).[14]
**The DQLQ is a reliable and valid questionnaire to assess quality of life in healthy individuals.[15]
The questionnaires are provided in Figures 1–3.

	Never	Rarely	Sometimes	Often	Always
I felt fearful	☐	☐	☐	☐	☐
I found it hard to focus on anything other than my anxiety	☐	☐	☐	☐	☐
My worries overwhelmed me	☐	☐	☐	☐	☐
I felt uneasy	☐	☐	☐	☐	☐

Figure 1 PROMIS Anxiety 4A questionnaire.
Notes: Participants were asked to respond to each question or statement by marking one box per row evaluating occurrence over the past 7 days.

	Never	Rarely	Sometimes	Often	Always
I felt worthless	☐	☐	☐	☐	☐
I felt helpless	☐	☐	☐	☐	☐
I felt depressed	☐	☐	☐	☐	☐
I felt hopeless	☐	☐	☐	☐	☐

Figure 2 PROMIS Depression 4A questionnaire.
Notes: Participants were asked to respond to each question or statement by marking one box per row evaluating occurrence over 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 food and beverages was less enjoyable	☐	☐	☐	☐	☐	☐	☐

Figure 3 DQLQ.
Notes: Participants were asked to respond to each question or statement by marking one box per row evaluating how often these events in your daily life were affected due to digestive events and experiences in the past 7 days.

Outcomes Measurements

The primary outcome measure of the overall study was an assessment of gastrointestinal health, based on the responses to a collective of validated GI questionnaires. This involves different questions, results and analyses, and has been previously reported.[12] Necessary description of the study methodology has been previously reported, in part.[12] Anxiety and depression as measured by the PROMIS questionnaires and the DQLQ were secondary and exploratory outcomes of the study and are reported here.

Statistical Analysis

Statistical analysis was performed on the dataset provided to the authors by Radicle Science. The statistical analyses were conducted using an intent-to-treat (ITT) protocol. An ITT protocol is a method of analyzing clinical trial data in which participants are included in the group they were assigned, regardless of adherence to the intervention, provided they

completed baseline assessments and at least one week of weekly questionnaires. The flow of participants through this process is shown in Figures 4 and 5 for the anxiety and depression questionnaires.

Due to the substantial dropout early in the study, we examined the use of the ITT protocol coupled with the Last Observation Carry Forward (LOCF) and other mixed effect models to impute missing data. Although LOCF is widely used and yielded similar results in preliminary testing, it also effectively doubled the sample size for each questionnaire. We determined that it artificially inflated the dataset and introduced biased estimates without enhancing data integrity or robustness. Therefore, we proceeded with the ITT analysis without carry-forward imputation. Despite the reduced participant count after the first week, the ITT dataset was found to be sufficient to detect meaningful differences and provide useful insights.

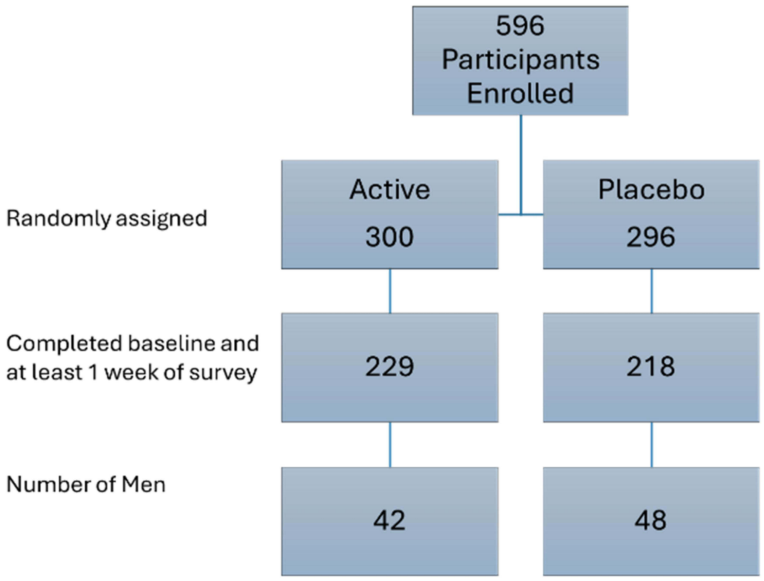

Figure 4 Participant flow through the study and analysis for the PROMIS Anxiety 4A questionnaire.

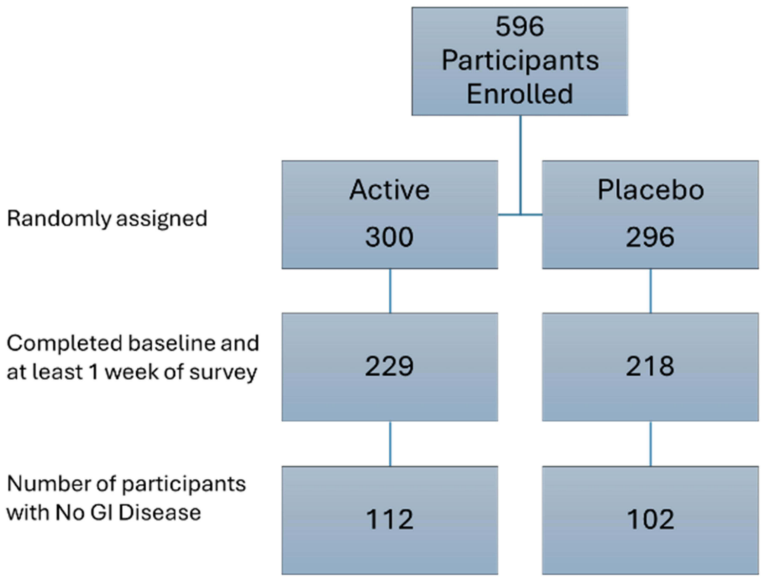

Figure 5 Participant flow through the study and analysis for the PROMIS Depression 4A questionnaire.

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.[16] MCID analysis examines the likelihood of meaningful improvements in participant questionnaires. 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.

The statistical analysis also considered specific demographic subsets of the study participants, allowing for a deeper understanding of how specific population subgroups responded to the intervention.

The core analysis and development for this study were conducted by using Python,[17] leveraging the following libraries:

Statsmodels: Used to fit the Poisson general linear model.[18]
Pandas: Used for the manipulation and preprocessing of raw study data.[19]
Bokeh: Used to create visualizations and charts for the study.[20]

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 cognitive outcomes while accounting for real-world variability and consumer adherence.

A small number of participants reported cannabinoid use. We found that there was no effect of cannabinoid use on the statistical analysis of the results presented here. We allowed for real-world consumption of alcohol and caffeine without bounds.

Results

Study Demographics

A total of 596 participants were enrolled in the study, with 229 completing the active arm and 218 completing the placebo arm. The study population consisted of 32% males and 68% females, with a mean age of 44 years (range: 21–79). Approximately 25% of the participants identified as racial minorities, and 36.4% self-reported having gastrointestinal (GI) disease. Participants were geographically diverse, representing 47 of 50 US states.

Study Compliance

Seventy-five percent of the enrolled participants completed the trial, reflecting moderate adherence in this real-world study format. Weekly survey completion rates ranged from approximately 60% to 90%, with similar rates observed across both arms. The analysis of weekly dropout shows a rapid decline in participation after the first week, followed by a moderate decline over the remainder of the study (Figures 6 and 7). The total number of participants answering each survey weekly is reported in Table 1. Demographic analysis of dropout shows uniform dropout rates between study arms, race, BMI, and gender. Younger participants had an elevated dropout rate compared to older participants.

Anxiety (PROMIS Anxiety 4A)

Statistically significant improvements were observed among male participants in the active arm, as measured by the PROMIS Anxiety 4A questionnaire. Among males, the rate of meaningful improvement was 27.38% higher in the active group compared to placebo, corresponding to a 2.14-fold greater likelihood of improvement ($p = 0.007$, risk ratio = 2.14 ± 0.28). Although the number of male participants was relatively small, the effect was robust and statistically significant (Table 2).

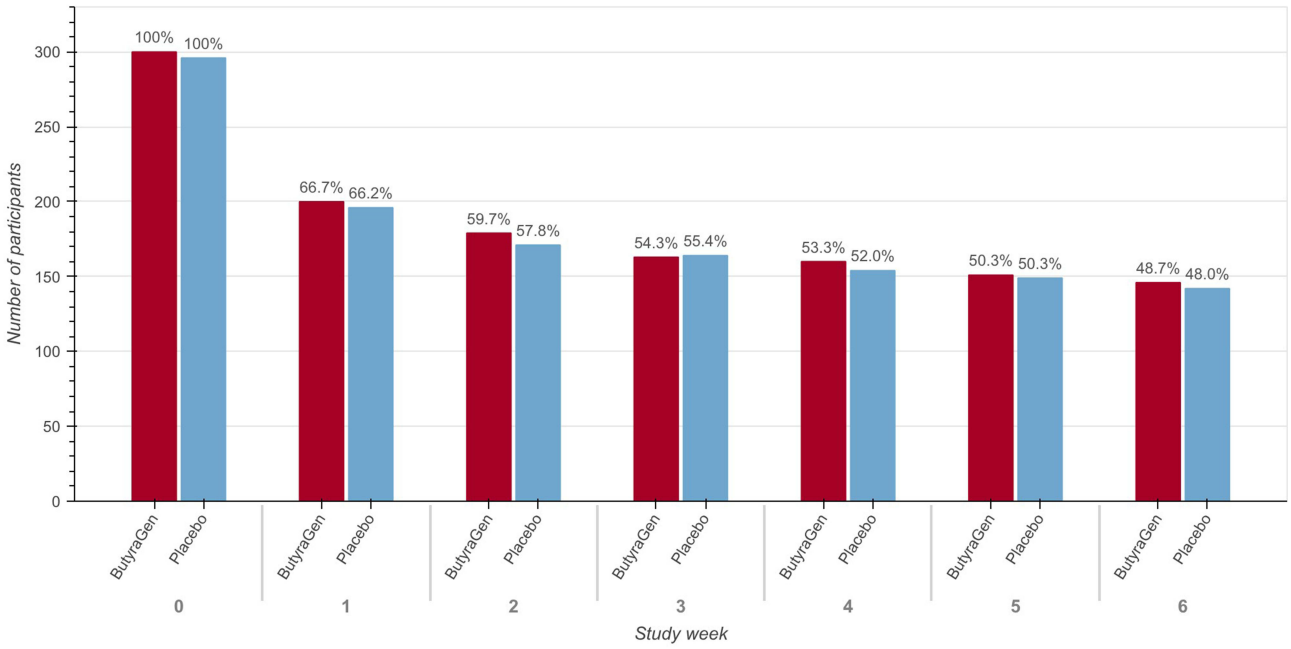

Figure 6 Weekly survey completion rates, by trial arm for participants who completed baseline.

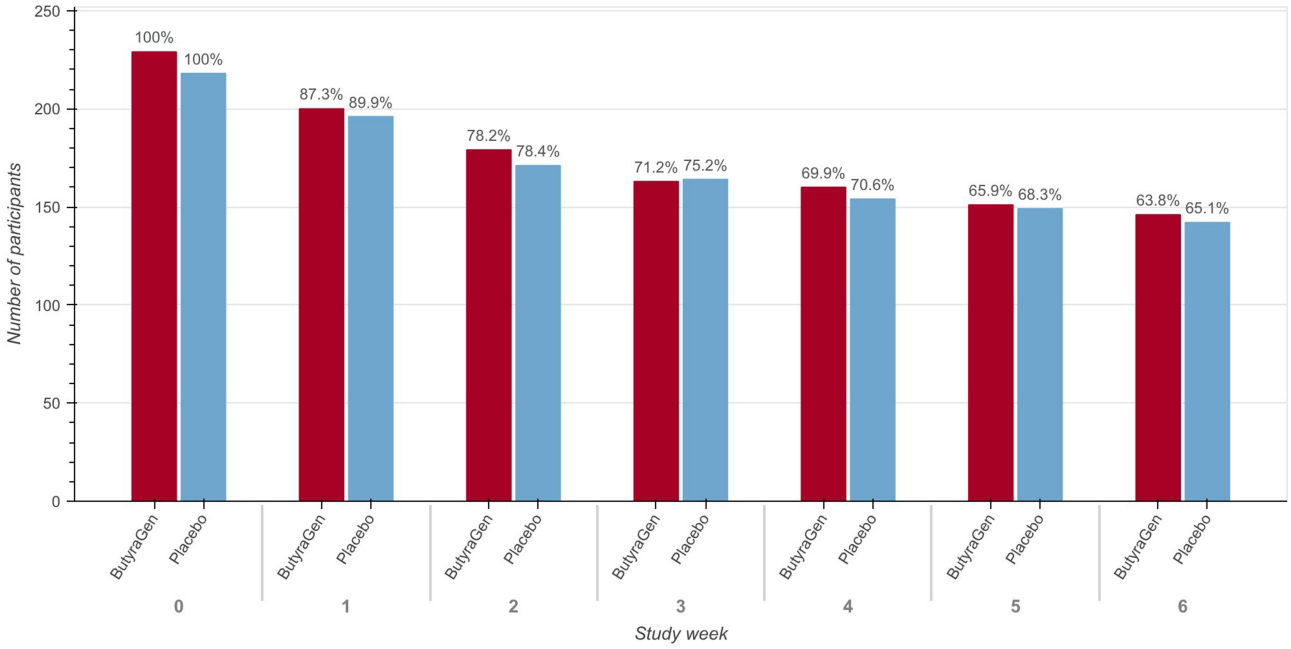

Figure 7 Weekly survey completion rates, by trial arm for participants who completed baseline and at least week 1 questionnaire (ITT).

In the overall population (combined males and females), the rate of meaningful improvement was 9.29% higher in the active group compared to placebo, corresponding to a non-significant 1.24-fold greater likelihood of improvement ($p = 0.140$). No improvement trends were observed among females. A weekly analysis of anxiety improvement, shown in Figure 8, indicates that male participants taking ButyraGen experienced a consistently greater reduction in anxiety compared to placebo throughout the study. Statistically significant differences were observed in week 2 ($p = 0.036$), week 3 ($p = 0.008$), week 4 ($p = 0.006$), and week 6 ($p = 0.007$). Anxiety improved rapidly (by week 2) and continued to improve with smaller p values through the study (except for week 5).

Table 1 Total Number of Participants Who Responded to Survey at Study Timepoints

Questionnaire	Subgroup	Treatment	Week	Participants
PROMIS Depression 4A	No GI disease	ButyraGen®	1	148
			2	134
			3	128
			4	119
			5	115
			6	112
		Placebo	1	141
			2	124
			3	121
			4	111
			5	107
			6	102
PROMIS Anxiety 4A	Male	ButyraGen®	1	57
			2	52
			3	46
			4	44
			5	40
			6	42
		Placebo	1	64
			2	59
			3	55
			4	53
			5	53
			6	48

Table 2 Anxiety Results for Males

Questionnaire	PROMIS Anxiety 4A
Population subset	Males
Total number of participants	90
Active male participants	42
Placebo male participants	48
MCID improvement p value	0.007**

(*Continued*)

https://doi.org/10.2147/NDS.S565532

Table 2 (Continued).

Questionnaire	PROMIS Anxiety 4A
MCID risk ratio (± 95% C.I.)	2.14 (± 0.28)
Difference in overall MCID (active – placebo)	27.38%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: ** $p < 0.01$.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

In order to explore this anxiety relationship further through post-hoc analyses, we looked for statistically significant improvements at the question level. The individual questions have also been validated by NIH as part of the validation of the overall questionnaire. The results are presented in Table 3.

The individual question results for "fearful" (I felt fearful) and "uneasy" (I felt uneasy) showed statistical significance compared to the placebo for the entire population (both males and females). The results for "focus" (I found it hard to focus on anything other than my anxiety) were significant compared to placebo amongst male participants. The "overwhelmed" question was not statistically significant for any meaningful population subset.

Depression (PROMIS Depression 4A)

For depression, no significant improvement was observed in the total population; however, among participants without self-reported GI disease, the active group had a 15.56% higher rate of improvement compared to placebo, corresponding to a significant 2.00-fold greater likelihood of improvement ($p = 0.011$; Table 4).

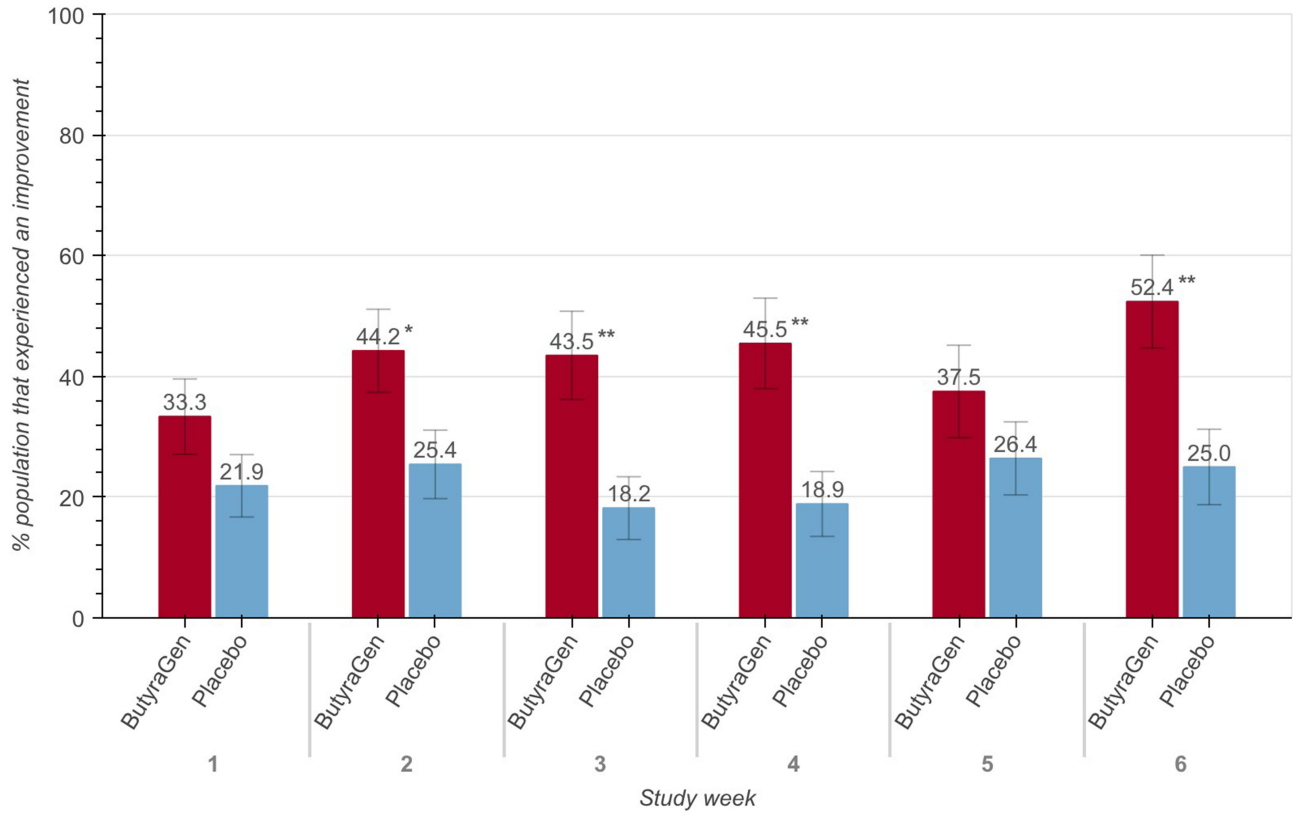

Figure 8 Temporal (week-to-week) changes in Anxiety 4A % male participant improvement over the course of the 6-week study.
Notes: MCID significant statistical differences compared to placebo and reported as mean±SEM: * $p<0.05$, ** $p<0.01$.
Abbreviations: MCID, minimal clinically important difference. SEM, standard error of the mean.

Table 3 Analysis of the Individual Questions in the Anxiety 4A Questionnaire

Questionnaire	**Anxiety 4A**			
Question	**"Fearful"**	**"Focus"**	**"Overwhelmed"**	**"Uneasy"**
Population subset	No Subset	Male	No Subset	No Subset
Total number of participants	288	90	288	288
Active participants	146	42	146	146
Placebo participants	142	48	142	142
MCID improvement p value	0.026*	0.005**	0.389	0.004**
MCID risk ratio (± 95% C.I.)	1.43 (± 0.16)	2.29 (± 0.29)	1.12 (± 0.13)	1.47 (± 0.13)
Difference in overall MCID (active – placebo)	12.90%	29.46%	6.40%	18.16%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: * $p < 0.05$, ** $p < 0.01$.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

Table 4 Depression Results for Those with No GI Disease

Questionnaire	**PROMIS Depression 4A**
Population subset	No GI disease
Total number of participants	214
Active participants	112
Placebo participants	102
MCID improvement p value	0.011*
MCID risk ratio (± 95% C.I.)	2.00 (± 0.27)
Difference in overall MCID (active – placebo)	15.56%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: * $p < 0.05$.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

These results are notable, as "no GI disease" refers to participants who self-reported no GI-related diseases in their baseline survey with no other exclusion criteria applied. With the broader inclusion criteria, the overall population (no subpopulation constraints) showed a non-significant 6.94% higher rate of improvement on the Depression 4A questionnaire in the active group compared to placebo, corresponding to a 1.29-fold greater likelihood of improvement ($p = 0.222$). The analogous temporal results for self-reported depression are shown in Figure 9. In this case the temporal effects were still significant by week 3 and at the end of the study in week 6. However, these depression temporal results were generally weaker in statistical correlation compared to the anxiety results.

In order to explore this mood relationship further, as captured through the PROMIS 4A Depression questionnaire, post-hoc analysis was conducted, to look for statistically significant improvements at the individual question level (also validated by NIH). The results are presented in Tables 5–7.

The post-hoc analysis of individual items in the Depression questionnaire revealed statistically significant effects for certain questions among groups of participants, such as those with no GI disease, younger participants (<40 years), and males. No other significant effects were observed in any subgroups with a total sample size (males and females) greater than 50. For the Depression questionnaire, the finding of statistical significance in participants with no GI disease is noteworthy and is discussed in detail later.

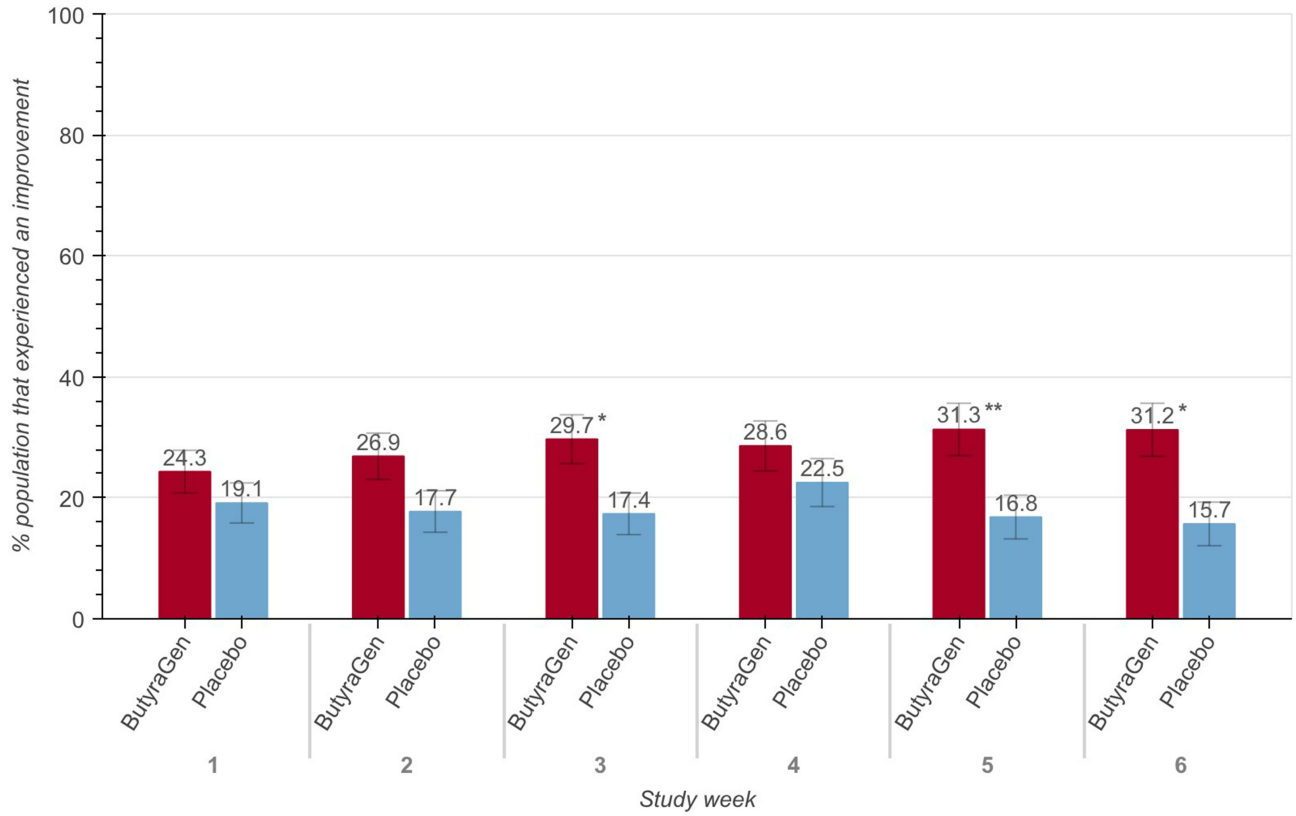

Figure 9 Temporal (week-to-week) changes in Depression 4A % participants with No GI Disorder improvement over the course of the 6-week study.
Notes: MCID significant statistical differences compared to placebo and reported as mean±SEM: * p<0.05, ** p<0.01.
Abbreviations: MCID, minimal clinically important difference. SEM, standard error of the mean.

DQLQ Questions

Two questions in the DQLQ relating to physiological actions "distracted" (I was distracted while doing certain activities), and "social" (social activities-spending time with friends or family, going out to eat etc., were unpleasant or avoided) were the only two questions in the post-hoc analysis of the DQLQ individual questions to show statistically significant improvement with a subset total population >50. The results are listed in Table 8.

Table 5 Analysis of the Individual Questions in the Depression 4A Questionnaire for Those with No GI Disease

Questionnaire	**Depression 4A**			
Question	**"Worthless"**	**"Helpless"**	**"Depressed"**	**"Hopeless"**
Population subset	No GI Disease	No GI Disease	No GI Disease	No GI Disease
Total number of participants	214	214	214	214
Active participants	112	112	112	112
Placebo participants	102	102	102	102
MCID improvement p value	0.059	0.012*	0.006**	0.007**
MCID risk ratio (± 95% C.I.)	1.50 (± 0.21)	1.68 (± 0.21)	1.73 (± 0.20)	1.93 (± 0.25)
Difference in overall MCID (active – placebo)	12.10%	16.56%	18.26%	17.98%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: * p < 0.05, ** p < 0.01.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

Table 6 Analysis of the Individual Questions in the Depression 4A Questionnaire for Those with Age < 40 Yrs

Questionnaire	**Depression 4A**			
Question	**"Worthless"**	**"Helpless"**	**"Depressed"**	**"Hopeless"**
Population subset	Age < 40 yrs	Age < 40 yrs	Age < 40 yrs	Age < 40 yrs
Total number of participants	98	98	98	98
Active participants	53	53	53	53
Placebo participants	45	45	45	45
MCID improvement p value	0.188	0.022*	0.040*	0.031*
MCID risk ratio (± 95% C.I.)	1.42 (± 0.27)	1.97 (± 0.36)	1.77 (± 0.28)	1.92 (± 0.30)
Difference in overall MCID (active – placebo)	14.17%	24.61%	20.50%	22.73%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: * $p < 0.05$.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

Table 7 Analysis of the Individual Questions in the Depression 4A Questionnaire for Males

Questionnaire	**Depression 4A**			
Question	**"Worthless"**	**"Helpless"**	**"Depressed"**	**"Hopeless"**
Population subset	Male	Male	Male	Male
Total number of participants	90	90	90	90
Active participants	42	42	42	42
Placebo participants	48	48	48	48
MCID improvement p value	0.234	0.399	0.115	0.014*
MCID risk ratio (± 95% C.I.)	1.46 (± 0.32)	1.30 (± 0.31)	1.63 (± 0.31)	2.67 (± 0.40)
Difference in overall MCID (active – placebo)	10.71%	8.63%	15.48%	23.51%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: * $p < 0.05$.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

Table 8 Participants Showing Significant Improvement in Their DQLQ Questions

DQLQ Post-hoc Analysis	**"Distracted"**	**"Social"**
Population subset	Baseline (sometimes / occasionally)	All
Total number of participants	109	288
Active participants	53	146
Placebo participants	56	142
MCID improvement p value	0.029*	0.034*
MCID risk ratio (± 95% C.I.)	1.25 (± 0.10)	1.39 (± 0.16)
Difference in overall MCID (active – placebo)	17.15%	12.16%

Notes: MCID used to analyze the results for meaningful clinical differences, and the associated MCID risk ratio (95% C.I.) was reported. Significant statistical differences compared to placebo: * $p < 0.05$.
Abbreviations: MCID, minimal clinically important difference. CI, confidence interval.

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Among participants who reported being distracted sometimes or occasionally at baseline, the active group had a 17.15% lower rate of distraction compared to placebo, corresponding to a significant 1.25-fold greater likelihood of reduced distraction ($p = 0.029$). All active arm participants had a 12.16% lower rate of social activity avoidance compared to placebo, corresponding to a significant 1.39-fold greater likelihood of engaging in social activities ($p = 0.034$) These questions relate to measurable physiological improvements in gut health.

Other Results

Other post-hoc anxiety and depression questionnaire statistical analyses were either not significant, marginally significant or were significant for smaller subset populations and are not considered here.

Discussion

This study provides new insights into the potential benefits of ButyraGen supplementation in helping regulate the gut-brain axis, highlighting its efficacy in specific populations and key physiological and psychological measures. The results demonstrate that ButyraGen, at a practical supplement level dose led to meaningful improvements in self-reported feelings of anxiety and depression, particularly among men, individuals without GI disease, and younger participants (<40 yrs). The results of this study suggest potential targeted applications for supporting brain function, managing psychological stress, and improving overall quality of life through the gut-brain axis. Notably, the response appears to be fast-acting, with statistically significant improvements observed in certain situations as early as 2 weeks, with more pronounced benefits by 6 weeks.

Potential Mechanisms

Before examining the specific results, it is important to consider two complementary pathways through which ButyraGen may influence the gut-brain axis. The first pathway is an indirect route, in which butyrate generation improves gut physiology, such as enhancing barrier integrity, modulating local inflammation, and reducing digestive distress, which in turn supports psychological well-being. This likely involves gut-to-brain communication via the vagus nerve, immune signaling, and hormonal mediators, where a healthier gut environment sends more favorable signals to the brain.[3] The second pathway is a more direct route, in which butyrate generated by ButyraGen influences brain function independently of gut symptom improvement. Butyrate is known to cross the blood-brain barrier and can modulate neuroinflammation, neurotransmitter synthesis, and gene expression via HDAC inhibition.[7] Through these neurochemical actions, butyrate may directly impact mood regulation, cognitive processes, and stress responses. While our study design did not include biochemical assays due to the virtual trial format, participant-reported outcomes allowed us to infer that both pathways seem to have been engaged. The distinctive capacity of ButyraGen to generate butyrate primarily in the small intestine, rather than relying solely on colonic fermentation from dietary fiber, may account for the dual effects observed, offering a unique therapeutic approach to targeting both gut and brain health through the gut-brain axis.

Anxiety

Anxiousness or feelings of anxiety is a common mental health condition linked to chronic stress, disrupted sleep, impaired cognitive function, and reduced executive processing. In this study, self-reported non-clinically diagnosed anxiety significantly improves in male participants following ButyraGen supplementation, with a steady reduction in anxiety scores from week 2 onward and statistically significant improvements at weeks 3, 4, and 6. The rapid onset and continuation of action suggests that ButyraGen may have both acute and sustained effects on psychological well-being. Anxiety in men can be caused by several factors, including work stress, family dynamics, traumatic events, and more. It also can stem from ongoing medical conditions like metabolic dysfunction and heart disease. Hormonal imbalances, particularly low testosterone levels, are also associated with increased anxiety and elevated cortisol levels, a key stress hormone. Interestingly, butyrate may be associated with higher testosterone in men, which could partially explain the observed improvements in anxiety.[21] Additionally, post-hoc analysis of individual items from the Anxiety 4A questionnaire revealed all participants (men and women) showed statistically significant improvements in being fearful and uneasy, while male participants specifically showed statistically significant improvements in focus. This suggests that

ButyraGen may support psychological balance in both males and females (all participants) but its impact on anxiety appears to be more pronounced in men.

Depression

The Depression 4A questionnaire in this study relied on self-reported assessment and was also not a formal clinical diagnosis of depression. The individual factors of depression which showed improvements were feeling fearful, unfocused, and uneasy. Depression score improvement appeared to correlate most strongly with participants who did not have self-reported GI disease, with statistically significant improvements observed in this subgroup. This suggests that, for these participants, the improvements in depression were not solely related to improvements in GI disease. This may be related to the second pathway discussed above – where ButyraGen generates butyrate independent of GI disease and impacts psychological and brain health.

In the post-hoc analysis, the "no GI disease" subgroup again showed improvements in depression related symptoms. Additionally, younger participants (<40 years) were found to have statistically significant improvements in feelings of helplessness, hopelessness, and depression. This could be attributed to better metabolic health in younger individuals, allowing butyrate to be more effectively utilized for neurological and psychological benefits rather than digestive health. Due to the limited sample size, exacerbated by the increased dropout rate of younger adults, we did not analyze multi-factorial subgroups, as the reduced participant count impacted statistical robustness.

DQLQ

As noted in the results section, the two significant questions from the DQLQ ("distracted" and "social") both relate to the physiological impact of the gut-brain axis. Each involves tangible, activity-based outcomes, such as difficulty concentrating or avoiding social activities, that can arise from underlying gut health challenges. Improvements in these measures suggest that direct butyrate generation led to physiological changes in the gut that, in turn, alleviated physical barriers to daily functioning. This perspective underscores the functional relevance of gut-brain axis health, moving beyond subjective feelings to show how gut improvements can restore the ability to engage in everyday physical and social activities. To our knowledge, this type of functional, real-world outcome has been underrepresented in prior gut-brain axis studies, which have largely focused on microbiota-driven butyrate production or direct supplementation, rather than on activity-level improvements linked to butyrate generation.

Insights and Opportunities

This study was conducted entirely virtually with no in-clinic visits, representing real-world conditions and a diverse US 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 helping to modulate the gut-brain axis and cognitive effects over the 6-week course for the 200 mg ButyraGen dose regimen. This study also 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 biomarkers. Additionally, ensuring participant compliance for remotely completing questionnaires is more challenging compared with an in-clinic setting, where investigators can provide direct oversight. Other factors not controlled for in the virtual nature of this study are diet and exercise. The 6-week study length is appropriate for capturing acute mood alterations. A longer-term study could investigate sustained cognitive effects. These limitations present opportunities for future research in order to provide definitive powered evidence of these outcomes.

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. Structural differences in brain regions have been observed between males and females, potentially influencing the way the brain responds

to various factors, including those related to the gut microbiome and its metabolites,[22] which may be relevant to understanding why we saw significant effects in men and not in women. In men, butyrate has also been associated in preclinical models with supporting healthy testosterone levels, possibly through anti-inflammatory effects and modulation of gut-derived signaling pathways that interact with the hypothalamic–pituitary–gonadal axis.[23] This could further explain the more pronounced metabolic benefits seen in male participants. Additionally, factors such as hormonal profiles, dietary patterns, age, and physical activity all modulate the gut–brain–hormone axis and butyrate's downstream effects. Collectively, these insights underscore the complexity of butyrate's systemic impact and the importance of designing targeted strategies that account for demographic and lifestyle differences to maximize benefits.

Conclusion

This study demonstrates the ability of ButyraGen, a direct butyrate generating supplement, to help modulate the gut-brain axis, which is hypothesized to occur through two interconnected pathways. First, direct butyrate generation led to measurable physiological improvements in gut health, which in turn supported meaningful improvements in psychological measures. Second, direct butyrate generation in the gut was associated with significant and fast-acting improvements in psychological markers, including non-clinical depression and anxiety. These improvements were particularly notable in men, as well as in both men and women without self-reported GI disease or under 40 years of age. By addressing both gut and brain health through these exploratory analyses, ButyraGen emerges as a promising intervention for helping modulate gut-brain axis health and cognitive health and also enhancing overall well-being across diverse populations.

Data Sharing Statement

The data obtained during this study contains personal information and will not be shared openly.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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