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New advances in dietary fibers and their role in metabolic, digestive, and immune health

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New advances in dietary fibers and their role in metabolic, digestive, and immune health

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Editorial: New advances in dietary fibers and their role in metabolic, digestive, and immune health

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Editorial on the Research Topic
New advances in dietary fibers and their role in metabolic, digestive, and immune health

Global health authorities including FDA, EFSA, Health Canada, etc. have defined dietary fiber as non-digestible carbohydrates that either naturally occur in foods of plant origin, or isolated or synthetic carbohydrates that have demonstrated physiological effects that are beneficial to human health (1). Differing by solubility, fermentability, and their structure, dietary fibers function through diverse mechanisms and pathways, and benefit human health both directly and/or indirectly. The gut microbiome plays a critical role in modulating the metabolic, digestive, and immune health of the host and, when disturbed, may lead to the development of diseases (2, 3). Fermentable dietary fibers provide the main source of energy for the colonocytes and gut microbes (2–4). Microbial fermentation of dietary fibers generates metabolites such as short-chain fatty acids (SCFAs), branched-chain amino acids, and neuro-active chemical substances, which act as paracrine or endocrine signaling molecules in initiating physiological responses (4). Despite a considerable number of studies on dietary fibers and their role in human health, the individual variation on the gut microbiome has introduced complexity when drawing accurate conclusions.

Therefore, new interventional studies or mechanistic investigations on dietary fibers and metabolic, digestive, and immune health will advance our knowledge of the function of dietary fibers in these areas. An augmenting pool of research will also enable health professionals to provide dietary recommendations based on health needs. With this, the aim of this Research Topic is to collect papers suitable to improve our knowledge and understanding on dietary fibers and their role in impacting metabolic, digestive, and immune health.

The diverse landscape of research on dietary fibers has seen remarkable advancements, as evidenced by the compelling array of publications in this Research Topic. In this Research Topic there are ten papers covering the above-mentioned aspects. From elucidating the impacts of low carbohydrate diets to exploring the immunomodulatory effects of specific dietary fibers, each study contributes to our understanding of the intricate interplay between nutrition and gastrointestinal wellbeing.

One noteworthy trend highlighted in these publications is the growing recognition of the pivotal role played by dietary fibers and microbial fermentation products, such as short-chain fatty acids (SCFAs), in maintaining gut homeostasis and fostering overall health (Ashique et al., Cheng and Zhou, Bacha et al., Li et al., Sheng et al., Singh and Bhardwaj, Qi et al.). In addition, there is a growing emphasis on understanding the intricate crosstalk between the gut and other organs (Cheng and Zhou, Li et al., Sheng et al., Singh and Bhardwaj, Qi et al.). This emerging focus underscores the profound impact of gut metabolites on systemic physiology and disease pathogenesis. Studies investigating the immunomodulatory effects of inulin and its intestinal metabolites, for instance, shed light on the intricate signaling pathways through which gut-derived compounds exert far-reaching effects on immune function and inflammatory processes beyond the confines of the gastrointestinal tract (Sheng et al.). In addition to inulin, studies examining the effects of other prebiotics including β-glucans, High-Amylose Maize Starch Butyrate (HAMSB), psyllium husk fiber, and a variety of viscous soluble dietary fibers, as well as synbiotics underscore their potential as valuable tools for modulating gut microbiota composition and bolstering immune function (Cheng and Zhou, Bacha et al., Lu K. et al., Singh and Bhardwaj). Furthermore, investigations into the mechanisms of butyrate, particularly its therapeutic potential in addressing colorectal disturbances, offer promising avenues for clinical intervention (Cheng and Zhou, Bacha et al., Sheng et al., Singh and Bhardwaj).

However, amidst these strides, certain studies also shed light on areas warranting further exploration and clarification. For instance, the findings regarding pea hull fiber supplementation in individuals undergoing hemodialysis emphasize the need for nuanced approaches tailored to specific populations (Fatani et al.), highlighting the complexity inherent in assessing dietary interventions across diverse health conditions. Furthermore, as highlighted by the investigation into the effect of viscous soluble dietary fiber on glucose and lipid metabolism in patients with type 2 diabetes mellitus, personalized nutrition approaches can offer tailored solutions to address specific metabolic imbalances and optimize health outcomes for individuals with distinct physiological profiles (Lu K. et al.). Another evidence-based study emphasized the role of synbiotics in supporting the management of ulcerative colitis (Ashique et al.). By integrating advanced omics technologies with comprehensive lifestyle assessments, personalized nutrition strategies can provide nuanced insights into the complex interactions between diet, genetics, and microbiome composition, paving the way for precision health interventions tailored to individual needs. Meanwhile, the study using NHANES data highlighted a benefit of consuming more fibers in combating inflammation and improving immune health (Qi et al.), indicating that the holistic impact of dietary choices on overall wellbeing cannot be overstated.

As we navigate this complex terrain, it is imperative to adopt an integrative approach that encompasses not only the direct effects of dietary components on gut health but also their broader implications for immune and metabolic health (Lu G. et al.). Furthermore, ongoing efforts to elucidate the underlying mechanisms driving these interactions will be essential for informing targeted dietary interventions and optimizing health outcomes across diverse populations.

In conclusion, the breadth and depth of research showcased in this Research Topic underscore the dynamic nature of the field of dietary fiber research. By synthesizing insights from these diverse studies, we move closer toward unraveling the intricacies of the gut microbiome and harnessing its therapeutic potential to promote optimal health and wellbeing.

Author contributions

JC: Writing – original draft. AS: Writing – review & editing. CH: Writing – review & editing. RK: Writing – review & editing. JZ: Writing – review & editing.

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JC and JZ are employed by Ingredion Incorporated.

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A bibliometric and visual analysis of low carbohydrate diet

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Introduction: Numerous studies have confirmed the effects of low carbohydrate diet (LChD) on metabolism and chronic diseases. However, there were no bibliometric studies on LChD. This study was conducted through a bibliometric analysis to investigate the current status, hotspots and frontiers trends.

Methods: We searched all research publications related to LChD from 2002 to 2021 on the Web of Scientific Core Collection (WoSCC). CiteSpace and VOSviewer software was used to analyze countries/regions, institutions, journals, authors, references, and keywords.

Results: A total of 6938 papers were included, with an increasing trend of annual publication. LChD categories mainly included nutrition, endocrinology, and neurosciences which reflected the interdisciplinary characteristics. USA was with the largest number and the world science center in LChD field. Universities were main research institutions and five of the top 10 institutions were from USA. Eric Heath Kossoff had 101 publications and ranked first. Nutrients was the leading journal. "A randomized trial of a low-carbohydrate diet for obesity" and "Obesity" were considered to be the most co-cited and cited reference respectively. The hotspots of LChD are four aspects, "ketogenic diet", "metabolism disease", "cardiovascular disease" and "cancer". We summarized that "oxidative stress", "gut microbiota", and "inflammation factors" are becoming frontiers trends of LChD research in the future and deserve further study.

Discussion: Over the past 20 years research on LChD has gained great attention. To better explore LChD field, multilevel mechanism studies will be required in the future.

KEYWORDS

bibliometric analysis, low carbohydrate diet, CiteSpace, VOSviewer, hotspots, frontiers trends

Introduction

Obesity-related complications affect almost all body systems and are significant risk factors for coronary heart disease, type 2 diabetes, cancers such as endometrial, breast, prostate, and skin cancers, as well as several other chronic non-communicable diseases. Diet therapy methods, theories, and applications are constantly updated as a result of ongoing research on the metabolism of the organism in normal and disease states (1). It has been demonstrated that consuming a diet high in carbohydrate increases the risk of developing metabolic and chronic diseases, and that lowering carbohydrate intake decreases the incidence of morbidity (2). The effects of LChD on health have garnered a lot of attention recently. There are many types of low carbon diet prescriptions according on the carbohydrate intake ratio. The American Diabetes Association recommended a conventional 2,000 calorie daily diet with <130 g of carbohydrates (3). The other study suggested consuming <40% of one's daily calories from carbohydrates (4). Anyway, the two prescriptions LChD above are powered by glucose first and then switch to ketone

bodies after fasting. Moreover, the ketogenic diet (KD) is another LChD that calls for a very low carbohydrate intake (<10%). KD, a sort of LChD, was initially used to cure epilepsy (5). Atkins, an American, wrote about an LChD in his 1972 book "*Dr. Atkins' New Diet Revolution*," in which the intake of carbohydrate was rigorously limited while the intake of protein and fat is raised (6). Currently modified Atkins diet, a easier KD, has showed very similar effects with KD (7). A LChD can lower excess body weight (8, 9), as well as the risk of diabetes, cancer, cardiovascular disease, and internal inflammatory responses brought on by obesity (10, 11). Of fact, some research has indicated that LChD can also produce negative health effects, such as gastric dysfunction (12), atherosclerosis (13), physical fatigue (14), etc.

Studies on LChD are becoming increasingly popular in recent years as a response of the academic community's intense interest in the disease's favorable health effects (15–17). Most of studies, nevertheless, have concentrated on how LChD affects certain disease locations. We require a thorough understanding of the development process and research trends in this subject given the rapid proliferation of research on LChD. However, there are no bibliometric and visual analysis article on LChD.

Bibliometrics, a mathematical and statistical tool for quantitatively analyzing all knowledge (18), has been used to assess distributions, collaboration, citation, keywords, hotspots, and frontiers trends (19). CiteSpace and VOSviewer are software for visualization for bibliometrics analysis (20, 21). These two software generate network maps that allow researchers to intuitively analyze the current status within the field, and determine the research hotspots and frontiers trends (22). Therefore, this study employs CiteSpace and VOSviewer software to analyze the publications on LChD from 2002 to 2021, to evaluate and analysis the research hotspots and frontiers trends. This has been the first study to use bibliometric strategies in the field of LChD. The study is expected to help researchers extract potential information for further research in the field of LChD research and offer them helpful advice in choosing ground-breaking subject matter by answering the following questions:

(i) Which countries, institutions, journals, authors, and references are the current status of research in the field of LChD ?
(ii) What are the current hotspots and major categories of LChD?
(iii) Where are in the future frontiers trends of LChD ?

Materials and methods

Data acquisition and search strategy

In this study, WoSCC was selected as the data source. As a high-quality digital literature resource database, WoSCC has been accepted by many researchers, and considered as the most suitable database for literature analysis (23). All publications were retrieved from the Science Citation Index Expanded (SCI-E) of the WoSCC database on November 12, 2022. We completed the search within the same day to avoid any bias caused by database updates. The following methods were conducted for search publications: topic words = ("low carbohydrate" OR "low-carbohydrate" OR "low carb" OR "low-carb" OR "ketogenic" OR "carbohydrate-restricted" OR "carbohydrate restricted" OR "restricted carbohydrate" OR "restricting carbohydrate" OR "carbohydrate restriction" OR "South Beach diet" OR "Atkins diet"). In order to more accurately analyze the current status, hotspots and frontiers trends of LChD, the publications from 2002 to 2021 were selected. Time span = January 1, 2002–December 31, 2021. To ensure the representativeness of the included studies, the types of publications were limited to "articles" and "reviews" (24). No languages limitation to avoid bias in the geographical distribution of publications. The content of literature records were "full records and cited references," downloaded and saved in plain text document format.

Statistical analysis

We used the CiteSpace (6.1.R3) and VOSviewer (1.6.18) for a bibliometric analysis of 6,938 publications on LChD from 2002 to 2021. The java-based program CiteSpace does bibliometric analysis of publications using distribution network maps, co-citation network maps, dual maps of journal overlay, and keyword burst citation maps (25). Nodes and links are included in the visual network diagram produced by CiteSpace. Every node is a factor, such as an author, an institution, or a country (26). Links between different nodes show a network of relationships involving co-operation, co-citation, or co-occurrence (27) A wider line indicates a more effective collaboration. The higher the centrality, the larger the circle is in terms of centrality. When a node has a purple circle around it, it has a high centrality score and is therefore an important node in the field (22). VOSviewer was used to form keyword co-occurrence of overlay visualization. The colors represent the years (28). The size of the node is proportional to the frequency of keyword occurrences (29). Data was managed, charts were made, and all data tables were created using Microsoft Excel 2021 software.

Results

Annual output and categories

A total of 6,938 publications including 5,350 articles and 1,588 reviews, related to LChD from 2002 to 2021 were retrieved by searching the WoSCC database. The flowchart was shown in Figure 1. The annual publications reflected the activities in the field and the attention given to certain areas of research (30). As seen in Figure 2, the number of annual publications on LChD showed an overall upward trend in spite of fluctuation slightly in some years over the past 20 years. It indicates that LChD research is becoming a research of great interest to scholars and has attracted great interest from scholars in recent years.

LChD publications in the past 20 years can be divided into 2 stages. The initial stage (2002–2010) was a steady growth period. The average number of publications was 188 publications every year, with the lowest number of publications being 72 publications

in 2002 and the highest number being 273 publications in 2009. In 1927, a low carbohydrate ketogenic diet had been reported for epilepsy (31). As an early study in 1948, LChD was used to control of dental caries (32). Since 2002, LChD was contributed to a variety of areas, including obesity (33), diabetes (34), and cardiovascular disease (35). Although the number of papers varied at this stage, the overall trend was one of consistent growth. The second stage (2011–2021) was a sustained growth period. The average number of publications annually was 476 publications. The number of publications reached 872 in 2021. Nutrition has a significant role in daily life, and it is crucial for the advancement of social development to support research on diet and health. LChD research has gained popularity as a nutritional approach and is rapidly developing into a research hotspot.

The categories refer to the disciplines covered by the dissertation research. At top 10 categories (Table 1), Nutrition Dietetics had 1,621 publications and ranked first, followed by Clinical Neurology (1,269 publications), Endocrinology Metabolism (960 publications), Neurosciences (734 publications) and Pediatrics (472 publications). LChD research mainly covered the fields of nutrition, endocrinology, and neurosciences, reflecting the multidisciplinary nature and comprehensive knowledge.

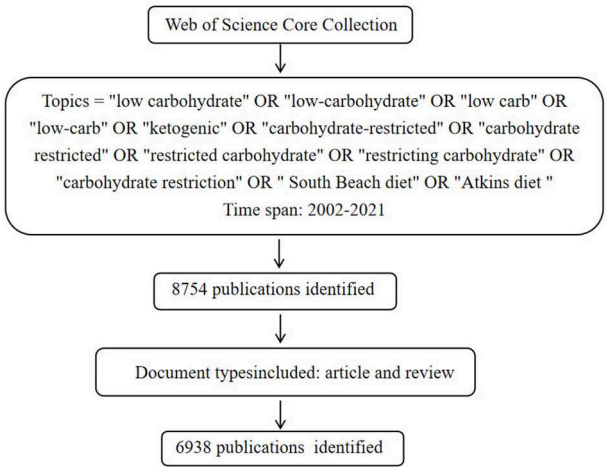

FIGURE 1
The flowchart searching papers in databases.

Analysis of countries/regions

In total, 112 countries/regions participated in 6,938 publications on LChD from 2002 to 2021. CiteSpace generated the countries/regions distribution map, and 112 nodes and 880 links were shown in the map (Figure 3). Table 2 presented the top 10 countries/regions published in LChD research field. USA had the highest number of publications, 2,862 papers, accounting for 41.25%. The Yuasa phenomenon states that the nation whose research output accounts for more than 25% of all scientific output at any given moment can be referred to as the world center of

TABLE 1 The top 10 categories on LChD from 2002 to 2021.

Rank	Category	Publications
1	Nutrition dietetics	1,621
2	Clinical neurology	1,269
3	Endocrinology metabolism	960
4	Neurosciences	734
5	Pediatrics	472
6	Biochemistry molecular biology	417
7	Medicine general internal	314
8	Medicine research experimental	282
9	Pharmacology pharmacy	269
10	Multidisciplinary sciences	227

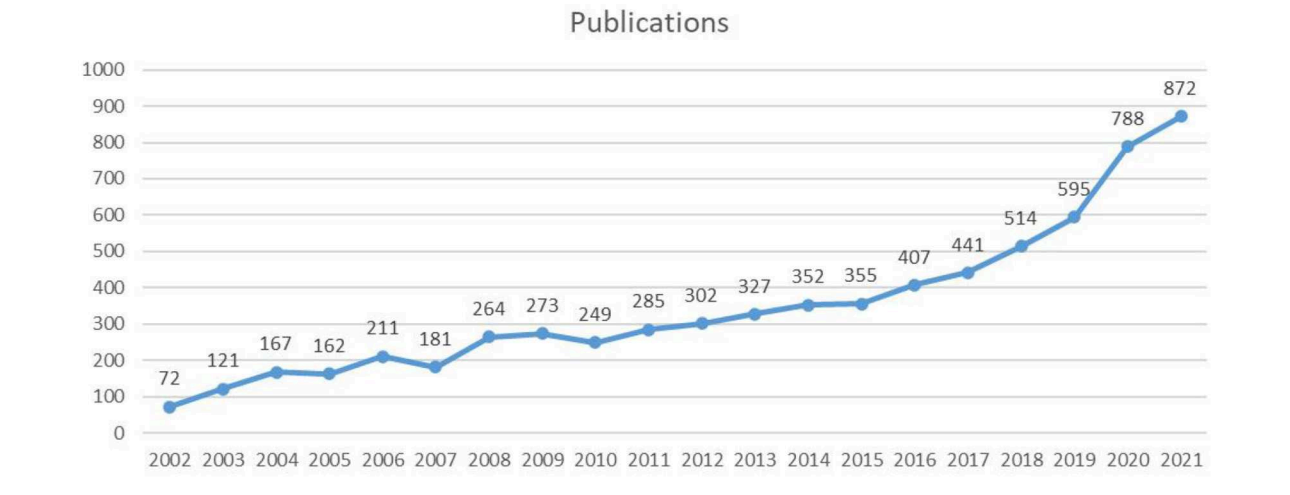

FIGURE 2
The annual number of publications on LChD from 2002 to 2021.

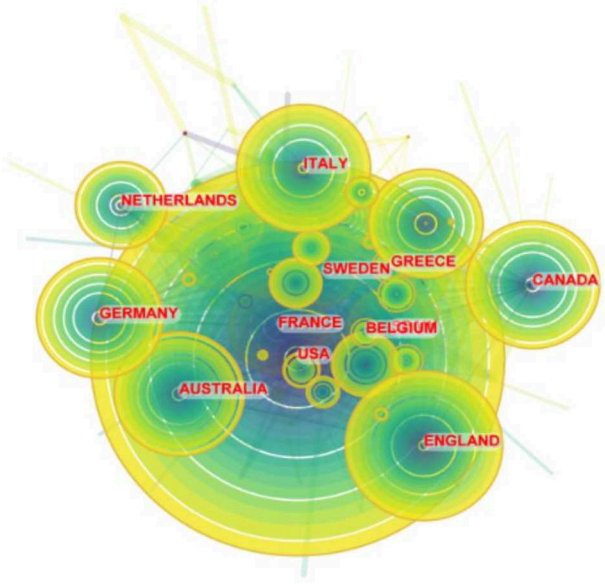

FIGURE 3
Map of countries/regions on LChD from 2002 to 2021.

TABLE 2 The top 10 countries/regions on LChD from 2002 to 2021.

Rank	Country/region	Publications	Centrality
1	USA	2,862	0.08
2	England	543	0.13
3	Italy	472	0.08
4	Germany	449	0.08
5	China	441	0.01
6	Canada	434	0.16
7	Australia	422	0.13
8	Japan	339	0.02
9	Spain	301	0.11
10	France	299	0.14

science during that time (36). As the leader in LChD research, USA published far more than a quarter of the total publications and was the world science center in the field of LChD. England (543 publications), Italy (472 publications), China (449 publications), and Germany (441 publications) followed closely behind. In terms of centrality, Canada (0.16) ranked first, followed by, Spain (0.14), Australia (0.13), England (0.13), France (0.11) and, which maintain close cooperation relationships. Countries/regions with centrality played an important role in LChD research. Germany, Canada, Australia and France each had <450 publications, but their research roles were important. In terms of publications, China had 449 papers, but the centrality was only 0.01. It demonstrated that despite having a high publications number, China had few connections and little influence over the network map. The level of LChD research in China therefore was raised effectively by deepening the field's research, advancing cross-disciplinary and

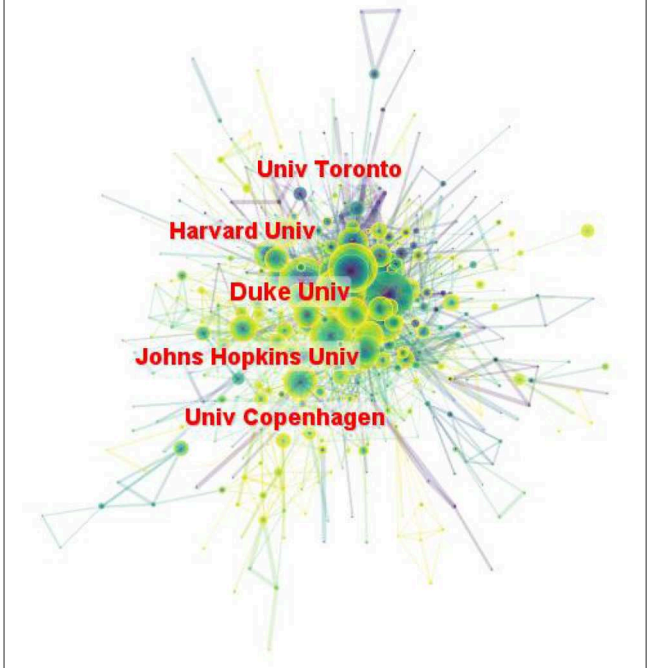

FIGURE 4
Map of institutions on LChD from 2002 to 2021.

TABLE 3 The top 10 institutions on LChD from 2002 to 2021.

Rank	Institution	Country	Publications
1	Harvard University	USA	451
2	University of California System	USA	258
3	Johns Hopkins University	USA	216
4	Udice, French research universities	France	191
5	University of London	England	174
6	Johns Hopkins Medicine	USA	146
7	Institut National de la Santé et de la Recherche Médicale	France	131
8	University of Toronto	Cananda	127
9	Assistance Publique Hopitaux Paris Aphp	France	125
10	University of Connecticut	USA	124

cross-field collaboration, and enhancing researchers' capacity for creative thinking and global communication.

Analysis of institutions

A total of 604 institutions provided research in the field of LChD. CiteSpace generated the institutions distribution map with 604 nodes and 2,103 links (Figure 4). The institutions

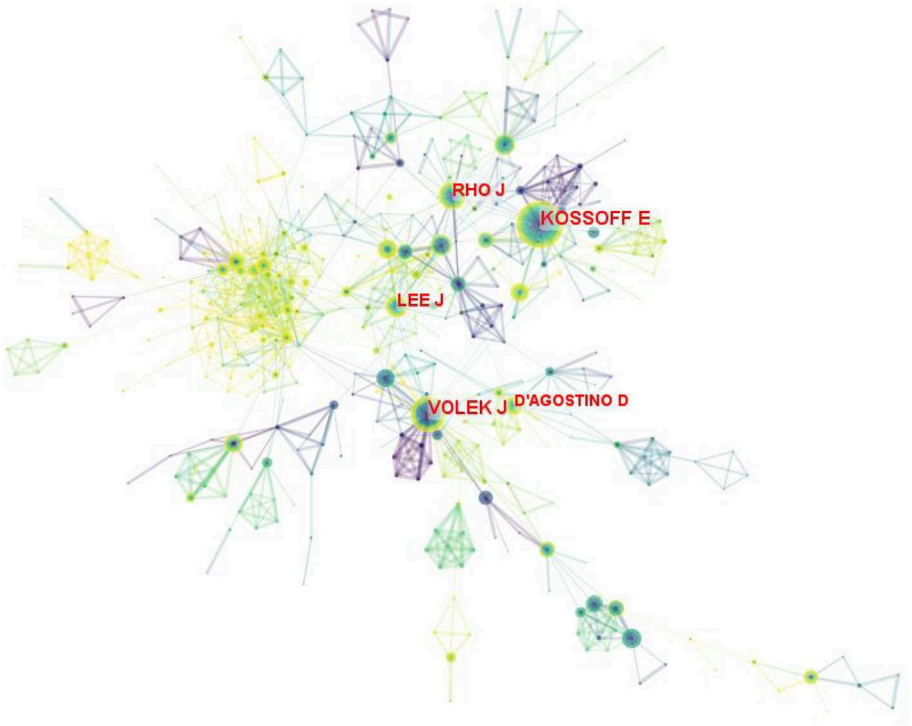

FIGURE 5
Map of authors on LChD from 2002 to 2021.

with large numbers of publications have been identified as influential institutions (37). Table 3 listed the top 10 institutions in publications, and they were the most influential institutions in LChD research. Universities were major institutions for LChD research. Harvard University ranking first, had 451 papers, followed by University of California System (258 publications), Johns Hopkins University (216 publications), Udice French research universities (191 publications), and University of London (174 publications). Five of the top 10 institutions were from USA, which further confirmed US predominance in the field of LChD research. Duke University, Harvard University, Johns Hopkins University and University of Toronto had close collaboration relationships.

Analysis of authors

In total of 890 authors participated in 6,938 publications on LChD from 2002 to 2021. CiteSpace generated the institutions distribution map with 890 nodes and 1965 links (Figure 5). The top 10 authors participating in the LChD research are shown in Table 4. The most productive authors were Eric Heath Kossoff (101 publications), Jeff Scott Volek (69 publications), Jong M. Rho (62 publications), William S. Yancy (45 publications), and Maria Luz Fernandez (43 publications). Eric Heath Kossoff ranked first in the number of publications devoted to the study of the effects of a high-fat, low-carb ketogenic diet on neurological disorders. He demonstrated that a high-fat, low-carb ketogenic diet reduced the number of seizures in refractory epilepsy and reported no cardiovascular or cerebrovascular events (38, 39).

TABLE 4 The top 10 authors on LChD from 2002 to 2021.

Rank	Author	Affiliations	Publications
1	Kossoff Eric H.	Johns Hopkins University	101
2	Volek Jeff	University System of Ohio	69
3	Rho Jong M.	University of Calgary	62
4	Yancy William S.	Duke University	45
5	Rodriguez Fernandez Maria Luz	University of Connecticut	43
6	Cross J. Helen	UCL Great Ormond St Inst Child and Lealth	43
7	Kim Heung Dong	Yonsei University Health System	40
8	Westman Eric	Lund University	39
9	Auvin Stéphane	University of California System	37
10	Clifton Peter Marshall	University of South Australia	37

In addition ketogenic diets are being applied to a range of neurological disorders from autism to Alzheimer's disease (40). Jeff Scott Volek was the second position of papers. He reported that in individuals with atherosclerotic dyslipidemia, a 12-week carbohydrate restriction diet improved postprandial vascular function more than a low-fat diet (41). An study revealed that

LChD (10%) not only decreased lipid deposition but avoided the buildup of plasma and aortic oxidation, decreased inflammatory cytokines within the artery wall, and prevented atherosclerosis (42). Jong M. Rho was in the third place in terms of number of publications. In addition to a high-fat, low-carbon-water ketogenic diet that improves epilepsy (43), he emphasized that a ketogenic diet enhances mitochondrial function and reduces autistic behavior in humans and rodent models of autism spectrum disorder (44, 45). The authors' collaboration displayed a geographical concentration and general decentralization.

Analysis of journals

Researchers can accurately understand the core journals in a topic by analyzing its source journals, which also serves as a reliable resource for further field research (46). A total of 1,545 academic journals published 6,938 publications in the field of research on LChD from 2002 to 2021. As shown in Table 5, the top 10 journals accounted for 17.93% of the total publications. The most productive journals were Nutrients (292 publications), Epilepsia (203 publications), Epilepsy Research (134 publications), PLoS One (116 publications), and American Journal of Clinical Nutrition (105 publications). Of the top 10 journals, eight journals' IF more than 3.0. With a maximum of 8.472, the top 2 journals had an IF >6.0. This shows that high IF journals are open to publishing LChD research.

TABLE 5 The top 10 journals on LChD from 2002 to 2021.

Rank	Journal	Publications	IF (2021)
1	Nutrients	292	6.706
2	Epilepsia	203	6.740
3	Epilepsy Research	134	2.991
4	PLoS One	116	3.752
5	American Journal of Clinical Nutrition	105	8.472
6	Epilepsy and Behavior	96	3.337
7	Journal of Child Neurology	78	2.363
8	British Journal of Nutrition	76	4.125
9	Seizure European Journal of Epilepsy	74	3.414
10	Nutrition	73	4.893

Figure 6 illustrated the dual- map overlay of journals that produced literature linked to the topic of LChD. On the map, the right labels represented the disciplines of the journals that published the cited papers, while the left labels represented the fields of the citing journals. Citation links can show the in and out of the citation dataset. Figure 6 showed 5 reference pathways. Three yellow pathways indicate articles published in molecular/biological/immunology journals mainly citing journals in the molecular/biological/genetics field. Two green pathways suggest that articles published in medicine/clinical journals mainly cite journals in the molecular/biology/genetics/health/nursing/medicine fields. One red pathway shows that the publications from neurology/sports/ophthalmology mainly cite journals in the in molecular/biology/genetics field.

Analysis of co-cited references

The co-cited reference analysis is one of the important indicators in bibliometric research and is usually used to explore research priorities in specific academic fields (47). CiteSpace generated the co-cited reference map, and 1,693 nodes and

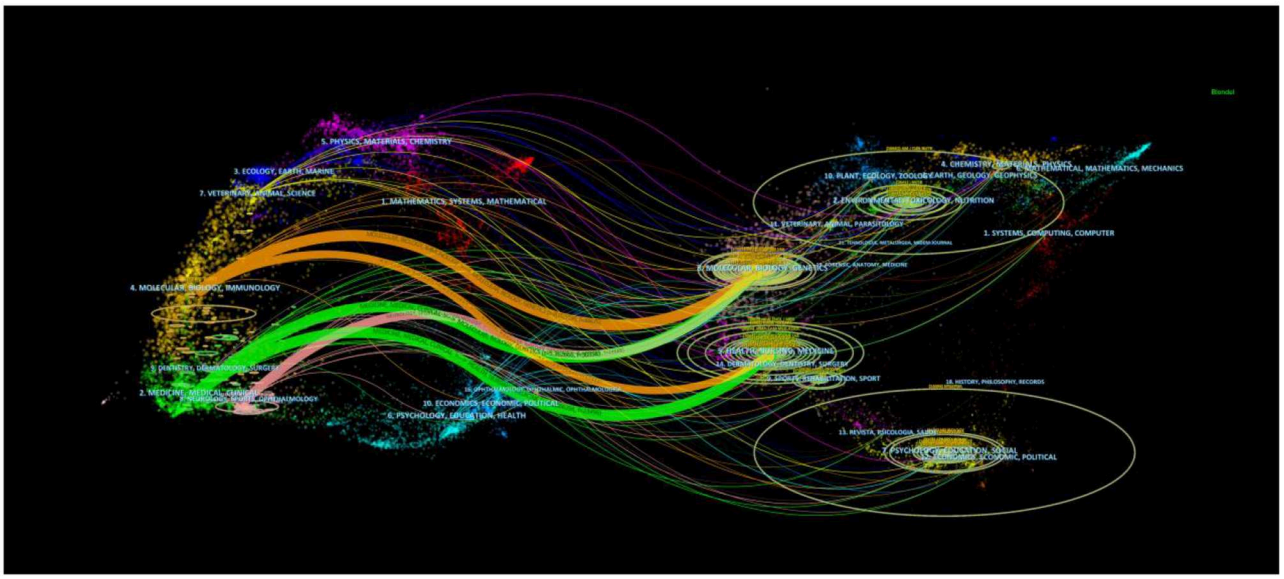

FIGURE 6
Dual-map overlay of academic journals from 2002 to 2021.

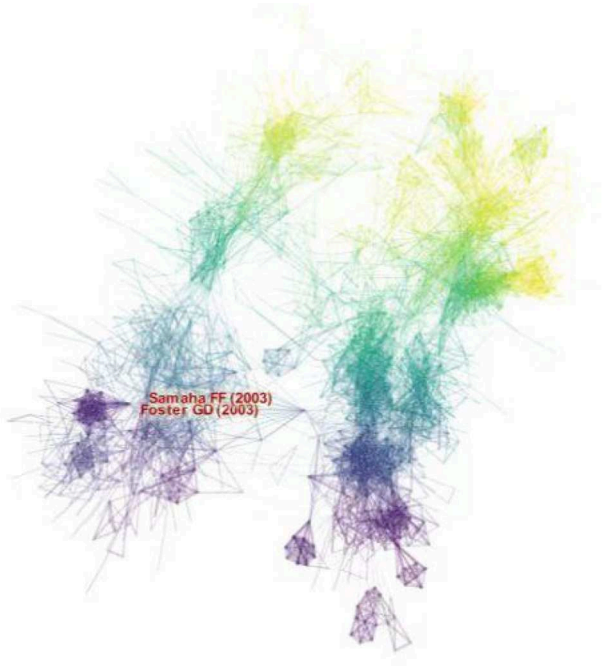

FIGURE 7
The map of co-cited reference on LChD from 2002 to 2021.

9,455 links were shown in the map (Figure 7). The top 5 co-cited references in terms of frequency were in Table 6. Analysis of co-cited references provided basic data for LChD research. Noteworthy were three publications from the New England Journal of Medicine and two from the Annals of Internal Medicine, both of which have significant academic influence. The five references were all clinical trials. In most co-cited reference, obese people were given Atkins diet, and lost more weight in the first 6 months (48). Additionally, high density lipoprotein cholesterol levels increased and triglyceride levels decreased more in Atkins diet participants than in control group, indicating that Atkins diet had a higher impact on the risk factors for coronary heart disease. The second-most co-cited reference reported that patients who received a carbohydrate-restricted diet with 30 g per day or less, lost more weight than control group did and had relative improvements in their insulin sensitivity and triglyceride levels (49). The third most co-cited reference of 132 obese people on who were restricted carbohydrate intake to <30 g per day showed more beneficial effects than those on conventional diets at 1 year; the effects of restricted carbohydrate on atherogenic dyslipidemia and glycemic control remained more favorable (34). Diet therapies were given to moderately obese subjects, and a low-carbohydrate diet and a Mediterranean diet were found to have beneficial effects on lipids and blood glucose, respectively (50). Individualized dietary regimens tailored to individual preferences and metabolism are recommended. A low-carbohydrate, ketogenic diet exhibited higher participant retention and more weight loss compared to low-fat diets in the literature with the sixth greatest co-citation frequency (51).

References analysis

High cited references lay the foundation and accelerate the development of research in the field (23). The top 10 cited references were listed in Table 7. Of the top 10 references, 7 references were articles and 3 were reviews. Three references were published in the New England Journal of Medicine and two were published in Lancet. "Obesity" published by Haslam et al. in 2005, was cited 3,136 times, and ranked first. Shai et al. published in 2008 in New England Journal of Medicine of "Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet" was cited 1,250 times, and ranked second. Foster et al. published in 2003 in "A randomized trial of a low-carbohydrate diet for obesity" in New England Journal of Medicinewas cited 1,124 times, and ranked third.

Analysis of keywords

The map of keywords can present the main research objects and the hot topics and frontiers trends. In this study, VOSviewer software performed the keyword co-occurrence of overlay visualization (Figure 8). A total of 9,750 keywords, 172 keywords met the thresholds when the minimum number of occurrences of a keywords was 20. From Figure 8, we found that the keywords research hotspots were categorized into "ketogenic diet," "metabolism disease," "cardiovascular disease" and "cancer." Bursts keywords were frequently used at a period time, reflecting the frontiers trends. We used CiteSpace software to map the top 32 keywords with the strongest citation bursts from 2002 to 2021 (Figure 9). We summarized that "oxidative stress," "gut microbiota," and "inflammation factors" are becoming frontiers trends of LChD research in the future.

Discussion

We performed a bibliometric analysis of the publications from WoSCC on LChD from 2002 to 2021 using CiteSpace and VOSviewer software. We then summarized the current status, hotspots and frontiers trends in this field.

A total of 6,938 publications including 5,350 articles and 1,588 reviews, related to LChD from 2002 to 2021 were retrieved by searching WOSCC database. The number of annual publications on LChD showed an overall upward trend in spite of fluctuation slightly in some years. LChD research mainly involved the categories of nutrition, endocrinology, and neurosciences, reflecting the multidisciplinary nature and comprehensive knowledge about LChD research. USA was with the largest number and the world science center in LChD field, and Australia, Canada, England, France and Germany maintained close cooperation relationships. Universities were major institutions for LChD research. Five of the top 10 institutions were from USA, which further confirmed US predominance in the field of LChD research. Duke University, Harvard University, Johns Hopkins University and University of Toronto had close collaboration relationships. The most productive authors were Eric Heath Kossoff, Jeff Scott Volek, Jong M. Rho, William S.

TABLE 6 The top 5 co-cited reference on LChD from 2002 to 2021.

Rank	Frequency	Cited reference	Source	Reference
1	287	A randomized trial of a low-carbohydrate diet for obesity	New England Journal of Medicine	(48)
2	234	A low-carbohydrate as compared with a low-fat diet in severe obesity	New England Journal of Medicine	(49)
3	178	The effects of low-carbohydrate vs. conventional weight loss diets in severely obese adults: 1-year follow-up of a randomized trial	Annals of Internal Medicine	(34)
4	171	Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet	New England Journal of Medicine	(50)
5	154	A low-carbohydrate, ketogenic diet vs. a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial.	Annals of Internal Medicine	(51)

TABLE 7 The top 10 cited references on LChD from 2002 to 2021.

Rank	Title	Author	Type	Journal	Year	Citations
1	Obesity	Haslam DW, et al.	Review	*Lancet*	2005	3,136
2	Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet	Shai I, et al.	Article	New England Journal of Medicine	2008	1,250
3	Hepatic fibroblast growth factor 21 is regulated by PPAR alpha and is a key mediator of hepatic lipid metabolism in ketotic states	Michael K Badman, et al.	Article	Cell Metabolism	2007	1,125
4	A randomized trial of a low-carbohydrate diet for obesity	Foster GD, et al.	Article	New England Journal of Medicine	2003	1,124
5	Comparison of the Atkins, Ornish, Weight watchers, and Zone diets for weight loss and heart disease risk reduction	Dansinger ML, et al.	Article	JAMA	2005	1,100
6	Nutrition recommendations and interventions for diabetes—a position statement of the American Diabetes Association	American Diabetes Association, et al.	Article	Diabetes Care	2008	1,074
7	Childhood obesity	Han JC, et al.	Review	Lancet	2010	1,010
8	Weight-loss outcomes: A systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up	Franz MJ, et al.	Review	Journal of the American Dietetic Association	2007	953
9	The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease	Youm YH, et al.	Article	Nature Medicine	2015	935
10	A low-carbohydrate as compared with a low-fat diet in severe obesity	Samaha FF, et al.	Article	New England Journal of Medicine	2003	844

Yancy, and Maria Luz Fernandez. The authors' collaboration showed a geographical concentration and general decentralization. The most productive journals were Nutrients, Epilepsia, Epilepsy Research, PLoS One, and American Journal of Clinical Nutrition. "A randomized trial of a low-carbohydrate diet for obesity" and "Obesity" were considered to be the most co-cited and cited reference respectively.

Based on the keywords the keyword co-occurrence of overlay visualization, we can explore the hotspots. From Figure 8, we summarized and analyzed four hotspots in LChD field. Here, we further analyzed the following aspects according to the application field of LChD: ketogenic diet, metabolism disease, cardiovascular disease and cancer.

(i) Ketogenic diet: The KD is a type of low-carb diet, characterized by high fat and very low carbohydrate. KD works on the basis of the biological principle of starvation, using fat as the main energy source of the body (52). KD initially achieved satisfactory results in pediatric refractory epilepsy and obesity (53). In recent years, research on KD has been extended to metabolic (54), cardiovascular (55), cancer (56), neurological (57), and respiratory (58) diseases with positive results, especially in hyperglycemia (59), hyperlipidemia (60), and insulin resistance (61). Of course there are some shortcomings. The long-term efficacy, such as weight regain (62), cardiovascular events (63), and bone metabolism (64), cannot be ignored. Therefore, the use of KD for disease treatment needs to be individualized according to different diseases and patients' data.

(ii) Metabolism disease: In this study, obesity and diabetes are common metabolic diseases. By consuming less carbohydrates, restricting the body's usage of exogenous glucose, and boosting lipolysis and fatty acid oxidation to meet the body's energy needs, low carbohydrate nutrition

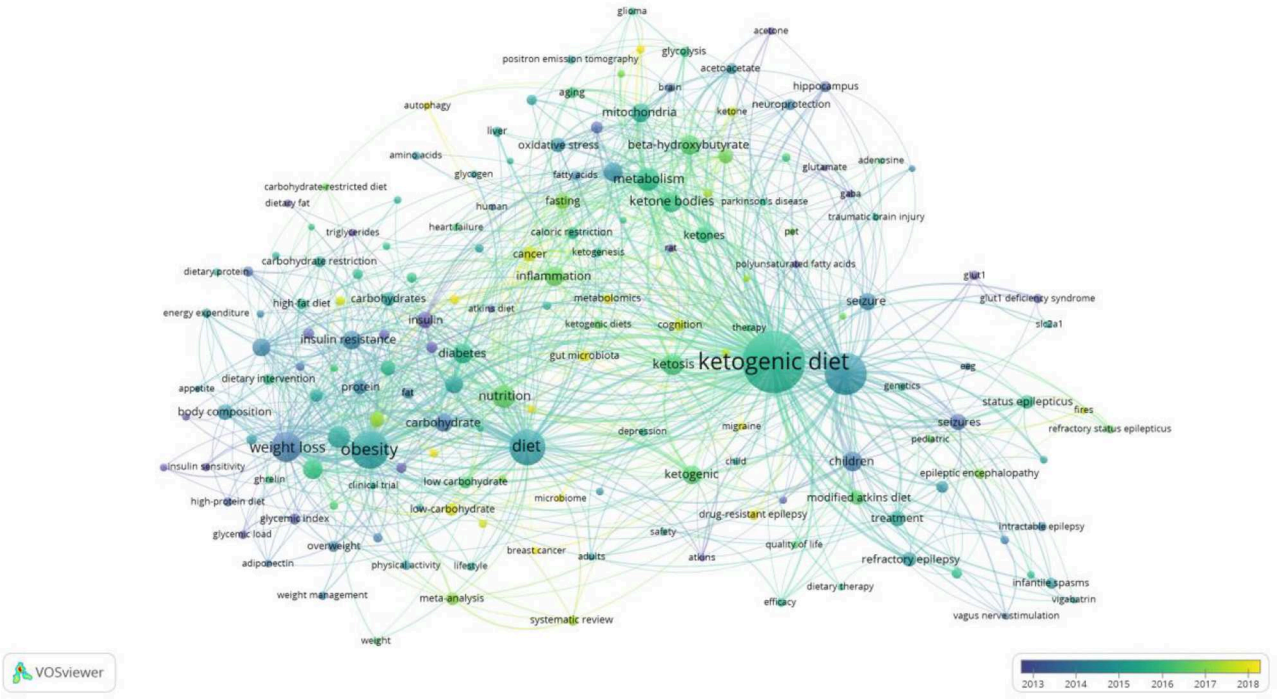

FIGURE 8
Map of keyword co-occurrence of overlay visualization on LChD from 2002 to 2021.

enable individuals to lose weight (65). The studies have shown that LChD are efficient in assisting obese individuals reduce their weight (16, 66). In addition, a low-carbohydrate-high-fat diet can reduce the risk factors for obesity-related diseases while improving obesity and have good prospects for healthy weight loss (67). According to type 2 diabetes epidemiological report, a rise in carbohydrates is largely responsible for the increased calories in patients. Glycemic management begins with dietary carbohydrate restriction (68). One study has shown that patients who have dietary carbohydrate restriction maintains lower levels of glycosylated hemoglobin after a year (69). Research already have proven to the positive effects of LChD on the metabolic diseases of diabetes, obesity, and hypertension. Along with the changes in lifestyle and work style currently occurring, the current research on dietary nutrition and metabolism diseases is going to become a popular topic.

(iii) Cardiovascular disease: The risk of cardiovascular disease rises with a high carbohydrate diet (70). The risk of cardiovascular disease can be decreased by a low carbohydrate nutrition (71). Blood pressure and cardiovascular disease morbidity and mortality are known to be strongly causally correlated. Several studies have shown that LChD can improve blood pressure by lowering diastolic and systolic pressure (72, 73). Increased levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol are crucial contributors to the development of atherosclerotic cardiovascular disease. Excessive levels of high-density lipoprotein cholesterol have a preventive impact, but elevated levels of total cholesterol and triglycerides are significant risk factors for atherosclerotic cardiovascular disease. A number of lipids, including triacylglycerol, total cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol, are improved by LChD (74, 75). A reasonable LChD program is good for cardiovascular health since it has a long-term positive impact on the prevention of cardiovascular disease.

(iv) Cancer: Nutrition is receiving increasing attention in oncology clinical research (76). Proper diet can prevent and treat cancer and reduce the incidence of cancer (77). Seyfried et al. (78) found that the rate of tumor growth is directly proportional to blood sugar levels. Reducing carbohydrate intake, especially KD, can make blood sugar at a low level and effectively inhibit tumor cell proliferation (79). Low-carb diet and KD can improve the quality of life, physical performance, body composition and metabolic health of cancer patients (80). Low-carb diet and KD may create an unfavorable metabolic environment for cancer cells. Therefore, LChD or/and standard therapy, enhance the potential of anti-tumor effects and improve quality of life (11).

Burst keywords can explore the future development trends. Therefore, we summarized the burst keywords into three aspects, and considered them to be frontiers trends of LChD field and anticipated to occur frequently in the future years.

(i) Oxidative stress: Oxidative stress is a negative effect produced by free radicals in the body, and it is considered to

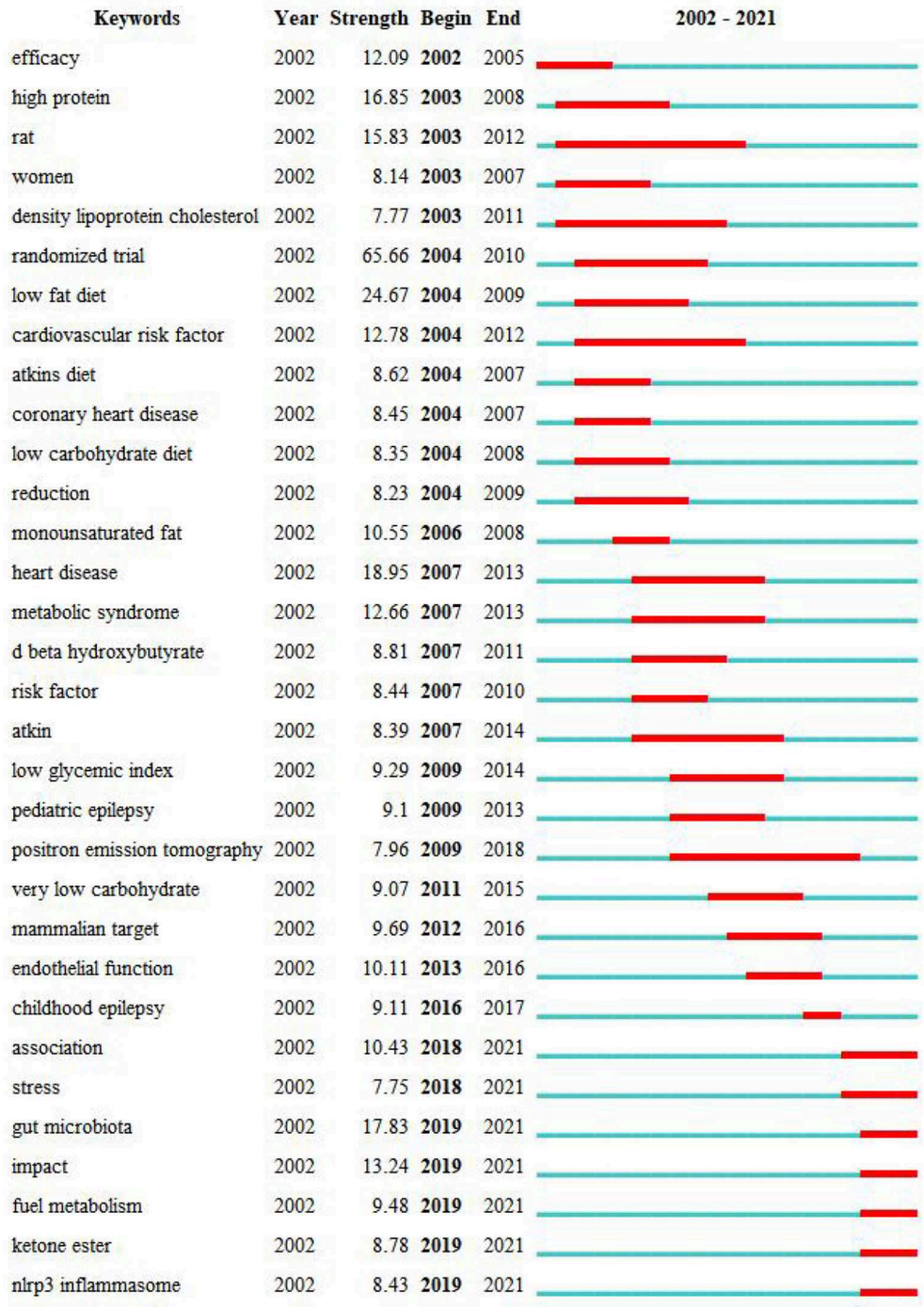

FIGURE 9
Map of keyword with the strongest citation bursts on LChD from 2002 to 2021.

be an important pathogenic factor, such as diabetes mellitus, obesity, heart disease, and cancer (81). The metabolite of the carbohydrate is glucose. The intake of excessive carbohydrates produces more glucose and increases the oxidative pressure on mitochondria, which increases the production of excessive reactive oxygen species, leading to the occurrence of disease (82). Low-carbohydrate intake reduces to reduce the occurrence of oxidative stress in the body, thus reducing the incidence of disease (83). A review showed that the low-carbon ketogenic diet-mediated

reduction in glucose levels and enhanced electron transport in the mitochondria further disrupt the energy metabolism of tumor cells, thus adversely affecting tumor cell proliferation (83).

(ii) Gut microbiota: Gut flora can regulate body metabolism and participate in the occurrence of diseases through a variety of mechanisms. With increasing research on gut microbiota, the dietary pattern was identified as one of the main drivers of gut microbiota change. Recently, carbon aquatic ketone diet has been shown to effectively treat neurological diseases (84), tumor (85, 86), metabolic diseases (87), inflammatory bowel disease (88), etc. Its effect source is related to the participation of intestinal flora in neurodevelopment (84), various pathways to hinder tumor cell growth (89, 90), inhibit the growth of bifidobacterium and reduce the inflammatory factor (89). The current research on intestinal microbiota has explained the action mechanism of low-carbon ketogenic diet to some extent, but the number of studies is too small, requiring further exploration in the future to provide a more solid theoretical basis for the application of low-carbon ketogenic diet.

(iii) Inflammation factors: With the development of life science and technology, the current literature also further explains the disease treatment and prevention of LChD from the inflammation level. Tumor necrosis factor-a, interleukin-6, lipocalin, and C-reactive protein are inflammation factors produced by adipose tissue (91–94). Dietary habits can influence immune function and have anti-inflammatory effects (95). The diets of 9.6% energy from carbohydrate (96) and carbohydrate <40 g/day (97) enhanced lipocalin and lowered C-reactive protein levels in obese patients. According to Jonasson et al. (98), type 2 diabetic individuals who took LChD of 20% energy from carbohydrate, had lower serum levels of interleukin-1 receptor and interleukin-6. An further experiment revealed that LChD lessens lipid deposition, avoids the buildup of plasma and aortic oxidation, lowers inflammatory cytokines in the arterial wall, and inhibits atherosclerosis (99). There will be a spectrum of levels at which research on LChD is conducted, with more inflammation factors becoming increasingly prevalent in the future.

Limitations

To the best of our knowledge, the present study is the first bibliometric analysis to assess LChD. However, it has many limitations. First, considering that the data difference and incompleteness of other database data, we only analyzed publications from the WoSCC. Next, to better present the analysis result and to ensure the quality of the included literature, we included only articles and reviews published in English. This may lead to some screening bias.

Conclusion

We searched all research publications related to LChD on the Web of Scientific Core Collection (WoSCC). CiteSpace software was used to analyze countries/regions, institutions, journals, authors, references, and keywords. LChD is a popular diet, attracting attention from scholars. The hotspots of LChD are three aspects, "metabolism disease," "cardiovascular disease," and "risk factor." We summarize that "research on prevention and treatment," "research on diet," and "research on molecular level" are becoming frontiers trends of LChD research in the future directions and deserve further study.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HP and GL: conceptualization. XH: methodology, writing-original draft preparation, and writing-review and editing. CL and LZ: software. LZ: investigation, data curation, and supervision. GL and XH: resources. GL and CL: visualization. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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β-glucans: a potential source for maintaining gut microbiota and the immune system

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The human gastrointestinal (GI) tract holds a complex and dynamic population of microbial communities, which exerts a marked influence on the host physiology during homeostasis and disease conditions. Diet is considered one of the main factors in structuring the gut microbiota across a lifespan. Intestinal microbial communities play a vital role in sustaining immune and metabolic homeostasis as well as protecting against pathogens. The negatively altered gut bacterial composition has related to many inflammatory diseases and infections. β-glucans are a heterogeneous assemblage of glucose polymers with a typical structure comprising a leading chain of β-(1,4) and/or β-(1,3)-glucopyranosyl units with various branches and lengths as a side chain. β-glucans bind to specific receptors on immune cells and initiate immune responses. However, β-glucans from different sources differ in their structures, conformation, physical properties, and binding affinity to receptors. How these properties modulate biological functions in terms of molecular mechanisms is not known in many examples. This review provides a critical understanding of the structures of β-glucans and their functions for modulating the gut microbiota and immune system.

KEYWORDS

β-glucans, gut microbiota, *Bifidobacterium*, *Bacteroides*, immune system

1. Introduction

Research of the current decade in the field of food science is paying close attention to studying microbiota and their associated health benefits (1). Diet is a critical modifiable factor and plays a crucial role in maintaining the microbiota and influencing their composition, proving the possibility of therapeutic dietary approaches to control microbial diversity, composition, and stability. The diet must also include non-digestible components, particularly dietary fibers, in addition to the necessary nutrients, including proteins, vitamins, lipids, and minerals, as diet strongly influences the composition of colonic microbiota and their metabolic products (2). β-glucans, a common component of the human diet, have several positive health effects (3, 4). Yeast, fungi (including mushrooms), certain bacteria, seaweeds, and cereals (oat and barley) contain β-glucans, and the polysaccharides of D-glucose monomers joined by β-glycosidic linkages (5, 6). β-glucans exist in different glycosidic linkages, such as β (1,3), (1,4), and (1,6) in either an unbranched or branched arrangement (7, 8). The abundant hydroxyl groups form hydrogen bonds with water which gives the molecule capacity to store water in both soluble and insoluble states, making it strongly hydrophilic. β-glucans' molecular weight (MW) depends on the source and varies between 10^2 and 10^6 Da (9). High MW and high viscosity features of β-glucan cause hypocholesterolemia and hypoglycemia (10, 11). Notwithstanding, when β-glucans are used

as a food factor, they are recognized for their capacity to change the functional aspects of food products, including viscosity, texture, rheology, and sensory qualities (12).

Because β-glucans are crucial to a healthy diet, the US Food and Drug Administration advises to consume 3 g β-glucan on a routine basis from cereal sources, such as barley or oats, to lower the risk of heart-related illnesses (13). Foods rich in β-glucans are a significant contender for a healthy diet due to their bioactive properties and numerous functional activities. Multiple features of β-glucan, including anticancer (14), anti-diabetic (15), anti-inflammatory, and a decrease in the glycemic index as well as serum cholesterol and triglycerides, have been demonstrated. β-glucans maintain the balance of blood glucose and cardiovascular diseases (16), enhance the immune system (17) and wound healing activities (18), and show antimicrobial (antibacterial and antiviral) properties (Figure 1). Proved antioxidant, wound healing, and moisturizing properties of β-glucan derived from microorganisms and cereal (19). These diverse activities of β-glucans attribute to their physical properties such as water solubility (20), viscosity, and gelation (21). Thus, the physical characteristics of bread and cakes enhance by adding β-glucan to the recipe (22).

β-glucans are known to show a robust immune stimulant (23) and mitigate benign and malignant cancers (24, 25). β-glucan acts as a pathogen-associated molecular pattern (PAMP) to inhibit the host's insusceptible reactions (26). When a fungal infection occurs, the host recognizes a crucial PAMP associated with β-glucan and then stimulates the host's immunological responses (27). Dendritic and macrophage cells are often regarded as the primary target cells of β-glucans that also stimulates neutrophils, B, T, and natural killer cells to attach pathogens (25, 28, 29).

Approximately 100 trillion microbial cells inhabit the gastrointestinal tract of the human, which encode 100 times more genes than the human genome (30). In addition, microbial cells are present in approximately 10 times the number of human cells (30, 31). This microbial community contains an estimated 5,000 bacterial species, mainly belonging to *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, and *Proteobacteria* (31). The gut microbiota studies understand the status of different gut conditions between diseases and healthy for improving gut integrity, controlling host immunity, safeguarding the host against microbes, harvesting energy, and unraveling dietary utilizing molecular mechanisms (32, 33). The gut bacterial community plays a significant role in maintaining human wellbeing by producing many fundamental health-benefiting substances, including synthesizing vitamin K (34), promoting angiogenesis, involving host metabolic processes, and altering the appetite signaling pathway (35–38). Recent evidence also revealed that gut microbiota is critical in advancing cerebrum capabilities connecting with uneasiness, sorrow, stress, and cognizance (39, 40). There is always an association between gut dysbiosis and numerous chronic diseases (41), such as cardiovascular, autism, cancer, and obesity (42, 43). Dietary supplementation with indigestible polysaccharides obtained from plants, fungi, and probiotics benefits health by promoting microbial community growth (44). Bacterial fermentation occurs when dietary fibers enter the colon (45), and as a result, they produce short-chain fatty acids (SCFAs such as acetic acid, propionic acid, and butyric acid) (46), biogenic amines (47), indole and tryptophan derivatives (48), and secondary bile acids, conferring a health benefit. Microbial-produced SCFAs play significant parts in the proliferation of immune cells, apoptosis, cell differentiation, chemotaxis, and gene expression (49). Bile acids (50) and tryptophan derivatives (48) also play a gene

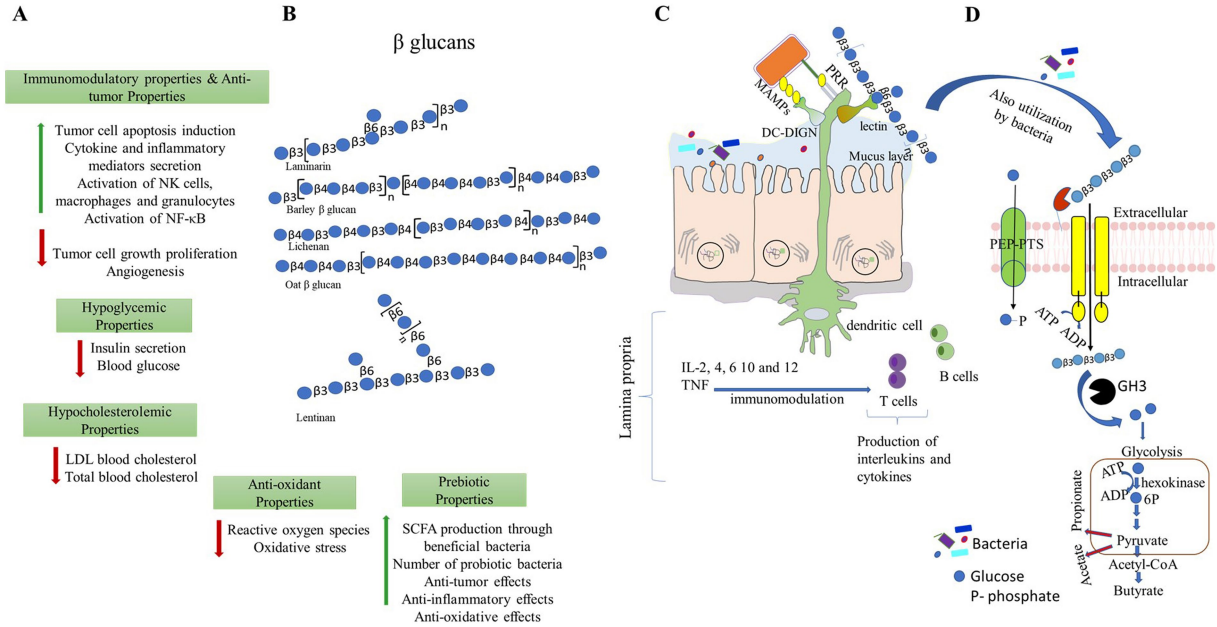

FIGURE 1
Different beneficial properties of β-glucans. **(A,B)** β-glucans can be extracted from different sources and have a wide range of applications for improving the health of the host. Representative structures of β-glucans are made as per symbolic nomenclature for glycans (https://www.ncbi.nlm.nih.gov/glycans/snfg.html). **(C)** β-glucans modulate immune responses by direct binding and recognizing receptors present in the immune cells. **(D)** β-glucans modulate gut microbiota, and in turn, they promote the production of short-chain fatty acids. PPR- pattern recognition receptors; MAMPs- microbe-associated molecular patterns and DG-SIGN- dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin.

regulatory role. The gut microbial communities harbor several unique genes that encode different enzymes to break down carbohydrates, including glycoside hydrolases, amino acid decarboxylases, carbohydrate esterases, and polysaccharide lyases (51). These highly diverse thousands of carbohydrate-active enzymes (CAZymes) of microbial communities are referred to as the host's secondary genome (52). Therefore, the usage of dietary fibers entirely depends on CAZymes.

Gram-negative microorganisms, sjuch as *Bacteroides*, possess many glycoside hydrolases and polysaccharide lyases (35), while Gram-positive bacteria, such as *Lactobacillus* and *Bifidobacterium* (36), primarily have glycoside hydrolases for breaking down different types of dietary fiber (53). How these microbes utilize dietary fiber and their produced metabolites modulate the immune system are outstanding questions among glycobiologists and biochemists. However, there are few known molecular mechanisms for digesting *β*-glucan by bacteria and structure-functional interactions between β-glucan and the immune system. The latest understanding of them is summarized here.

2. Microbial composition in the human GI tract and capability for digesting the dietary fiber

Microorganisms in the intestine of humans fluctuate from a few hundred to 100 trillion, such as 10^3 cells/g in the stomach, ~ 10^7 cells/g in the small intestine with a more significant part of facultative anaerobes, and ~ 10^{13} cells/g in the colon which obligate anaerobes make up the majority fraction (54, 55). The gut microbiome populace overwhelmingly incorporates the individual from phyla *Bacteroidetes* (primarily *Bacteroides* and *Prevotella*), *Firmicutes* (primarily *Clostridia genus*), *Fusobacteria*, *Actinobacteria*, and *Proteobacteria* (56, 57). *Bacteroidetes* and *Firmicutes* are the major phyla that account for approximately 90% of the total bacteria in the adult gut. *Bacteroidetes* and *Proteobacteria* regulate the immune system, formation of the gut microbiome, and defense against pathogen invasion (58). They maintain the microbiome and immune systems through an integrated metabolic energy-harvesting process based on dietary fiber cross-feeding (syntrophy) and co-metabolism, including polyphenols (36, 59).

Individuals from the *Bacteroidetes*, a predominant phylum in the human gut, have polysaccharide utilization loci (PUL) to focus on a wide variety of complex glycans. The arrangement of genes centered on tandem susC/susD homologs that code for the TonB-dependent transporter (TBDT) and the cell-surface glycan-binding protein (SGBP) (60). Extra colocalized and co-regulated SGBP(s), susC/susD, and a transcriptional regulator typically make up machinery that detects, imports, and upregulates a PUL in the presence of glycans (61). Only a few PULs have been now biochemically characterized in *Bacteroidetes*, despite a massive number of them being recognized (62). For utilizing *β*-(1,3)-glucans, bacteria use activities of *β*-(1,3) glucanases (EC 3.2.1.6 and EC 3.2.1.39) and *β*-(1,3) glucosidase (EC 3.2.1.58) that have a place with the glycoside hydrolase families, GH5, GH16, GH17, GH55, GH64, GH81, GH128, and GH158 (63). *β*-(1,3)-glucanases break-down the glycan with internal glucoside bonds and make oligosaccharides. *β*-(1,3)-glucosidases act on the non-reducing ends of *β*-(1,3)-glucans and discharge glucose from oligosaccharides (64). Some endo-acting *β*-(1,3)-glucanases have carbohydrate binding domains to enhance their capacity to bind substrates that are not soluble in water (65), while *β*-(1,6)-glucanase (EC 3.2.1.75), a member of the GH30 family, is necessary to dissect *β*-(1,6) linked branched chains (33).

3. Impact of *β*-glucan on gut microbiota

Diet is a major factor in regulating the diversity and activity of gut microbiota, including how ingested diet is shared among the microbial communities at different syntrophic levels. This interaction determines the balancing of the gut microbiota and preventing of non-communicable diseases (66, 67). Indeed, *β*-glucan is a non-digestible carbohydrate and acts as a substrate for improving colonic microbes as they are permitted to go through the small intestine due to their resistance to absorption (68, 69). Several studies on *β*-glucans that modulate gut microbiota are presented in Table 1.

β-glucan increases the growth of *Lactobacillus casei, Lactobacillus acidophilus,* and *Bifidobacterium animalis* subsp. *lactis* both *in vivo* and *in vitro* (81). Cereal *β*-glucans were fed to experimental rats for 3, 6, and 7 weeks, and the results showed that the population of *Bifidobacterium* and *Lactobacillus* was enhanced (82). *Clostridiaceae* (*Clostridium orbiscindens* and *Clostridium* sp.), *Roseburia hominis, Ruminococcus* sp., and low levels of *Firmicutes* and *Fusobacteria* were found to be more abundant after the consumption of whole grain barley pasta and durum wheat flour rich in *β*-glucan (74). In yogurt, *β*-glucans of oats and grains were found to expand the development and reasonability of *Bifidobacterium animalis* subsp. *lactis* (83). An increased gut-bacterial population has positive functional consequences, such as ensuring adequate digestion and preventing constipation, diarrhea, and inflammatory bowel disease (IBD) (84, 85). In addition, integrating high molecular weight oat *β*-glucan into milk brings cholesterol down and decreases calories in dairy items (86). Oat and barley-β-glucans increase beneficial bacterial communities' population and promote microbial metabolites such as 2-methyl-propanoic, butyric acid, propionic acid, and acetic acid (87–89).

Bacterial communities of our gut reveal the diverse digestion capability of dietary glycans. For instance, *Bifidobacterium* cannot digest complex glycan, such as pectin. Thus, they rely on *Bacteroides* to produce oligosaccharides from pectin before they can grow. This type of cooperation is known as a syntrophic system, and different bacteria have adjusted their genome through evolution to maintain gut microbial homeostasis (90). *Bifidobacterium* and *Bacteroides* are essential members of the gut microbiota, where they occupy approximately 80% of microbial space in infant and adult gut, respectively, involving in the utilization of dietary glycans. Therefore, *Bacteroides* and *Bifidobacterium* are primary and secondary degraders for utilizing complex and simpler glycans, respectively. Some of the known glycan-utilizing mechanisms are mentioned below pertaining to *β*-glucans.

TABLE 1 Modulation of gut microbiota by β-glucans.

Source of β-glucan *Study model*	Molecular weight or/and composition of material that was used	Modulation of Gut microbiota	References
Cereal-β-glucan			
Tibetan hulless barley-*in vitro* fermentation of human fecal	3.45×10^4 Da	↑ *Pantoea, Megamonas, Bifidiobacteria, Prevotella* ↑ Concentrations of SCFA (acetate, propionate, butyrate)	Nie et al. (70)
Barley *in vivo* - human patient with high risk for metabolic syndrome development	Experimental β- glucans bread was prepared with wheat flour and β-glucans-enriched barley flour (Valechol)	↑ *Bifidobacterium* spp. and *Akkermansia municiphila*	Velikonja et al. (71)
Barley-*in vivo* in rat	LMW- β- glucans were partially prepared by cellulase MW 12 kDa	↑ *Bifidobacterium* and *Bacteroides* ↑ Total SCFAs, particularly ↑ Acetate and n-butyrate.	Aoe et al. (72)
Barley-*in vivo hypercholesterolemic rat*	LMW barley	↑ *Bifidobacterium*	Mikkelsen et al. (73)
Barley-*in vivo* study on human	Granoro's Cuore Mio pasta was made by using a mixture of durum wheat flour (75%) and whole-grain barley flour (25%)	↑ *Roseburia hominis, Ruminococcus* ssp. *Clostridiaceae* spp. ↓ *Fusobacteria* and *Firmicutes*	De Angelis et al. (74)
Barley-*in vivo* randomized individual study	HMW barley (1,349 kDa)	↑ *Bacteroidetes* and ↓ *Firmicutes*	Wang et al. (75)
Oat-*in vitro* fermentation of colonic microbiota	PepsiCo, Inc. (Barrington). Used different oat ingredients	↑ *Bifidobacterium, Roseburia, Lactobacillus* spp.	Van den Abbeele et al. (76)
Mushroom-β-glucan			
Pleurotus eryngii in vitro – fermentation of human fecal sample	Mushroom cultivation was conducted in substrates consisting of wheat straw or beech sawdust, and in their mixtures in various ratios (w/w) with grape marc or olive prunings and of olive leaves with two-phase olive-mill waste. Therefore, used mushroom may have contamination of used substrates.	↑ *Bifidobacterium* spp. and *F. prausnitzii* populations. ↑ Acetate, propionate and butyrate concentration.	Boulaka et al. (77)
Fungal β-glucan			
Grifolan (Grifola frondosa)-in vitro-colorectal cell lines		↑ *Lactobacillus* and *Bifidobacterium.* ↑ Lactic, succinic, and valeric acid concentrations.	De Giani et al. (78)
Polysaccharide form *Ganoderma lucidum-in vivo* obesity mice model	MW: 133.1 KDa	↑ Ratio *Bacteroides* to *Firmicutes*, *Bacteroides ovatus* and *B. uniformis.* ↑ Acetic, propionic, butyric and valeric acid.	Chang et al. (79)
Yeast β-glucan			
Zymosan-*in vitro* fermentation model		↑ *Bifidobacterium, Faecalibacterium, Prevotella* ↓ *Escherichia-Shigella* ↑ Acetic acid and propionic acid	Pi et al. (80)

Cereal and oat-β-glucan: Mixed-linkage (1,3) and (1,4)-linked β-D-glucans, and fungal-β-glucan: (1,3) and (1,6)-linked β-D-glucans.

3.1. Trapping of β-glucan by some gram-positive and gram-negative human gut bacteria

Gram-negative bacteria, such as *Bacteroidetes,* can access and grow on a broad spectrum of complex glycans, which they encounter in the gastrointestinal tract of humans (91). As aforementioned, they comprise a starch utilization system (Sus), which is a hallmark distributed across their phylum (92). PULs have enabled human gut *Bacteroidetes* to utilize xylan (93), arabinoxylan (94), rhamnogalacturonan I (95) and II (96), and various other plant polysaccharides (97, 98), and their detailed molecular mechanism has been characterized by comprehensive functional analyses.

The degradation of β-glucan mainly occurs extracellularly by outer membrane-bound enzymes which produce oligosaccharides upon digestion of complex polysaccharides. Utilization of

oligosaccharides by Gram-negative bacteria depends on an outer membrane protein complex consisting of an extracellular SGBP and an integral membrane SusC-like TonB-dependent transporter. Crystal structures of two practically distinct SusCD complexes purified from *B. thetaiotaomicron* have derived a standard model for substrate translocation (99). The TBDT forms homodimers, with each β-barrel protomer tightly capped by SGBP. The single-channel electrophysiology revealed a 'pedal bin' mechanism in which SGBP (SusD homolog) moves away from TBDT (SusC homolog). In the absence of oligosaccharides, the SusD lid of the empty transporter is free and undergoes conformational changes. In the presence of glucan, TonB binds to the TonB box of the transporter to initiate the conformational changes in the plug, extracellular loops of SusC, that lead to oligosaccharide release and the creation of a transport channel into the periplasmic space (99). The TonB promotes the dissociation of glucan into periplasmic space, and then, the transporter (SusC) returns to its open state conformational. The required energy is governed by ExbBD–TonB system, which is equivalent to pressing the pedal to open the SusCD (99). These mechanistic insights into how the outer membrane nutrients are imported inside the periplasm and cytoplasm by microbiota members provide outlines of understanding human–microbiota symbiosis.

For example, a mechanism for the utilization of barley-β-glucan was established in *B. ovatus* ATCC 8483 and *B. uniformis* JCM 13288 (61, 100). These studies demonstrated through synteny analyses that the mixed-linkage glucan utilization locus (MLGUL) is widely present among human gut microbiota. The presence of homolog genes within or without locus enables selective *Bacteroides* species in the gut microbiota to cleave barley-β-glucan. The locus consists of outer membrane-bound GH16 and a periplasmic GH3 that acts as exo-β-glucosidase. The GH16 cleaves high MW barley-β-glucan and produces mixed-linkage β-(1,3)/β-(1,4) glucan-oligosaccharides. Those oligosaccharides are converted into monomeric units by GH3 in periplasmic space. The GH3 is a part of the locus or is present in another site of a genome in *B. ovatus* ATCC 8483 and *B. uniformis* JCM 13288, respectively (61, 100). Locus outer membrane-bound non-catalytic SGBPs plays essential roles in recruiting and capturing high MW barley-β-glucan, and the SusD allows mixed-linkage β-(1,3)/β-(1,4) glucan-oligosaccharides to enter in periplasmic space in concert with cognate TonB-dependent transporters (TBDTs) as shown in Figure 2.

Polysaccharide degradation is accomplished by Gram-positive bacteria by different cellular mechanisms that may not involve TonB-dependent transporter systems. These symbionts encode polysaccharide degrading functions with genetic loci that encode ATP binding cassette (ABC), proton symporters, or phosphoenolpyruvate phosphotransferase system (PEP-PTS) transporters. These are co-expressed with associated degradative enzymes (101, 102). Oligosaccharides are internalized by ABC transporter-coupled ATP hydrolysis serving as the primary transport system in *Bifidiobacterium*. In PEP-PTS, phosphoenolpyruvate serves as the phosphate donor to the recipient monosaccharide, and PTS internalizes monosaccharide and concomitant phosphorylation. Although these systems are present in various bacteria, all *Bifidiobacterium* do not possess them in their genomes.

Interestingly, *g*ram-*p*ositive *p*olysaccharide *u*tilization *l*oci (gpPULs) are present in families of *Roseburia* and *Eubacterium rectale* that ideally consist of ABC transport proteins, transcriptional regulator genes, and glycoside hydrolases (103). Such gpPULs are found to involve in the utilization of konjac glucomannan and spruce acetylated galactoglucomannan (104) and xylan and arabinogalactan utilization (103). We have identified β-glucan utilizing gpPUL in *Blautia producta* ATCC 27340 and widely presence in many species of *Lachnospiraceae* (98). Distinct from Gram-negative bacterial PUL, gpPUL does not encode SGBP, but the glycan-binding function is likely to perform by carbohydrate-binding modules associated with endo-acting enzymes. The gpPUL also encodes a transcriptional regulator gene that seems to control the expression of the locus in the presence of suitable carbon (105).

In a symbiosis system between *Bacteroides* and *Bifidobacterium*, it was observed that *Bacteroides cellulosilyticus* and *Bacteroides ovatus* share β-(1,3)/(1,6)-glucooligosaccharides with *Bifidobacterium breve* UCC2003 and *Bifidobacterium bifidum* (102). Zhao and Cheung (106) suggested that *B. infantis*, *B. longum*, and *B. adolescentis* can ferment β-glucans obtained from mushroom sclerotia, seaweed, bacteria, and barley. Among them, *B. infantis* produces double amount of SCFAs than other two *Bifidobacterium*. However, a systematic evaluation of β-glucans utilization is required to use species for mitigating gut-related syndromes through appropriate modulation.

3.2. β-Glucan sensing by bacteria

The capability of gut *Bacteroidetes* to reckon and respond to diverse glycans in their environment is bestowed in many extracellular sensor-regulator systems that are closely associated with the PUL they encode. The most biochemically and structurally well-characterized system in *Bacteroidetes* is the hybrid two-component system (HTCS) (107). HTCS is a cytoplasmic membrane-spanning protein that comprises all domains of a classical two-component system in one polypeptide (N-terminal extracellular sensor, cytoplasmic histidine kinase, and response regulator). Signal recognition in HTCS takes place *via* the direct binding of oligosaccharide fragments to the periplasmic sensor domain. These oligosaccharides are products of polysaccharide degradation at the outer membrane cell surface-tethered PUL-encoded endo-acting enzymes. The produced oligosaccharides were earlier transported into the periplasm by the TBDT (SusC homolog). In some cases, oligosaccharides process further *via* periplasmic enzymes before acting as activating signals (108).

Although the sensing system in Gram-positive bacteria is not extensively known as in Gram-negative bacteria, there are few transporters known to mediate glucan uptake and can readily utilize them through highly conserved sequences of the solute binding protein (Figure 2B). For instance, in *Bifidobacterium animalis* subsp. *lactis* to overcome the need for HTCS has the presence of an ABC transport system that allows the tethering and uptake of complex glycan such as arabinoxylan. The solute-binding protein, such as *Bl*AXBP, of ATP-binding cassette (ABC) transporter mediates the uptake of arabinoxylan–oligosaccharides with exceptionally broad specificity for tri-saccharides and tetra-saccharides of undecorated xylo- and arabinose-decorated-oligosaccharide (109). Crystal structures of *Bl*AXBP suggested that a spacious binding pocket and the conformational flexibility of a lid-like loop facilitate the binding of decorated oligosaccharides. The *Bl*AXBP is highly conserved within *Bifidobacterium* and highlights the gut microbiota metabolic

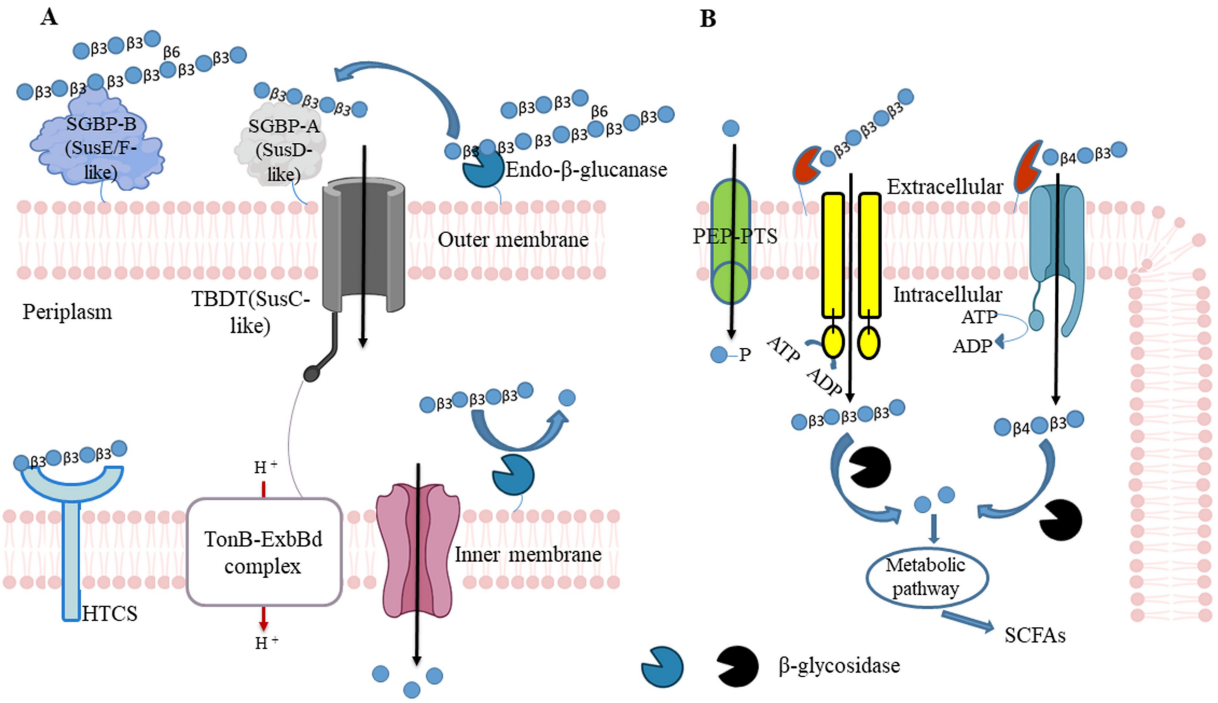

FIGURE 2
β-glucan utilizing mechanisms in Gram-negative bacteria and Gram-positive. **(A)** Gram-negative bacteria can hydrolyze polysaccharides at the outer membrane tendered glycoside hydrolase (GH) and convert into oligosaccharides. Those polysaccharides first recognize by surface glycan-binding protein and facilitate endo-acting enzymes to cleave them. Generated oligosaccharides are again caught by SusD, and it allows them to enter the periplasmic space through TonB - dependent transporter (SusC homolog). Entered oligosaccharides further cleave into monosaccharide contents by periplasmic GH. Those monosaccharides pass to the cytoplasm *via* Major Facilitator Superfamily (MFS) transporter and produce bacterial metabolites (including SCFAs) through fermentation to promote the host's health. HTCS, hybrid two-component system sensor/regulator. **(B)** Similarly to Gram-negative bacteria, Gram-positive bacteria hydrolyze polysaccharides at the outer membrane tendered GH and convert into oligosaccharides. Generated oligosaccharides enter into cytoplasmic space through MFS, the phosphoenolpyruvate (PEP)- carbohydrate phosphotransferase system (PTS), or ATP-binding cassette (ABC) transporters couple ATP hydrolysis. SCFAs: short-chain fatty acids.

syntrophy with other species. The occurrence of transport systems is a prerequisite for utilizing glucan- oligosaccharides and xylooligosaccharides. Solute-binding protein is also identified in *Limosilactobacillus reuteri* ATCC 53608 and *Blautia producta* ATCC 27340 for utilizing xylooligosaccharides (105).

The expression of the locus or gene involved in utilizing available carbon sources is suppressed by the presence of a preferred glycan. It is controlled by carbon catabolite repressor (CCR), a regulatory system in most bacteria (92). It is accomplished by different regulatory mechanisms, including the regulator of translation by an RNA-binding protein in diverse bacteria. The CCR-related metabolism was first seen in *B. animalis* subsp. *lactis* (110). It was also observed in other members of *Bifidobacterium* that can control the expression of genes involved in the utilization of raffinose, sucrose, or oligofructose (111).

The mechanism by which a glycan is utilized by *Bifidobacterium* is not yet well established as it is known for *Bacteroides*. Due to their usage in probiotics, detailed emphasis should be given to how specific genes/enzymes sense, break-down, and import complex glycans inside the cytoplasmic space by gram-positive bacteria. Novel pathways from *Bifidobacterium* would clearly elucidate the metabolites that play a role in maintaining gut homeostasis. The members of *Lachnospiraceae* express a gpPUL that consists of the transcriptional regulator (103, 104); however, the defined function of the such regulator is not yet known. The function of such a regulator should exploit by further studies.

4. Immunomodulatory effects of *β*-glucan

The gut microbiota constantly interacts with the immune system aiding diverse processes such as behavior, digestion, as well as the maturation of the immune system (Table 2); thus, it shows a symbiotic relationship with the host (138, 139). The immune system is also acknowledged as one of the most critical factors that affect the composition of the gut microbiota through cross-talk between immunity and microbiome (140). The colonization of gut microbiota can mediate and influence the production of antimicrobial peptides/bacteriocins through epithelial cells and pattern recognition receptors encoded by intestinal layers (141).

In addition to the interaction of immunity and microbiome, *β*-glucans are considered one of our diet's active ingredients that show immunological benefits. They can interact with various immunological receptors, including Dectin-1, complement receptor (CR3), and toll-like receptors (TLR) 2/6. This causes several immune cells to be triggered, such as dendritic cells, macrophages, neutrophils, monocytes, and natural killer cells (142). *β*-glucans can modulate innate and adaptive responses, and they can also improve opsonic as well as non-opsonic phagocytosis (143, 144). The intricacy of their structure governs diverse *β*-glucan immune functions. Stronger immune-modulating and anti-cancer actions are correlated with

TABLE 2 Immunological studies of-β-glucan.

Type of β-Glucan	Source	Immunomodulation effects/ properties *in vitro* and *in vivo*	References
Lantinan	*Lentinula edodes*	Enhances the phenotypic and functional maturation of dendritic cells with significant IL −12 productions.	Wang et al. (112)
		Reduction in anti-inflammatory cytokines such as IL-4, IL-10. It significant increases weight gains, blood cells, monocytes, circulatory cytotoxic T-cells. It increases in cage-side health of acute myeloid leukemia demonstrated in animal studies in Male BN/RijHsd rats.	McCormack et al. (113)
		Increases NK cell-mediated killing of Yac-1 cells both *in vitro* and *in vivo*.	Vetvicka et al. (114)
		Enhances cytotoxic activity and inflammatory cytokines of macrophages and RAW 264.7 cell lines.	Chan et al. (115)
		Increases anti-tumor activity in BALB/c mice inoculated with S-180 cells.	Zhang et al. (116)
		Lentinan-activated macrophages and dendritic cells indirectly activate T cells *via* IL-12 and IFN-γ.	Murata et al. (117)
		Increases T cell functions in cancer patients.	Yoshino et al. (118)
Laminarin	Fronds of *Laminaria*	The increased population of B, T and macrophage cells due to the administration of laminarin in the normal mice as compared to BALB mice, demonstrated in *in vivo* studies.	Shang et al. (119)
	Laminaria digitata	Induces anti-cancerous effect by activating dendritic cells, antigen-specific T cells in the C57BL/6 rodents, and releases pro-inflammatory cytokines such as TNF-α, IL-12 and IL-6 in B16 melanoma cells.	Song et al. (120)
	Laminaria digitata	Enhancement in the expression of IL-6 and IL-8 in response to *ex vivo* LPS-induced in pigs due to 600 ppm dietary inclusion of laminarin.	Smith et al. (121)
	Brown algae	Interleukin (IL-6 and IL-1β) and TNF-α have been expressed in RAW 264.7 cells under *in vitro* conditions.	Lee et al. (122)
		Induces apoptosis *via* Fas pathway and blocks the insulin-like growth factor-I (IGF-1, which has a role in cancer development) receptor in human colon adenocarcinoma H29 cells.	Park et al. (123)
		Strong binding efficiency for Dectin-1 in macrophages isolated from C57BL/6 mice under *in vitro* conditions.	Brown et al. (124)
Zymosan	*Saccharomyces. cerevisiae*	Activates TLR 2 and Dectin-1 on macrophages.	Dennehy et al. (125)
		Increases cytokine production such as TNF-α and IL-12 *via* NF-kB signaling. Increases production of monocyte chemo-attractant protein-1.	Lebron et al. (126)
Schizophyllan	*Schizophyllum commune*	Increases the expression of cytokines and activity of NK cells.	Yoneda et al. (127)
	Fungal Schizophyllan	Inhibited spread of the virus in the lungs. Augmented protective immune responses induced by low doses of a live *Sendai virus* vaccine. It was determined through animal studies.	Hotta et al. (128)

(Continued)

TABLE 2 (Continued)

Type of β-Glucan	Source	Immunomodulation effects/ properties *in vitro* and *in vivo*	References
Polysaccharide ganoderma	*Ganoderma lucidum*	Increases MAPKs and Syk-dependent TNF-α and IL-6 expressed in CHO cells RAW264.7 cells. It also increases anti-tumor activity.	Guo et al. (129)
		It induces human peripheral blood mononuclear cell proliferation and produces cytokines like IL-10 and IL-12.	Chan et al. (115)
Pleuran	*Pleurotus ostreatus*	Increases proliferation of lymphocytes.	Mitsou et al. (130)
PGG glucan	*Saccharomyces cerevisiae*	Induces activation of NF-κB like nuclear transcription factor in purified human neutrophils, and enhances neutrophil anti-microbial function.	Wakshull et al. (131)
Algal β-glucan	*Durvillaea antarctica*	Increases activation of CD19+ B lymphocytes under *in vitro* studies.	Bobadilla et al. (132)
Phycarine	Seaweed	Stimulate both humoral and cellular branches of immune reactions to cure gastrointestinal diseases under *in vitro* studies.	Vetvicka et al. (133)
	Laminaria digitata	Significantly stimulates phagocytic activity in animal studies.	Vetvicka and Yvin (134)
Scleroglucan	*Sclerotium rolfsii*	Increases in TNF-α in human monocytes.	Falch et al. (135)
Ulvan	*Ulva intestinalis*	Releases cytokines such as IL-1β, IL-4, IL-6, IL-10, IL-11, IL-12, IL-13 and TNF-α, and activation of RAW 264.7 cells under *in vitro* conditions.	Tabarsa et al. (136)
		Expresses anti-tumor activity as inhibited the cell growth of breast cancer cell line by the *U. lactuta*. It decreases the anti-apoptotic marker (BCL-2) and tumor suppressor gene (P53) under *in vitro* conditions.	Lahaye and Robic (137)

higher structural complexity (145). The direct binding of β-glucans to particular immune cell receptors raises the possibility of an immunological modulatory action independent of microbes (146).

The ability of an innate immune system to rapidly recognizing and reacting to invasive pathogens is crucial for infection control. β-glucan guards against illness brought on by bacteria, viruses, and other harmful microbes (147). During *in vivo* investigations, β-glucans were tagged with fluorescein to monitor their oral uptake and digestion (148). The orally administered β-glucans bind to the Dectin-1, a type II transmembrane β-glucan receptor, on the macrophages and get taken up by the cell (149). It was demonstrated that low MW β-glucans bind strongly with Dectin-1 as compared with HW ones (150). It was subsequently moved to the bone marrow, lymph nodes, and spleen. Large β-(1,3)-glucans get degraded by macrophages within the bone marrow and produce smaller, soluble β-(1,3)-glucan fragments (Figure 3). This soluble β-(1,3)-glucan fragments were then recognized through CR3 of the circulating monocytes, macrophages, and granulocytes (151). These granulocytes with CR3-bound β-glucan-fluorescein when enrolled to a site of complement activation were enabled CR3 to trigger cytotoxicity of inactivated complement 3b (iC3b)-opsonized tumor cells, covered in monoclonal antibodies (mAb) (148). Yeast β-(1,3)/(1,6)-glucan and barley β-(1,3)/(1,4)-glucan potentiated the action of anti-tumor mAb, leading to more robust tumor regression and survival (148). When a lentinan, a type of β-glucan, binds to dectin-1, it activates Syk kinase that regulates COX2 expression, modulating immune responses.

Lentinan isolated from the fruiting bodies of *Lentinus edodes* is a popular medication with anti-infective and anti-tumor activities. RAW264.7 cell line's cytotoxic activities and inflammatory cytokine production were enhanced by lentinan (29, 152). The dendritic cells show enhanced phenotypic and functional maturation and produce a considerable amount of IL-10 and IL-12 due to the presence of lentinan (115). Lentinan acts as a vaccine adjuvant, enhancing the virus-specific CD8(+) T cell functions generated by DNA vaccination in HBcAg (pB144) in mice (112) and raising T cell functions in mice with tumors (113) and malaria-infected mice. Lentinan-induced dendritic and macrophage cells indirectly activate T cells by producing IL-12 and IFN-γ (117). Lentinan increases NK cell-mediated death of Yac-1 cells in both *in vitro* and *in vivo* experiments (114).

In contrast to the Dectin-1-Cox2 signaling axis, mannan/β-(1,6)-glucan-containing polysaccharides (MGCP) facilitate regulatory T (Treg) cell differentiation from naïve T cells. Additionally, it confines Th1 differentiation of effector T cells based on a TLR2-dependent mechanism through suppressing IFN-γ expression. Thus, the administration of MGCP exhibits a strong suppressive capability toward investigational colitis and autoimmune encephalomyelitis in mice models. It highlights the potential therapeutic utility of MGCP against clinically related autoimmune diseases (153). β-glucan-based

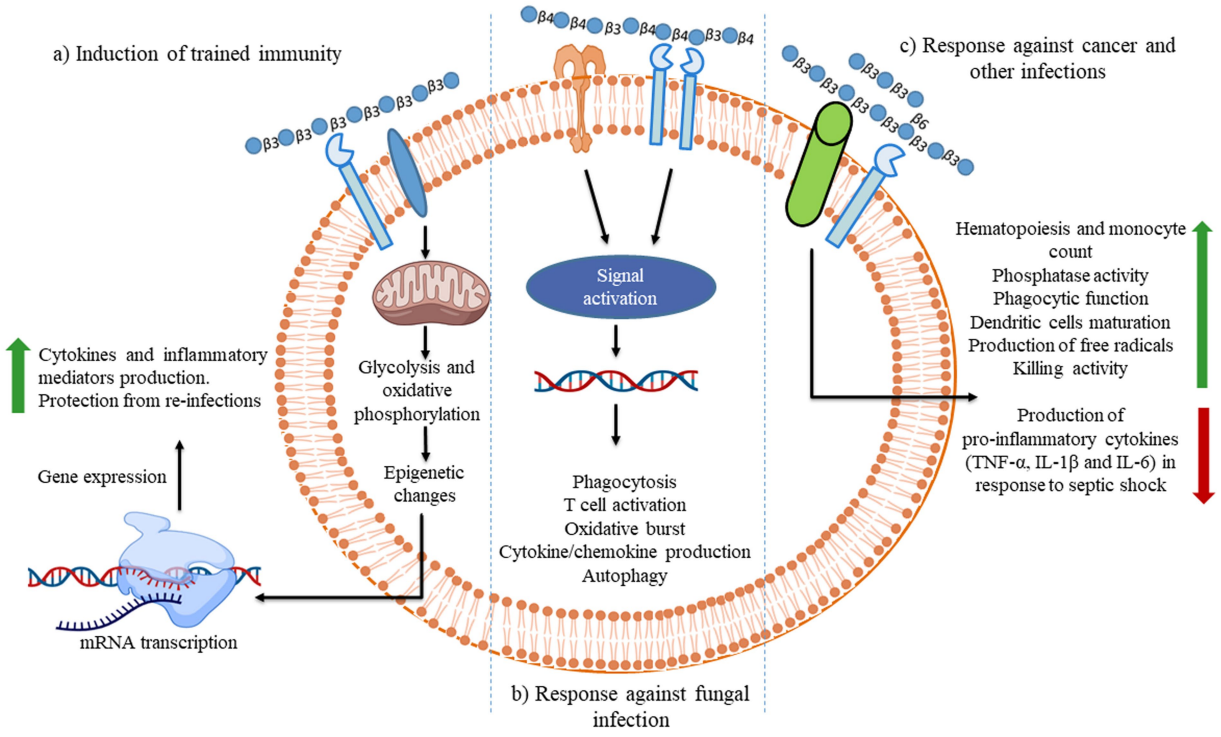

FIGURE 3
Illustration of the different consequences of *β*-glucan recognition by immune cells in the context of fungal infection. The recognition of different *β*-glucans triggers innate immune memory.

immunological responses that trigger through receptors are summarized below as demonstrated in Figures 3–5.

4.1. *β*-Glucan receptors

Pattern recognition receptors (PRRs) are the typical cell surface receptor possessed by immune cells, including macrophages and dendritic cells that recognize PAMPs and other naturally occurring ligands, such as *β*-glucans (156). Dectin-1 and toll-like receptor (TLR) are major PRRs for *β*-glucans (157). Several receptors, such as CR3, scavenger receptors (SR), Dectin-1, the TLR, and lactosylceramide (LacCer) are involved in recognizing *β*-glucans. When these receptors connect to *β*-glucans, a signaling cascade activates immune cells (158).

4.1.1. Dectin-1

It is a type II trans-membrane protein receptor (C-type lectin receptors, CLRs), and its structure consists of four parts such as (1) a carbohydrate recognition domain, (2) a single trans-membrane region, (3) a short stalk region, and (4) a cytoplasmic tail consisted of immunoreceptor tyrosine-based activation motif (ITAM) (159–161). It is expressed in macrophage, dendritic, and neutrophil cells, which are responsible for an innate immune response (149, 162). Dectin-1 recognizes explicitly and binds *β*-(1,3) and *β*-(1,6) glucans from bacteria, seaweeds, fungi, and plants (142, 160, 163). The binding of Dectin-1 with *β*-glucans can start and control the innate immune response (142, 162), such as phagocytosis, inflammatory cytokines production, ROS production, and pro-inflammatory factors production, leading to the elimination of infectious agents (158, 164, 165). Dectin-1 contains six cysteine residues among 244 amino acids, particularly Trp221 and His223 are situated close to the fourth cysteine residue, which is especially important for *β*-glucan binding (166–168). On the cytoplasmic tail, an ITAM-like motif (YxxI/Lx7YxxL) communicates through the spleen tyrosine kinase (Syk) in cooperation with TLR 2 and 6 (161).

Upon *β*-glucan binding, Src family kinases phosphorylate the tyrosine in the ITAM sequence *via* interacting with Syk's two SH2 domains (Src homology 2) (169). For the enzyme activation, the YxxL sequences must be spaced apart to engage both of the SH2 domains of Syk family kinase (Figure 5). It has been known that Dectin-1 multimerizes upon ligand binding and then provides a binding site for the Syk kinase (170). The recruited Syk activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and CARD9-Bcl10-MALT1 pathways to induce dendritic cell maturation, co-stimulatory molecules, and inflammatory cytokines (171). Additionally, it also promotes Th1 and Th17 responses to arrange immunity to pathogens (172). Ligand-binding Dectin-1 activates phospholipase C*γ* *via* phosphorylation and then activated phospholipase C*γ* generates inositol trisphosphate and diacylglycerol for triggering an intracellular Ca^{2+} flux in dendritic cells (173). Elevated concentration of Ca^{2+} is crucial for secreting IL-2, IL-6, IL-10, IL-12, IL-23, and TNF α. Dectin-1 also modulates the expression of cytokines *via* activating the nuclear factor of activated T cells (NFAT) that regulates IL-2, IL-10, and IL-12 p70 production (174).

A 2.8 Å high-resolution crystal structure of murine Dectin-1 was obtained with a laminaritriose and revealed higher order complex formation between Dectin-1 and *β*-glucans (142). It comprises two antiparallel β-sheets and two α-helices with domain integrity

maintained by three disulfide bridges. It has been postulated that hydrophobic contacts might play a key role in *β*-glucan binding (142, 175). Alanine mutations confirmed that Trp221 and His223 at the surface groove are critical in the formation of the *β*-glucan binding site on Dectin-1, and this site finds to be conserved among Dectin-1 of murine, chimpanzee, rhesus monkey, cow, and humans (168). It was further proposed that a minimum length of the ligand should be 10 to 11 of *β*-linked glucose residues (163), and Dectin-1–*β*-glucan complex might get more robust in the presence of divalent ions (142). Takano et al. (176) observed that low-valency β-glucan (such as fucan, a seaweed) only activates human Dectin-1 but not murine Dectin-1, and this specificity is determined by intracellular domain rather than a ligand-binding domain. Therefore, a complex structure of β-glucan can activate both types of Dectin-1.

A previous study by Brown, O'Callaghan (142) theoretically suggested that CTLD of Dectin-1 undergo oligomerization and form a quaternary structure when ligand binding to CTLD. It was proposed based on the Syk kinase's binding to the cytoplasmic parts of two nearby Dectin-1 monomers as part of a signaling pathway. Dulal et al. (177) further reinforced this evidence by demonstrating laminarin binding. The study observed that it forms a tetramer of CTLD when four laminarin molecules bound to four CTLD cooperatively. The formation of oligomerization seems to be physiologically relevant in triggering intracellular signaling. This formation is quite appropriate for eliminating fungal pathogens through phagocytosis and triggering a pro-inflammatory immune response. This reckoning can be used for the rational design of *β*-glucans-based immunomodulatory therapy.

4.1.2. TLRs

TLRs are the vital mediators of inflammatory pathways in the gut that play a major role in orchestrating the immune responses to a wide range of PAMPs and the link between innate immunity and adaptive immunity. TLRs are the type I transmembrane receptors that belong to glycoproteins. TLRs have three domains as follows: (A) an intracellular Toll-interleukin 1 receptor (TIR) domain, which is essential for downstream signal transduction, (B) a single transmembrane domain, and (3) an extracellular domain (consisting of leucine-rich repeats) that recognizes specific PAMPs (178). They are present in dendritic cells, endothelial cells, macrophages, B cells, and T cells. Microbes such as bacteria, fungi, viruses, and protozoa can get recognized by TLRs (179). The ligand-receptor binding activates several signaling pathways, including TRIF-mediated and MyD88-mediated signaling that are associated with the recruitment of neutrophils through fast mobilization (180). TRIF-mediated and MyD88 signaling also cause NF-κB activation and MAPK signaling (131, 181). NF-κB is a predominant transcription factor, which is intricate in the TLR-mediated production of cytokines (Figure 4).

β-Glucans modulate the signaling of TLR2 and TLR4 (182). It was found that *β*-glucans suppressed TNF-α and IL-6 production by microglia *via* binding to Dectin-1 (183). Zymosan binding with Dectin-1 enhances TLR2/4/6-mediated production of IL-10, TNF-α, and ROS through NF-κB signaling from macrophage and dendritic cells (182). Dectin-1 activation by particulate *β*-glucans was shown to promote the production of pro-inflammatory cytokines IL-6, IL-8, and TNF-α in THP-1 macrophages *via* co-binding with TLR2 and TLR4 (184), as also shown in Figure 4. The study also suggested that particulate *β*-glucan exhibited a stronger immune response than soluble. In addition to zymosan, barley-*β*-glucan is also interacted with TLR2 and Dectin-1 and induces inflammatory responses in *Leishmania donovani*-infected macrophages (158). Thus, these studies demonstrated that *β*-glucans are an immune regulatory ligand for TLR2 and TLR4 and can be manipulated in the clearance of pathogens.

4.1.3. Scavenger receptor

They are a family of proteins with a variety of structural variations and various biological activities. SRs are expressed on endothelial, epithelial, and myeloid cells (185). SR is classified into classes A, B, D, E, F, G, H, and I based on their structures (186). Numerous ligands including HDL (187), LDL (188), selected polyanionic compounds of microorganisms (189), and *β*-glucan recognized by SR (190). These receptors were initially described as mediating cholesterol uptake in cultured macrophages but can be reprogrammed to kill tumor cells (191, 192). Yeast *β*-glucan can be recognized by SR type A and increased their uptakes in macrophages (J774 cells) (193). Kim (194) studied SR type B1 for phagocytosis of *Coriolus versicolor* and observed that SR-B1 is not mandatory for uptaking this fungus. The binding of *β*-glucan by SR affects the polarization of adaptive immune responses; however, the proper mechanism of recognizing *β*-glucan by SR has to be known yet.

4.1.4. Lactosylceramide

Lactosylceramide (LacCer) (CDw17 and Gal4Glc1Cer) is highly expressed on the plasma membranes of human neutrophils and indispensable for many cellular processes, including innate immune functions, as they act as PRR (195). It comprises a hydrophobic ceramide and a hydrophilic sugar moiety. LacCer recognizes numerous microorganisms and pathogens, including fungi such as *Saccharomyces cerevisiae, Candida albicans,* and *Cryptococcus neoformans* (196, 197). It is also identified as a *β*-glucan receptor (198). *β*-glucans isolated from *Candida albicans* encourage chemotaxis of neutrophils through LacCer-enriched microdomains (197). Under *in vitro* circumstances, the interaction of LacCer with *β*-glucan caused various cellular responses (199). *Pneumocystis carinii* isolated *β*-glucan can induce the production of macrophage inflammatory protein-2 and TNF-α *via* NF-κB and PKC signaling pathways in alveolar neutrophils (200). It can also enhance anti-microbial properties by increasing myeloid progenitor proliferation and the neutrophil oxidative burst response (131). CDw17 can bind with *β*-glucan of *Candida* and promotes their non-opsonized phagocytosis through neutrophils (201). Overall, LacCer plays a vital role in the protection against fungal pathogens.

4.1.5. CR3

Activated CR3 (also called CD11b/CD18) mediates another mechanism of *β*-glucan. They are exclusively expressed in natural killer (NK) cells, macrophages, and neutrophils (202). CR3 is the major receptor on human neutrophils for *β*-glucan (202). The two chains that make up the heterodimeric transmembrane integrin CR3 are CD11b (α m) and CD18 (β2). CD11b contains two binding sites in which the C terminus of CD11b contains a binding site for *β*-glucan, while iC3b (cleaved component 3 fragment of serum complement system) attaches within the N-terminus of it (203). CR3 is peculiar among other integrins in consisting of a lectin-like domain that binds *β*-glucan of the fungal pathogen and assists as the central receptor for reckoning fungal pathogens by human granulocytes. When *β*-glucan binds to the C-terminal lectin-binding domain, it increases adherence

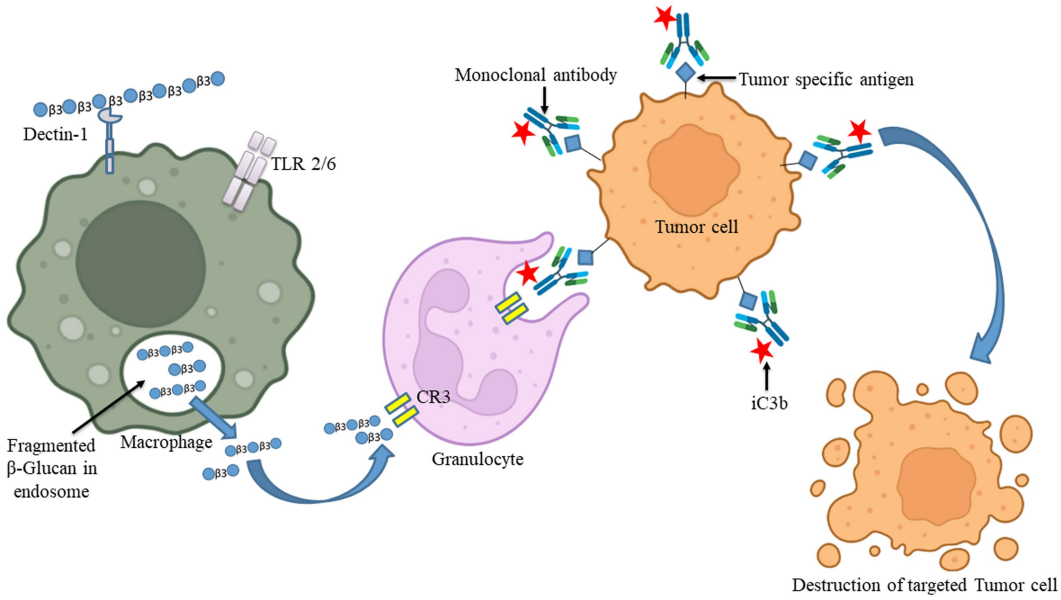

FIGURE 4
The uptake of β-glucan polysaccharides by macrophages and subsequent actions of β-glucan- oligosaccharides on immune cells. β-glucans are captured by macrophages through Dectin-1/TLR-2/6. The polysaccharides form of β-glucans gets internalized by the macrophages. Afterward, those are fragmented into oligosaccharides, which are subsequently released from macrophages. The circulating granulocytes eventually take these oligosaccharides by the complement receptor (CR)-3. The immune response will then be turned on and will be released by several monoclonal antibodies. Those released monoclonal antibodies have destroyed monoclonal antibody-tagged tumor cells. Images were prepared in BioRender.

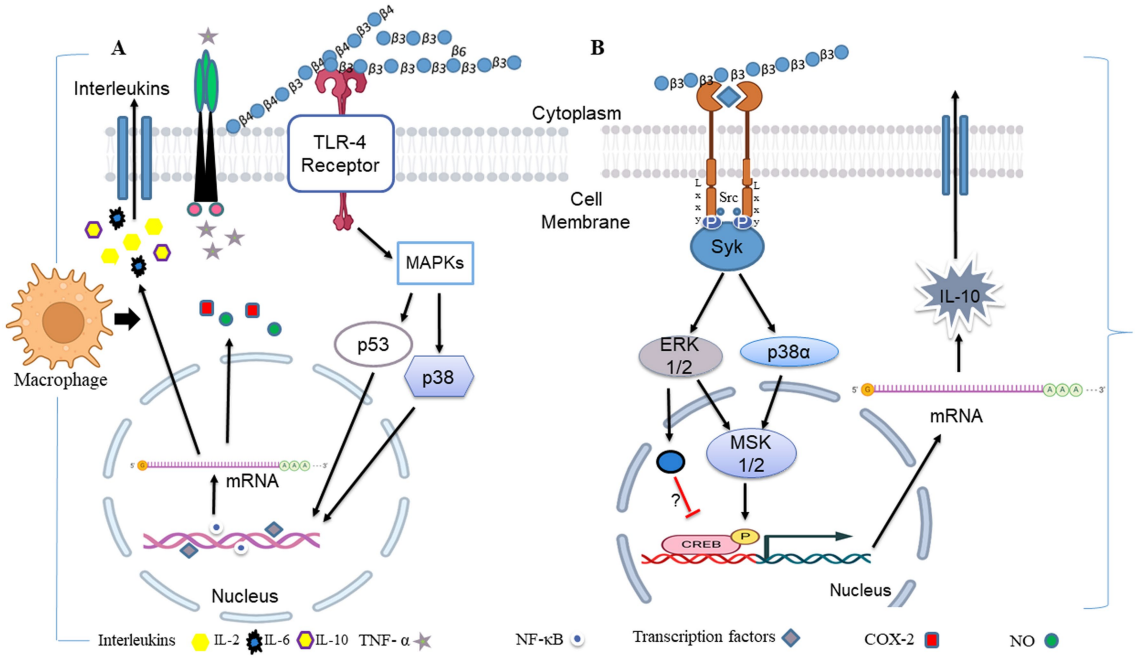

FIGURE 5
During fungal pathogen infection, the innate immune system recognizes fungal β-glucans as pathogen-associated molecular patterns through Dectin-1 and toll-like receptor 4. **(A)** Dectin-1 activation by zymosan was shown to promote the production of pro-inflammatory cytokines IL-6, IL-8 and TNF-α and anti-inflammatory IL-10 in macrophages *via* co-binding with TLR 2/4 through NF-κB signaling. **(B)** Dectin-1 receptor forms clustering when it binds with β-glucans and subsequently forms a phagocytic synapse. It allows Syk to bind at ITAM like the cytoplasmic domain of dectin-1. It then activates downstream signaling, including p38α MAPK and the ERK1/2 cascades. Both p38α and ERK1/2 phosphorylate and activate the protein kinases (MSK1 and 2). MSK1 and 2 switches on CREB through phosphorylation on the IL-10 gene promoter that promotes IL-10 mRNA transcription. In addition, ERK1/2 also inhibits IL-10 mRNA transcription *via* MSK and p38 independent pathways, although this mechanism is not precisely known yet (154). Dectin-1 also activates NF-κB, which inducts IL-2, 6, and 10 transcriptions. Produced cytokines activate monocytes and circulatory cytotoxic T-cells (113). Activates circulatory cytotoxic T-cells to destroy cancerous and fungal pathogenic cells through MAPKs signaling and p53 (155).

of microbial cells and activates iC3b pathways that cause tumor cytotoxicity (204). Additionally, numerous cellular responses including adhesion, cytotoxicity, phagocytosis, and migration (205) mediate upon ligand and CR3 binding (206). The CR3-containing neutrophil and circulating cells have stimulated by β-glucans that cause cell lysis on iC3b-coated tumor cells (207). Thus, CR3 may provide an alternative way for developing therapeutic β-glucans for the clearance of tumor cells and fungal pathogens. Interestingly, CR3 is also recognized in low MW (1,3)-β-glucans, generated from high MW (1,3)-β-glucans through the actions of macrophages and other cells. CR3 was initially anticipated to be the main (1,3)-β-glucan receptor on leukocytes but the ability of CR3-deficient leukocytes to still reckon and respond to (1,3)-β-glucans and the discovery of Dectin-1 suggests that CR3 may only display a minor role for macrophage and dendritic cells (reference herein).

Based on immunological studies, β-glucans considered active compounds to induce immune effects and initiate anti-microbial immune responses and anti-tumor activities. β-glucans emerged as an effective immunomodulatory as it acts on various immunological receptors, namely, Dectin-1, CR3, LacCer, SR, and TLR-2/6. It triggers immune cells such as macrophages, neutrophils, dendritic cells, monocytes, and natural killer cells. These results induce several immune reactions against the pathogen, such as phagocytosis, inflammatory cytokines production, ROS production, and pro-inflammatory factors production. Overall, these lead to the elimination of infectious agents. Thus, β-glucans are essential in controlling the host's immunity, resulting in a healthy individual.

5. Biological application of β-glucans

5.1. β-Glucan impacts epithelial integrity *via* gut microbiota

The gut microbiota impacts epithelial homeostasis and is known to encourage epithelial integrity and proliferation. The integrated relationship of gut microbial communities provides the host with structural, metabolic, and protective functions, necessary for sustenance. *In vitro* study found that the fermentation of barley and oat β-glucan by human fecal samples show variations in SCFAs production and the bacterial populations of *Clostridium histolyticum* and the ratio of *Bacteroides–Prevotella* species (88, 208, 209). Absorption of these SCFAs by the gut epithelial cells helps in regulating cell differentiation, proliferation, apoptosis, and gene expression (210). Butyrate increases the protein expression of tight junctions such as ZO-1 and claudin-1, resulting in enhanced intestinal barrier function (211).

5.2. β- Glucan lowers the level of cholesterol

The effects of β-glucans in reducing cholesterol are widely accepted. The soluble β-glucans help in various activities such as lowering the total level of low-density lipoprotein (LDL), preventing the transit of triglycerides and cholesterol across the gut (Table 3), and prolonging gastric emptying by forming viscous solutions (227). Diet enhanced with β-glucan-rich grain affirmed the hypocholesterolemic impacts of glucans in the broiler chicks (228). A high-fat meal was used to increase the production of β-glucans from the *Aureobasidium pullulans* in the hamster experimental animal model of hyperlipidemia (212). A subsequent study showed that glucan lowered triglyceride levels, total cholesterol by 32% and malondialdehyde levels by 45% (229). LDL and total cholesterol levels considerably decreased when Granoro's Cuore Mio pasta was supplemented with barley-β-glucans (3 g/100 g) (230). Supplementing with oat β-glucans decreased the amounts of LDL and very LDL by 25–31% and 0.2–2.3%, respectively. It also reduced total cholesterol and triglyceride levels and increased the high-density lipoprotein, HDL (231). Oat β-glucans lower cholesterol through gut microbiota by producing SCFA, particularly propionate. As the ratio of propionate to acetic acid (the primary substrate for cholesterol production) rises, the rate of cholesterol biosynthesis declines (232). Concerning this, an intriguing study has highlighted that in Caco-2/TC-7 enterocytes, propionic and butyric acids decreased the mRNA levels of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-Co-A), the rate-limiting enzyme of cholesterol production (233).

5.3. β-Glucans are effective cardio protectors through gut microbiota

Cardiovascular disease (CVD) is proven to increase drastically globally and is one of the leading causes of death. The pathogenesis of CVD is heavily influenced by microbial dysbiosis (234). Microbial communities control CVD and atherosclerosis by regulating the production of trimethylamine *N*-oxide (TMAO) (43). Trimethylamine is a precursor of TMAO, which is formed in the gut *via* peculiar bacterial choline trimethylamine (TMA) lyases. Specifically, TMA moieties (such as choline, phosphatidylcholine, and L-carnitine) containing fatty acids are converted to TMA by bacterial TMA lyase *via* various metabolic pathways (235). Formed TMA is transported to the liver, where it is converted into TMAO by hepatic flavin monooxygenase 3, FMO3 (236). A previous study showed that oat-β-glucan promotes the expansion of *the Verrucomicrobia* population (such as *Akkermansia muciniphila*), which has a prebiotic impact on alterations in circulatory lipids and decreases the number of plaques in the aortic walls as compared with simvastatin (is an oral antilipemic agent) (213). In addition, oral administration of live *A. muciniphila* minimizes the expansion of atherosclerotic lesion formation and systemic inflammation in the aortic as well as enhanced intestinal integrity in atherosclerotic Apoe$^{-/-}$ mice (213). Plovier, Everard (237) used pasteurized *A. muciniphila* in mice model experiments and observed that their administration could suppress HFD-induced expression of FMO3 compared with control diet-fed mice. This evidence specifies that *A. muciniphila* protects against CVD development in live or pasteurized conditions. The *Firmicutes* to *Bactereoidetes* ratio is significantly higher in persons at risk for cardiovascular disease, in which *Prevotella* and *Klebsiella* species are abundant in assessing the fecal microbiota of atherosclerotic CVD patients (238). Overall, it has been highlighted that maintaining gut microbiota, especially *A. muciniphila* population, is essential for mitigating CVD by taking an adequate amount of β-glucan as a dietary supplement.

TABLE 3 Studies for evaluating impact of β-glucan on colorectal cancer, diabetes mellitus, cholesterol, epithelial integrity, and inflammation.

β-Glucan	Model system	Impact	References
In vivo studies			
Polycan (*Aureobasidium pullulans*)	Male hamsters (age, 7 weeks)	Decreases HFD-induced hyperglycemia and associated atherosclerosis, with relatively good protective effects on liver damage.	Lim et al. (212)
Oat-β-glucan	Clinical study in patients	Increases *Akkermansia muciniphila* and leads to reduction of total cholesterol	Li et al. (213)
Barley-β-glucan	Adult male Sprague–Dawley rats	Reduction in colon inflammation	Kopiasz et al. (214)
Yeast β-glucans	Male C57BL/6J mice	Improves insulin sensitivity and hepatic lipid metabolism	Mitchelson et al. (215)
Glucooligosaccharides (GOS)-supplemented HFD	C57Bl/6 male mice	Change in gut microbiota population that shows an essential role of GOS in controlling diabetic metabolic phenotype.	Serino et al. (216)
Barley-β-glucan	50 healthy subjects without a prior diagnosis of diabetes mellitus were included. Out of them, 44 were completers who were administered beverages containing placebo (control), a lower dose (3 g/d), or a higher dose (6 g/d) of reduced viscosity barley-β-glucan extract.	Improve insulin sensitivity among hyperglycemic individuals with no prior diagnosis of diabetes mellitus.	Bays et al. (217)
Oat-β-glucan	A total of 100 free-living hypercholesterolemia subjects were locally recruited and 89 completed the study.	Increases the population of *Bacteroides–Prevotella* species and propionate and butyrate ratio during *in vitro* studies. It improves insulin levels and maintains glucose homeostasis.	Biorklund et al. (218)
Oat β-glucans	A total of 16 male, well-controlled type 2 diabetes patients	A greater increase in HDL cholesterol and larger decreases in the hemoglobin A1c, weight, and body mass index were found.	Reyna et al. (219)
6% oat-β-glucan concentrate	Pig	Significant decreases in glucose levels and increases in the levels of SCFAs and insulin.	Braaten et al. (220)
β-glucan	A total of 19 adult females and males.	It reduces DNA damage substantially in colorectal cancer patients and shows anti-mutagenic effects.	Benlier et al. (221)
β-glucan	Mice	It significantly decreased the TNF-α level and down-regulated three genes (*hmgcs2*, *fabp2*, and *gpt*) that are associated with inflammation and cancer. It increases the relative abundance of *Parabacteroides*.	Qi et al. (222)
Lentinan	Clinical study in patients	Increases host defense mechanisms against murine and human tumors. Induces the production of IL-12 and the binding ability of peripheral blood monocytes. Shows a positive effect on long-term survival and the improving quality of life status.	Hazama et al. (223)

(Continued)

TABLE 3 (Continued)

β-Glucan	Model system	Impact	References
Aminated β-1,3-D-glucan and interferon-gamma	Syngeneic mice	Inhibited the growth of liver metastases significantly.	Sveinbjornsson et al. (224)
β-glucans (paramylon and its isomer amorphous paramylon)	Mice	Shows preventive effects against colon cancer.	Watanabe et al. (225)
In vitro studies			
Oat-β-glucan	Caco-2 cell line and HT29-MTX-E12 cell line	Increases 28% butyrate production that promotes the tightness of the gut barrier.	Pham et al. (36)
Barley-β-glucan	Caco-2 cells and dendritic cells	Reduction of proinflammatory markers in the colon	Bermudez-Brito et al. (226)

5.4. β-Glucan can regulate type 2 diabetes by promoting gut microbes

Type 2 diabetes (T2D) is a metabolic disorder that is categorized by hyperglycemia resulting from failings in insulin secretion from β cells of the pancreas and insufficient insulin action. This is typically characterized by symptoms such as polyuria, polyphagia, polydipsia, and weight loss (239). T2D links to a modified gut microbial population that exhibits less diversity and resilience (240).

β cells are responsible for insulin production, and produced insulin is stored in secretory granules. High glucose concentrations in the blood mainly trigger insulin release; however, it can also induce by the availability of fatty acids and amino acids in the blood (241). A solute carrier protein called glucose transporter 2 (GLUT2) primarily serves as a glucose sensor for β cells in rodents (242), while GLUT1 is suggested to take a significant role in glucose uptaking by many cells in humans including β cells (243). When circulating glucose level increases, β cells mostly absorb glucose *via* the GLUT2 (244, 245). The glucose catabolism is activated when the glucose enters into β cells. Cytoplasmic glucose immediately converts into phosphorylated glucose and enters into a glycolysis cycle to produce pyruvate. Pyruvate transports to mitochondria, which processes in the Krebs cycle to generate ATP. It increases the intracellular ATP/ADP ratio, stimulating the plasma membrane's ATP-dependent potassium channels to close. It causes the membrane to depolarize and the voltage-dependent Ca^{2+} channels to open, letting Ca^{2+} into the cell. The increased intracellular Ca^{2+} causes the secretory insulin-containing granules to prime and fuse to the plasma membrane, leading to insulin exocytosis (246). Additionally, ryanodine receptors (RYR), primarily associated with the endoplasmic reticulum and on the secretory vesicles, can amplify Ca^{2+} signals and are involved in increasing the secretion of insulin when the channel is sensitized by the influx of messenger molecules (247, 248). Such a process is called Ca^{2+}-induced Ca^{2+} release (CICR). Many glycolytic intermediates, such as ATP, cAMP, cyclic ADP ribose, nitric oxide (NO), long-chain acyl CoA, and high luminal Ca^{2+} concentration, have been shown to sensitize RY receptors (248). Perhaps, the most significant messenger promoting insulin released is cAMP, thereby increasing intracellular Ca^{2+} concentration (249, 250).

Chronic hyperglycemia and hyperlipidemia are vital causative factors for T2D, disrupting endoplasmic reticulum homeostasis to induce unfolded protein response (UPR) activation. If homeostasis cannot revert to customary conditions, the ER recruits death signaling pathways, which leads to β-cell death (251). High levels of saturated free fatty acids can cause ER stress that activates the UPR pathway by various mechanisms, such as inhibition of the enzyme that mobilizes ER Ca^{2+} (i.e., ER Ca^{2+} ATPase), activation of IP3 receptors, and/or directly impairing ER homeostasis (241). During high blood glucose levels, proinsulin biosynthesis and islet amyloid polypeptides (IAAP) are significantly increased in β cells. These abrupt changes in glucose levels lead to the accumulation of misfolded insulin and IAAP in β cells. It ultimately increases the production of oxidative protein folding-mediated reactive oxygen species (ROS) (251). Therefore, physiological ER Ca^{2+} mobilization gets altered by these effects, which favors the degradation of proinsulin mRNA and pro-apoptotic signals. ROS promotes releasing of interleukin (IL)-1, which attracts macrophages and intensifies local islet inflammation (252).

Reduction in SCFAs synthesis due to intestinal dysbiosis encourages pancreatic β-cell proliferation, insulin production, and glucose tolerance, showing that these impacts are dependent on short-chain fatty acid receptors FFA2 and FFA3 in mouse model system (253). Synthesis of additional metabolites, including TMA and branched amino acids, can also cause dysbiosis, disrupt glucose homeostasis, and trigger the development of T2D (241). A study including 277 non-diabetic Danish people discovered that the human gut microbiome populations have an effect on serum metabolome and are linked to insulin resistance (254). Butyrate-producing bacteria having anti-inflammatory properties such as *Clostridium*, *Roseburia*, and *Faecalibacterium* species, have reduced significantly in T2D patients, while the population of gram-negative bacteria, such as *Escherichia,* increases high levels of lipopolysaccharides, LPS. LPSs are responsible for low-grade inflammation, causing glucose metabolism abnormalities in T2D patients (255). *Prevotella copri* and *Bacteroides vulgatus* were found to be the primary species driving the relationship between the branched-chain amino acids (BCAAs) biosynthesis and insulin resistance in a Danish cohort of non-diabetic males. The study further stated that *P. copri* can cause insulin resistance, exacerbate glucose intolerance, and increase mouse circulating BCAA levels (254, 256). It suggests that intestinal microbiota could be an essential resource for increased levels of BCAAs and display a key role in insulin resistance.

Through SCFA receptor GPR43, the gut bacteria inhibit insulin-mediated fat storage. In particular, SCFA-mediated activation of GPR43 in adipocytes reduces insulin signaling, resulting in the prevention of fat accumulation and an increase in the metabolism of lipids and glucose (257). Pigs given 6% oat β-glucan significantly reduced blood glucose levels and increased insulin and SCFA levels (220). Products high in β-glucan can lower glucose levels and insulin responses more than those low in dietary fiber (217). The C57BI/6 mouse was fed with β-glucan and observed that they were evolved to have a diabetic metabolic phenotype despite possessing the same genetic determinants, suggesting that the alteration in the gut microbiota population may be a significant factor in the development of diabetic metabolic phenotype (258). β-glucans can play an essential role in increasing the viscosity of a meal during digestion in the intestine, slowing down gastric emptying, limiting the absorption of macronutrients, and entrapping cholesterol and bile acids (259). Thus, β-glucans lower cholesterol and serum sugar levels in T2D (Table 3).

5.5. β-Glucans can prevent colon cancer by modulating gut microbiota

Dysbiosis in the gut microbiota causes human colorectal cancer (CRC). CRC is the third most common type of cancer with about 2 million new cases every year, and the gut microbiome can modulate a crucial role in their progression or prevention (260). The presence of healthy or altered gut microbiomes determines the formation and progression of CRC (261). The altered gut microbiome during dysbiosis negatively impacts CRC treatments with chemotherapy and immunotherapy (262, 263). A few bacteria, namely, *Bacteroides fragilis, Fusobacterium nucleatum*, *Parvimonas micra*, *Porphyromonas asaccharolytica,* and *Prevotella intermedia*, are suggested to associate with CRC conditions (264). These bacteria can cause initial inflammation and modulate different signaling pathways for the progression of CRC (265–267). The biological action of gut microbiota interrupts the control of the cell cycle by generating genotoxins which may lead to oxidative stress and a chronic inflammatory state (268). Bacterial metabolites, such as SCFAs, can also suppress the development of CRC. Among other SCFAs, butyrate is considered an essential metabolite and plays a vital role in inhibiting colon cancer because of its capacity to renew the intestinal epithelial cells (267, 269). It improves the tight junction of the epithelial cells, thereby minimizing the translocation of bacteria and metabolites in lamina propria that trigger inflammation (270). In response to dietary intake of fiber-rich foods, species from the *Lachnospiraceae, Bifidobacteriaceae,* and *Ruminococcaceae* families produce butyrate, lowering the risk of CRC. Butyrate can reduce tumors through a variety of mechanisms, including apoptosis induction, epigenetic alteration in gene expression, reduction of cell proliferation, and manipulation of cytokine levels and inflammatory responses during *in vitro* studies (271, 272). In particular, Donohoe, Collins (273) decisively demonstrated that due to undergoing the Warburg effect, colon cancerous cells primarily rely on uptaking glucose instead of butyrate as a primary carbon source for the production of lactate. Because of that effect, butyrate continuously collects in the cells and at certain physiological concentrations, it acts as an inhibitor of histone deacetylases, leading to the death of the cancerous cells. In addition to butyrate, bacteriocins produced by gut bacteria can prevent CRC through their cytotoxic activities as it was demonstrated by clinical studies (274). Phenylpropanoid-derived metabolites are also associated with the prevention of CRC (275).

Dietary non-digestible carbohydrates enhance the protection against CRC (276). β-glucans, such as lentinan, schizophyllan, scleroglucan, and grifolan extracted from mushrooms, have been studied for controlling CRC *via* modulation of gut microbiota and regulation of immune genes (223, 277) (Table 3). β-glucans reduce the risk of CRC bt activating leukocytes, synthesizing anti-inflammatory cytokines, and activating immune cells (Figure 3). β-glucans were found to be an immunomodulatory agent and can be beneficial for breast cancer patients as a supplemental or adjuvant therapy (278, 279). β-glucans had less impact on white blood cells, significantly reducing the level of IL-4 in breast cancer patients, while IFN-γ and β-glucans together have completely stopped liver metastasis from growing cancerous cells (224). The frequently used chemotherapeutic medicines to prevent liver metastases are 5-fluorouracil and mitomycin. These performed better when used in association with lentinan (a β-glucan) as compared with what they did when used separately. Thus, a better understanding of the roles of β-glucans in preventing cancer at the mechanism level would be helpful in developing nutraceutical therapy.

6. Conclusion

β-glucan is an essential food ingredient in controlling metabolic dysregulations linked to metabolic syndrome. Nevertheless, the impact of β-glucan is shaped by their dose, style, MW, and glucoside linkage. Given the intimate symbiotic link between the host and the gut microbiota, it is not surprising to see a divergence from the typical microbiota composition (usually referred to as dysbiosis) in a variety of illness states, ranging from chronic GI diseases to neurodevelopmental disorders. Additionally, β-glucans have a very minimal probability of having any unfavorable side effects and are reasonably inexpensive. Human gut bacteria display diverse molecular mechanisms for utilizing those β–glucans and support other bacteria that cannot utilize complex structural β-glucans. The impacts of β-glucan on different diseases, such as cancer, diabetes, cardiovascular, and low immunity, have been examined by several researchers. Notwithstanding, how β-glucan exerts these many biological actions at the defined and molecular levels is still unclear. Perhaps, immunostimulation may be the initial mechanism governing the β-glucan activity. Specifically, binding of β-glucan to certain receptors in cells such as macrophage and dendritic cells can trigger the production of different cytokines, which indirectly activates other immune cells, including T and B cells in *in vivo* setting. The primary method for inhibiting the development of cancer cells and infectious microorganisms in the host may involve systemic immunostimulation. Many β-glucan receptors in macrophages and dendritic cells including Dectin-1 and TLRs are essential for recognizing β-glucans, but the precise signaling pathways that lie downstream from each receptor are unknown. Future research should seek to gather this knowledge to help us to use β-glucans to treat future patients rationally and efficiently.

Author contributions

AB and RS designed this research, collected different articles, wrote, edited, and reviewed the manuscript. Both authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pea hull fiber supplementation does not modulate uremic metabolites in adults receiving hemodialysis: a randomized, double-blind, controlled trial

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Background: Fiber is a potential therapeutic to suppress microbiota-generated uremic molecules. This study aimed to determine if fiber supplementation decreased serum levels of uremic molecules through the modulation of gut microbiota in adults undergoing hemodialysis.

Methods: A randomized, double-blinded, controlled crossover study was conducted. Following a 1-week baseline, participants consumed muffins with added pea hull fiber (PHF) (15g/d) and control muffins daily, each for 4 weeks, separated by a 4-week washout. Blood and stool samples were collected per period. Serum *p*-cresyl sulfate (PCS), indoxyl sulfate (IS), phenylacetylglutamine (PAG), and trimethylamine *N*-oxide (TMAO) were quantified by LC–MS/MS, and fecal microbiota profiled by 16S rRNA gene amplicon sequencing and specific taxa of interest by qPCR. QIIME 2 sample-classifier was used to discover unique microbiota profiles due to the consumption of PHF.

Results: Intake of PHF contributed an additional 9g/d of dietary fiber to the subjects' diet due to compliance. No significant changes from baseline were observed in serum PCS, IS, PAG, or TMAO, or for the relative quantification of *Akkermansia muciniphila, Faecalibacterium prausnitzii, Bifidobacterium,* or *Roseburia,* taxa considered health-enhancing. Dietary protein intake and IS (r=−0.5, p=0.05) and slow transit stool form and PCS (r=0.7, p<0.01) were significantly correlated at baseline. PHF and control periods were not differentiated; however, using machine learning, taxa most distinguishing the microbiota composition during the PHF periods compared to usual diet alone were enriched *Gemmiger*, *Collinsella,* and depleted *Lactobacillus*, *Ruminococcus*, *Coprococcus,* and Mogibacteriaceae.

Conclusion: PHF supplementation did not mitigate serum levels of targeted microbial-generated uremic molecules. Given the high cellulose content, which may be resistant to fermentation, PHF may not exert sufficient effects on microbiota composition to modulate its activity at the dose consumed.

KEYWORDS

microbiota, hemodialysis, uremia, fiber, *p*-cresyl sulfate, indoxyl sulfate, stool form, dietary protein

Introduction

Loss of kidney function leads to an accumulation of uremic molecules, such as *p*-cresyl sulfate (PCS) and indoxyl sulfate (IS), associated with poor quality of life (1) and increased mortality (2). Given that several gut-derived uremic molecules are protein-bound and, thus, dialysis offers limited removal of these toxins, alternative therapies are needed to mitigate uremia. Diet therapy has been proposed, as diets lower in protein and higher in fiber, favoring saccharolytic vs. proteolytic fermentation in the gut (3), mitigate the production of the microbially generated metabolites contributing to uremia (4–6). Whereas protein restriction may diminish uremic toxin levels in the earlier stages of chronic kidney disease (CKD) (7), this is not a viable therapeutic option for the dialysis population due to their increased protein needs (8) and high risk of malnutrition (9). Enhancing fiber intake may be a potential dietary therapy to reduce uremia.

Plant-based diets, providing plenty of diverse fiber substrates for gut microbiota, are increasingly being recommended for the CKD population (10, 11) for the mitigation of inflammation (12, 13) and the reduction of uremic symptoms (14). Although a vegetarian diet has been shown to reduce uremic toxin levels (15), implementing such plant-based diets in the dialysis population is met with the challenges of poor appetite (16–18), food preferences (19), perceptions of dietary restrictions (20, 21), food insecurity (22) and in some cases limited access to fresh foods (23). Additionally, recommending plant-based diets may exacerbate the time, convenience, and financial barriers to dietary change experienced by hemodialysis patients (24). Isolated fibers, which can be easily incorporated into common foods, particularly baked goods, offer alternative sources of fiber for the dialysis population without significant additions of potassium, phosphorus, and sodium – minerals of concern.

Prebiotic fibers, at 9–20 g/day, have shown efficacy for reducing blood levels of uremic toxins such as PCS in some studies (25, 26) but not in others (27, 28). However, highly fermentable prebiotic fibers such as inulin and fructooligosaccharides may contribute to gastrointestinal symptoms (27, 29), mitigate appetite, and enhance satiety (30), which may contribute to the risk of malnutrition in the dialysis population. In contrast, fibers that are resistant to fermentation, such as hull and bran fibers (31) or which are more slowly fermented, such as resistant starches (32), may be better tolerated while still reducing uremia. However, there are limited data to support the efficacy of resistant starch in reducing uremic toxins (33), and intact bran or hull fibers have been unexplored in dialysis patients.

Pea hull fiber (PHF), the finely-ground outer hulls of yellow field peas, is rich in dietary fiber, and bioactive components, including polyphenols (34). PHF, a non-viscous fiber ingredient, consists of soluble and insoluble fibers, primarily cellulose (65%) and pectin (16%), with oligosaccharides, hemicelluloses, and lignin as minor components (34), a profile that suggests reduced fermentability compared to typical prebiotics. However, in older adults, PHF has been shown to modulate fecal microbiota without appetite suppression (35), suggesting it may be fermentable and also an appropriate fiber for hemodialysis patients at nutritional risk. PHF at 10 g/d, in combination with 15 g of inulin, led to lower blood levels of PCS in adults with stage 3 chronic kidney disease (36). PHF may elicit uremic suppressing effects through saccharolytic fermentation if microbially accessible or, if resistent, may decrease colonic transit time through stool bulking, as do bran fibers (37), thereby lessening proteolytic fermentation (38). The primary aim of this study was to determine the effect of PHF supplementation on serum levels of PCS in adults undergoing hemodialysis. The secondary aims were to determine the effect of PHF supplementation on serum levels of IS, phenylacetylglutamine (PAG), and trimethylamine *N*-oxide (TMAO), and fecal microbiota composition. Additionally, to monitor the safety and tolerance to PHF supplementation in the hemodialysis patient population, blood chemistry, gastrointestinal function and symptoms, inflammatory markers, dietary intake, appetite, and quality of life were monitored.

Materials and methods

Study design

A 13-week, randomized, double-blind, controlled crossover study was conducted in adults undergoing hemodialysis and included a one-week baseline, two 4-week interventions (PHF and control), and a 4-week washout period between interventions (Figure 1). At the beginning of each period, pre-dialysis blood and fecal samples were collected, and quality of life, weight, blood pressure, and dietary intake were monitored. Height, body composition, and hand-grip strength were measured at baseline. The study protocol was approved by the Institutional Review Board of the University of Florida (IRB201701457), and the trial was registered at ClinicalTrials.gov (NCT03354364). Written informed consent was obtained from all study participants, and study procedures were in accordance with the Declaration of Helsinki.

Participants

Adults undergoing hemodialysis were recruited from a single center in Florida, United States. Participants were excluded for food allergy, lactation, pregnancy, previous or current treatment for any gastrointestinal disease (gastric ulcers, Crohn's disease, celiac disease, ulcerative colitis, etc.), use of medications for diarrhea or constipation, or non-continuation of any probiotic supplements.

Randomization and intervention

An unaffiliated researcher completed the randomization scheme. Randomization was performed by a sealed envelope method; the study coordinator opened a sealed envelope containing the allocated treatment regimen at the time of randomization.

Study foods

PHF (Best Cooking Pulses; Portage la Prairie, MB, Canada; total dietary fiber 92%; insoluble 85%, soluble 7%) was added to vanilla, lemon, cinnamon, and chocolate chip mini muffins with each muffin providing 5 g of PHF and 70 kcal. A sensory evaluation panel was carried out to test the acceptability of the chocolate chip muffin. Both muffins were rated acceptable; however, the control muffin rated higher for overall liking and texture liking, with no differences for flavor, sweetness liking, or dryness (39). Participants were provided

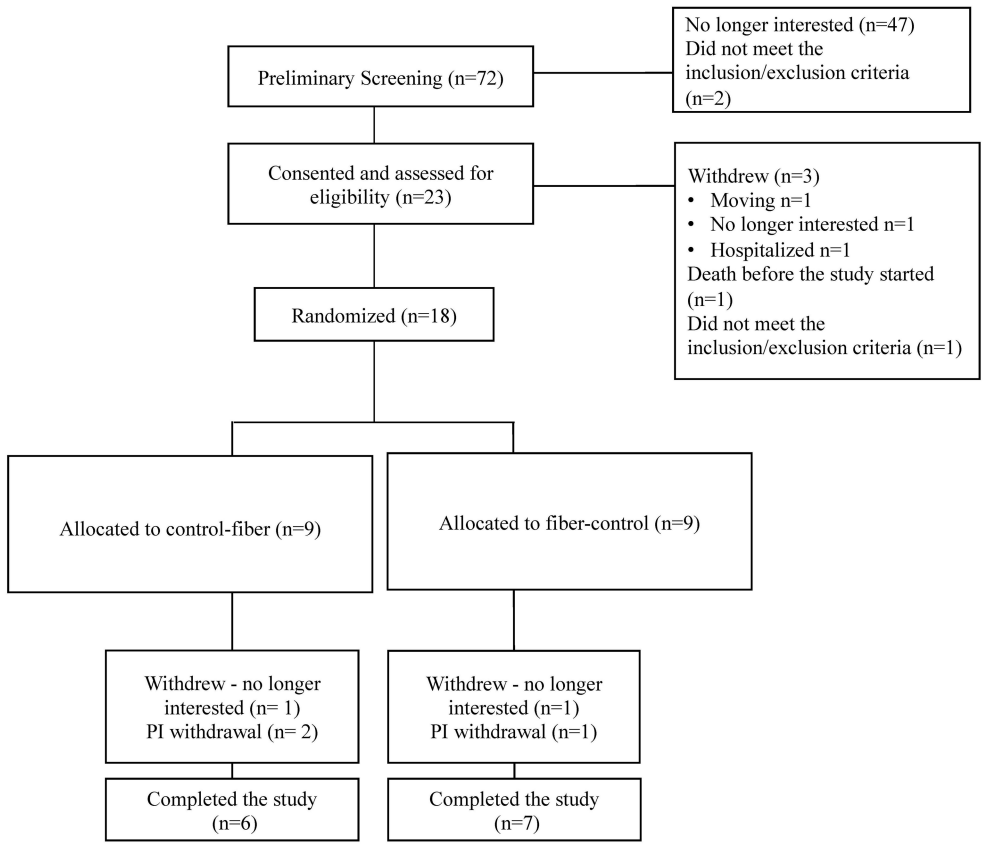

FIGURE 1
Participant recruitment, randomization, and study completion. Principal Investigator (PI) withdrawals due to hospitalizations.

frozen muffins every 2 weeks, asked to thaw and consume three muffins daily (providing ~15 g/day of PHF), and to return any uneaten muffins. Control muffins, also consumed at 3 per day, provided 0.9 g of fiber and 67 kcal per muffin. A daily question in the participants' study diary assessed compliance with muffin intake for the entirety of the intervention period.

Clinical assessments

Post- and pre-dialysis weight in duplicate were measured at each visit, and height was measured at baseline using a flat scale and portable stadiometer (Seca® models 217 and 874, Mount Pleasant, SC), respectively. Blood pressure was measured at each visit using the monitor connected to the hemodialysis machine. At baseline, post-dialysis, hand-grip strength was assessed using the Jamar® Plus+ digital dynamometer (Patterson Medical, Warrenville, IL, United States) (40) and body composition by Bioelectrical Impedance Analysis Xitron Hydra ECF/ICF 4200 equipment (Xitron Technologies, San Diego, CA, United States). In their study diary, participants recorded their daily stool frequency and stool form using Bristol Stool Form Scale (BSFS) (41). Gastrointestinal symptoms were assessed weekly using the Gastrointestinal Symptom Response Scale (GSRS) (42). The Simplified Nutritional Appetite Questionnaire (SNAQ) (43) was assessed weekly. A SNAQ score of >14 of 20 points indicates no risk of weight loss, whereas a score of 14 or less indicates a significant risk of at least 5% weight loss within 6 months. During each period, the KDQOL-36 with its five subscales (Physical Composite, Mental Composite, Burden of Kidney Disease, Symptoms and Problems of Kidney Disease, and Effects of Kidney Disease) was administered, and the total score was calculated using the standardized formulae (range from 0 to 100), with a higher score indicating a better health-related quality of life (44).

Pre-dialysis blood samples were collected at baseline and on the first day of each period via the hemodialysis vascular access. The components of a comprehensive metabolic profile were analyzed by standard laboratory methods. TNFα, IL-6, and IL-10 were measured using the Bio-Plex Pro™ Human Cytokine Standard 27-plex, Group 1 (Lot #64103331) (Bio-Rad Laboratories, Hercules, CA, United States) with the Bio-Plex 200 suspension array system (Bio-Rad Laboratories) and Bio-Plex Manager software (v6.2), according to the manufacturer's instructions. Serum samples were thawed at 4°C overnight and then vortexed and centrifuged; the collected supernatants were transferred into new 1.5 mL tubes. The samples were diluted at 1:4 by using the diluent provided with the kit. The Quick Guide supplied with the kit was followed to finish running the assay.

Dietary assessment

A team of trained dietetic graduate students assessed dietary intake at baseline and the final week of each period. Participants were

typically interviewed for their 24-h dietary recalls on dialysis days, and thus intake generally reflected non-dialysis days. Three 24-h recalls for each period were analyzed for nutrition composition using Food Processor Nutrition Analysis Software (ESHA version 11.3.2). Diet quality at baseline was assessed using the Healthy Eating Index (HEI-2015), which applies the recommendations of the 2015–2020 Dietary Guidelines for Americans as previously described (45).

Quantification of uremic metabolites

The uremic molecules PCS, IS, PAG, and TMAO levels were measured, as were trimethylamine (TMA) and carnitine, to elucidate TMAO metabolism. Ten mL Vacutainer® Plus SST™ serum separation tubes were centrifuged for 10 min at 4°C in a Hettich Instruments ROTINA 420R centrifuge at 800 x g (1,500 rpm). Serum was aliquoted in 250 µL tubes and stored at −80°C until analyzed. PCS and IS were obtained from Alsachim (Illkirch-Graffenstaden, France), and PAG was sourced from LGC (Middlesex, United Kingdom). Carnitine, trimethylamine hydrochloride, TMAO, $_{L}$-carnitine-methyl-d_3 hydrochloride (carnitine-d_3), trimethylamine-d_9-*N*-oxide (TMAO-d_9), and *p*-toluene sulfonic acid sodium salt were sourced from Sigma-Aldrich (St. Louis, MO, United States). Trimethylamine-d_9 hydrochloride (TMA-d_9) was obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). Acetonitrile, water, ammonium formate, and formic acid were sourced from Fisher Scientific (Fair Lawn, NJ, United States) and were of liquid chromatography-mass spectrometry (LC–MS) grade. For standard solutions preparation, stock solutions of individual analytes and internal standards were prepared at a concentration of 1,000 µg/mL in acetonitrile or water-acetonitrile (1:1) and stored at −80°C until used for analysis. Standard mixture solutions used for calibration curves were prepared as follows: for PCS, IS, and PAG analysis, diluting concentrated solutions with water-acetonitrile (1:1), and for carnitine, TMA, and TMAO analysis, diluting concentrated solutions with just acetonitrile.

Following standard preparation, 20 microliters of serum were aliquoted into a 1.5 mL Eppendorf tube and mixed with acetonitrile that contained internal standards (1 µg/mL of *p*-toluene sulfonic acid, 0.75 µg/mL of carnitine-d_3, 0.5 µg/mL of TMA-d_9, and 0.15 µg/mL of TMAO-d_9) to reach a total of 1 mL. Samples were then vigorously vortexed for 10 min, followed by sample centrifuging at 25000 g at 4°C for 5 min. After centrifugation, the supernatants for carnitine, TMA, and TMAO were directly injected into a liquid chromatography with tandem mass spectrometry (LC–MS/MS) system, while the supernatants for PCS, IS, and PAG were first diluted with the same volume of water before they were injected into the LC–MS/MS system. The individual samples were extracted in triplicates ($n = 3$).

The PCS, IS, and PAG quantified in serum were done by LC–MS/MS analyses, applying an Ultimate 3,000 LC system coupled to a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, United States). Separation of PCS, IS, and PAG was performed using a Waters Acquity BEH C18 column (2.1 × 50 mm, particle size 1.7 µm) at a column temperature of 25°C using a gradient elution with 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B). The gradient program was set as follows: 0–3 min 10–50% B, 3–4 min 50–95%, and 4–6 min 95% B. The column was re-equilibrated in 3.5 min using the initial composition of the mobile phase. The injection volume was 2 µL with a flow rate of 0.2 mL/min. The mass spectrometer was equipped with an electrospray ionization (ESI) interface, operating in both positive and negative ionization modes. The spray voltage was set as 3,500 V for positive mode and 2,500 V for negative mode. Other ESI parameters were set as follows: ion transfer tube temperature, 325°C; vaporizer temperature, 275°C; sheath gas, 35 Arb; aux gas, 10 Arb; and sweep gas, 0 Arb. Selective reaction monitoring mode was used when the MS/MS detection was operated. Dwell time was 100 msec, and CID gas was set at 2 mTorr. MS/MS parameters for each analyte (PCS, IS, and PAG) were optimized using flow injection analysis of individual standards. Xcalibur software (Ver. 3.0) was utilized for data processing and instrument control.

Carnitine, TMA, and TMAO were analyzed using the same LC–MS/MS described above, with a different chromatographic system (hydrophilic interaction liquid chromatography, HILIC). These compounds were separated using a Thermo scientific Accucore HILIC column (2.1 × 100 mm, particle size 2.6 µm). The column temperature was maintained at 30°C using gradient elution with 15 mM ammonium formate (pH 3.5) (eluent A) and acetonitrile (eluent B). The gradient program was set as follows: 0–4 min 90–60% B and 4–6 min 60% B. The initial composition of the mobile phase was used to re-equilibrate the column in 4 min. The injection volume was 2 µL with a flow rate of 0.4 mL/min. The mass spectrometer was equipped with an ESI interface, operating in the positive ionization mode. The positive spray voltage was 3,500 V, and the ion transfer tube temperature was maintained at 340°C. The other ESI parameters were set as follows: vaporizer temperature, 350°C; sheath gas, 45 Arb; aux gas, 15 Arb; and sweep gas, 1 Arb. The selective reaction monitoring mode was used to operate the MS/MS detection. Dwell time was 100 msec, and CID gas was set at 2 mTorr. MS/MS parameters for every analyte were adjusted using flow injection analysis of individual standards. Xcalibur software (Ver. 3.0) was used for data processing and instrument control.

Microbiota analyses

Stool collections were made using a plastic container with a lid (Fisherbrand™ Commode Specimen Collection System) during the baseline and the last week of each period. Upon receipt, one sample was homogenized for microbiota analysis, aliquoted into 5 mL tubes, and stored at −80°C. Total DNA was isolated from the 250–350 mg homogenized stool sample, as previously described (46). The QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) was used, and the manufacturer's instructions were followed with the following modifications. Sodium phosphate buffer as two 50 mmol/L was utilized for washing preceding the addition of InhibitEX (Qiagen) and a 0.1-mm zirconia/silica bead beating step (~250–350 mg/tube, 4 m/s for 1 min × 3) following the incubation with InhibitEx. Then, Nanodrop was used to determine the concentration of the DNA. Samples were stored at −20°C until additional analysis. Before the process of qPCR analysis, molecular biology-grade water was used to dilute the samples fivefold.

A previously reported method was followed to analyze gut microbiota composition and diversity (47). In brief, gene-specific primers for the V4 hypervariable region of the 16S ribosomal RNA gene

were used for DNA amplification and tagged with unique identifiers. As previously described (44), for the bacterial 16S ribosomal RNA gene libraries preparation, Illumina's"16S Metagenomic Sequencing Library Preparation" guide (part #15044223 Rev. B) was used. The Qiagen HotStarTaq MasterMix was utilized for the first PCR (amplicon PCR) at 25 cycles with heat at temperatures of 55°C. However, only 50% of the reagent volumes were used for the second (index PCR) PCR. The template-specific primers, 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), flanked with appropriate overhang adapter sequences, were utilized. Samples were diluted, pooled, and sequenced using a 500-cycle MiSeq Reagent Kit v3 before loading on an Illumina MiSeq.

Taxonomic attribution of the amplicon sequence variants (ASVs) was used to generate the taxonomic profiles for each sample using the QIIME™ 2 feature-classifier machine learning-based tool and the database, GreenGenes (47). The taxonomic profiles for each participant in each period were generated on individual taxa and strains. Principal coordinate analysis (PcoA), weighted and unweighted UniFrac, alpha diversity (evenness and faith) profiles, and individual taxonomic profiles were produced and assessed for each participant in each period using QIIME™'s visualization tools. QIIME™ 2 sample-classifier was used to discover any unique microbiota profile that may be determined because of PHF consumption and distinguish the taxa differing in abundance. For each participant, both treatments were compared to the baseline or washout, depending on the period before the treatment.

Given their positive associations with health (48–51), relative quantification of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii, Bifidobacterium,* and *Roseburia* was performed. 16S ribosomal DNA Universal Bacterial primers were used for DNA normalization. The $2^{-\Delta\Delta CT}$ algorithm method was used to analyze the changes in the relative fold gene expression when comparing baseline to the other study periods. The *A. muciniphila* and *F. prausnitzii* primer sequences and assay conditions were obtained from previous studies (52, 53). However, the primers for *Bifidobacterium* were designed in-house as described previously (forward: TGG AAG GTC TCG ATG GAG GT and reverse: CTG GAC AAG CCG TTC CTG AT) and utilized the same assay and cycling conditions as listed for the primers used in strain quantification. A dissociation curve analysis (60°C to 95°C) was also performed to ensure primer specificity for all assays. The epMotion5075tc liquid handling robot and Select SYBR Mastermix (Thermo Fisher Scientific) were used for all qPCR reaction preparations. The analysis was performed on the CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories).

Statistical analysis

The sample size was calculated based on expected PCS change using data from our previous study of fiber supplementation in adults with CKD (54). The expected PCS mean difference was 4 mg/L with a standard deviation of 6 mg/L. Assuming an alpha = 0.05 and power = 0.80 (80%), a sample of 21 was needed. To account for an expected 20% dropout rate, 25 participants were targeted.

Linear mixed models with fixed factors treatment (PHF, Control, Baseline, Washout), sequence (PHF-Control, Control-PHF), and the interaction between the two was used. The interaction tests were for order effects of treatment. The random effects in the model included random ID (participant) and an auto-regressive correlation structure. For uremic molecules analysis, the data were log-transformed for analysis, and variance was allowed to differ between time points. Additional analysis was conducted to investigate the association between some variables and uremic molecules using the Pearson correlation coefficient test. Alpha was set at 0.05. Data are presented as mean ± SE unless otherwise indicated.

Individual symptoms evaluated by the GSRS were averaged into syndrome scores. For the GSRS symptoms analysis, the data were log-transformed for analysis and variance. For BSFS analysis, data were divided into three categories to detect the transit time, following a previously published method (55). Stool forms were grouped as slow transit, types 1 and 2, normal transit, types 3, 4, and 5, and fast transit, types 6 and 7. The frequency procedure was used to detect the frequency of each transit type during the whole study period. Then, the GIMMIX procedure was conducted to examine the effect of treatments on transit time.

The Mann–Whitney test was performed to compare the relative quantification of each treatment group for each type of bacteria (*A. muciniphila, F. prausnitzii, Bifidobacterium,* and *Roseburia*). Kruskal-Wallis test was used to determine the differences between treatments for evenness α-diversity and faith α-diversity. The Quantitative Insight Into Microbial Ecology-2 (QIIME 2) software suite was used as described previously (56) to determine the results for α-diversity and principal component analysis (weighted and unweighted UniFrac). A machine learning model was used to predict PHF vs. control or usual diet (baseline/washout). The accuracy of the results of the Sample Classifier was visualized using a confusion matrix (as it is more informative than the Accuracy Ratio alone), showing the prediction made on the Test Dataset (a subset of ~1/3 of the full dataset) after algorithmic training on the Training Dataset.

Results

Study population

From December 2017 to January 2019, 23 adults undergoing hemodialysis were recruited from a single hemodialysis center in Florida, United States. The study flow is shown in Figure 2. Post-consent inclusion/exclusion criteria excluded one participant; one died before the initiation of the intervention, five participants withdrew, and the principal investigator withdrew three due to hospitalizations. Table 1 presents the baseline characteristics of the study participants. Most participants were non-Hispanic Black and had been undergoing hemodialysis for more than 2 years. Compliance with the interventions was 61.9% for PHF (+ 9 g/d fiber) and 81.6% for the control muffins.

Clinical assessments

Clinical assessments were generally unaffected by the interventions. The baseline hand-grip strength is reported in Table 1. According to the Matos et al. (57) criteria, 27% of study participants exhibited hand-grip strength below the cut point for a higher risk of death, and another 20% fell below the cut point for increased risk of frailty (58). According to the SNAQ score, 67% of the participants were at no risk of weight loss, whereas 33% were at high risk.

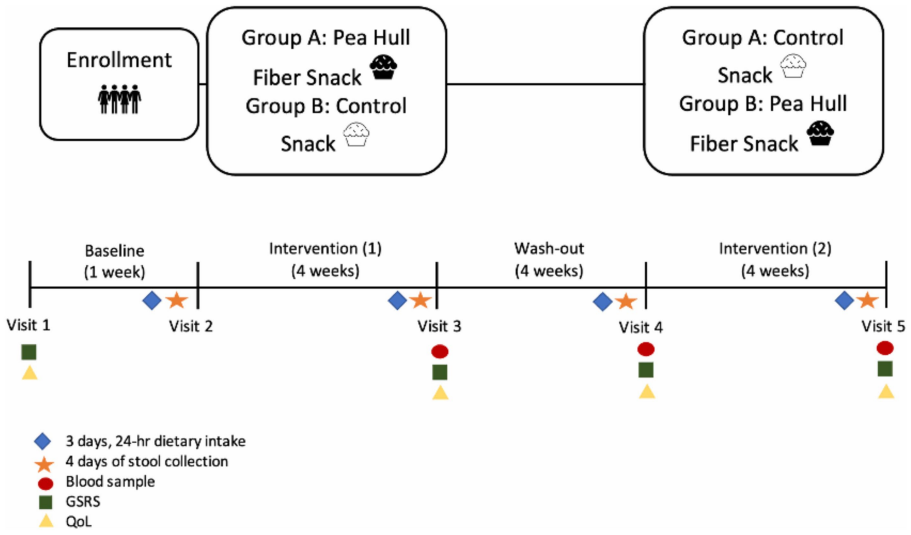

FIGURE 2
Study design for the 13-week double-blinded, randomized, crossover trial investigating the effects of pea hull fiber (PHF) on uremic toxins. GSRS: Gastrointestinal Symptoms Rating Scale. QoL: Kidney Disease Quality of Life 36-Item Short-Form Survey.

Baseline stool frequency (Table 1) was reported at 5.2 ± 0.6 stools per week and differed from PHF (9.8 ± 1.3), control (10.2 ± 1.4), and washout (10.0 ± 1.2) periods ($p < 0.01$). Stool forms at baseline were 30.2% slow transit (BSFS 1 and 2), 39.7% normal transit (BSFS 3–5), and 30.1% fast transit (BSFS 6 and 7) and did not differ with interventions. The baseline GSRS syndrome scores of abdominal pain (1.5 ± 0.2), reflux (1.6 ± 0.2), indigestion (2.0 ± 0.2), diarrhea (1.9 ± 0.2), constipation (1.9 ± 0.3) were unchanged with interventions.

The KDQOL-36 components at baseline (Table 1) of SF-12 Physical Composite (35.1 ± 2.3), SF-12 Mental Composite (50.2 ± 3.1), KDQOL-36 Burden of Kidney Disease (45.5 ± 6.6), KDQOL-36 Symptoms and Problems of Kidney Disease (77.2 ± 3.8), and KDQOL-36 Effects of Kidney Disease (70.8 ± 5.1) showed no differences between periods (data not shown).

Biochemical measurements of the complete metabolic panel at baseline are shown in Table 1; there were no differences between periods (data not shown). Of the targeted cytokines (Table 1), there were no significant differences between study periods for TNF-α. However, for IL-6, the washout was higher compared to other study periods. Very low, out-of-range values for IL-10 precluded statistical analysis.

Dietary intake

Energy, macronutrient, and select mineral intakes of the background diet during each period, shown in Supplementary Table S1, remain unchanged from baseline. At baseline, the mean energy intake of study participants was 1786 ± 143 kcal providing 23.8 kcal/kg body weight. Protein intake at 68 ± 7 g/day (0.9 g/kg body weight) and fiber at 11 ± 1 g/day (6.2 g/1000 kcal) provided a protein-to-fiber ratio of 6 to 1. This ratio was reduced to 3.2 to 1 during the PHF period. Carbohydrate intake averaged 224 ± 19 g/d and total fat, 67 ± 7 g/day. The participants' background diet at baseline was approximately 50% carbohydrate, 15% protein, and 34% fat and showed no significant changes during the study. The mean HEI-15 score was 44.2 ± 2.5 out of the maximum score of 100, indicating poor diet quality. Due to compliance issues, consumption of the study muffins contributed 130 kcal/day during the PHF period and 161 kcal/day during the control period.

Uremic molecules

The results for PCS, the primary outcome, and IS, PAG, and TMAO are presented in Table 2. No significant differences were observed in serum levels of these uremic molecules (individual data pre and post-PHF are shown in Supplementary Figure S1) nor for TMA or carnitine (data not shown). At baseline, there was a negative correlation between protein intake and serum IS level ($r = -0.47$ and $p = 0.05$) and a positive correlation between the number of slow transit stools, as assessed by BSFS, and serum PCS level ($r = 0.68$ and $p < 0.01$).

Microbiota composition

At the genus level, the relative abundance was highest for *Bacteroides* but was very high (30 to 50%) for some participants and near 0% for others (Supplementary Figure S2). No significant differences in alpha diversity or evenness were seen between PHF and control periods (Supplementary Figure S3), nor between treatments for the relative fold change in the relative quantification of *A. muciniphila, F. prausnitzii, Bifidobacterium,* and *Roseburia* by qPCR (Figure 3).

The weighted and unweighted UniFrac PCoA from QIIME™ of the hemodialysis participants' datasets visualized using Emperor according to treatment period showed significant overlap (Supplementary Figure S4). PHF may have influenced the microbiota composition profiles. The confusion matrix showed strong accuracy scores on the main diagonal and a good accuracy result of 83% for the control and an accuracy of 100% for the PHF predictions alone compared to baseline/washout periods. Only six important taxa were used to predict PHF compared to participants' usual diet (baseline +

TABLE 1 Demographic characteristics and biochemical data of participants receiving hemodialysis.

Characteristics		Biochemical	
Gender M/F, *n*	10/8	Albumin, g/dL	4.0 ± 0.1
Age, y, median (range)	52 (21–71)	Albumin/Globulin, (calc)	1.3 ± 0.1
Race, *n* (%)		Anion Gap, mmol/L	130.1 ± 12.2
African American	12 (67)	BUN/Creatinine ratio, (calc)	5.9 ± 0.4
Asian	0	Calcium, mg/dL	9.1 ± 0.2
White	4 (22)	Carbon dioxide, mmol/L	23.4 ± 0.7
More than one race	2 (11)	Chloride, mmol/L	97.6 ± 0.7
Ethnicity, *n* (%)		Creatinine, mg/dL	9.6 ± 0.7
Hispanic	0	eGFR, ml/min/1.73M^2	6.6 ± 0.7
Non-Hispanic	18 (100)	Glucose, mg/dL	139.8 ± 22.3
Dialysis, *n* (%)		Magnesium, mg/dL	2.3 ± 0.1
<1 y	3(17)	Phosphorus, mg/dL	5.7 ± 0.4
1–2 y	2 (11)	Potassium, mmol/L	4.7 ± 0.2
>2 y	13 (72)	Sodium, mmol/L	136.9 ± 0.7
Body Mass Index (BMI), *n* (%)		Total protein, g/dL	7.4 ± 0.2
Underweight (<18.5)	2 (11)	Urea nitrogen, mg/dL	55.0 ± 3.6
Normal (18.5 to 24.9)	8 (44)		
Overweight (25 to 29.9)	3 (17)	Cytokines	
Obese (>30)	5 (28)	IL-10, pg./mL	3.3 ± 1.8
Body Fat (%) mean ± SE	26.4 ± 3.0	IL-6, pg./mL	5.9 ± 1.1
Hand-grip strength, (kg) mean ± SE	31.3 ± 2.7	TNF-α, pg./mL	130.1 ± 14.3

TABLE 2 Serum uremic molecules by period for participants receiving hemodialysis.

Metabolite (μmol/L)	Baseline	Fiber	Control	Washout
p-Cresyl sulfate	3256.3 ± 505.3	3309.7 ± 668.5	2858.8 ± 464.2	3365.5 ± 558.6
Indoxyl sulfate	166.0 ± 23.4	185.4 ± 26.3	148.8 ± 20.8	166.7 ± 27.4
Phenylacetylglutamine	36.4 ± 5.8	33.6 ± 8.1	43.1 ± 8.0	51.2 ± 11.0
Trimethylamine-*N*-oxide	95.9 ± 12.2	128.3 ± 18.7	140.2 ± 52.8	100.0 ± 13.3

Data presented as mean ± SE.

washout), whereas 49 taxa predicted control. PiratePlots of the relative abundance of the six important taxa that predicted PHF consumption include *Coprococcus*, *Lactobacillus*, *Ruminococcus*, *Gemmiger*, *Collinsella*, and the family, Mogibacteriaceae; only *Gemmiger* and *Collinsella* showed higher abundance (Supplementary Figure S5). The algorithm using the QIIME 2 classifier was unable to differentiate between fiber and control; the prediction was lower than by chance. Similarly, no differentiation was seen for intervention (fiber + control) compared to usual diet intake (baseline/washout), suggesting no 'muffin' effect. Of note, no participants reported being prescribed an antibiotic during the study.

Discussion

The study findings confirm that adults undergoing hemodialysis have inadequate intakes of dietary fiber, in agreement with previous reports (59–62). This fiber deficit may be in part due to long-standing dietary recommendations to restrict plant foods higher in phosphorus (whole grains and legumes) and potassium (legumes, fruits, and vegetables). As assessed by the HEI-2015, study participants consumed almost exclusively refined grains and had very low intakes of fruit, a dietary pattern contributing to their dietary fiber deficit. Given the questionable effectiveness of restricting plant foods, with their lower phosphorus bioavailability, to manage serum phosphorus levels (63), a concerted effort may be needed to enhance diet quality, particularly regarding whole-grain intake, and thus increase dietary fiber intake of hemodialysis patients and the CKD population in general. With many lower potassium options available, enhanced fruit and vegetable intake also is needed to improve fiber intake in this population (64). However, as low socioeconomic status is associated with low fruit and vegetable consumption (65) and end-stage kidney disease (66), this may be a challenging long-term approach. In the short term, recommending the replacement of refined grain-based foods with sensorily acceptable, fiber-fortified versions may be a feasible approach to improving the fiber intake of the hemodialysis patient population.

Although in the US, there is no clinical practice guideline for fiber intake for adults undergoing hemodialysis (8), their low fiber intake strongly suggests a lack of microbial-available carbohydrate substrate in the colon, which facilitates uremic molecule generation, particularly in an environment of reduced protein digestion (67). Although the

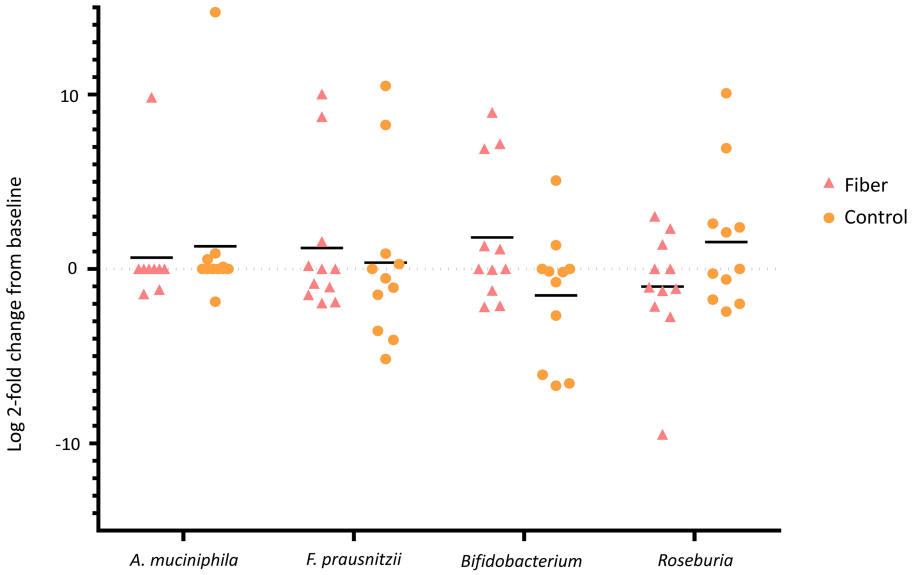

FIGURE 3
Comparison of the relative fold change of the relative quantification by qPCR of *Akkermansia muciniphila, Faecalibacterium prausnitzii, Bifidobacterium* spp., and *Roseburia* spp. of fecal samples from participants receiving hemodialysis during the pea hull fiber (Fiber) and control periods. No statistically significant differences were observed.

PHF intervention enhanced fiber intake by approximately 9 g/day and improved the protein-to-fiber ratio from 6:1 to 3:1, PHF was ineffective in suppressing serum levels of targeted uremic toxins. Additionally, inflammatory cytokines remained unchanged, although higher dietary fiber intake is associated with less inflammation in individuals with CKD (68, 69). Reported reduced serum PCS with an escalating dose of 20 g/d of oligofructose-enriched inulin in an open-label study in hemodialysis patients (26), had suggested that the prebiotic inulin may be a driver of uremic molecule reduction. However, Poesen et al. (28) showed no effects on the serum levels of PCS, *p*-cresol glucuronide, IS, or PAG in adults with CKD after 4 weeks of 20 g/day of the prebiotic arabinoxylan oligosaccharides, even though in healthy subjects, arabinoxylan oligosaccharides decreased urinary PCS excretion after only 2 weeks of supplementation (70). Similarly, an intervention of 12 g/day of fructooligosaccharides showed no significant effects on serum or urinary PCS in non-dialysis-dependent adults with CKD (71), nor did 10–15 g/day inulin supplementation in hemodialysis patients (27). Alternatively, participants' protein intake, with its expected incomplete digestion (67), may have contributed more to uremic molecule levels by specifically providing aromatic amino acid substrates. Surprisingly, baseline protein intake was not associated with serum PCS or PAG, and a negative correlation was seen for IS.

BSFS of 1 and 2 comprised more than 30% of the reported stool forms suggesting a significant level of slow gut transit in this patient population. Examining individual data, the number of slow transit stools per week was associated with the serum levels of PCS. This finding is in agreement with Ramos et al. (72), who reported that a BSFS score of <3 was associated with higher serum PCS but not IS in a cross-sectional analysis of CKD patients. Similarly, Pereira et al. (73) reported that peritoneal dialysis patients with a BSFS of <3 had higher levels of total and free PCS and indole-3-acetic acid but not IS. Indeed, slow transit time, which depletes microbially available carbohydrates, may contribute to proteolytic fermentation and PCS production. As insoluble, less fermentable fiber sources such as wheat bran have been shown to mitigate slow transit time (74), interventions with adequate doses of such fibers may decrease PCS. PHF, although an insoluble hull fiber, did not modulate BSFS. This may be due to its fine particle size, which may have enhanced fermentability, an effect shown with wheat brans (75), thereby reducing its potential impact on transit time. Low consumption of fruit may have contributed to the high percentage of slow transit stool forms, as increased fruit intake has been associated with a lower risk of constipation in adults receiving hemodialysis (76).

It is well established that dysbiosis of CKD is worsened by hemodialysis (77). In the present study, relative abundance was highest for the genus *Bacteroides*. Similarly, *Bacteroides* was the most abundant genus in Chinese (78) and Taiwanese (79) hemodialysis patients, specifically *Bacteroides ovatus*, *Bacteroides caccae*, and *Bacteroides uniformis* in the latter study. PHF supplementation did not modulate relative fold changes of *A. muciniphila, F. prausnitzii, Bifidobacterium,* and *Roseburia,* taxa considered health enhancing (48–51). However, the machine learning model confusion matrix showed strong accuracy scores, and the relative abundance of *Coprococcus*, *Lactobacillus*, *Ruminococcus*, *Gemmiger*, *Collinsella*, and Mogibacteriaceae predicted PHF. Of note, *Coprococcus* showed higher relative bacterial proportions by LDA effect size (LefSe) comparison (as well as *Methanobrevibacter* and Peptostreptococcaceae) with PHF supplementation in a subgroup of older adults who exhibited increased gastrointestinal symptoms, a proxy for fermentation, within 2 weeks of PHF consumption (35). In contrast, a 12-week study of adults with overweight or obesity showed that PHF decreased the relative abundance *of Actinomyces*, *Holdermania,* and *Oscillospira,* and increased *Lachnospira* over time, the latter a possible outcome confounded by weight loss (80). Although antibiotic use was not an exclusion criterion, none was reported during the trial and therefore did not affect outcomes. In summary, there is insufficient evidence to support that PHF exerts a consistent effect on fecal microbiota composition.

A notable limitation of this study was the high withdrawal and dropout rate. Given the high levels of morbidity in the hemodialysis population, a higher level of over-sampling is needed for future

studies. An additional limitation was the less-than-optimal compliance with study snacks consumption, which may have been due to the lower acceptability of the PHF muffins compared to the control muffins; some participants commented that the muffins were somewhat dry. Furthermore, providing a variety of fiber-fortified foods may lead to better compliance, as previously demonstrated (54). Low adherence may also have been due to poor appetite commonly experienced by individuals receiving dialysis. Additionally, given that dysbiosis is exhibited in hemodialysis patients, a more extended intervention period may be needed to modulate fecal microbiota and, thus, serum levels of uremic toxins.

Although fiber fortification with PHF did not mitigate uremic molecule generation, higher doses or supplementation with mixed sources of fiber may demonstrate efficacy. More aggressive dietary interventions may be needed to effect changes in the microbiota composition and activity. However, adherence to a healthful Mediterranean dietary pattern failed to explain PCS and IS levels in a CKD cohort (81). Fecal microbiota transplantation (82) and intensive intestinal interventions (83) have been proposed as therapies for uremia toxin reduction but lack practicality. Alternatively, select targeting of microbes contributing to uremic toxins generation requires exploration. However, research is first needed to elucidate the relationships between the microbiome and uremic toxins levels, given the dramatic between-subject variations observed in hemodialysis patients.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA934347.

Ethics statement

The study involving human participants were reviewed and approved by University of Florida IRB1. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AF, KA, and YW performed the experiments. AF, MS, and WD contributed to the conception and design of the study. AF, JA, and WD analyzed the data. AF, JS, JA, and WD wrote the original draft. AF, JS, JA, KA, YW, MS, and WD reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author JA, employed with Lallemand Health Solutions Inc., assisted with the analysis and interpretation of the microbiota data and in writing the microbiota methods and results.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1179295/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Serum levels of *p*-cresyl sulfate (PCS), indoxyl sulfate (IS), phenylacetylglutamine (PAG), and trimethylamine *N*-oxide (TMAO) pre- and post-pea hull fiber. No statistically significant differences were observed.

SUPPLEMENTARY FIGURE 2
Taxonomic classification by genus level by study timepoint: baseline (1K), intervention 1(2K), washout (3K), intervention 2 (4K).

SUPPLEMENTARY FIGURE 3
Evenness (Pielou) and alpha-diversity (Faith) metrics between treatments; Pea hull fiber (Fiber) vs. Control. No statistically significant difference was observed.

SUPPLEMENTARY FIGURE S4
Beta diversity by Weighted **(A)** and Unweighted **(B)** UniFrac Principal Coordinates Analysis (PCoA) of the fecal microbiome data sets of participants receiving hemodialysis received fiber snacks and control snacks. Large overlap represents similar beta diversity between **A**) control (red) and **B**) pea hull fiber (blue).

SUPPLEMENTARY FIGURE S5
PiratePlots of all taxa distinguishing the consumption of pea hull fiber (Fiber) from usual diet during baseline and washout. The taxa are listed as follows: **(A)** *Coprococcus*, **(B)** *Lactobacillus*, **(C)** *Ruminococcus*, **(D)** *Gemmiger*, **(E)** *Collinsella*, **(F)** Mogibacteriaceae.

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Recent updates on correlation between reactive oxygen species and synbiotics for effective management of ulcerative colitis

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Ulcerative colitis (UC) is presently considered a multifactorial pathology, which may lead to persistent inflammatory action of the gastrointestinal tract (GIT) because of an improperly managed immunological reactivity to the intestinal microbiota found in the GIT. The immune response to common commensal microbes plays an essential role in intestinal inflammation related to UC synbiotics, and it is an important element in the optimal therapy of UC. Therefore, synbiotics, i.e., a mixture of prebiotics and probiotics, may help control the diseased state. Synbiotics alleviate the inflammation of the colon by lowering the reactive oxygen species (ROS) and improving the level of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD). Prebiotic supplementation is not a common practice at the moment, despite numerous research findings proving that the benefits of both probiotics and prebiotics encourage their continued existence and positioning in the GIT, with positive effects on human health by managing the inflammatory response. However, the fact that there have been fewer studies on the treatment of UC with different probiotics coupled with selected prebiotics, i.e., synbiotics, and the outcomes of these studies have been very favorable. This evidence-based study explores the possible role of ROS, SOD, and synbiotics in managing the UC. The proposed review also focuses on the role of alteration of gut microbiota, antioxidant defense in the gastrointestinal tract, and the management of UC. Thus, the current article emphasizes oxidative stress signaling in the GI tract, oxidative stress-based pathomechanisms in UC patients, and UC therapies inhibiting oxidative stress' effects.

KEYWORDS

ulcerative colitis, synbiotics, gut microbiota, inflammation, oxidative stress, superoxide dismutase

1. Introduction

Ulcerative colitis (UC) is a gastrointestinal inflammatory condition characterized by bloody and mucous diarrhea, rectal bleeding, and gastrointestinal pain (1). According to current projections, UC affects around 5 million people worldwide and is characterized by recurring and repatriating irritation of the intestine's mucous membrane. In UC, inflammation sticks to the mucus layer (2, 3).

Ulcerative colitis is associated with impaired mucosal barrier function, which allows luminal bacteria to generate a prolonged and uncontrollable inflammatory response. UC is a type of sickness classified as one of the "inflammatory bowel diseases (IBDs)." It is defined by a prolonged inflammatory response of the "intestinal lamina propria" that might start in the rectum and progress across the colonic mucosa. Clinically, UC and Crohn's diseases frequently contribute significantly to global mortality, particularly in the Western world (4).

Unlike Crohn's disease, where the mucosa surrounding the ulcers may or may not be inflamed, ulcers in UC are virtually invariably accompanied by mucosal inflammation (5). Interleukins (ILs), important components of the cytokine profile observed in the gastrointestinal mucosa in UC, have therefore been emphasized as potential targets for targeted therapeutics in the future. The ILs chosen for consideration have the highest promise as future targeted treatments. Furthermore, investigating several of the most recently investigated ILs involves their potential significance in UC (6). In addition to the standard proinflammatory cytokines like IL-1, IL-6, and TNF-, a complex network of Th2 cytokines, including IL-10 and IL-13, play an important role in the pathogenesis of UC. This network is crucial because it regulates the immune response.

In comparison to Crohn's disease, UC affects a smaller geographic region. The sickness only invades (inflames) the inner lining of the gut tissue, and it usually only affects the colon (large intestine), including the rectum and anus. The prevalence of UC is far greater than that of Crohn's disease. North America and northern Europe have the highest UC prevalence and incidence rates (7). The incidence rates vary between nine and twenty cases per 100,000 people per year, whereas the prevalence rates range between 156 and 291 instances per 100,000 people. The etiology and pathophysiology of UC are both complex. The cause of UC is assumed to be an imbalance between the intestinal microbiota and mucosal immunology, which causes excessive inflammation in the digestive system (8). As a result, an imbalance in the digestive tract microbiota has a role in the pathogenesis of UC. The intestinal microbial population and gut bacteria's functional diversity and stability are all affected in individuals with UC. Certain Firmicutes bacteria are declining, while Bacteroidetes bacteria and facultative anaerobes are increasing (9). Dysbiosis has been observed in UC patients (10), albeit to a lesser extent than in Crohn's disease patients. Patients with UC have been reported to have lower biodiversity (11), with fewer Firmicutes and a higher proportion of Gamma-proteobacteria and *Enterobacteriaceae* in their gut microbiomes. Furthermore, individuals with the illness have more sulfite-reducing Delta-proteobacteria in their colons (12).

Probiotics are living microorganisms with a wide range of beneficial features which play an important role in GIT protection (13). However, probiotics have a wide range of impacts on the human body, including the skin, oral cavity, respiratory tract, urinary tract, and reproductive tract. Clinical trials have investigated probiotics' health advantages in children, adults, the elderly, and immunocompromised patients (14). Probiotics work through several methods, including gut flora alteration, intestinal mucosa barrier strengthening, pathogen colonization decrease, inhibition of enhanced immune response, and generation of short-chain fatty acids, amino acids, vitamins, and enzymes, among others. The use of probiotics as part of a UC treatment approach is becoming more widespread. Probiotics are living microorganisms that do not cause sickness. Lactobacillus, Bifidobacterium, and Enterococcus are examples of probiotic bacteria. Probiotic bacteria have been found in studies to be beneficial, particularly to the gut and immune systems (15). Probiotics have been proven to boost both local and systemic immunity, repair the function of a disrupted mucosal barrier, correct an imbalance in the intestinal microbiota, reduce competition between potential pathogens, and encourage intestinal barrier function (15, 16). Probiotics can effectively induce and prolong remission in UC patients, indicating that UC care should emphasize favorable gut flora (17–21).

On the other hand, a synbiotic is a mix of both a probiotic and a prebiotic, a carbohydrate that functions as a food source for the probiotic and allows it to develop more efficiently in the gut. As a result, a synbiotic does not include living microbes. Synbiotics include both probiotics and prebiotics; this combination is thought to be more helpful in gut health and function than either probiotics or prebiotics alone (22). As a result, considering the numerous possible combinations, the use of synbiotics, in which probiotics and prebiotics act together to give a synergistic effect, is considered promising (22, 23).

The purpose of synbiotic involvement, on the other hand, is uncertain. More research is needed to determine the synbiosis in both prebiotics and probiotics that could modify inflammatory reactivity primarily through inflammatory cytokines and innate immune activation, as well as the development of reactive oxygen species (ROS)-inhibiting short-chain fatty acids (SCFAs) associated with intestinal mucosal stabilization, T cell initiation, advancement of anti-inflammatory cytokines efflux, and inflammation reduction (3).

Abbreviations: UC, ulcerative colitis; NF-κB, nuclear factor kappa-B; IKK, inhibitor of nuclear factor-κB (IκB) kinase; IκB, inhibitor of nuclear factor kappa-B; GIT, gastrointestinal tract; ROS, reactive oxygen species; CAT, catalase; GPX, glutathione peroxidase; SODs, superoxide dismutase; SCFA, short-chain fatty acids; HOCl, hypochlorous acid; H_2O_2, hydrogen peroxide; ETC, electron transport chain; COXs, cyclooxygenases; NOS, nitric oxide synthase; MPO, myeloperoxidase; XO, xanthine oxidase; NOX, NADPH oxidase; IECs, intestinal epithelial cells; ICAM, intracellular adhesion molecule; NF-kB, nuclear factor-kappa B; CAMs, complementary and alternative medicines; APC, antigen-presenting cells; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; RCTs, randomized controlled trials.

2. Pathogenesis of ulcerative colitis

Ulcerative colitis is a "chronic IBD" that affects the rectum and the colon. Several factors, including genetic profile, environmental and gastrointestinal conditions, and mucosal immune dysfunction, are thought to impact the genesis of UC. Despite its broad prevalence, the pathophysiology of UC is complicated and poorly understood. Nonetheless, the newly accessible data allow for constructing a current working model of the disease's pathophysiology. This model incorporates several aspects and components that contribute to illness development. UC is an intestinal barrier disorder caused by a breakdown in an epithelial cell or the fundamental epithelial architecture of the gastrointestinal tract. UC can be caused by various factors, which might eventually lead to immunological difficulties.

Furthermore, infected individuals may risk getting a disease caused by commensal gut microorganisms (24). Alternatively, the barriers might be disrupted by highly inflammatory chemicals and cells in the lamina propria, leading to the barrier's rupture; this inflammatory cascade would then contribute to the illness's chronicity. The pathogenesis of UC is complex, with several factors. SCFAs are generated by probiotic gut bacteria from a fiber-rich diet that cannot be taken directly (25). SCFAs, including acetate, propionate, and butyrate, are essential metabolites for sustaining intestinal homeostasis (26). SCFAs with significant anti-inflammatory action can reduce ROS production, which may modulate the immunological function and prevent an excessive immune response, delaying the clinical development of IBD. SCFAs are essential for fueling intestinal epithelial cells and are known to maintain gut barrier function. SCFAs contribute to the formation and development of UC (25–27). In UC, the mucous membrane fails to generate as much intestinal mucin. A barrier breach is produced by changed microbiota and a weaker mucous membrane, which allows the microbiota to permeate the epithelial barrier more easily. Apoptotic foci and altered tight junction protein expression damage the intestinal epithelium, enabling germs to get through, activating macrophages and antigen-presenting cells (APCs), and promoting the production of chemokines that attract neutrophils. Neutrophil extracellular traps act as the first line of defense, and immune cells infiltrate by sticking to blood vessel endothelial adhesion molecules. Type 1 T-helper (TH1) cells become polarized as a result of invading monocytes that grow into macrophages producing tumor necrosis factor (TNF), interleukin (IL)-12, IL-23, and IL-6.

Furthermore, IL-36 generated by the epithelium inhibits regulatory-T (Treg) cells, causing IL-9-producing T-helper (TH9) cell polarization. IL-13, released by natural killer (NK) T cells, also causes barrier dysfunction. Changes in barrier function in UC patients can involve cytolytic destruction to the epithelial layer by NK-T cells and a more subtle modification caused by the actions of IL-13. Consequently, this cytokine may have a dual pathogenic effect, one influencing epithelial cells directly and the other as a stimulator of NK T cell cytotoxicity (28).

3. Alteration of gut microbiota in ulcerative colitis

Numerous investigations have shown that UC patients have impaired gut microbiota regarding composition and structure. There is a reduction in several bacteria, including *Akkermansia muciniphila*. It is a common element of the mammalian gut microbiota, accounting for 1 and 5% of all human intestinal microorganisms. According to the observations of numerous studies, there is a connection between UC and *A. muciniphila*. Together with the Roseburia bacteria, the *A. muciniphila* levels were found diminished in patients with UC (29). Patients with UC typically notice changes in their gut microbiota composition. Furthermore, several investigations suggest that abnormalities of the gut microbiota are strongly associated with UC (30). The gut microbiota significantly influences the gut mucosal immune system (21, 31–33).

4. Oxidative stress signaling in the gastrointestinal tract

ROS are necessary for mammalian cell survival. Free radicals such as hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), peroxyl (RO_2), hydroxyl radicals (H.O.), superoxide (O_2^-), and others are referred to as ROS (34). ROS and RNS are two important components in the cell that cause harm to nucleic acids, lipids, proteins, and carbohydrates and regulate the gene transcription that triggers immunological activities in the GIT (35). Intrinsic ROS is mostly produced in cell constituents such as the cytosol, nucleus, peroxisomes, mitochondria, and endoplasmic reticulum. The electron transport chain (ETC) primarily contributes to ROS production. Also, intracellular ROS is produced by a variety of enzymes, including cyclooxygenases (COXs), nitric oxide synthase (NOS), myeloperoxidase (MPO), glucose oxidase, xanthine oxidase (XO), NADPH oxidase (NOX), and peroxidases. Various cytokines produced by Th2-type T cells, such as IL-13, IL-10, IL-5, and IL-4, help to suppress UC. Numerous opposing effects maintain GI epithelial stability while destroying UC and gastric ulceration (36). Xenobiotics, medications, alcohol consumption, antigens (luminal), smoking, chemotherapy, and radiation are some of the external variables that cause ROS generation in UC. Due to its antioxidant capabilities, the cells can tolerate a certain quantity of ROS under ordinary circumstances, which is critical for GI equilibrium. However, an excessive oxidant payload promotes increased ROS production, irritation, transmembrane permeability, DNA destruction, and eventually UC (34–37) (Figure 1).

5. Antioxidant defense in the gastrointestinal tract

Uncontrolled oxidative stress harms the GIT and the human body's antioxidative defensive mechanism (s) that could protect individuals from the consequences of too much ROS. The defense mechanism indicates that the number of ROS the human body produces can be managed without causing damage. Numerous enzymatic antioxidants, including CAT, GPX, and SODs, are found in the exogenous antioxidant defense systems. Multiple diagnostic outcomes have previously demonstrated that IBD sufferers have three SOD isoforms, with SOD2 being significantly highly expressed, SOD1 being less impacted, and SOD3 being decreased mostly in intestinal epithelial cells (IECs). SOD performance is

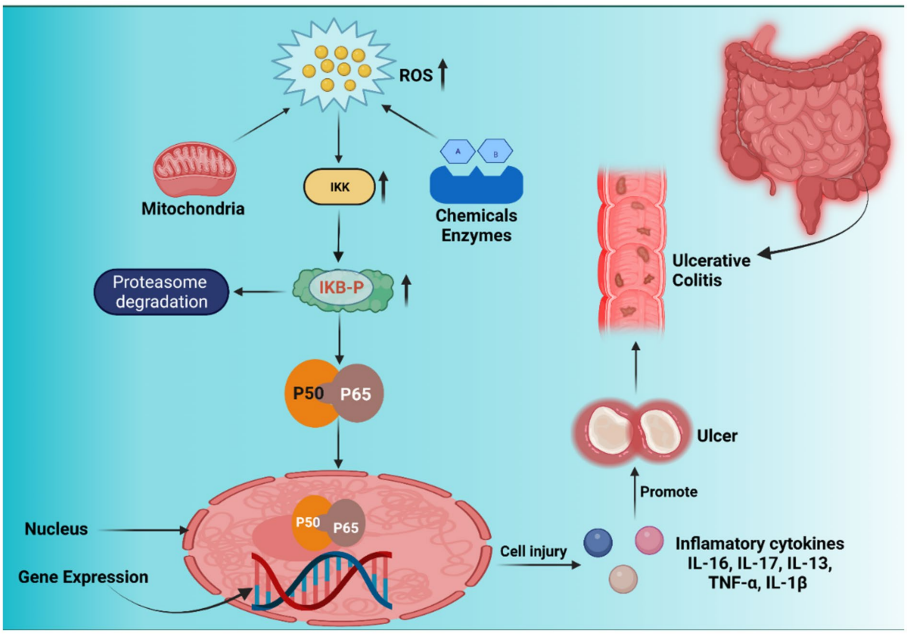

FIGURE 1
Nuclear factor kappa-B (NF-κB) signaling pathway, inflammation, and ROS-induced carcinogenesis. Inhibitor of nuclear factor-κB (IκB) kinase (IKK) is first activated by xenobiotics, excessive reactive oxygen species (ROS) from the mitochondrial membrane, and enzyme reactions. IκB is phosphorylated by activated IKK, which causes IκB (inhibitor of nuclear factor kappa-B) to be ubiquitinated and broken down by the proteasome, releasing NF-κB proteins (p50 and p65). Free p50 and p65 shift target gene expression in nuclei identical to inflammatory cytokines, resulting in inflammatory lesions and carcinogenesis.

usually associated with the degree of UC in IBD patients, wherein S.O.D. activity is increased during IBD pathophysiology and promotes oxidative injury (35).

6. Pathomechanisms of oxidative stress in ulcerative colitis patients: role of gut microbiota

Though several factors are responsible for UC, such as genetic perceptivity, alterations in IECs, immune dysregulation, microbiota resistance, and ecological variables – all contribute to the progression of IBD. However, our major focus is the correlation between ROS and UC.

GIT is vulnerable to ROS assault. However, it is exposed to the external ecosystem, which includes the immune system, intestinal flora, and dietary components, all of which are significant sources of ROS. The GIT generates ROS primarily via two biochemical responses: the NADPH oxidase mechanism and the HX/XO pathway. Elevated levels of ROS can disrupt cell components (38, 39), especially structural proteins, and eventually increase gut permeability and disrupt the GIT membrane, resulting in GIT irritation. The microflora is linked to various defensive, architectural, and biochemical functions and is essential to gut equilibrium and recipient health. Gut bacteria regulate the development of pathogenic bacteria in the GI system, activate immunity, regulate vitamin and mineral utilization and host metabolic activity, produce SCFAs, disrupt carbohydrates and proteins important for mucous membrane & cell function, and the production of anti-inflammatory IL (40). Recent research has shown that the equilibrium between the intestinal microbiome in the GIT and human defense mechanisms is critical in the etiology and persistence of UC (41). Dysbiosis (alterations in the makeup of commensal microorganisms or instability y in the microbiota environment) has been documented in UC individuals, and current therapies also impact the microbiome (42). As a result, an investigation into establishing synbiotic-associated therapeutics for UC is currently proceeding. In systemic inflammation, intestinal microbes activate the formation of NO and NOS via host macrophage stimulation, initiating DNA damage. Oxidative stress causes DNA damage, protein deposition, and cell wall disorganization and activates the beginning of inflammatory reactivity via supportive remarks, triggering supplementary ROS development and further membrane/tissue injury (43).

7. Targeting oxidative stress in ulcerative colitis

Proinflammatory cytokines like NOS and ROS are more involved in the genesis and progression of UC (44). In the acute inflammatory lamina propria of individuals affected by UC connected to the endothelium, a noteworthy infiltration of white blood cells and improved myeloperoxidase (MPO) concentration were assessed (45). In UC, iNOS is the primary key element responsible for increased NO synthesis in the epithelial cell and iNOS-derived NO promotes TNF-α-expansions in the intermediate and proximal intestine, which increases leukocyte infiltration primarily through activation of the formation of "intracellular adhesion molecule" (ICAM) and P-selectin,

resulting in intestinal cellular injury (46). The influx of neutrophils and the activation of important genomic signal transduction pathways, including "AP-1" and "nuclear factor-kappa B (NF-kB)," increase inflammatory activity and tissue destruction (47).

8. Therapies inhibiting oxidative stress in ulcerative colitis

IBD is a severe gastrointestinal condition characterized by immunological dysregulations, supporting the hypothesis that IBD therapies should primarily focus on reducing inflammation. In addition, anti-inflammatory medicines such as corticosteroids, infliximab, mesalazine, and sulfasalazine are used in conventional therapeutic approaches to combat irritation promptly and alleviate IBD discomfort. These bio-actives work by inhibiting NF-κB-or TNF-α-associated inflammation, resulting in various health consequences such as GIT issues, anemia, allergy, and medication resistance. Another category of therapeutics, immune modulators like thiopurines and cyclosporine, can also be used for managing IBD via immunosuppression (48). These immune modulators are frequently associated with anti-inflammatory chemicals. Most have free radical-scavenging abilities, with some resulting from TNF-α-induced downstream antioxidant effects. Because of the significant growth in the complexity of UC and colon cancer, there is a greater need for innovative treatment methods for UC. Various experimental studies have identified one of the key oxidative stress pathways in IBD, and modulating Nrf2 signaling and blocking ROS development by inhibiting mitochondria and NOX are both critical therapy options for IBD. As a result, several promising alternative treatment modalities with antioxidant properties, such as ROS blockers, responsive nutritional approaches, and naturally derived agents that prevent apoptosis and activate antioxidant activity, have received considerable focus as "complementary and alternative medicines" (CAMs) in the management of UC (49–56) (Table 1).

9. Role of probiotics for the management of ulcerative colitis

Probiotics have beneficial effects on the host microbiota and have attracted greater research interest in managing UC. Probiotics serve as an inhibitor of ROS production, the development of antioxidative enzymes, metal chelation, and enzyme suppression. Probiotics have increased GPX, CAT, SOD, and GSH levels while reducing NO and MPO functions (57). The multifaceted activity of nutrition, nutritious dietary compounds, and healthy gut microbiome has bolstered the use of probiotics, which have been shown to have favorable effects on the human intestinal microbiota (58). Many microbiomes have been studied for discarding gut microbiota. Microbes such as (*Streptococcus*, *Bifidobacterium*, and *Lactobacillus*) were used in the synthesis of probiotic strains associated with more positive treatment responses on GIT inflammation and the ability to maintain a better and healthier intestinal microbiota, and probiotics are often used to assess the efficacy of living microorganisms in reducing IBD manifestations (59). Scientific studies using probiotics to treat IBD are widespread (60). As a result, whenever the gut ecosystem has been damaged by illness, ill-nutrition, or drugs, better knowledge is required when selecting a particular probiotic strain that might alter the patient's health. Probiotics have been offered as a novel preventative and therapeutic alternative in cancer management and may inhibit cancer development. Thus, probiotics could provide a fresh approach to studying the active ingredients found in various probiotic strains. With few traditional therapies available, there is a need for new options. One such strategy is the delivery of chemotherapeutic drugs via nanocarriers employing nanotechnology (61).

Mesalamine and probiotic "(*Saccharomyces boulardii* and *Lactobacillus acidophilus*)" encapsulated pectin microparticles embedded with cellulose acetate phthalate (CAP) were synthesized by Singh et al. in 2021. The main issue with this research is the strong NO scavenging capacity of *Saccharomyces boulardii*, which was validated by the NO test. According to the FT-IR interpretation, no chemical interaction between the medication and CAP was seen. According to the *in vitro* drug release kinetics of coated microparticles, the synthesized formulation can release the medicine and probiotics at the colonic site (62). To control UC, Singh et al. (63) have developed and characterized "enteric-coated pectin pellets" consisting of mesalamine and *S. boulardii* for precise colon-targeted drug delivery. Mesalamine and *S. boulardii* pellets were created utilizing the extrusion spheronization method, pectin, and microcrystalline cellulose (MCC) and were then decorated with cellulose acetate phthalate (CAP). Experimental studies have demonstrated that mesalamine and *S. boulardii*-coated pellets dramatically alleviated the sick conditions in Wistar rats (63).

TABLE 1 Antioxidative and anti-inflammatory effects of therapeutics used to manage ulcerative colitis (UC).

Agents used in UC management	Outcomes	References
N-acetylcysteine	Decreased lipid peroxidation, enhanced GSH and SOD in ulcerative colitis, and decreased iNOS activity in UC	(49)
Tetradecylthioacetic acid	Reduced iNOS, TNF-α, and IL-6 mRNA in ulcerative colitis UC	(50)
Mesalazine	Decreased $O_2^{\bullet -}$, H_2O_2 in UC, ↓IL-6, Il-8, reduced GSH, TNF-α in UC	(51)
Glucocorticoids	Reduced MPO and neutrophil elastase in pediatrics IBD	(52)
Infliximab	Reduced TNF-α in the colonic mucosa, reduced INF-γ mRNA in inflammatory cells in colitis	(53, 54)
Tributyrin	Enhanced TGF-β and IL-10 in lamina propria	(55, 56)

9.1. Mechanism of probiotics against ulcerative colitis

Abundant pathogenic microorganisms, depletion of protein bindings and junctions, and a thinner mucus membrane cause inflammatory consequences in UC. Although the APCs identify microorganisms, T-lymphocytes form proinflammatory cytokines that trigger inflammatory mediators NF-κB, which produce reactive nitrogen species (RNS) and ROS, resulting in the irritated intestinal mucosa. Being overweight causes abnormalities between microorganisms and commensal bacteria and a significant irritation effect. An increased ω-3 triggers inflammatory reactions to ω-6 fatty acid equilibrium in the diet. The use of probiotics aids in the maintenance of functional gut flora by preserving barrier function and the mucus barrier. Probiotics and antioxidants decrease irrational immune function and ROS-associated inflammatory responses modified by antioxidants (64) (Figure 2).

10. Selective therapies aiming at microbiota manipulation in ulcerative colitis

Probiotics, prebiotics, antibiotics, gut microbiome transplants, and a nutritious diet may all assist in maintaining a balanced gut microbiota habitat. Antibiotics are excellent in eliminating pathobionts, but their non-selective antibacterial activity disrupts gut equilibrium by destroying beneficial microflora, reducing their usage in managing colorectal cancer.

10.1. Studies on the use of prebiotics and probiotics in the management of ulcerative colitis

Host bacteria utilize nutritional prebiotics to give therapeutic advantages to target tissues. Prebiotics are thought to increase intestinal irritation by promoting beneficial gut microbiota formation, intestinal vulnerability, and SCFA production. Probiotics are live microorganisms that can provide therapeutic effects to humans whenever administered at sufficient levels. They are usually made up of one or even more strains of bacteria (65–72) (Table 2).

11. Cross-links between reactive oxygen species and microflora in the gastrointestinal tract

ROS, which comprises radical variants (superoxide) and non-radical peroxide forms (H_2O_2), are short-lived, highly electrophilic entities that originate from the partial reduction of molecular oxygen. Extremely reactive ROS, particularly superoxide, may cause macromolecular destruction to crucial biological constituents, including membrane phospholipids and nucleic acids.

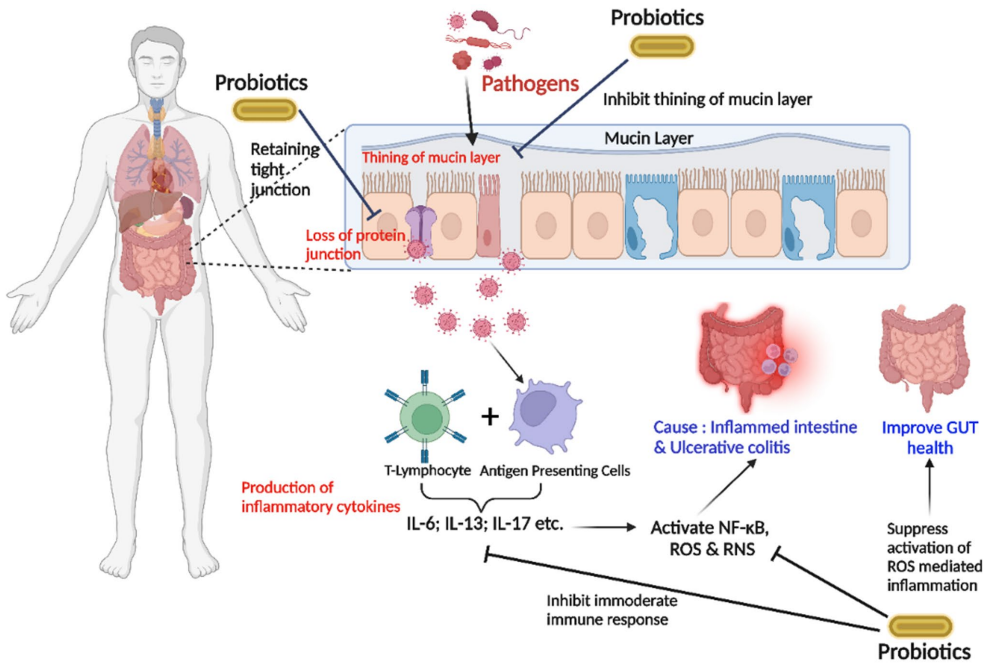

FIGURE 2
The strategy of probiotics' action on the gut lumen is associated with inhibiting the inflammation in ulcerative colitis (UC). The entry of an overwhelming number of pathogenic microorganisms, the degradation of cell-junction proteins, and the thin mucin layer trigger inflammatory reactions. T-lymphocytes produce proinflammatory cytokines after pathogens are identified by "antigen-presenting cells (APC)," stimulating inflammation-inducing NF-κB and producing reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in an acute inflammatory intestinal lumen. Probiotics inhibit excessive immunological responses and attenuate ROS-induced inflammatory responses (Interleukin-6; Interleukin-13; Interleukin-17).

TABLE 2 Several findings of probiotics and prebiotics against ulcerative colitis (UC).

Prebiotics and probiotics	Outcomes against UC	References
Lactulose	Reduced inflammation	(65)
Oligofructose enriched Inulin	Levels of fecal calprotectin increased in the prebiotic-administered group than in the placebo-taken group	(66, 67)
UC treated with different doses of oligofructose-enriched inulin	Prebiotic courses were found to be higher butyrate levels	(68)
Meta-analysis of *Escherichia coli* Nissle 1917, *Bifidobacterium longum* 356, *Lactobacillus rhamnosus G.G.*, a multi-strain probiotic containing a combination of lactic acid bacteria, *Streptococci*, and *Bifidobacterium* probiotics	The use of probiotics reduced adverse events	(69)
Meta-analysis of randomized controlled trials (RCTs) examining the effects of probiotics, prebiotics, and synbiotics on human UC	Patients suffering from active UC who took *Bifidobacterium*-containing probiotics were more likely to be in remission than the placebo group	(70)
Supplementation of *Bifidobacterium*-fermented milk	Exhibited anti-inflammatory properties by protecting mucosal barrier integrity and maintaining gut microbiota homeostasis	(71, 72)

Another important finding from scientific studies was the evidence that certain species of commensal gut bacteria in humans cause the quick, "deliberate" production of physiological amounts of ROS in human epithelial cells (35). Additionally, epithelial cells co-cultured with specific bacteria demonstrated an increment in the oxidation of soluble redox sinks like glutathione and thioredoxin, as well as an enhancement in redox-stimulated transcriptional stimulation, both of which reflect a cellular response to elevated ROS. Contacting cells may produce significantly varying ROS levels in response to commensal bacterial strains. Although all examined microorganisms have some capacity to change the intracellular redox state, *Lactobacilli* are potent inducers of ROS production in cultivated cells. High ROS-stimulating microorganisms, like *Lactobacilli*, may have membrane elements or even release substances that stimulate cellular ROS generation. Bacteria that produce high levels of ROS may have improved adhesion or the capacity to permeate mucous membranes, providing them with more proximal accessibility to cellular receptors (e.g., FPRs and TLRs). FPRs are potential candidates and are reported to substantially induce ROS generation, located on apical surfaces and in epithelial cells and phagocytes. The gut has a variety of unique cell varieties that transmit proinflammatory and immune-tolerance messages in response to commensal bacteria and pathogens. The generation of ROS by mucosa-resident cells or freshly recruited innate immune cells is crucial for antimicrobial responses and the control of signal transduction pathways, including phenomena associated with wound healing. Crohn's disease and pancolitis have been linked to decreased ROS production due to patient variations that render NADPH oxidases as passive sources of ROS. However, ileitis and UC have been related to increased ROS production due to upregulated oxidases or modified mitochondrial features and functions (35, 73).

Its pathogenesis is multifactorial, including environmental factors, genetic susceptibility, epithelial barrier defect, symbiotic flora imbalance, and dysregulated immune response. Thus far, although immune cells have become the focus of most research, it is increasingly clear that intestinal epithelial cells play an important role in the pathogenesis and progression of UC. Notably, apoptosis is a vital catabolic process in cells, which is crucial to maintain the intestinal environment's stability and regulation of intestinal ecology (74). ROS have been recognized as a common mechanism in UC (75). Either antioxidants or free radical scavengers are reported as effective therapeutic agents for UC (76, 77).

Moreover, due to the relatively high ROS concentration in UC patients' tissues, ROS-responsive systems may specifically release drugs in inflamed colon tissues. Long-term irreversible damage to the GI structure and function in patients with IBD increases the risk of colon cancer. Current treatment strategies include corticosteroids, aminosalicylic acid (ASA), immunomodulatory drugs, Janus kinase inhibitors, and biological agents-monoclonal antibodies against TNF-α, IL-12/23 (78).

12. Role of microbiome, synbiotics, and xylooligosaccharides in the management of ulcerative colitis

Several studies have revealed that UC is closely linked with disturbance in gut microbiota (79). Gut microbiota plays a main role in a healthy gut mucosal immune system (80). Scaldaferri et al. (81) established that the most severe inflammatory sites in the gut of UC patients are also the sites with the highest abundance of bacteria. When the dominant bacterial species in the gut is altered, this results in instability of the gut microbiota and an immune reaction within the gut mucosa (82). Microbial disorders can cause deviations in the metabolism of bacteria, inducing gut inflammation. Variations to innate gut microbiota characteristics may be used as a diagnostic marker and a prognosticator of UC (83).

Numerous strains of probiotics or prebiotics, in varying ratios, may be used to sustain healthy microbiota. Synbiotics are a mixture of prebiotics and probiotics that are more effective than individual prebiotics and probiotics. Synbiotics synergistically impact the intestinal microbiota, enhancing certain advantageous probiotic strains' durability and physiological functions. Bifidobacteria, Lactobacilli, inulin, oligosaccharides, and fibers as prebiotic elements are most often utilized in synbiotic combinations. Due to their stronger potential to increase SCFAs developing bacteria counts and substrates for fermentation, the synbiotics get a more substantial anti-inflammatory impact, either probiotics or prebiotics individually (22, 23, 25).

UC is intimately linked to gut microbial dysbiosis. Prebiotic treatment is a viable strategy for managing UC, particularly

sustaining remission. "Xylo-oligosaccharide (XOS)" is an effective prebiotic with several clinically documented medical advantages and few adverse outcomes. Prebiotic Xylo-oligosaccharide (XOS), which improves gut flora, is more effective than conventional prebiotics (84). The term "prebiotic" describes non-viable dietary components like "fructan (also known as "inulin") indigestible polysaccharides, galactooligosaccharides (GOS), oligosaccharides, or fructooligosaccharides (FOS), Xylo-oligosaccharides (XOS)" that preferentially promote the growth of a small number of health-promoting microorganisms in the gut and have beneficial impacts on the GIT, cognitive abilities, cardiovascular wellness, and bone density (85, 86). To explain the potency of prebiotics and Xylo-oligosaccharides, numerous research works were carried out to show the beneficial effect of prebiotics and xylo-oligosaccharides in the maintenance of gut and UC.

A synbiotic is a good option for reducing UC-related inflammation since it contains both probiotic and prebiotic components. To ascertain the additive effect of the probiotic "*Bifidobacterium infantis* (*B. infantis*)" and the prebiotic "xylooligosaccharide (XOS)" against ulcerative colitis, Sheng et al. (87), conducted a study on the synbiotic supplementation containing "*Bifidobacterium infantis* and xylooligosaccharides." For this, "*B. infantis*, XOS, or synbiotic (a mix of *B. infantis* and XOS)" were administered to "C57BL/6 mice" for 21 days. "Dextran sulfate sodium (DSS)" solution in water was given to the mice during the last 7 days of therapy to cause colitis. The "disease activity index (DAI)" and pathological scores were all reduced by all treatments, suggesting that synbiotic therapy was more effective than either probiotic or prebiotic treatment alone. All treatment groups significantly reduced the proinflammatory cytokines "TNF-α and IL-1β" compared to the DSS-induced colitis group. All treatments reduced oxidative stress, and the mRNA levels of the "tight junction (TJ) molecules zonula occludens-1 (ZO-1), occludin, and claudin-1" were elevated in the colon tissues. As a result, the reported effectiveness of synbiotics against colitis may be explained by the additive interaction of the probiotic and prebiotic components' direct anti-inflammatory actions and their capacity to strengthen the integrity of the colonic epithelial barrier. According to research findings, synbiotics are a viable dietary supplement or functional food for the efficient treatment of UC (87). Another research used an *in vitro* fermentation model to examine the prebiotic impacts of XOS on the fecal microbiota of individuals with UC who were in clinical remission. The research included five UC patients in clinical remission and five healthy participants. Fresh feces specimens from UC patients were diluted and inoculated in "yeast extract, casitone and fatty acid (YCFA) medium," either alone or in combination with XOS. Samples were obtained for "16S rDNA" sequencing to examine the makeup of the gut microbiota after 48 h of fermentation. Using original fecal samples, differences in the gut microbiota between healthy individuals and UC patients in clinical remission were found. The effects of XOS on the gut microbiota of UC patients were then shown by comparing the differences between the YCFA medium alone or with XOS samples. The fecal samples of UC patients were different from those of healthy volunteers in "principal coordinate analysis (PCoA) and principal component analysis (PCA)." The relative abundances of "g Roseburia and g-Lachnospiraceae ND3007 group" were greater in healthy volunteers than in UC patients, but "o-Lactobacillales" abundance exhibited the reverse tendency, according to a "linear discriminant analysis effect size (LEfSe) analysis." The abundances of the "g-Eubacterium halli group" and "g-Lachnospiraceae ND3007 group" were greater in the healthy volunteers than in the UC patients (P 0.05) according to the Wilcoxon rank-sum test bar plot. The Wilcoxon rank-sum test also revealed that XOS fermentation in UC patients boosted the development of bacterial groups such as "g-Roseburia, g-Bifidobacterium, and g-Lactobacillus," which is advantageous for the recovery of intestinal disorders. These findings point to XOS as a potential prebiotic material for sustaining clinical remission by alleviating dysbiosis in the feces of UC patients

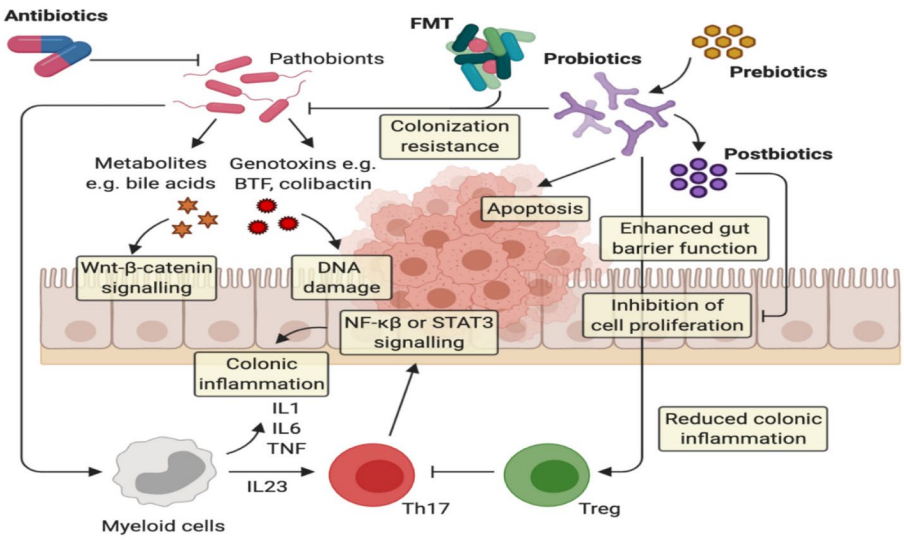

FIGURE 3
The implication of prebiotics and probiotics in ulcerative colitis. Probiotics act via several anti-cancerogenic mechanisms: (i) probiotics can inhibit the colonization of pathogenic bacteria, (ii) they can improve the barrier function by enhancing mucin production and tight junction protein appearance, and (iii) they Improve homeostatic immune responses, providing the extension of anti-inflammatory responses via Treg cells and the modification of proinflammatory cytokine release, (iv) Increase apoptosis on inflamed cells.

TABLE 3 Summary of essential studies involving synbiotics treatments in ulcerative colitis (UC).

Treatment	Dosage	Duration of treatment	Subjects	Outcomes	References
Bifidobacterium longum plus inulin-oligofructose; Treatment time: 1 month	Probiotics: 2×10^{11} (CFU) freeze-dried viable *Bifidobacterium longum* and 6 g of prebiotic fructooligosaccharide/inulin mix	1 month/4 weeks	18 patients with active UC	Sigmoidoscopy scores decreased, TNF-α, IL-1βreduced	(92)
Bifidobacterium longum plus *psyllium*; Treatment time: 4 weeks	Probiotics: *Bifidobacterium longum*-2 × 10 (9) (CFU), Prebiotics: 8.0-g doses of *psyllium*	4 Weeks	120 patients with UC	IBDQ (total, bowel, systemic, emotional, and social functional scores) increased	(93)
Lactobacillus Paracasei B 20160 + XOS; Treatment time: 8 weeks	6 g of lyophilized powder with 5×10^9 CFUs of *Lactobacillus paracasei* B 21060	8 weeks	18 patients with mild-to-moderate UC	Serum IL-6, IL-8 inhibited	(94)
Bifidobacterium breve strain Yakult plus galactooligosaccharides; Treatment time: 1 year	1 g of the probiotics powder 10 (9 CFU/g), 5.5 g of GOS	12 months/52 weeks	21 patients with mild to moderate UC	MPO reduction, *Bacteroidaceae* decreased, reduced fecal pH	(95)
Lactobacillus acidophilus LA-5®, *Lactobacillus delbrueckii* subsp. *bulgaricus* LBY-27, *Bifidobacterium animalis subsp. lactis* BB-12® and *Streptococcus thermophilus* STY-31™ plus oligofructose; Treatment time: 1 month	Probiotics: 4×10^9 CFU and pre-biotics 15 g of oligofructose powder	8 weeks	8 patients with UC	Microflora spectrum improved	(96)
Enterococcus faecium, *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Bifidobacterium longum* plus fructooligosaccharide; Treatment time: 8 weeks	Six probiotic strains: 3×10^9 CFU	8 weeks	40 patients with mild to moderate UC	CRP reduced	(97)
Streptococcus faecalis T-110 JPC, *Clostridium butyricum* TO-A, *Bacillus mesentericus* TO-A JPC, *Lactobacillus sporogenes* plus prebiotic; Treatment time: 3 months	The synbiotic capsule contained *Streptococcus faecalis* T-110 JPC: 60 million, *Clostridium butyricum* TO-A: 4 million, *Bacillus mesentricus* TO-A JPC:2 millions, *Lactobacillus sporogenes*: 100 millions	24 Weeks	32 patients with UC	Reduced severity score, steroid intake reduced, relapse during follow-up (3 months) decreased; duration of remission improved.	(98)

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(88). Another study by Le et al. in 2022, compared the effects of soymilk inoculated with "*Lactobacillus rhamnosus* GG (LGG) and *Weissella cibaria* FB069 (FSMXW)," reported a synbiotic fermented soymilk fortified with XOS, on the growth of colon cancer cells. FB069 and LGG could expand in soy-based products, and fermentation quickly lowered their pH. In fermented soymilk inoculated with *W. cibaria* FB069, adding XOS dramatically increased the acidification rate, viscosity, and total cell concentration. However, after receiving the LGG vaccine, the same result was not seen. The synbiotic FSMXW also had increased "dextran, folate, GABA, and aglycone" levels. Lowering the transcription of "MD2, TLR4, MyD88, and NF-κb, FSMXW" reduced the growth of the Caco-2 and HCT-116 cell lines. The synbiotic soymilk containing XOS and *W. cibaria* FB069 through fermentation increases nutrients and useful compounds. The research outcome indicated that *W. cibaria* and XOS may be used to create functional foods and healthcare items (89). The data also imply that XOS can treat dysbiosis in individuals with UC who have achieved clinical treatment; hence, XOS may constitute a viable prebiotic for the therapy of UC.

Symbiotic medication is a unique way to improve the operating performance of any immune-related illness, and further therapeutic, prospective studies are needed to confirm positive results in UC. Various animal investigations have lately been undertaken to assess the effectiveness and safety of synbiotics on human health, and multiple areas have been investigated, with encouraging findings in the suppression of oxidative stress in UC (90). Several studies also demonstrated the significance of probiotics in initiating tolerogenic immune responses and suppressing inflammatory conditions (91). The existence of microbiome composition and species discovered an effective function in attempting to control gut immune response (Figure 3).

12.1. Role of synbiotic formulation in the treatment of ulcerative colitis

Few research studies have examined the impact of prebiotic therapy on UC patients so far. The most referenced synbiotic investigations for UC treatments are included in Table 3 (92).

13. Conclusion

UC is a persistent inflammatory illness with several causes. The principal reason for UC is increased oxidative stress caused by increased ROS production and reduced SOD concentrations. Due to a functioning antioxidant defense mechanism, SOD bioactivity was higher in UC patients. SODs are the primary catalysts that regulate RNS and ROS quantities by directly associating with superoxide and, thus, are essential signaling mediators. As a result, antioxidants may be used in combination with other treatments for UC. Synbiotics function via increasing SOD concentrations, which are primarily accountable for UC.

Author contributions

SA: writing—original draft, review and editing, and artwork. NM, KKK, ShuR, PS, and PKG: conceptualization, visualization, and supervision. AG, SD, LSW, NA-D, and ShaR: writing—review and editing. BZS, PT, and GR: artwork—figures and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The roles and applications of short-chain fatty acids derived from microbial fermentation of dietary fibers in human cancer

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Dietary fibers (DFs) and their metabolites attract significant attention in research on health and disease, attributing to their effects on regulating metabolism, proliferation, inflammation, and immunity. When fermented by gut microbiota, DFs mainly produce short-chain fatty acids (SCFAs), such as acetic acid, propionic acid, and butyric acid. As the essential nutrients for intestinal epithelial cells, SCFAs maintain intestinal homeostasis and play essential roles in a wide range of biological functions. SCFAs have been found to inhibit histone deacetylase, activate G protein-coupled receptors, and modulate the immune response, which impacts cancer and anti-cancer treatment. Notably, while extensive studies have illuminated the roles of SCFAs in colorectal cancer development, progression, and treatment outcomes, limited evidence is available for other types of cancers. This restricts our understanding of the complex mechanisms and clinical applications of SCFAs in tumors outside the intestinal tract. In this study, we provide a comprehensive summary of the latest evidence on the roles and mechanisms of SCFAs, with a focus on butyric acid and propionic acid, derived from microbial fermentation of DFs in cancer. Additionally, we recapitulate the clinical applications of SCFAs in cancer treatments and offer our perspectives on the challenges, limitations, and prospects of utilizing SCFAs in cancer research and therapy.

KEYWORDS

dietary fiber, gut microbiota, short-chain fatty acids, cancer, immunotherapy

Introduction

Recent research has highlighted the significant impact of dietary fibers (DFs) on human health (1, 2) influencing the risk of chronic diseases, such as cancer, obesity, type 2 diabetes, and cardiovascular diseases (3, 4). DFs encompass soluble and insoluble fibers, which are a group of carbohydrates that cannot be digested or absorbed in the small intestine (3, 5). Soluble fibers, including oligo galactose, oligofructose, inulin, β-glucan, resistant starch, and pectin, are widely recognized as prebiotics (6). When fermented by gut microbiota, soluble fibers mainly produce short-chain fatty acids (SCFAs), such as acetic acid, propionic acid, and butyric acid (7).

SCFAs, which serve as essential nutrients for colonocytes and gut microbes, play a crucial role in maintaining intestinal and systemic homeostasis, impacting lipid and glucose metabolism, cell proliferation, inflammation, and immune system functionality (7, 8). In particular, the roles of butyric acid and propionic acid have been extensively investigated, revealing their contributions to health and diseases, including human cancers. It is suggested that butyric acid and propionic acid act as histone deacetylase inhibitors (HDACIs) to epigenetic modulate gene expression, influencing cell growth, proliferation, and apoptosis (9–12); act as ligands for G protein-coupled receptors (GPCRs), regulating cell proliferation, apoptosis, and immune response (11, 13); furthermore, they exhibit anti-inflammatory and immunomodulatory effects by regulating inflammatory factors and cytokines and promoting the differentiation and migration of immune cells (10, 11, 14–16).

Notably, while extensive studies have illuminated the roles and applications of SCFAs in colorectal cancer (CRC) (17–22), limited evidence is available for other types of cancers. This restricts our understanding of the roles of SCFAs in tumors outside the intestinal tract and the complex mechanisms underlying the regulation of the tumor-immune microenvironment (TIME). In this study, we provide a comprehensive summary of the latest evidence on the roles and mechanisms of SCFAs, with a focus on butyric acid and propionic acid, derived from microbial fermentation of DFs in cancer. Additionally, we recapitulate the clinical applications of SCFAs in cancer treatments and offer our perspectives on the challenges, limitations, and prospects for utilizing SCFAs in cancer research and therapy.

The roles and mechanisms of SCFAs in cancer

Functioning as epigenetic modificators

SCFAs as HDACIs play a crucial role in the epigenetic regulation of gene expression, influencing cell survival, proliferation, and differentiation (23, 24). Numerous *in vitro* studies have demonstrated that SCFAs presented HDACI activities in various cancer cell lines, including (9, 25–27) breast (28), gastric (29), and cervical cancer (30). SCFAs have been shown to inhibit cell proliferation, induce cell cycle arrest at G0/G1 or G2/M phase, trigger apoptosis mediated *via* the mitochondrial pathway, promote autophagy, and increase the accumulation of reactive oxygen species (ROS). In a study of BALB/c nude mouse model with HCT-116 cells inoculation by Ma et al. (31), sitosterols feeding elevated diversity of gut microbiota, increased levels of SCFAs in fecal samples, and restrained CRC cell growth. The study further revealed that SCFAs induced tumor apoptosis through the PI3K/Akt pathway and altered the expression levels of apoptosis-related proteins, such as Bad, Bcl-xl, and cytochrome C (31). Hence, SCFAs by acting as HDACI show potential as attractive targets for developing novel therapeutic strategies, as discussed in Section 3.

Acting as G protein-coupled receptor ligands

SCFAs are natural ligands for the G protein-coupled receptors (GPCRs), including GPR43 (also termed free fatty acid receptor, FFAR2), GPR41 (also termed FFAR3), and GPR109A (13, 32). In colon cancer cells, by combining these receptors, SCFAs inhibit cell proliferation, induce apoptosis, and cycle arrest *via* the NF-κB, MAPK, ERK1/2, PI3K, and Wnt signaling pathways (13, 32). For instance, SCFAs induced cell proliferation inhibition, apoptosis, and invasion inhibition, mediated by GPR43 in colon cancer cells (9, 33), HeLa cells (34), BaF3 leukemia cells (35), and breast cancer cells (36). Propionate and butyrate are high-affinity ligands for GPR43, dual-coupled to the pertussis-sensitive Gαi/o and Gq protein, and reduce cAMP levels (37). Similarly, Yonezawa found that both GPR41 and GPR43 were expressed in breast cancer cell lines; while combining with SCFAs, they raised intracellular concentration of Ca^{2+} and activated the p38 MAPK pathway, thereby inhibiting cell proliferation (38) (Table 1). In an intestinal cancer model, Kim et al. (40) observed that the SCFA-GPR43 axis suppresses the Th17-driven inflammatory response and intestinal carcinogenesis. In addition, GPR109A binds only to butyrate and reduces cAMP through Gαi/o proteins (37). GPR109A mediated butyrate anti-cancer activity in colon cancer cell lines by inhibiting the activation of NF-kB, downregulating anti-apoptotic genes, and upregulating pro-apoptotic genes (9, 41). Moreover, propionate and butyrate could activate GPR41 which was coupled through Gαi/o proteins to reduce cAMP (37), increase the intracellular concentration of Ca^{2+}, and inhibit the MAPK signaling pathway to lower the invasion of breast cancer cells (36).

Regulating TIME

SCFAs play essential roles in the host immune system, such as influencing the differentiation of myeloid and lymphocytes (42–44). SCFAs exert their immunomodulatory effects through two primary mechanisms: acting as HDACIs and interacting with GPCRs (43) (Figure 1). For example, *in vitro* and *in vivo* investigations involving C57BL/6 mice, various gene-deficient mouse models [*Rag1(−/−), GPR41(−/−), GPR43(−/−), IL-10(−/−)*] and T cell lines ($CD4^+$, $CD8^+$) showed that SCFAs promoted the differentiation of naive T cells into effector cells (Th1 or Th17) or regulatory T cells (Tregs). This regulation influences the production of IL-17, IFN-γ, and IL-10, thereby affecting immunity or immune tolerance (45). Additionally, SCFAs regulate the MAPK signaling pathways (ERK, JNK, and p38) to modulate immune and endothelial cells, leading to the suppression of inflammation and tumors (16). SCFAs have been observed to suppress inflammatory cytokines IL-1β, IL-2, IL-3, IL-5, IL-6, IL-8, IL-12, IL-17, IL-21, IL-23, TNF-α, TNF-β, NOS, and COX2, while increasing the expression of anti-inflammatory cytokines IL-10 and IL-18. This reduction in inflammation contributes to the suppression of CRC development (9, 11, 16, 22, 32). Furthermore, SCFAs affected both innate and adaptive immune responses by stimulating B

TABLE 1 SCFA receptors and related signaling pathways.

GPCR	Ligands	Tissue/cell expression	Signaling pathways	References
GPR41 (FFAR3)	Propionate butyrate	Adipose tissue, colon, spleen, lymph nodes, and bone marrow	Increase histone acetylation and involve in the regulation of acetylation-related cellular processes; reduce cAMP through Gαi/o; increase in intracellular Ca^{2+}; inhibit MAPK signaling pathway	(32, 36, 38, 39)
GPR43 (FFAR2)	Propionate butyrate	Immune cells, neutrophils, monocytes, gastrointestinal epithelial cells, adipocytes, enterocytes, and endocrine	Reduce cAMP through Gαi/o and Gαq proteins; p38 MAPK/HSP27 pathway; inhibit the Hippo-Yap pathway and increase E-cadherin to inhibit invasion; ↓ *Bcl-2*, ↓*Survivin*, ↓*cyclinD1/D3*, ↓*CDK1*, ↓*CDK21*, ↓*PCNA*, ↑*p21*, activate caspases-3/6/7/8, G0/G1 cell cycle arrest; suppress Th17-driven inflammatory response and intestinal carcinogenesis	(33, 34, 36, 38, 40)
GPR109A (HCAR2)	Butyrate	Adipocytes, immune cells (neutrophils, dendritic cells, and macrophages), retina, and colon	Reduce cAMP through Gαi/o proteins; ↓*Bcl-2*, ↓*Bcl-W*, ↓*Bcl-xL*, ↓*Bfl-1*, ↓ *cyclin D1*, ↑*FAS-L*, ↑*FAS-R*, ↑*FADD*, ↑*TNF-R1*, ↑*PTEN*, ↑*PPARγ*, ↑*Foxo3A*, inhibit NF-κB; activate caspase-3/8/9	(32, 41)

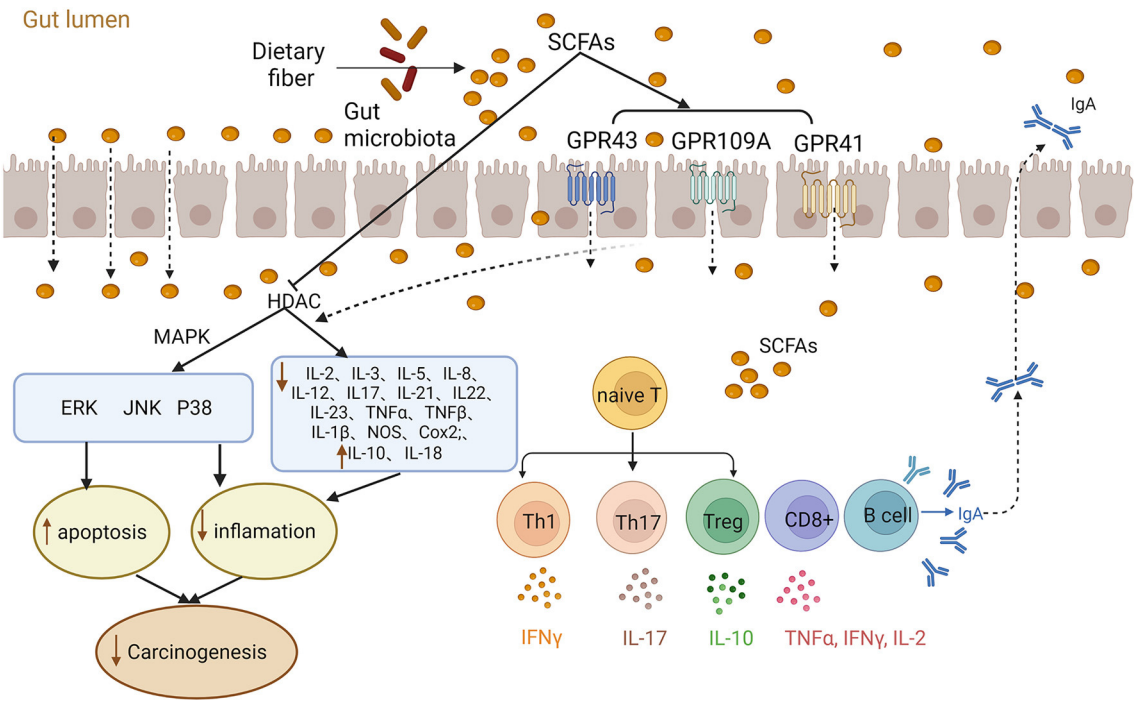

FIGURE 1
Role of short-chain fatty acids (SCFAs) in inflammation and immunity. The gut microbiota ferments dietary fibers producing SCFA that exert their effects through various mechanisms. SCFAs can act as histone deacetylase inhibitors (HDACIs) and activate G protein-coupled receptors (GPCRs) to modulate cellular responses. For instance, SCFAs regulate MAPK signaling pathways (ERK, JNK, and p38) and stimulate the release of inflammatory factors, contributing to inflammation and carcinogenesis. Additionally, SCFAs play a vital role in immune regulation by promoting the differentiation of naive T cells into effector cells and regulatory T cells (Tregs). Moreover, SCFAs influence cytokines release, impacting intestinal homeostasis and immunity.

cells to secrete intestinal immunoglobulin A (IgA) (13, 46, 47). Notably, a study by Luu et al. (48) demonstrated that SCFAs enhanced the secretion of cytokines (including IL-2, TNF-α, and IFN-γ) by modulating $CD8^+$ T cells, thereby improving cancer immunotherapy.

Carcinogenic effects of SCFAs

While SCFAs have commonly been recognized as tumor-suppressive metabolites, it is noteworthy that under certain conditions, SCFAs can promote tumorigenesis (49–51). Matsushita

et al. (52) conducted research using prostate-specific *Pten* knockout mice (*Pb-Cre*$_{\beta}$; *Pten*$^{fl/fl}$) and prostate cancer cell lines (DU145, 22Rv1) to demonstrate that SCFAs supplementation promoted prostate carcinogenesis by increasing insulin-like growth factor-1 production. Another study reported that long-term consumption of fiber-enriched foods in dysbiosis mice resulted in hepatocellular carcinoma (HCC) (53). In addition, a mouse model with colon cancer driven by mutations in the mismatch repair gene *Msh2* and *Apc* gene showed that butyrate promoted the development of CRC (54). Okumura et al. (55) have currently described that the overgrowth of *Porphyromonas* species in an *Apc*$^{\Delta 14/+}$ mouse model is casually related to colorectal cancer due to butyrate-engaged senescence. Notably, scientists have long debated these opposing observations and dubbed the phenomenon "butyrate paradox" (51). Given the "Warburg effect" (56), it has been widely accepted that butyrate provides energy to normal cells to promote cell growth. In contrast, cancerous cells instead relied on aerobic glycolysis; therefore, butyrate accumulated and functioned as an HDACI to halt cell cycle progression. Surprisingly, growing evidence in colon cancer cells showed that butyrate could directly combine and change the metabolic enzymes, leading to an anti-tumor effect without following the "Warburg effect" (57–59). Moreover, GPR41 could decrease butyrate-induced histone acetylation and negatively regulate butyric-induced anti-proliferative and apoptosis (39). Thus, it would be narrow to define butyrate or SCFAs simply as onco-metabolites or tumor-suppressive metabolites, given their complex effects that are waiting for exploration.

Advancements of gut microbiota-derived SCFAs in cancer treatment

SCFAs gained attention in the 1980s when butyrate was reported to modulate the malignant biological behavior of cultured colon cancer cells (60, 61). Sodium butyrate has been shown to inhibit the growth of hepatocellular carcinoma (HCC) cells in both *in vitro* using the HuH-7 human HCC cell line and *in vivo* utilizing an HCC tumor-bearing mice model (62). These inhibitory effects are likely mediated by a p21-dependent mechanism. In addition, sodium butyrate has demonstrated the ability to hinder the G1-S transition of human glioma cells, as evidenced by increased expression of p21 and cyclin D1, and reduced phosphorylation of pRb (63). It has also been found to impede cell proliferation in the MCF-7 human breast cancer cell line, reflected by increased expression levels of p21WAF1 and RARβ (64). Moreover, sodium butyrate induced AMPK-mTOR-mediated autophagy and ROS-mediated apoptosis of bladder cancer cells (T24, 5637, and SV-HUC-1 bladder cancer cell lines) (65), induced DAPK-mediated apoptosis in human gastric cancer cell lines (AGS, Kato III, etc.) (66), and triggered mitochondrial-mediated apoptosis in colon cancer cell line (Caco-2 cell line) (67). However, the translation of SCFAs to clinical applications has been impeded by their low concentration in peripheral blood and rapid plasma clearance (68), which will be further discussed in Section 4. Two decades later, with the iteration of sequencing technology, the association between gut microbiota-derived SCFAs and their role as anti-cancer agents once again captured scientists' attention for SCFAs as anti-cancer agents.

The investigations of the association between SCFAs and cancers fell into several research modes as follows:

1) ***In vitro* studies.** Nakkarach et al. (69) isolated the bacterial strain (*Escherichia coli* KUB-36) from fecal samples collected from healthy individuals which demonstrated the highest production of SCFAs. The researchers applied the metabolites and individual SCFA to various tumor cell lines, including breast cancer, colorectal cancer, and leukemia. Remarkably, all treatments exhibited inhibitory effects on tumor cell growth, with breast cancer cells showing the greatest sensitivity to the treatments (69). Additionally, Zheng et al. indicated that secretions of *C. butyricum* induced cytotoxic effects on CRC cells, including human CRC cell lines HCT116 and HT29, as well as the mouse CRC cell line CT26. However, the subsequent addition of butyrate kinase inhibitors impaired the cytotoxic effects specifically in CT26 cells, providing strong evidence that the anti-cancer effect of *C. butyricum* was mainly attributed to the secretion of butyrate (70).
2) ***In vivo* studies.** In a recent study, it was demonstrated that the concentration of intestinal SCFAs concentration in mice with HCC can be increased by administering a probiotic mixture named Prohep. Prohep, composed of Lactobacillus rhamnosus GG, *Escherichia coli* Nissle 1917, and VSL#3, was found to confer tumor suppression effect. This effect was associated with alterations in the composition and diversity of gut microbiota and an increase in SCFA-producing bacteria in the group of mice treated with the probiotic mixture (71). The intervention with Prohep appeared to be relevant to the downregulation of IL-17, the reduction of Th17 polarization, and the differentiation of Treg/Tr1 (72). In another study, the effect of SCFAs on extra-intestinal tumor progression was investigated in a mouse model of lung metastasis from melanoma. Supplementation with VSL#3, a registered probiotic formula consisting of eight different strains of probiotic bacteria, resulted in an increased amount of propionate and butyrate in plasma and fecal samples. Subsequent analysis showed that these SCFAs significantly decreased the volume of tumors, possibly by recruiting Th17 cells to the lung tissue through the chemokine ligand 20/chemokine receptor 6 axis (73).
3) **Multi-omics analysis.** Multi-omics analyses have emerged as novel approaches, integrating metagenomic, transcriptomic, proteomic, metabolomic, and lipidomic analysis. These comprehensive investigations shed further light on the host's response to probiotics at multiple levels (74). For instance, in a mouse model of HCC treated with probiotics, researchers utilized metagenomic analysis to identify altered pathways and corresponding biological functions (71). Notably, they observed significant changes in pathways involved in SCFAs synthesis within tumor cells. Furthermore, applying metabolomic analysis provides valuable insights into the modulation of metabolite profiles following probiotic intervention (75). In a study that combined metagenomics and metabolomics (using gas chromatography-mass spectrometry, GC-MS), researchers screened for phages associated with CRC promotion (mainly

Fusobacterium nucleatum) and inhibition (mainly *Clostridium butyricum*). Through gene ontology enrichment analysis, differentially expressed genes were found to be enriched in apoptosis and autophagy, uncovering the potential mechanism. Additionally, GC-MS analysis of *C. butyricum's* secretome revealed that butyrate played a prominent role in the cytotoxic effects on CRC cells (70).

While many studies regarding SCFAs in cancer management provided valuable insights into their potential effects and mechanisms, these preclinical studies were limited *in vitro* and *in vivo*. It is essential to conduct well-designed clinical trials (including double-blinded or triple-blinded studies) to further investigate the efficacy and safety of SCFAs in human subjects.

SCFAs combined with chemotherapy and radiotherapy

Recently, SCFAs have been studied as a sensitizer for radiotherapy and chemotherapy. Sodium butyrate combined with cisplatin has been described to promote apoptosis in different tumor cells, such as gastric cancer (76) and cervical cancer (77) *in vitro* and *in vivo*. In the tumor-bearing mouse model of gastric cancer, butyrate plus cisplatin inhibited tumor growth *via* the mitochondrial apoptosis-related pathway, surpassing other groups with monotherapy (76). The combination of butyrate and cisplatin has been reported in the cervical cancer model (Hela and Siha cell lines and tumor cell-inoculation mice) that inhibited cell migration and invasion by blocking the nuclear conversion of β-catenin, reversing epithelial–mesenchymal transition, upregulating the expression of E-cadherin and downregulating matrix metalloproteinase (MMP)2, MMP7, and MMP9 (77). In addition, Park et al. (78) investigated the effects of radiotherapy combined with butyrate, propionate, and acetate in organoids. Among them, butyrate showed radio-sensibilization and weak toxicity to normal mucosa and inhibited the proliferation of organoids. Data on the safety and efficacy of the combination in animal studies and clinical trials are yet to come.

SCFAs combined with immunotherapy

Several studies focused on patients with different types of cancer receiving immune checkpoint inhibition (ICI) therapy and collected patients' fecal samples (20, 79, 80). They suggested that the concentration of SCFAs in fecal samples might be associated with the efficacy of anti-programmed cell death protein 1 (PD-1) and anti-programmed death-ligand 1 (PD-L1) immunotherapy. These findings prompt that gut microbiota links to ICI therapeutic efficacy through SCFAs, which show the potential to be a response marker. Animal studies found that SCFAs had diverse effects on different ICI therapies (81, 82). In a CRC mouse model, researchers found that the dietary supplement of pectin increased butyrate production in the gut, promoted T-cell infiltration, and enhanced the anti-cancer effect of anti-PD-1 drugs in CRC mice (81). Another mouse model CRC/fibrosarcoma reported that butyrate restrained anti-CTLA-4 response through downregulating CD80/CD86 on dendritic cells and Inducible costimulatory on T cells and preventing the accumulation of tumor-specific T cells, memory T cells, and IL-2 (82).

SCFAs in the comprehensive management of cancer

SCFAs have therapeutic potential in treating intestinal inflammation induced by chemotherapy or radiotherapy. They reconstruct the intestinal epithelium barrier and regulate intestinal immunomodulatory function (83). In addition, direct administration of SCFA-producing bacteria (probiotics) can restore intestinal ecology and inhibit the secretion of proinflammatory cytokines (84). In the perioperative management of resectable tumors, the application of SCFA-producing bacteria (probiotics) could decrease the incidence of postoperative complications (85, 86). For CRC patients, adding butyrate before the operation helps to improve the integrity of the intestinal barrier (87).

SCFAs from dietary fibers supplementation in cancer treatment

Numerous studies support the health-promoting effects of DFs from daily food (88, 89), including the anti-tumor effect. Pectin and inulin have been reported to enhance the immune response to tumors in mouse models. Pectin supplementation was associated with an improved response to immunotherapy in mice with CRC (81). Another study suggested a potential link between SCFAs derived from inulin fermentation and the anti-tumor activity of ICIs (90). Nevertheless, pectin has been shown to accelerate carcinogenesis in *Apc*-deficient mice (91), while dietary inulin supplementation may induce gut microbiota-dependent hepatocellular carcinoma (53). In addition to animal experiments, clinical research has indicated that adequate DFs intake can improve the prognosis of cancer patients. A cross-section study revealed that sufficient DFs intake was associated with significantly improved PFS and response to ICIs in melanoma patients, compared to a combination of DFs and probiotics (92). However, the study did not find a significant association between DF proportions and the SCFA levels in the gut. Furthermore, SCFAs play a critical role in the health-promoting effect of vegetarian and Mediterranean diets, which are characterized by high DF content (93–95). Nevertheless, the absence of relevant cohort studies makes it uncertain whether cancer patients can benefit from these dietary patterns. These findings highlight the need to carefully evaluate the potential benefits of DFs in future studies, considering their potential risks.

Challenges and limitations

Challenges as a therapeutic approach for cancer

The anti-cancer drug usually requires a comprehensive understanding of its pharmacology, toxicology, and high specificity

on its target molecules. SCFAs have been found ambiguity effects on tumor progression: suppression and promotion, which challenges the further application of SCFAs in anti-cancer treatment. Donohoe et al. (96) reported decreased production of butyrate and increased butyrate nuclear accumulation in a microbiota- and butyrate-dependent mouse model with colon tumor cells. These phenomena were associated with enhanced apoptosis and reduced proliferation in tumors. Another mouse model with colon cancer driven by mutations in the mismatch repair genes *Msh2* and *Apc* showed that butyrate drove the hyperproliferation of *Msh2*-deficient epithelial cells and promoted the development of CRC (54). Noteworthy, tumor genetics and butyrate concentrations were considered the key factors that led to the opposite effects of SCFAs on carcinogenesis between these investigations mentioned above (49, 50). So far, the questions about which are the responsible mutations and what is the cut-off concentration still need to be answered. It indicates that researchers should be aware that SCFAs may play more complex and comprehensive roles in cancer than we used to understand. Thus, we urge that more efforts be put into unraveling the spectrum of SCFAs' biological effects on cancer.

Limitations of distribution and plasma clearance

SCFAs serve as the primary energy source for intestinal epithelial cells; therefore the systemic absorption of butyrate is low (51). Their concentrations significantly differ between enteral and abenteric environments (butyrate concentration is 29 μM in portal vein vs. 4 μM in peripheral circulation) (68, 97). To engage their anti-tumor effects, SCFAs shall maintain different effective concentrations continuously in a patient's circulation given cancer types. For example, butyrate concentration in circulation should reach at least 0.5 mM to induce tumor cell differentiation in CRC (98) and breast cancer (28), However, butyrate at the concentration of 0.5 mM did not significantly affect the gastric cancer cell viability *in vitro* experiments (76). In addition, butyrate has a rapid plasma clearance in the human body with only a 6 min half-life. Once absorbed, SCFAs are transported to the liver *via* portal circulation and become the substrate for longer-chain fatty acids (51). Researchers reported that the peak concentration of butyrate in plasma among patients with acute leukemia was merely 0.05 mM by intravenous infusion (99). The insufficient concentration and short half-life of SCFAs in human circulation challenge their application. Current efforts have been made to innovate drug administration and explore stable derivatives:

1) **Drug administration.** Oral administration of solid lipid nanoparticles (SLN) (100) is an attempt to deliver butyrate across the intestinal barrier to target organs using a sustained-release drug delivery system. SLN is not absorbed by the gastrointestinal tract and cannot pass through the blood–brain barrier. Cholesteryl-butyrate SLN has been confirmed to increase the stability and efficacy of butyrate in a mouse glioma model (100).
2) **Stable derivatives.** Researchers tried to use prodrugs of SCFAs [Trybutirin (101), phenylbutyrate (102), and pivaloyloxymethyl butyrate (Pivanex, AN-9) (103, 104)] and explore their effects on tumors [leukemia (102), non-small cell lung cancer (104), and prostate cancer (105)]. These prodrugs had not only similar effects as butyrate in inducing apoptosis (101) and anti-angiogenesis effects (106) but also longer half-life and higher stable plasma concentrations (107). Notably, the doses were still insufficient to exert consistent anti-tumor effects (108).

To sum up, exploring various local delivery methods (such as enema, nasal spray, aerosol inhalation, intravaginal administration, and bladder irrigation) or developing new drug delivery systems may be the direction of future translational research.

Conclusion and future perspectives

Although astounding clinical successes in anti-cancer treatments have been achieved, cancer remains the second leading cause of death worldwide and dramatically affects the quality of life of cancer survivors. In the present review, we summarize advancements in the roles of the microbial fermentation of DFs-derived SCFAs in cancer and recapitulate the up-to-date evidence on the applications of SCFAs in cancer treatment. Additionally, we notice that SCFAs present the potential to mediate a wide range of biological effects beyond function as HDACIs, GPCRs, and TIME modulators, resulting in both tumor suppression and promotion. It highlighted the challenges of applying prebiotics, probiotics, and microbial metabolites to a therapeutic modality for cancer. We urge more effort to be put into unraveling the spectrum of SCFAs' biological effects and their functional organizing network, which is the prerequisite for better management of cancer.

Moreover, SCFAs might influence carcinogenesis and inflammation similarly in other regions beyond the gut, such as the reproductive tract, respiratory tract, and urinary tract. A fiber-rich diet can increase the production of SCFAs by altering the composition, diversity, and abundance of the microbiome to promote health. Hence, we might regulate SCFAs by prebiotics or probiotics to alter the commensal microbiome and modulate the desirable concentration of SCFAs in particular regions. To test these hypotheses, future investigations are warranted to explore the associations between commensal microbiota and its metabolites in various body sites and various types of cancer, consequently developing novel therapeutic approaches for improving prognosis and quality of life among cancer patients.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Immunomodulatory effects of inulin and its intestinal metabolites

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"Dietary fiber" (DF) refers to a type of carbohydrate that cannot be digested fully. DF is not an essential nutrient, but it plays an important part in enhancing digestive capacity and maintaining intestinal health. Therefore, DF supplementation in the daily diet is highly recommended. Inulin is a soluble DF, and commonly added to foods. Recently, several studies have found that dietary supplementation of inulin can improve metabolic function and regulate intestinal immunity. Inulin is fermented in the colon by the gut microbiota and a series of metabolites is generated. Among these metabolites, short-chain fatty acids provide energy to intestinal epithelial cells and participate in regulating the differentiation of immune cells. Inulin and its intestinal metabolites contribute to host immunity. This review summarizes the effect of inulin and its metabolites on intestinal immunity, and the underlying mechanisms of inulin in preventing diseases such as type 2 diabetes mellitus, inflammatory bowel disease, chronic kidney disease, and certain cancer types.

KEYWORDS

inulin, short-chain fatty acids, intestinal immunity, intestinal microbiota, intestinal epithelial cells, intestinal immune cells

Introduction

"Dietary fiber" (DF) is defined as carbohydrate polymers containing ≥ 10 monomeric units that resist digestion by endogenous enzymes in the small intestine. DF includes edible carbohydrate polymers that exist naturally in food, and carbohydrate polymers that are synthesized by physical, chemical, or enzymatic methods (1). DF can be divided into "soluble DF" (SDF) and "insoluble DF" (IDF) according to solubility, and "partially fermentable fiber" and "completely fermentable fiber" by its fermentability (2). The microfibrils formed by the inter- and intra-molecular hydrogen bonds can hinder the degradation and utilization of partially fermentable fiber, which prevent its fermentation in the intestine (3). The health benefits of DF are manifested mainly by altering gut microbiota composition and microbial metabolites.

Inulin is one kind of SDF. It is a type of fructan derived mainly from plants such as chicory, ginger, garlic, onion, and asparagus. "Inulin" is a generic term covering all β- (2, 1) linear fructans, and inulin-type fructans must have β-(2,1) linkages, which give inulin

unique structural and physiological properties, making it resistant to enzymatic hydrolysis by human saliva and small intestinal digestive enzymes (4). Most inulin-type fructans have an average degree of polymerization of 10-12 and a chain length of 2-60 units of molecular distribution (5). Oligofructose can be hydrolyzed from inulin by inulinase into a chain length from 2 to 10. Therefore, the sugar chain of inulin is longer compared with that of oligofructose, resulting in slower fermentation and gas production. Inulin has been used widely as a prebiotic, fat substitute, sugar substitute, texture modifier, and in the development of functional foods (6). The US Department of Agriculture recommends consuming 25-36 g of fiber daily (or 14 g for every 1000 calories per day) (7). In 2003, the US Food and Drug Administration (FDA) categorized inulin as "generally recognized as safe". The daily effective intake is 5 g, and the recommended maximum daily intake is 15-20 g (8). Nausea, bloating, and flatulence are the most common adverse effects of taking inulin. Inulin consumption under 40 g per day in healthy adults is safe. However, inulin can cause serious side effects in patients with inflammatory bowel disease (IBD) or allergies.

The intestine is the front-line of the body's defense, and is exposed to many pathogens and bacteria. As the largest immune organ of the body, the intestinal immune system (also known as the mucosal immune system) is composed mainly of intestinal epithelial cells (IECs), lamina propria-lymphocytes, intraepithelial lymphocytes, and the Peyer's patch. An inulin-rich diet has been reported to improve the function of the intestinal barrier and modulate the immune system (9).

The aim of this review is to focus on the immunomodulatory effects of inulin and its intestinal metabolites. In this way, we hope to provide a comprehensive overview of the role of inulin and its metabolites in different diseases.

Intestinal metabolites of inulin

As mentioned above, the unique β-configuration in the monomeric isomer C2 of fructose prevents inulin-type fructose from being hydrolyzed by digestive enzymes (including α-glucosidase, maltosidase, and sucrase) (10). Upon the fermentation of intestinal bacteria, inulin produces lactate and short-chain fatty acids (SCFAs), including acetate, butyrate and propionate, as well as gases that are excreted from the body eventually (11–15) (Table 1). Notably, lactate does not usually accumulate in the healthy gut because microbes can convert it further to propionate, butyrate, or acetate (25). The degree of fermentation of DF is closely correlated with its composition. SDFs such as inulin are usually more fermentable than IDFs and produce more gas and SCFAs (16, 26). In addition, the fermentation properties of inulin are related to the length of its sugar chain; short-chain inulin is more soluble in water than long-chain inulin. Muthyala and colleagues reported changes in fecal SCFA levels in mice of different ages after inulin ingestion. They found butyric acid to be the main metabolite in middle-aged mice, whereas the fecal level of propionic acid showed an age-dependent decrease. Those evidences suggest that age is an important factor influencing inulin metabolism by the intestinal microbiota (27).

More interestingly, inulin and gut microbiota are mutually interacted. Gut bacteria ferment inulin to produce the corresponding metabolites. Likewise, the gut microbiome responds to inulin treatment and exhibits significant structural alterations. Inulin treatment promotes the growth of certain beneficial bacteria as well as bacteria that promote the production of SCFAs, such as *Bifidobacterium* spp (28). SCFAs can act locally in the intestine and be used as energy sources by intestinal mucosal

TABLE 1 Metabolites induced by inulin fermentation.

Interventions	Duration	Models or subjects	Metabolites with significantly upregulated expression	References
Inulin (10 g/L)	24 h	Fresh stool samples from 9 healthy humans (*ex vivo* system)	Acetate, propionate, and butyrate	(14)
FOS (12 g/d)-enriched inulin supplementation	0, 12, 24, and 48 h	Fecal cultures from pigs (*in vitro* fecal fermentation)	Succinate, lactate, propionate and butyrate	(16)
Inulin (24 g) plus glucose (75 g)/water (300 mL)	0-6 h	25 adults with BMI of 20-35 kg/m^2	Propionate and butyrate	(15)
Inulin (24 g) plus high-fructose corn syrup (56 g)/drinks (400 mL)	4-6 h	12 healthy humans	Serum acetate, propionate, and butyrate	(17)
U-^{13}C-inulin (0.5 g)/inulin (24 g) in a high-fat milkshake	7 h	14 healthy, overweight to obese men	Plasma propionate, butyrate, acetate	(18)
Inulin-type fructans	6 weeks	25 patients with type 2 diabetes mellitus	Significantly increased fecal concentrations of total short-chain fatty acids, acetic acid and propionic acid	(19, 20)
Water with 20% sucrose and 5% inulin (*w/w*)	6 weeks	Male Sprague–Dawley rats (6 weeks)	Propionate and butyrate; fecal contents of indole-3-acetic acid and kynurenic acid	(21)
Basal diet containing 0.5% inulin	21 days	20 growing-pigs	Acetate and butyrate concentrations in cecum	(22)
Control diet with 20% inulin	3 weeks	BALB/c mice (6–8 weeks)	Fecal acetate, propionate and butyrate	(23)
High-fat/high-sucrose diet containing inulin (7.5% kcal)	12 weeks	Male C57BL/6J mice (8 weeks)	Acetic acid in jejunum; succinic acid, acetic acid and propionic acid in the rectal feces and portal vein serum	(24)

cells to promote barrier function and maintain mucosal immunity, and provide energy substrate for colonic cells (29, 30). SCFAs can also enter the circulation through the hepatic portal vein and act as signaling molecules, thereby regulating systemic immune function (31, 32). The G protein-coupled receptors (GPCRs) GPR43 and GPR41 were the first GPCRs to be identified as activated by SCFAs, and were subsequently renamed as the specific free fatty acid receptors (FFARs) FFAR2 and FFAR3, respectively. Recently, three additional GPCRs, GPR109A, Olfr78 and Olfr558, were identified as receptors for SCFAs (33). SCFAs are involved in the regulation of inflammatory responses because they interact with these receptors expressed on innate immune cells (34). Notably, FFAR2/3 has been found to be expressed mainly in enteroendocrine cells and immune cells. Expression of FFARs on colonic regulatory T (T_{reg}) cells has been shown to be significantly higher than that on other tissues (34, 35), suggesting a potential role of SCFAs in maintaining intestinal immune homeostasis.

Effects of inulin and its metabolites on intestinal microbiota

The human intestinal microbiota is divided into four major phyla covering more than 90% of the total bacterial population, i.e., *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and other minor phyla, including Warty microbes and *Clostridium* (36). The phylum *Firmicutes* and *Bacteroidetes* are the two most abundant microbial phyla in the human intestinal microbiota. The *Firmicutes* are low GC Gram-positive bacteria, and *Clostridium* spp. and *Lactobacillus* spp. are the dominant component, while the leading members of *Bacteroidetes* are *Bacillus* spp. and *Prevotella* spp. Increased ratio of Phylum *Firmicutes*/*Bacteroidetes* is usually considered to be associated with obesity-associated dysbiosis (37, 38). Inulin intake has been reported to significantly reduce the ratio of *Firmicutes* and *Bacteroidetes*, as well as levels of several bacteria associated with a pro-inflammatory state (27). Bastard and colleagues also found that changes in intestinal microbiota after inulin supplementation decreased the relative abundance of *Bacteroidetes*, and increased levels of *Bifidobacterium* spp., *Anaerostipes* spp., *Enterococcus faecalis*, and *Lactobacillus* spp (39).. Inulin also promotes an increase in the abundance of bacteria of the genera *Phascolarctobacterium*, *Blautia*, *Akkermansia*, *Ruminococcus*, and the family *Lachnospiraceae*, which are also responsible for SCFAs production (21, 40, 41). We have summarized some major changes in intestinal microbiota after at least 4 weeks or even 3 months of inulin supplementation in different models or individuals, as shown in Table 2.

In addition to promoting SCFAs production, *Bifidobacteria* spp. are also considered to be probiotics that inhibit the proliferation of pathogenic bacteria. Dietary inulin supplementation increases the relative abundance of *Bifidobacteria* spp. and consequently brings a series of beneficial alterations defined as "bifidogenic effects" (28). Thus, inulin use inhibits harmful bacteria or opportunistic pathogens by promoting the proliferation of beneficial bacteria. In addition, pathogenic bacteria tend to colonize in the intestine with an alkaline environment. Inulin lowers intestinal pH after enterobacterial fermentation, which also contributes to the

TABLE 2 Examples of microbiota modulation after inulin ingestion.

Treatment	Duration	Models or subjects	Altered gut microbiota level		Reference
			Up-regulation	Down-regulation	
Diet of inulin (2.5 g or 5 g/100 g	4 weeks	*ob*/*ob* mice	*Ruminococcaceae*, *Lachnospiraceae*, *Bacteroides*, and *Bifidobacterium*	–	(42)
Inulin (16 g/d)	3 months	Obese patients	*Bifidobacterium*, *Catenibacterium*, *Erysipelotrichaceae incertae sedis*, *Escherichia/Shigella*, *Lact-bacillus*, and *Dorea*	*Desulfovibrio*, *Roseburia*, *Butyricimonas*, *Clostridium* cluster XIVa, and *Clostridium sensu stricto*	(43)
An inulin-containing semi-purified, irradiated regular diet	6 weeks	Male C57BL/6J mice	*Akkermansia*, *Roseburia*, *Bacteroides*	*Lactococcus*, *Ruminiclostridium_9*, *Ruminococcaceae* and *Streptococcaceae*	(27)
Vilof™ soluble dietary fiber powder (3 g/kg bodyweight/d) containing 91% inulin-type fructans	12 weeks	Model of diabetes mellitus in rat	*Lactobacillus*, Lachnospiraceae, *Bacteroides*, and *Phascolarctobacterium*	*Desulfovibrio*	(40)
Water with 20% sucrose and 5% inulin (*w*/*w*)	6 weeks	Male Sprague-Dawley rats	*Bifidobacterium*, *Actinobacteria*, *Blautia* and *Phascolarctobacterium*	*Proteobacteria*	(21)
Lieber–DeCarli liquid diets containing inulin (0.5 g/L)	6 weeks	Female C57BL/6J mice	*Allobaculum*, *Lactobacillus* and *Lactococcus*	*Parasutterella*	(44)
Inulin-propionate ester (20 g/d)	42 days	Overweight or obese adults not suffering from diabetes mellitus	*Actinobacteria*	*Clostridia*	(45)

inhibition of pathogenic bacteria (26). Furthermore, treatment with inulin has been shown to significantly reduce the abundance of lipopolysaccharide (LPS)-producing *Desulfovibrio* spp. in rats and obese patients (40, 43), which may protect the intestinal barrier from endotoxin damage.

Inulin has been reported to be beneficial for a series of diseases through modulating intestinal microbiota. A reduced abundance of *F. prausnitzii* has been observed in patients with nonalcoholic fatty liver disease (NAFLD). Inulin can provide carbon sources for the transporter of the fructose phosphotransferase system. This action enhances the fructose-absorption activity of *F. prausnitzii* and increases the abundance of *F. prausnitzii* in the gut (46). In a model of alcoholic fatty liver disease, chronic exposure to alcohol resulted in decreased abundance of the genera *Allobaculum*, *Lactobacillus*, and *Lactococcus*, but increased abundance of *Parasutterella* species. Inulin could reverse these alterations and reduce the number of macrophages (44). In addition, dietary supplementation with inulin has been found to restore the diversity of intestinal microbiota in a mouse model of obesity based on high-fat-diet (HFD) consumption (47).

Effects of inulin and its metabolites on IECs

Reduced intestinal mucosal tolerance promotes immune-mediated inflammatory diseases. The most important part of the intestinal mucosal barrier is the intestinal mucosal mechanical barrier. The latter is a defense layer composed of intestinal mucosal epithelial cells and tight junctions (TJs) that protects against pathogens (48). IECs are differentiated from intestinal stem cells and can be divided broadly into "absorptive enterocytes" and "secretory enterocytes" (goblet cells that secrete mucus, Paneth cells that secrete antimicrobial peptides and immunomodulatory proteins, and enteroendocrine cells that secrete hormone) (49, 50).

Inulin-type fructans extracted from different plants have been shown to have direct immunomodulatory effects on IECs. For example, inulin-type fructans from *Platycodon grandiflorus* have been shown to stimulate transcription of the anti-inflammatory factors interleukin (IL)-4 and IL-10 in a dose-dependent manner in a porcine jejunum epithelial cell line (IPEC-J2) (51). The inulin fractions from *Codonopsis pilosula* and *Codonopsis tangshen* are natural sources of potential antioxidants, which can increase intestinal levels of glutathione peroxidase, superoxide dismutase and catalase, but reduce the levels of malondialdehyde and lactate dehydrogenaseto enhance the antioxidant defense of IECs (52). Moreover, *Ruminococcus bromii*-producing butyrate is a major source of energy for colonocytes, which contributes to enterocyte proliferation (53, 54).

In addition to promoting the function of IECs, inulin can promote intestinal barrier function by regulating TJ proteins. Dietary supplementation with inulin can restore the integrity and function of the intestinal barrier by promoting the expression of zonula occludens (ZO)-1, claudin-1 and occludin (21). Chen et al. found that long-chain inulin-type fructans enhanced expression of the intestinal-barrier TJ proteins occludin and claudin-2, antimicrobial peptides β-defensin-1, cathelicidin-related antimicrobial peptide, and SCFAs production (55). In another animal experiment, inulin supplementation increased villus height and ZO-1 expression, reduced secretion of IL-6 and tumor necrosis factor (TNF)-α, and increased IECs apoptosis in the ileum and cecum (22). Mucin 2 (Muc2) is the main component of mucus. Muc2 can constrain the immunogenicity of antigens by forming a non-specific physical barrier. A previous study showed that Muc2 can be ingested by dendritic cells (DCs), and reduce the number of inflammatory DCs by inhibiting gene transcription through nuclear factor-kappa B (NF-κB). Therefore, Muc2 can increase the tolerance of the intestine (56). Inulin has also been found to promote the secretion of Muc2 and secretory immunoglobulin A (sIgA) in the ileum (57). sIgA is involved in important mucosal immune functions against external antigens on human mucosal surfaces. Thus, inulin intake facilitates the protection of IECs from luminal bacteria and food antigens, and enhances intestinal homeostasis and tolerance to prevent inflammation. In addition, the effect of inulin on host defense in Paneth cells may be mediated (at least in part) by SCFAs produced by inulin fermentation. Supplementation with inulin has been shown to induce expression of α-defensin and matrix metalloproteinase (MMP)-7 from Paneth cells in an obese mouse model. Moreover, organoid culture of small intestinal crypts revealed that the fermentation products of inulin induced α-defensin expression from Paneth cells (58). Butyrate has also been found to enhance the intestinal barrier by activating adenosine monophosphate-activated protein kinase to promote TJ assembly in monolayers of Caco-2 cells (59).

In general, consumption of an inulin-containing diet is beneficial for intestinal health. However, some studies have reported contradictory evidences. One study showed that a moderate dose of inulin (50 mg per mouse) was beneficial against food allergy, whereas high-dose inulin supplementation (80 mg per mouse) increased serum levels of allergic inflammation-related factors and an intestinal inflammatory response. Further profiling indicated that the altered intestinal TJ proteins and T cell homeostasis seen in hyperinulin-treated mice might be related to the high production of SCFAs by bacteria of the family *Ruminococcaceae* and *Bifidobacterium* spp (60). In addition, long-term intake of inulin also exacerbated intestinal damage and inflammatory responses in the progeny of rats in a dextran sodium sulfate (DSS)-induced colitis model (61).

Effects of inulin and its metabolites on intestinal immune cells

Many types of immune cell, such as T cells, innate lymphoid cells (ILCs), and macrophages, are present in the lamina propria of the intestine. Mucus and antimicrobial peptides secreted by goblet cells, as well as immunomodulatory proteins secreted by Paneth cells, can help to prevent the adhesion of pathogenic bacteria and viruses in the intestinal lumen (62, 63). Several studies have shown

that the role of inulin in regulation of immune cell activation and cytokine secretion is largely dependent on its intestinal metabolites, such as SCFAs. The latter can act directly on host T cells by reprogramming their metabolic activity and epigenetic status to control the differentiation of effector T (T_{eff}) cells and T_{reg} cells (64). More importantly, SCFAs can also enter the circulation and regulate the function of immune cells in other tissues (65). The effects of inulin and its metabolites on immune cells is summarized in Figure 1.

T_{eff} and T_{reg} cells

T cells are critical mediators of adaptive immunity. When T cells recognize pathogens through T-cell receptors, together with costimulatory signals provided by antigen-presenting cells, T cells expand clonally and traffic to tissues, thereby triggering an adaptive immune response. However, an excessive immune response usually leads to severe tissue damage. In contrast, T_{reg} cells can limit the immune response from T_{eff} cells to avoid overwhelming inflammatory responses, a process known as "immune tolerance" (66). Several recent studies have shown SCFAs to be critical factors in balancing adaptive immunity and immune tolerance (28, 67). SCFAs produced by inulin fermentation maintain immune homeostasis by suppressing excessive innate responses and stimulating specific adaptive immunity.

The metabolic and functional changes of cluster of differentiation (CD)8^+ T cells are partially mediated by inulin and SCFAs. Inulin treatment promotes the infiltration $CD8^+$ T cells in tumors of several mouse models, and induces a shift to a pro-inflammatory tumor microenvironment (68–70). Furthermore, SCFAs (e.g., butyrate) can regulate the metabolism of $CD8^+$ T cells by acting on FFAR3, thereby ensuring rapid and sustained activation of T_{eff} cells during viral infections (28). In contrast, the mechanisms by which inulin and SCFAs limit autoimmune responses by regulating T_{reg} cells differentiation are more complex. Butyrate promotes production of extra-thymic T_{reg} cells in an intronic enhancer CNS1-dependent manner if administered systemically, but increases only intracolonic T_{reg} cells production if administered locally via an enema (71). Conversely, acetate and propionate promote the accumulation of intracolonic T_{reg} cells in an FFAR2-dependent manner (66).

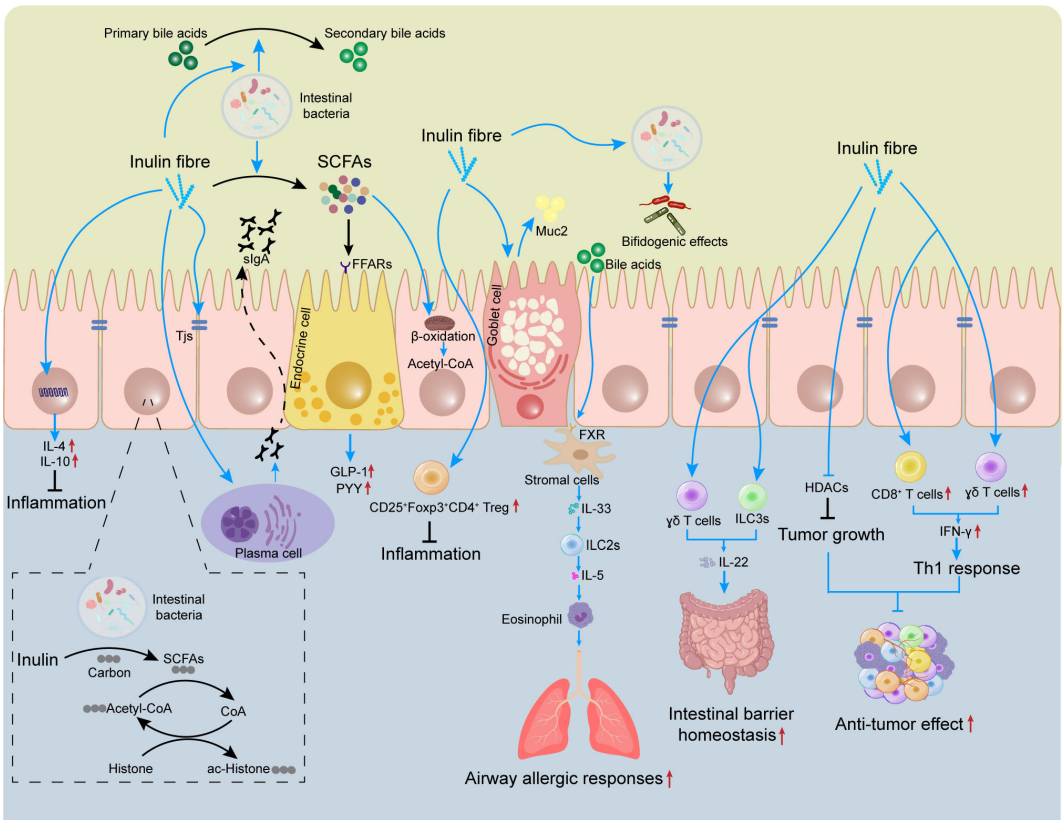

FIGURE 1
Effects of inulin on the mucosal immune system. The gut contains many immune cells. Inulin can regulate the differentiation and proliferation of these immune cells (e.g., T_{reg}) to limit intestinal inflammation. Inulin promotes expression of TJ proteins and induces secretion of sIgA and Muc2 by plasma cells and goblet cells, which helps to maintain intestinal-barrier homeostasis. Inulin promotes IL-22 secretion by γδ T cells and ILC3s, which also helps to improve the intestinal barrier. However, inulin increases the circulating level of bile acids and triggers ILC2s to induce eosinophils, thereby exacerbating airway allergic responses. Inulin provides carbon sources for histone acetylation, regulates epigenetics, and inhibits tumor growth. In tumors, inulin can also promote the infiltration of $CD8^+$ T cells and γδ T cells to enhance the anti-tumor effect. SCFAs, short-chain fatty acids; sIgA, secretory immunoglobulin A; FFARs, free fatty acid receptors; Muc2, mucin 2; TJ, tight junction; GLP-1, glucagon-like peptide 1; PYY, peptide YY, FXR, farnesoid X receptor; HDAC, histone deacetylase; ILC, innate lymphoid cell.

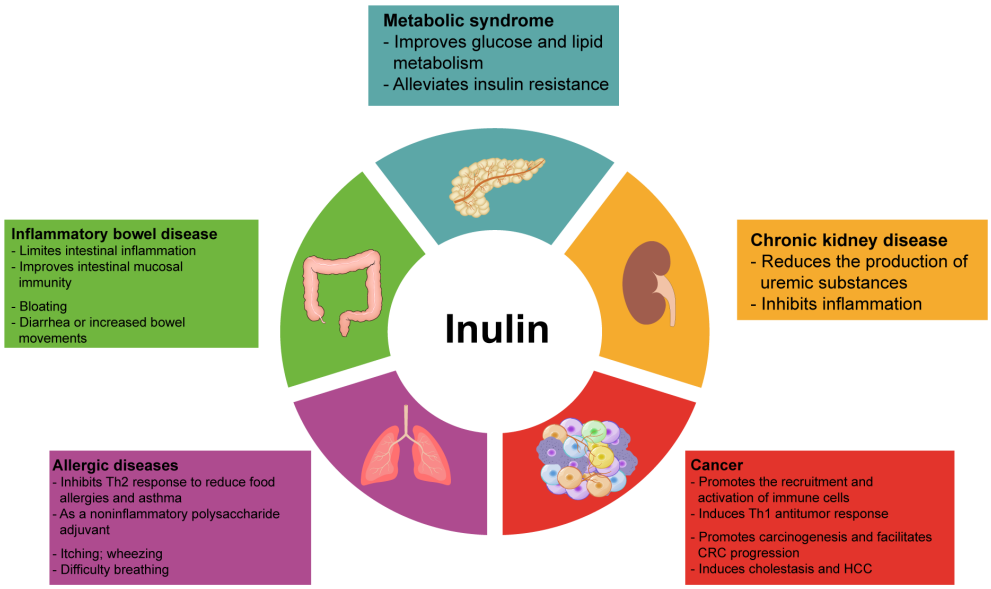

FIGURE 2
Overview of the involvement of inulin in disease. Inulin and its metabolites regulate energy metabolism and immune function, thus ameliorating various disease. However, inulin can also cause some side effects, such as nausea, bloating, flatulence, itching, and heartburn. In addition, people with inflammatory bowel disease or allergies should be more cautious about inulin intake to avoid serious adverse events.

IL-10 is a key cytokine for T_{reg} cells to exert anti-inflammatory effects. IL-10 is secreted by T_{reg} subsets that express the transcription factor forkhead box P3 (*Foxp3*). Thus, T_{reg} cells expressing *Foxp3* are crucial in limiting intestinal inflammatory responses (34). An independent study showed that supplementation with long-chain inulin-type fructans promoted the proliferation of $CD25^+$ $Foxp3^+$ $CD4^+$ T_{reg} cells and reduced the number of $IL17A^+$ $CD4^+$ T-helper (Th)17 cells, thereby modulating T cell responses and suppressing intestinal inflammatory responses (55). In addition, metabolites of inulin (e.g., propionate) regulate the proliferation and differentiation of $CD25^+$ $Foxp3^+$ T_{reg} cells (66, 72). Propionate also improves angiotensin II-induced inflammatory responses by modulating T_{reg} cells (73).

Histone acetyltransferase (HAT) and histone deacetylase (HDAC) are important for regulating gene expression. Usually, a high acetylation level indicates active transcriptional activity. A low acetylation level is associated with transcriptional repression. In the intestine, if HDAC is overexpressed, the balance of gene expression is disrupted and cell proliferation is abnormal, which eventually leads to tumorigenesis (74). In contrast, the gut microbiota can inhibit HDAC activity by fermenting inulin into SCFAs, thereby regulating the acetylation level of histones and affecting epigenetic changes in immune cells (75, 76). An independent study showed that inulin dietary treatment inhibited HDACs activity (including HDAC2 and HDAC8), and induced protective epigenetic changes in mouse mammary tumor cells (77). In another animal experiment, consumption of an inulin diet increased the level of SCFAs (especially butyrate), which enhanced the host antimicrobial program by inhibiting HDAC3 (23). Similarly, Fernández et al. found that administration of inulin-rich products reduced the number of colon polyps in two animal models of colorectal cancer (CRC), which may be related to HDACs regulation (78). Furthermore, a study using isotope tracing revealed inulin-derived SCFAs to provide carbon sources for histone acetylation (76).Notably, SCFAs are also involved in regulating epigenetic changes and energy metabolism of B cells by suppressing the activity of HDACs (79–82).

In particular, SCFAs contribute significantly in the fight against intestinal inflammation by promoting T_{reg} cells differentiation through the inhibition of HDACs. Butyrate has also been found to be a potent inhibitor of HDACs (83). Furusawa and colleagues found that butyrate produced by obligate anaerobic bacteria improved colitis by promoting histone H3 acetylation in the promoter and conserved non-coding sequence regions of the *Foxp3* locus, thereby supporting differentiation of T_{reg} cells and enhancing intestinal immune tolerance (84). In addition to directly promoting the differentiation of $CD4^+$ T-cell precursors into T_{reg} cells, butyrate and propionate can induce the differentiation of extrathymic T_{reg} cells and reduce expression of pro-inflammatory cytokines within DCs by inhibiting HDACs activity (71). Propionate enhances histone acetylation in colonic T_{reg} cells, drives the proliferation and differentiation of T_{reg} cells, and enhances T_{reg} cell-mediated inhibition of colitis. These effects of propionate appear to be dependent on activation of FFAR2 (66). Nevertheless, whether the inhibitory effect of SCFAs on HDACs is dependent on expression of FFAR2 and FFAR3 is controversial, because SCFAs seem to enter cells directly through membrane transport proteins on the cell surface (85). Furthermore, although butyrate and propionate can inhibit HDACs and promote the proliferation and differentiation of T_{reg} cells, acetate appears to lack this inhibitory activity towards HDACs (71).

Immune cells and cytokines are crucial in the development and regression of inflammatory responses. Inflammation is characterized by excessive infiltration of immune cells (e.g.

macrophages, neutrophils), which subsequently release pro-inflammatory cytokines. Simultaneously, regression of inflammatory responses requires the release of anti-inflammatory factors (e.g. IL-10) by immune cells. Inulin and its metabolites can selectively support the development of Th1 and Th17 effector cells and IL-10$^+$ T_{reg} cells, depending on the cytokine milieu and immunological context. SCFAs promote the differentiation of IL-10$^+$ CD4$^+$ T cells under a physiological state. Once the immune response is initiated, SCFAs turns to support the proliferation of T_{eff} cells, such as Th1 and Th17 cells (85). Therefore, inulin and its metabolites modulate the balance of the immune response, setting a reasonable "immune tension" that allows T cells to clear harmful substances but avoids exaggerating the level of tissue damage.

Innate lymphoid cells

ILCs are an important subpopulation of natural immune cells. ILCs (like B cells and T cells) develop from common lymphoid progenitor cells, and share some common characteristics with T cells. However, ILCs do not express antigen-specific receptors (e.g., T-cell receptors, B-cell receptors). In addition, ILCs do not undergo thymic selection, clonal selection, or clonal expansion. Therefore, ILCs respond rapidly to tissue infection and pathogens, but the effector molecules produced are the same as those in Th cells. ILCs can be classified into four categories according to the cytokines they secrete: ILC1s secrete interferon (IFN)-γ; ILC2s secrete IL-5, IL-9, and IL-13; ILC3s secrete IL-22, IL-17A/F, and granulocyte macrophage-colony stimulating factor; regulatory ILC cells secrete IL-10 (86, 87). Multiple GPCR receptors are expressed on the surface of ILCs, and SCFAs have been found to activate GPCR receptors on ILCs and promote tissue repair and host defense, which contributes to regulating adaptive immunity (88–91).

Allergens lead to type-2 inflammatory responses, which are mediated by Th2 cells, ILC2s, and their secreted cytokines. Type-2 inflammatory responses can stimulate B cell proliferation to produce antibodies, mediate humoral immunity, participate in barrier immunity at mucosal surfaces, and play a part in counteracting parasitic infections and allergic diseases (92). An inulin fiber diet promotes type-2 immune responses after spirochete infection in an eosinophil-dependent manner (93). Furthermore, an increased activity of ILC2s plays a key part in asthma development (94), and inulin intervention has been reported to reduce the number of airway eosinophils and improve asthma by suppressing HDAC9 expression in people suffering from asthma (95). Furthermore, direct supplementation with SCFAs can also suppressed ILC2s and lung-related allergic reactions (96).

However, in contradiction to previous evidence, Arifuzzaman and colleagues showed that inulin increased systemic levels of bile acids (particularly cholic acid), which led to an increased IL-33 level via activation of the farnesoid X receptor (FXR) pathway. IL-33 secretion caused subsequent activation of ILC2s and the production of IL-5, leading to increased eosinophilia which exacerbated airway allergic responses (93). SCFAs-mediated FFAR2 expression has been shown to trigger phosphoinositide 3-kinase (PI3K), signal transducer and activator of transcription (Stat)3, Stat5, and mammalian target of rapamycin pathways to promote ILC2s proliferation. However, SCFAs also seem to inhibit the proliferation of ILC2s through a non-FFAR2-mediated mechanism (88). Thus, inulin may maintain optimal amounts of ILC2s in peripheral tissues to modulate type-2 immune responses during infections through multiple pathways. The immunological outcomes of consuming an inulin fiber diet are dependent upon the interactions between various microbiota-derived metabolites and different immunomodulatory pathways (93).

As a member of the IL-10 family, IL-22 has an important role in intestinal immune regulation. IL-22 has been reported to promote epithelial cell proliferation and induce the production of Reg3γ and other antimicrobial peptides (97). ILC3s, γδT lymphocytes and CD4$^+$ T cells are the main cell types that secrete IL-22 in the gut (98). Inulin has been reported to promote colon epithelial remodeling by increasing γδT lymphocyte-induced IL-22 production (99). In addition, HFD consumption disrupts enterocyte proliferation, leading to an impaired intestinal barrier, low-grade inflammation, and metabolic syndrome. Conversely, inulin supplementation in a HFD impacts microbiota and promotes IL-22 expression in an ILC3s-dependent manner, which fortifies the intestine, thereby resulting in reduced microbiota encroachment and expression of pro-inflammatory genes (100). Several studies have also shown that SCFAs, which are products of the fermentation of DF (including inulin), promote the proliferation of ILC3s and CD4$^+$ T cells and subsequent production of IL-22 through several mechanisms (101, 102).

Monocytes and macrophages

Toll-like receptors (TLRs) recognize different pathogen-associated molecular patterns and trigger the production of pro-inflammatory factors in macrophages (103). However, sustained production of pro-inflammatory cytokines and chemokines can lead to disruption of immune homeostasis. As a TLR4 ligand, inulin activates TLR4 and regulates expression of inflammatory factors in monocytes (104). Butyrate has been shown to reverse the abnormal expression of ZO-1 and reduce LPS translocation as well as inhibit macrophage activation, pro-inflammatory cytokine production, and neutrophil infiltration, thereby reducing liver injury in rats (105). Similarly, Qiao et al. found that butyrate inhibits the production of TNF-α and IL-6 and myeloperoxidase activity by blocking NF-κB activation in Kupffer cells (106). In ulcerative colitis (UC), butyrate also inhibits NF-κB activation in macrophages and reduces mucosal inflammation (107). In a *Staphylococcus aureus*-induced mastitis model, intake of high-dose inulin was shown to inhibit HDAC3 by promoting butyrate production in mice, thereby activating the macrophage-mediated antimicrobial defense program (23). Moreover, butyrate administration in influenza-infected mice remodeled bone-marrow hematopoiesis, promoted production of Ly6c$^-$ monocytes, and enhanced alternative macrophage activation, thereby inhibiting CXCL1 production, neutrophil recruitment, and the immune response during infection (28). Recently, the protective effect of butyrate was also observed in a peripheral blood mononuclear cell (PBMC) model of gout, in which butyrate

downregulated the production of the pro-inflammatory cytokines IL-1β, IL-6, and IL-8 by inhibiting HDACs (108). In conclusion, these evidences suggest a protective role of inulin and SCFAs in regulating monocyte-macrophage-mediated protection in inflammatory responses and immune processes.

Regulation of inulin and its metabolites of energy metabolism

DF can slow down the absorption of glucose as well as impede the uptake of dietary lipids and cholesterol to enhance satiety and improve insulin resistance. Due to these properties of DF, inulin shows unique protective effects on the metabolism of glucose, lipids and amino acids, involving multiple mechanisms and partly related to the immune response (17, 19, 24).

First, inulin has been shown to increase the number of L cells (which are responsible for secreting glucagon-like peptide 1 (GLP-1)), suggesting a potential effect of inulin on glucose metabolism (100). Second, maternal mice supplemented with inulin during pregnancy and lactation improved glucose tolerance in their offspring exposed to a maternal HFD by modulating DNA methylation and gene expression of *Wnt5a* and *Pi3k* (109). Inulin supplementation may alleviate hepatic steatosis by increasing adipose triglyceride lipase activity on hepatic lipid droplets and inhibiting expression of cannabinoid receptor-1 and patatin-like phospholipase-3 in the liver (110, 111).

Beek et al. traced inulin-derived SCFAs using a stable isotope tracer. They found that inulin intake increased plasma concentrations of propionate, butyrate, and acetate. This phenomenon may explain how inulin improves metabolism in obese men because SCFAs regulate the balance between the synthesis, oxidation and catabolism of fatty acids (18). Similarly, Guo et al. found that inulin-induced remodeling of the intestinal microbiota resulted in increased production of SCFAs that promoted expression of angiopoietin-like protein 4, which contributed to the improved metabolism of glucose and lipids (42). Furthermore, Zhao et al. demonstrated that inulin-induced SCFAs interact with FFARs expressed on L cells, promoting the secretion of intestinal peptides (including GLP-1 and fasting peptide YY (PYY)), thereby improving glucose metabolism and insulin resistance (112, 113).

Tryptophan is one of the eight essential amino acids. It is the only amino acid that contains an indole (bicyclic compound) structure. Tryptophan can be obtained only from the diet. The usual tryptophan metabolic pathways are kynurenine, indole, and serotonin (114). Tryptophan metabolites and kynurenine inhibit activated T cells, B cells and natural killer (NK) cells selectively under physiological status, and promote immunomodulatory effects by activating aryl hydrocarbon receptors (115). Indole-3-acetate can activate ILC3s (116). Indole can upregulate the expression of TJ protein mucin and anti-inflammatory factor protein IL-10 in IECs, and downregulate expression of the pro-inflammatory factor IL-8 (117, 118). Dietary supplementation with inulin can increase levels of alistipes and indole-3-acrylic acid, which are involved in tryptophan metabolism and improve obesity (47). Tryptophan metabolism is one of the key metabolic pathways affected by changes in intestinal microbiota and is closely related to intestinal immune regulation (115, 119). A metabolomic analysis targeting tryptophan metabolism showed that inulin intervention upregulated fecal levels of indole-3-acetate and kynurenine in rats with NAFLD, while downregulating levels of kynurenine and 5-hydroxyindoleacetic acid (21). Thus, inulin can mitigate pro-inflammatory effects.

Role of inulin in disease

Inulin can regulate the metabolism of glucose, lipids, and amino acids in addition to intestinal immune and systemic immunomodulatory effects. Its intestinal metabolites also exert beneficial functions. Therefore, inulin can improve the symptoms of many diseases, such as metabolic syndrome, IBD, and chronic kidney disease(CKD), associated with intestinal inflammation and intestinal dysbiosis, as well as allergic diseases and tumors related to immune imbalance (Figure 2). In Table 3, we have summarized some information about clinical trials on inulin use in different diseases, and the adverse effects caused by inulin.

Metabolic syndrome

Metabolic syndrome is largely caused by physical inactivity and excess caloric intake. Patients with metabolic syndrome often have abdominal obesity, insulin resistance, hyperglycemia, hyperlipidemia and hypertension. Growing evidence suggests that obesity and metabolic disorders are associated with ecological dysbiosis of the gut microbiota, and that increased intake of DF is beneficial in improving ecological dysbiosis (131, 132). Thus, dietary inulin is a potential agent for improving disorders of glucose and lipid metabolism (20, 125, 128).

Studies have demonstrated that inulin intake modulates ecological dysbiosis, reduces the level of fasting glucose, attenuates insulin resistance, and improves lipid disorders (126, 127). However, some patients may suffer from mild gastrointestinal discomfort, including bloating and loose stools (45, 120, 121, 123). In a mouse model of a Western diet (42% of calories from fat, 43% of calories from carbohydrates, and 15% of calories from protein)-induced dysbiosis colonized with human vegan microbiota, inulin supplementation rendered a shift from protein hydrolysis to glycolytic fermentation of the gut microbiota. This action resulted in fewer sulfur-containing compounds and more SCFAs, which contributed to improved lipid denaturation and glucose homeostasis (133). The same improvement in the metabolism of glucose and lipids was also observed in *ob/ob* mice upon inulin treatment (42). Inulin also shows a significant improvement in type I diabetes mellitus (T1DM) in addition to T2DM. Disruption of the gut barrier leads to activation of pancreatic islet-reactive T cells and triggers autoimmune T1DM (134, 135), and diet is one of the most important factors in affecting

TABLE 3 Effects of inulin ingestion in human studies.

Treatment	Duration	Subjects	Effects	Adverse effects	Reference
Inulin (10 g/d)	3 months	Diabetes *n*=27	Overall improvement in glycemic index, increased serum 10 g/d of butyric acid and propionic acid	–	(120)
Inulin (10 g/d)	6 weeks	Adults at risk of T2DM *n*=24	Increased *Bifidobacterium* abundance in gut	Mild gastrointestinal side effects, including bloating and loose stools	(121)
10 g/d of inulin or maltodextrin	8 weeks	Women suffering from obesity or depression *n*=45	No significant beneficial effects on depressive symptoms, gut permeability, or inflammatory biomarkers	Gastrointestinal complaints (flatulence, soft stools)	(122)
Inulin (60.2%) in 20-g formula	5 weeks	Healthy adults *n*=8	Suppressed postprandial glycemic response	–	(123)
Inulin-type fructans (16 g/d)	6 weeks	Patients with T2DM *n*=35	No significant effects on appetite hormones, subjective feeling of appetite or energy intake	Gastrointestinal symptoms (flatulence)	(124)
Inulin-type fructans (16 g/d)	6 weeks	Patients with T2DM *n*=25	A significant bifidogenic effect and increased fecal concentration of SCFAs	–	(19)
Butyrate (600 mg/d) + high-performance inulin (10 g/d)	45 days	Patients with T2DM *n*=60	A significant increase in expression of miR-146a and miR-9, and antioxidant capacity	–	(125)
45 g of milk powder with inulin and resistant dextrin	12 weeks	Older patients with T2DM *n*=99	Reduced systolic BP, diastolic BP, fasting and 2-h postprandial plasma glucose level, serum level of glycosylated proteins, and insulin resistance index; increased 2-h postprandial insulin level and β-cell function index	No serious adverse events	(126)
Inulin (1.7 g/d) in enriched seafood sticks (50 g/d)	12 weeks	Abdominally obese individuals *n*=120	Reduced postprandial atherogenic triglyceride concentrations and potential protection against T2DM	–	(127)
Inulin (16 g/d)	3 months	Obese participants *n*=61	BMI decrease, reduced liver stiffness and plasma levels of AST and cholesterol, and improved glucose intolerance	Rumbling, cramps, bloating and flatulence, which could be improved by physical activity	(128)
16 g/d inulin	3 months	Obese patients *n*=106	Improved bodyweight, AST level and insulinemia, decreased abundance of *Desulfovibrio* and *Clostridium*, and increased abundance of *Bifidobacterium*, but without gut microbiota changes or metabolic improvements after metformin treatment	Nausea, cramp, reflux and rumbling	(43)
Inulin-propionate ester (20 g/d)	42 days	Overweight and obese adults not suffering from diabetes mellitus *n*=12	Improved insulin resistance, increased abundance of Actinobacteria and decreased abundance of Clostridia	Stomach discomfort, nausea, bloating, flatulence, belching, heartburn	(45)
10 g/d of a mixture of inulin and oligofructose	12 weeks	Patients undergoing continuous ambulatory peritoneal dialysis *n*=16	Changes in the composition of intestinal microbiota, reduction of the serum levels of uric acid, and increase in fecal degradation of uric acid	–	(129)
Inulin-type fructans (10 g/d)	3 months	Patients undergoing continuous ambulatory peritoneal dialysis *n*=22	Altered composition of intestinal microbiota	No adverse effects	(130)

T2DM, type 2 diabetes mellitus; SCFAs, short-chain fatty acids; BP, blood pressure; BMI, body mass index; AST, aspartate transaminase.

gut homeostasis. Several studies have shown that an inulin-rich diet can promote a beneficial gut microbiota composition, and increase expression of TJ proteins and mucins, thereby preventing and/or treating T1DM (55, 136, 137). The improvement of T1DM by inulin is dependent on its modulation of the intestinal metabolic profile because the fermentation of inulin by gut microbiota promotes SCFAs production and a subsequent increase in the number of $Foxp3^+$ T_{reg} and IL-10^+ Tr1 cells, which may limit activation of pancreatic islet- reactive T cells (138).

Intriguingly, another study found that mice supplemented with inulin undertook more locomotive activity than those supplemented with cellulose. Those data suggested that inulin intake intensified the willingness of mice to exercise and promoted energy expenditure in obese mice (139). However, the mechanisms behind these changes are largely unknown and may be related to the regulation of the nervous system by inulin metabolites. Guo et al. found that inulin could modulate neurological disorders through the microbiome-gut-brain axis (140). In addition, Shulman and colleagues reported that a HFD induced an increase in acetate production in the intestine of mice, and then the increased acetate level led to activation of the parasympathetic nervous system and promoted secretion of growth hormone-releasing peptide and glucose-stimulated insulin. In that study, direct stimulation of isolated pancreatic islets with acetate failed to promote insulin secretion. However, these changes were not observed when the parasympathetic nerves were cut off, which indicated that parasympathetic nerves in the gut-brain-pancreatic-β-cell axis might be involved in the regulation of inulin or its metabolites (141). However, other researchers have reported no significant effect on appetite after inulin intake

Inflammatory bowel disease

IBD includes Crohn's disease and UC. Chronic intestinal inflammation is the typical feature of IBD. IBD development is associated with environmental factors, genetic conditions, faults in the immune system, and changes in the microbiota (142). Inulin has been reported to limit intestinal inflammation, modulate the intestinal microbiome, and improve intestinal barrier function. A randomized controlled trial supported the notion that oligofructose-enriched inulin can improve gastrointestinal symptoms in patients with active UC without significant side effects (143). Therefore, inulin is also being used increasingly for IBD treatment (144, 145).

The ameliorative effect of inulin on IBD is related mainly to its: reshaping of intestinal microbiota structure; promoting the growth of beneficial bacteria; inhibiting expression of inflammatory factors; improving the intestinal mucosal barrier. As mentioned above, inulin significantly increased the abundance of beneficial bacteria such as *Bifidobacterium rhamnosus*. In an animal model induced by DSS, inulin combined with *Lactobacillus rhamnosus* increased the abundance and diversity of intestinal microbiota, decreased expression of pro-inflammatory cytokines, and relieved UC (146). In a study comparing the differences between inulin and another type of DF, the authors found that inulin had a modulatory effect on the microbiota of mice with DSS-induced colitis, reduced expression of pro-inflammatory cytokines significantly, and improved intestinal barrier function (147). Those results support that the notion that DFs (especially inulin) are promising dietary supplements to alleviate intestinal inflammation. In addition, inulin can be used as an immune- system modulator for the treatment and management of IBD, and its mechanism is related to the promotion of secretion of antimicrobial peptides and improvement of intestinal mucosal immunity (148).

Results in animal IBD models and humans suggest that inulin intake can help to improve the intestinal mucosal barrier and suppress intestinal inflammation (149), but some research teams have reached opposite conclusions. For example, Armstrong and colleagues found that unfermented inulin induced secretion of pro-inflammatory cytokines in a subset of IBD intestinal biopsies cultured *ex vivo* (150). In several other animal studies, researchers have found that dietary supplementation with inulin may be beneficial for low-grade inflammation and associated metabolic disease, but that it also exacerbates the severity of DSS-induced acute colitis (151–153). Furthermore, treatment with an "antibiotic cocktail" led to intestinal ecological dysregulation and induced colitis in mice, whereas supplementation with inulin-type fructans delayed the recovery of this antibiotic-induced intestinal inflammation and decreased the recovery of T_{reg} and B cells in the lamina propria. Moreover, although supplementation with inulin-type fructans inhibited expression of certain pro-inflammatory genes in the colon (e.g., inducible nitric oxide synthase, TNF-α), it also reduced sIgA secretion in the colon. Inulin also increased the serum level of LPS, reduced secretion of the anti-inflammatory mediator transforming growth factor-β1, and promoted secretion of the pro-inflammatory cytokine IL-17A (154). In a study on the anti-tumor effect of inulin, inulin promoted the infiltration of γδ T cells and production of IFN-γ in tumors, but also led to expression of several inflammation-related genes in IECs, including TNF-α, cyclooxygenase-9, and MMP-9, thereby exacerbating inflammation in the intestine, but this seems to be associated with immune surveillance. Inulin also triggered the expression of macrophage inflammatory protein-2, IL-22 and the transcription factor Foxp3 in $CD45^+$ cells in the lamina propria, and these were beneficial in suppressing inflammation. Those results suggest that an inulin diet triggers activation of γδ T cells in epithelial lymphocytes and immune surveillance in IECs, as well as induction of tissue repair signals and tolerance in cells of the lamina propria (69).

Overall, inulin is beneficial for IBD because it reshapes the intestinal microbiota structure, suppresses intestinal inflammation, and improves intestinal cellular and mucosal immunity. However, the gas produced by fermentation of inulin may aggravate the gastrointestinal symptoms of patients, thus limiting its beneficial effects (155). In two randomized controlled clinical trials, inulin ingestion did not change appetite, intestinal permeability, or levels of inflammatory biomarkers, but caused flatulence and soft stools (122, 124).

Chronic kidney disease

Urea accumulation associated with CKD can affect the composition of the gut microbiome and increase the permeability of the intestinal epithelial barrier. If the intestinal barrier is breached, uremic substances, including indole sulfate, para-cresol sulfate, and trimethylamine N-oxide (TMAO), can lead to endotoxemia and systemic inflammation (156, 157). Recently, modification of the gut flora by supplementation with prebiotics has been considered to be a potential therapeutic strategy to reduce uremic toxins of intestinal origin and inflammation. For example, inulin supplementation changed the composition of intestinal microbiota, reduced serum levels of uric acid, and increased degradation of fecal uric acid in patients with renal failure (129). Moreover, intake of inulin-type fructans limited the production of indoles (precursors of indoxyl sulfate) in patients undergoing peritoneal dialysis (158). Similar results were observed by Mitrović et al. They found that inulin treatment reduced the serum level of indoxyl sulfate, improved the glomerular filtration rate, and reduced the level of high sensitivity C-reactive protein levels by altering the gut microbiota composition in patients with CKD (159). In addition, long-term consumption of inulin-containing fructan water reduced serum levels of glucose, total cholesterol, uric acid and creatine kinase in mouse offspring, suggesting that inulin-type fructans contribute to a reduced risk of kidney disease (160).

However, in another study, intervention with inulin-type fructans (10 g/day) for 3 months altered composition of gut microbiome, but did not reduce the plasma TMAO level in patients undergoing peritoneal dialysis (130). This observation may be related to the duration and dose of the intervention. Furthermore, according to the results of a prospective cohort study, higher dietary inulin intake also failed to reduce the incidence of CKD and cardiovascular disease in the population, but prevented hypertension and T2DM, which are major risk factors for cardiovascular and renal events (161). Therefore, given that inulin showed an overall benefit or a neutral effect, inulin is considered to be a safe and reliable strategy to improve the uremic toxin and micro-inflammatory state in patients with CKD (159).

Allergic diseases

ILC2s and Th2 cells are among the key effector cells in allergic diseases, and the cytokines they secrete (IL-4, IL-5, IL-13) mediate the allergic immune response (162). Inulin and its intestinal metabolites (SCFAs) may be involved in mediating the amelioration of allergic diseases by regulating ILC2s and Th2 cells.

Several studies have shown that inulin supplementation in mice during gestation or lactation induced the growth of beneficial bacteria in the intestine of maternal mice. These beneficial bacteria could also be transferred to their offspring, enhance their intestinal barrier function, and increase the number of B-cell and T_{reg} subpopulations in lymph nodes. These actions shaped a more tolerogenic immune environment that suppressed Th2 responses to alleviate food allergy (163, 164). Furthermore, in airway allergic responses, propionate ameliorates inflammation by altering bone-marrow hematopoiesis in mice via FFAR3, promotes the production of DC precursors, and inhibits the differentiation capacity of Th2 cells (72). Several studies have demonstrated that inulin diets exhibit benefits for allergic diseases (including asthma), but inulin itself can cause rare allergic reactions (e.g., itching, rash, swelling, wheezing, difficulty in breathing, unconsciousness) (165–167).

In addition, inulin (especially delta inulin) has been used as an adjuvant to enhance the immune response (168). Venom immunotherapy is effective in improving anaphylactic reactions to stings from Hymenoptera spp, but it can also cause severe (and even life-threatening) immune reactions. Plant-based polysaccharide delta inulin is a new adjuvant with low reactogenicity that can enhance vaccine immunogenicity and antigen-sparing. A randomized controlled trial reported the benefit of delta inulin as an immune adjuvant in patients with bee-venom allergy, and found that delta inulin increased the levels of specific IgG_4 significantly during the early induction phase (169). In conclusion, even though inulin may cause allergic reactions, the function of inulin as a dietary supplement to alleviate allergic diseases (e.g., food allergies, asthma) or as an adjuvant to enhance vaccine efficacy has been demonstrated widely and utilized.

Cancer

Dietary supplementation with whole grains and DF usually reduces the incidence of tumors as well as the risk of postoperative oncologic complications and tumor-related mortality (170, 171). Studies have observed the tumor growth inhibitory effects of inulin, though the mechanisms remain largely unexplored (172, 173). Nevertheless, several mechanisms pertaining to the activity and regulation of inulin in anti-tumor immunity have been elucidated in recent years.

Perhaps the anti-tumor effects of inulin rely largely on its ability to promote immune cell recruitment to the tumor microenvironment. Two studies found increased infiltration of immune cells in the tumor bed after supplementation with an inulin-rich diet (69, 70). Upon subcutaneous injection of a syngeneic B16- ovalbumin melanoma tumor, inulin uptake promoted infiltration of $CD4^+$ and $CD8^+$ T cells and increased IFN-γ production, thereby triggering an effective Th1 anti-tumor response and inhibiting tumor growth. Meanwhile, inulin treatment increased expression of chemokines (CCL4, CCL8), inflammatory vesicle-related genes (TLR3, TLR7) and antigen presentation-related genes (CD40, Stat1, ICOS), induced anti-tumor immunity, and inhibited the growth of colon tumors. Moreover, either alone or in combination with SCFAs, inulin affected tumor growth, indicating that the anti-tumor effects of inulin were not dependent on SCFAs (70). Notably, in addition to B16-OVA melanoma tumors, the anti-tumor effects of inulin were also confirmed in tumor models of MCA205 fibrosarcoma and MC38 colorectal cancer (CRC) cell lines, and such effects were associated with the response of Th1 cells (69). In addition, γδ T cells are unconventional T cells that recognize metabolism-related molecules

and have potent anti-tumor activity. Inulin can activate γδ T cells via γδ T cell receptor signaling, and promote IFN-γ production (174). In mice with 1,2-dimethylhydrazine-induced colon cancer, the amelioration of colon cancer in mice by inulin involved modulation of Janus kinase-1/β-catenin signaling (175).

In liver-associated tumors, the anti-tumor effect of inulin is associated with its metabolites and subsequent immunomodulation. In mice with hepatocellular carcinoma (HCC), an increased acetate level by fecal-bacterial transplantation or direct administration of acetate inhibited the activity of HDACs, increased acetylation of sex-determining region Y-box transcription factor 13 (Sox13) at site K30, and decreased expression of Sox13, thereby reducing IL-17A production by ILC3s and retarding tumor growth. In addition, a combination of acetate with blockade of programmed death (PD)-1/PD-1 ligand promoted anti-tumor immunity significantly and enhanced the treatment efficacy of PD-1 (176).

Inulin has shown protective and tumor-suppressive effects in most CRC studies, but other reports have indicated that inulin intake promotes CRC development. The reason for this discrepancy may be due to differences in gut microbial composition. Inulin supplementation led to increased colonization of polyketide synthase-positive (*pks*$^+$) *E. coli* strain NC101, whereas *pks*$^+$ *Escherichia coli* can promote carcinogenesis and facilitate CRC progression through the production of colistin (a genotoxin that induces double-stranded DNA breaks) (177). Therefore, given the prevalence of *pks*$^+$ *E. coli* in healthy and CRC populations, individuals colonized with *pks*$^+$ bacteria should use inulin with caution (178). Furthermore, supplementation of inulin can induce cholestasis and HCC, which may be due to inulin fermentation (179).

Conclusions

DFs are indispensable supplements in daily life. Inulin and its metabolites (SCFAs) have key roles in lowering blood glucose, reducing bodyweight, and improving insulin resistance. The fermentation of inulin by intestinal microbiota can promote the proliferation of beneficial flora, regulate intestinal pH and maintain the homeostasis of the intestinal ecological environment. Therefore, dietary intake of inulin may serve as a simple but effective way to improve intestinal and systemic immune function and prevent diseases, and sufficient intake of inulin fiber is recommended. However, inulin ingestion may cause gastrointestinal symptoms, allergies or even more serious adverse effects, so it should be consumed under the supervision of healthcare professionals.

Author contributions

LZ conceptualized the manuscript, WS collected the literature and drafted the manuscript, LZ and GJ revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effect of viscous soluble dietary fiber on glucose and lipid metabolism in patients with type 2 diabetes mellitus: a systematic review and meta-analysis on randomized clinical trials

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Background: The effect of viscous soluble dietary fiber on glucose and lipid metabolism in type 2 diabetes mellitus (T2DM) remains controversial, and the dose–response relationship of its effect on blood glucose and blood lipid level is still unclear.

Methods: We conducted comprehensive searches in several databases up to 17 January 2023. We conducted a dose–response analysis of randomized controlled trials (RCTs) to investigate the effect of viscous dietary fiber on glucose and lipid metabolism in patients with T2DM.

Results: Statistical significance was observed in the decreases of glycosylated hemoglobin (HbA1c) (mean difference) [MD = −0.47; 95%CI: (−0.66, −0.27)], fasting blood glucose (FBG) [MD = −0.93; 95%CI: (−1.46, −0.41)], total cholesterol (TC) [MD = −0.33; 95%CI: (−0.46, −0.21)], and low-density lipoprotein and cholesterol (LDL-C) [MD = −0.24; 95%CI: (−0.35, −0.13)]. Contrarily, no difference was observed regarding the level of high-density lipoprotein cholesterol (HDL-C) or triglyceride (TG). In addition, the effect on fasting insulin remains unclear. Results from the subgroup analyses showed that an intervention duration longer than 6 weeks had a significant effect on the HbA1c level; a treatment dosage higher than 8.3 g/day had a significant effect on the FBG level.

Conclusions: Supplementation of viscous dietary fiber is beneficial to control blood glucose and blood lipid in T2DM.

KEYWORDS

viscous soluble dietary fiber, glucose and lipid metabolism, blood glucose, blood lipid, RCTs, meta-analysis

1. Introduction

Type 2 diabetes mellitus (T2DM) is a non-communicable chronic disease that is prevalent worldwide. The incidence of T2DM is increasing, posing a heavy burden on the global healthcare system. As estimated, there were 463 million people with diabetes worldwide in 2019, and the number of people with diabetes is estimated to reach 578

million and 700 million by 2030 and 2045, respectively (1). The main characteristic of T2DM is glucose metabolism disorder. In addition, it is often accompanied by other metabolic disorders, such as obesity, hyperlipidemia, hypertension, and kidney disease, which lead to neuropathy, as well as microvascular and macrovascular complications. Therefore, it is of great significance to control and manage blood glucose and blood lipid levels in patients with T2DM to prevent its potential complications.

Nutritional intervention is one of the key approaches to managing T2DM (2, 3). Notably, dietary fiber could contribute to improving gastrointestinal health, which could further impact lipid metabolism (4). Dietary fiber intake has been linked to a lower risk of T2DM (5). Dietary fiber is classified as soluble or insoluble, based on its solubility in hot water (6). It has been suggested that soluble dietary fiber could reduce energy intake and delay the hydrolysis and absorption of nutrients in the small intestine by increasing satiety (7, 8). It has also been reported that soluble dietary fiber could improve glucose metabolism and lipid distribution in patients with type 2 diabetes (9–11). However, some studies reported contradictory results (12–14). Soluble fiber can be divided into non-viscous fiber and viscous fiber, according to its viscosity. Major types of viscous fiber include psyllium, guar gum, β-glucan, glucomannan, and Cassia tora (6). Previous studies have shown that some highly viscous soluble fibers, such as guar gum, psyllium, and β-glucan, have a significant effect on lowering blood glucose or glycemic index (GI), and the effect is positively correlated with viscosity (15). The underlying mechanism was thought to be the water-holding ability of viscous fibers, which can form a gel matrix and slow down gastric emptying. Simultaneously, this gel matrix thickens the small intestinal contents, slows down the small intestinal transit time, and reduces the contact of nutrients with digestive enzymes, thereby reducing blood glucose levels (16). However, other studies have reached inconsistent conclusions. A study investigating the effect of cereal fiber and fruit fiber on type 2 diabetes (6) suggested that sticky soluble dietary fiber (from fruit sources) had a weak protective effect on the risk of T2DM, and this conclusion was based on a cohort study. In this meta-analysis, we selected RCTs, which are generally more controllable and have a higher level of evidence than cohort studies. In addition, some RCTs also reported consistent results, suggesting that sticky soluble dietary fiber has no effect on glucose and lipid metabolism (17–21). The benefits of sticky fiber in people with T2DM are controversial. This may be related to the experimental design, small sample size, insufficient dose, and other factors. Therefore, we conducted a meta-analysis to expand the sample size by including recent studies, aiming to draw a solid conclusion.

2. Materials and methods

The study followed the 2020 Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.

2.1. Literature search strategy

We systematically searched for relevant articles published until 17 January 2023: PubMed, Web of Science, Embase, and Cochrane Library. The keywords were related to the research objectives. In our search strategy, we combined MeSH and non-mesh keywords without date or language restrictions. The search terms were as follows: (diabetes mellitus, type 2 OR type 2 diabetes OR diabetes mellitus, type II OR type 2 diabetes mellitus OR type 2 diabetes OR diabetes, type 2 OR diabetes mellitus, non-insulin dependent OR NIDDM OR diabetes mellitus, non-insulin dependent OR non-insulin-dependent diabetes mellitus OR non-insulin dependent diabetes mellitus OR diabetes mellitus, maturity onset OR diabetes mellitus, and adult onset OR T2DM or DMT2); (dietary fiber OR dietary fibers OR fibers, dietary OR fiber, and dietary); and (randomized controlled trial OR randomized controlled trial OR controlled clinical trial OR clinical trial, randomized OR randomized, and trial OR randomized OR intervention OR controlled trial OR random OR placebo). In addition, we manually searched the reference lists of each study to supplement relevant studies that may have been overlooked.

2.2. Inclusion and exclusion criteria

This study strictly included original research according to the following criteria: (1) the study was an RCT with a parallel or crossover design with an experimental period of more than 2 weeks; (2) the study subjects were adults with T2DM (aged $\geq$ 18 years); (3) the intervention of interest was the supplementation of viscous soluble dietary fiber (such as psyllium, guar gum, β-glucan, glucomannan, Cassia tora, and other viscous soluble dietary fibers), with a placebo, insoluble fiber, or without fiber supplementation in the control group; (4) the outcome indicators included at least one blood glucose control indicator (HbA1c, fasting blood glucose, or fasting insulin) and one blood lipid control indicator (TC, LDL-C, HDL-C, or TG); and (5) the data were complete and could provide the basis for subsequent analyses.

We excluded literature based on the following criteria: (1) studies with subjects who had type I diabetes, gestational diabetes, or metabolic syndrome; studies with subjects who were adolescents or children; (2) experiments with a study period that was too short (<2 weeks); (3) studies that combined interventions or could not separate the effect of viscous soluble dietary fiber on blood glucose or blood lipids; (4) cytological studies, animal experiments, non-controlled trials, or non-clinical studies; and (5) trials with incomplete or irrelevant data.

2.3. Data extraction

Two researchers independently evaluated the included literature and extracted the following data by reading the full text: name of the first author, year of publication, types of study design, country, sample capacity, gender, average age of participants, BMI, duration of diabetes, type of fiber in the experimental group, substance in the control group, daily intake of viscous soluble dietary fiber, and cycles of intervention. In addition, the mean and standard deviation of blood glucose control indicators (HbA1c, FBG, or fasting insulin) and blood lipid control indicators (TC, LDL-C, HDL-C, or TG) in each literature were extracted. If not

reported, they were converted based on available data (95% CIs, SEM, or median).

2.4. Quality assessment of studies

The literature included in the study was evaluated for risk bias using the Cochrane Bias Risk Tool, which assesses seven validity questions as follows: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias. The bias risk of each validity question is divided into low risk, high risk, and unclear risk.

2.5. Statistical analysis

Excel 2010 was used to collect and organize literature data and to standardize the different units of blood glucose and blood lipid levels: FBG was converted to mmol/L (18.0 mg/dL = 1 mmol/L), fasting insulin was converted to μIU/mL (1 μIU/mL = 6.00 pmol/L, 1 mU/L = 1 μIU/mL) (22), and blood lipid units were converted to mmol/L (TC, LDL-C, HDL-C: 1 mmol/L = 1 mg/dL × 0.02586, TG:1 mmol/L = 1 mg/dL × 0.01129). Subsequent data were analyzed using Review Manager 5.4 and Stata 15.1. Mean differences (MD) and standard deviations (SD) of each outcome variable before and after intervention were calculated using the formula: $SD_{change} = (SD^2_{baseline} + SD^2_{endpoint} - 2R \times SD_{baseline} \times SD_{endpoint})$ 1/2, with a correlation coefficient R = 0.5 (23). The heterogeneity of the studies was evaluated by conducting a chi-square test and using the I^2 index. When the *p*-value of the chi-square test was <0.10 or I^2 was >50%, significant heterogeneity among the studies was considered to exist, and a random-effects model was used. Subgroup analysis was conducted based on region, study type, fiber type, dosage, and duration to explore possible sources of heterogeneity. Sensitivity analysis was performed by removing one or two studies to assess the stability of the overall statistical results. In addition, this study used a funnel plot and Egger's regression test to evaluate possible publication bias. Meta-regression was used for dose–response analysis.

3. Results

3.1. Literature search and study characteristics

The specific process of literature retrieval and screening is shown in Figure 1. According to the search strategy, 2,044 studies were retrieved from the four databases; 724 duplicated studies were removed and 1,246 irrelevant studies were excluded based on the title and abstract, of which 104 were systematic reviews and meta-analyses. Then, the remaining 74 studies were evaluated as a whole. Among them, 57 studies were excluded due to the following reasons: unavailability of the full text or incomplete data, combined intervention or inability to separate the effect of viscous soluble dietary fiber on blood glucose or blood lipids, non-randomized controlled trials, and short intervention period. Finally, 17 studies (24–40) met the inclusion criteria (10 parallel studies and 7 crossover studies). It should be noted that in two studies the data were divided into two groups. In one study, we selected the data at the end of week 16, while in other studies, we extracted the data at the end of the study. Therefore, we finally included a total of 19 datasets in the meta-analysis.

3.2. Study characteristics and risk of bias assessment

The characteristics of each study are shown in Supplementary Table 1. Among the included studies, 4 studies were conducted in North America (Canada-1 and America-3), and 5 studies were conducted in Asia (China-1, Korea-1, Palestine-1, and Iran-2). Ten studies were conducted in Europe (France-1, Greece-1, Italy-1, Finland-3, and the UK-4). A total of 642 participants were included in the studies, with a mean age of 51.9 to 66.5 years. The duration of diabetes was stable for more than 1 year in all the studies, except for one where diabetes was newly diagnosed and another with the duration unknown. The supplementation dose of viscous soluble dietary fiber ranged from 3 to 21 g/day, and the supplementation period was from 3 to 16 weeks.

The risk of bias assessment of the included studies is shown in Table 1. Most studies were considered to have a low risk of bias, while random sequence generation and other biases were unknown because not enough information is available for risk rating.

3.3. Results of meta-analysis

A total of 14 datasets from 12 studies were included in the HbA1c analysis, and the random effect model showed that the supplementation of viscous soluble dietary fiber could significantly reduce the HbA1c level [MD = −0.47; 95%CI: (−0.66, −0.27), $p < 0.001$, $I^2 = 57.3\%$, $p = 0.004$, Figure 2A]. In addition, in the non-linear dose–response analysis, we did not observe a significant effect of the dosage of viscous soluble dietary fiber supplementation on HbA1c (p-non-linearity = 0.2104, Figure 3A). Furthermore, no significant effect of the supplementation period on HbA1c was observed (p-non-linearity = 0.4660, Figure 4A).

Based on the 19 datasets from 17 studies, the reduction of FBG by viscous soluble dietary fiber was found to be statistically significant [MD = −0.93; 95%CI: (−1.46, −0.41), $p = 0.001$, $I^2 = 76.0\%$, $p < 0.001$, Figure 2B]. However, we found an insignificant non-linear relationship between FBG and supplementation dose (p-non-linearity = 0.2600, Figure 3B) and the supplementation period (p-non-linearity = 0.6922, Figure 4B) of viscous soluble dietary fiber.

Across 7 datasets from 6 studies, we found a significant effect of viscous soluble dietary fiber on fasting insulin under the random-effects model [MD = −3.64; 95%CI: (−6.98, −0.30), $p = 0.033$, $I^2 = 84.4\%$, $p < 0.001$, Figure 2C]. The non-linear dose–response relationship between fasting insulin and supplementation dose (p-non-linearity = 0.8965, Figure 3C) and

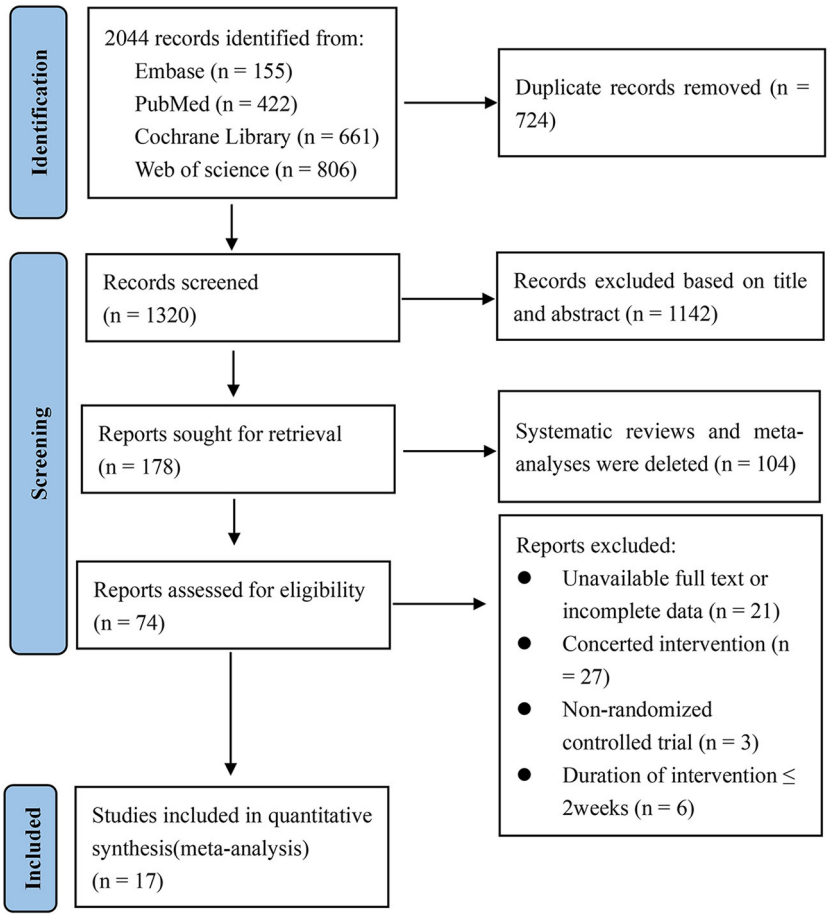

FIGURE 1
Flow chart of literature selection.

supplementation period (p-non-linearity = 0.8094, Figure 4C) was not statistically significant.

In 13 studies of 14 groups of data analyzed for the TC level, the effect of viscous soluble dietary fiber on the TC level was statistically significant [MD = −0.33; 95%CI: (−0.46, −0.21), $p < 0.001$, $I^2 = 19.0\%$, $p = 0.246$, Figure 5A]. However, in the non-linear dose–response analysis, the supplementation dose (p-non-linearity = 0.7934, Figure 6A) and supplementation period (p-non-linearity = 0.1340, Figure 7A) had no significant effect on the TC level.

In the LDL-C analysis, we found that viscous soluble dietary fiber had a significant effect on the reduction of LDL-C from 12 datasets obtained from 11 studies [MD = −0.24; 95%CI: (−0.35, −0.13), $p < 0.001$, $I^2 = 37.2\%$, $p = 0.094$, Figure 5B]. In addition, we failed to find a significant relationship in the non-linear dose–response relationship between LDL-C and the dosage of viscous soluble dietary fiber supplementation (p-non-linearity = 0.8693, Figure 6B). However, we observed a statistically significant non-linear relationship between LDL-C and the supplementation period (p-non-linearity = 0.0083, Figure 7B).

As for the effect variable of HDL-C, 13 groups of data from 12 studies were included in the analysis. According to the analysis results, the effect of viscous soluble dietary fiber on HDL-C was statistically significant [MD = 0.02; 95%CI: (−0.02, 0.06), $p = 0.367$, $I^2 = 26.3\%$, $p = 0.179$, Figure 5C]. In addition, we found that concerning the non-linear dose–response relationship analysis, the effect of the supplementation dose (p-non-linearity = 0.3020, Figure 6C) and supplement period (p-non-linearity = 0.5388, Figure 7C) on HDL-C was not statistically significant.

The analysis of the TG level was based on 12 groups of datasets from 11 studies. We failed to observe a significant effect of viscous soluble dietary fiber on the TG level [MD = −0.11; 95%CI: (−0.22, 0.00), $p = 0.060$, $I^2 = 0.0\%$, $p = 0.901$, Figure 5D]. Similarly, we also found that neither the supplementation dose (p-non-linearity = 0.2364, Figure 6D) nor the supplementation period (p-non-linearity = 0.3445, Figure 7D) had a non-linear dose–response relationship with the TG level.

3.4. Subgroup analysis

The heterogeneity of HbA1c, FBG, and fasting insulin was observed to exceed 50%, indicating significant heterogeneity. Therefore, a subgroup analysis was conducted, and the results are shown in Supplementary Table 2.

The subgroup analysis results showed that the heterogeneity of HbA1c between studies disappeared when grouped by region ($I^2 = 0.0\%$, $p = 0.712$), study type ($I^2 = 0.0\%$, $p = 0.963$), fiber type ($I^2 = 0.0\%$, $p = 0.947$), dose of viscous soluble dietary fiber ($I^2 =$

TABLE 1 Risk of bias assessment.

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Anderson et al. (25)	U	L	L	L	L	L	U
Aro et al. (26)	U	L	L	L	L	L	U
Abutair et al. (27)	U	U	H	U	L	L	U
Chen et al. (28)	U	L	L	L	L	L	U
Cho et al. (29)	U	L	L	L	L	L	U
Cugnet-Anceau et al. (24)	U	L	L	L	L	L	U
Feinglos et al. (40)	U	L	L	L	L	L	U
Fuessl et al. (30)	U	L	L	L	L	L	U
Ghalandari et al. (31)	U	L	L	L	L	L	U
Lalor et al. (32)	U	L	L	L	L	L	U
Liatis et al. (33)	L	L	L	L	L	L	U
Niemi et al. (34)	U	L	L	L	L	L	U
Peterson et al. (35)	U	U	H	U	L	L	U
Reimer et al. (36)	U	L	L	L	L	L	U
Uusitupa et al. (37)	U	U	H	U	L	L	U
Vuksan et al. (38)	U	L	L	L	L	L	U
Ziai et al. (39)	U	L	L	L	L	L	U

L, low; H, high; U, unclear.

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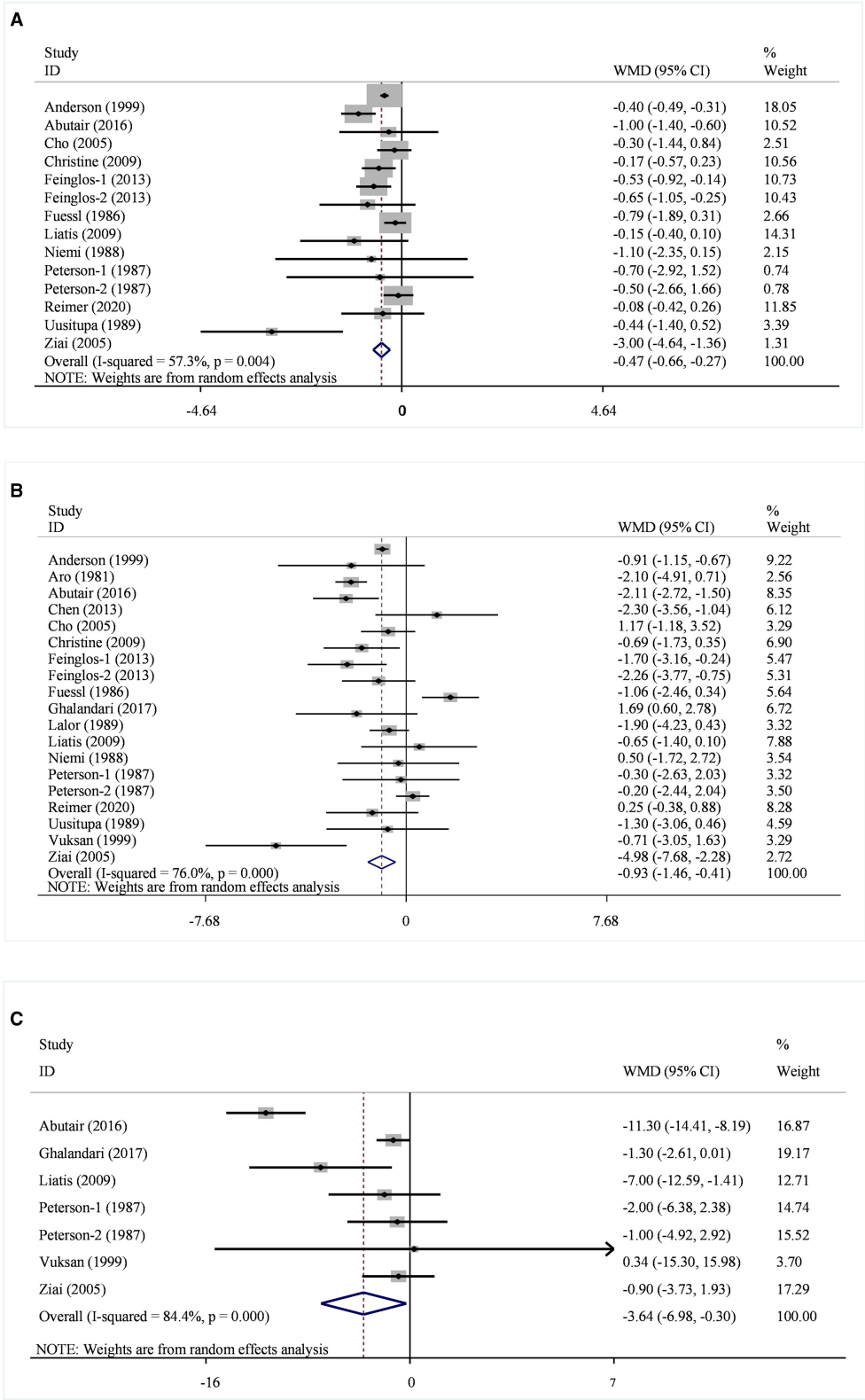

FIGURE 2
Effects of viscous soluble dietary fiber on HbA1c **(A)**, FBG **(B)**, and fasting insulin **(C)**. HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose; WMD, weighted mean difference.

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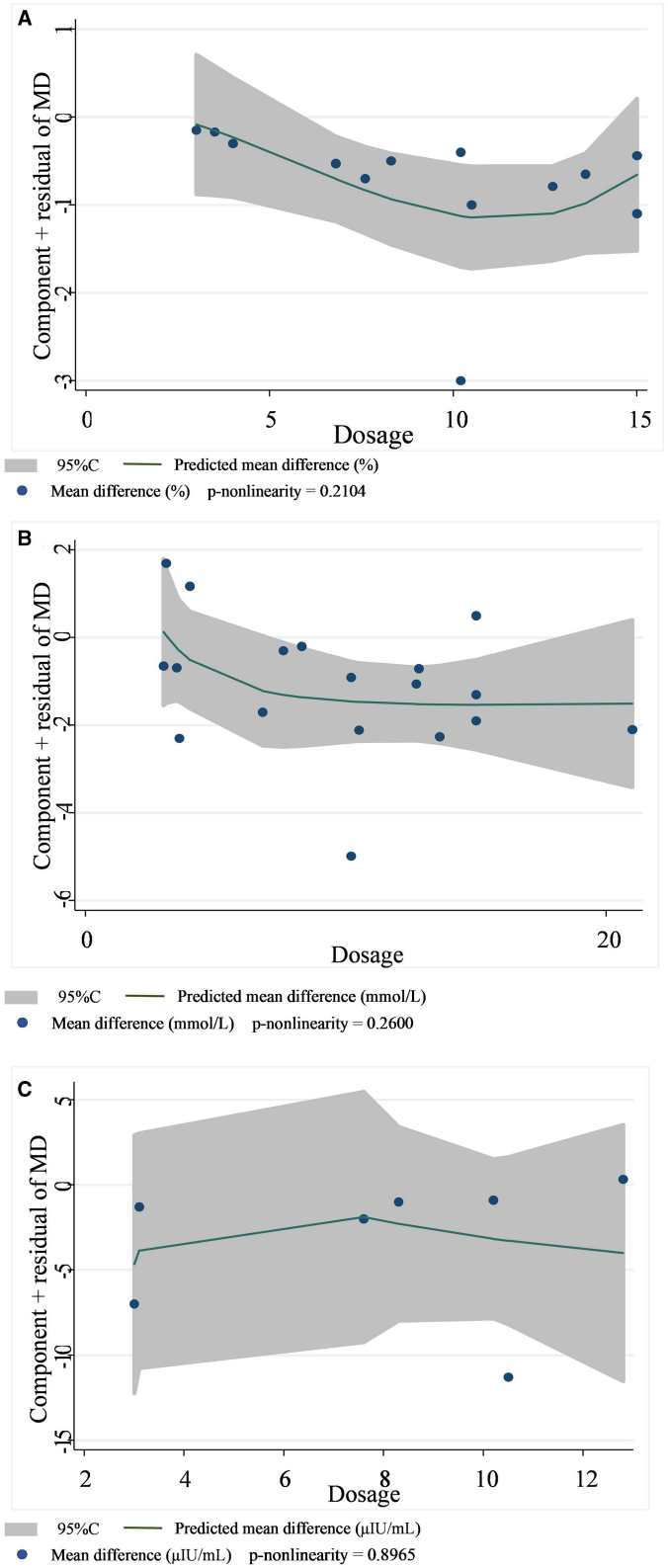

FIGURE 3
Non-linear dose–response relationships between viscous soluble dietary fiber (g) and the nonstandard mean difference (g/day) in HbA1c **(A)**, FBG **(B)**, and fasting insulin **(C)**. 95%CI is displayed in shaded areas. HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose.

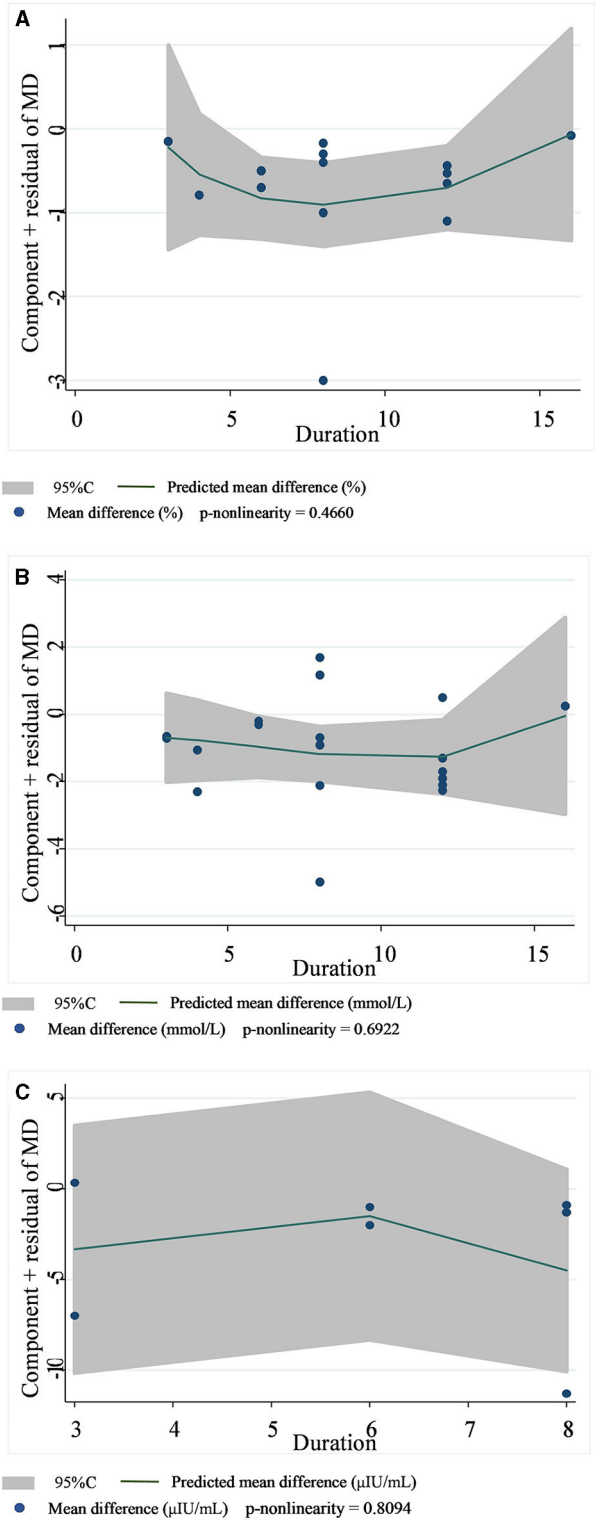

FIGURE 4
Non-linear dose–response relationships between supplementation duration (weeks) and the nonstandard mean difference (g/day) in HbA1c **(A)**, FBG **(B)**, and fasting insulin **(C)**. 95%CI is displayed in shaded areas. HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose.

0.0%, $p = 0.706$), and intervention period ($I^2 = 0.0\%$, $p = 0.679$). From these analyses, we observed significant changes in HbA1c in T2DM subjects in trials in which the fiber type was psyllium [MD = −0.72; 95%CI: (−1.08, −0.37), $p < 0.001$] or guar gum [MD = −0.70; 95%CI: (−1.28, −0.12), $p = 0.018$] and those in which the duration of viscous dietary fiber was >6 weeks [MD = −0.52; 95%CI: (−0.76, −0.28), $p < 0.001$].

For FBG, the inter-study heterogeneity disappeared when subgroup analysis was performed by the study region ($I^2 = 0.0\%$, $p = 0.907$), study type ($I^2 = 6.4\%$, $p = 0.381$), fiber type ($I^2 = 0.0\%$, $p = 0.701$), and the intervention period of viscous soluble dietary fiber ($I^2 = 14.0\%$, $p = 0.325$). It remained high when subgroup analysis was performed by supplementation dosage ($I^2 = 75.3\%$ or 76.4%, $p < 0.001$). In addition, viscous soluble dietary fiber was effective in reducing FBG in trials conducted in North America [MD = −0.94; 95%CI: (−1.83, −0.05), $p = 0.038$] and Europe [MD = −0.76; 95%CI: (−1.22, −0.29), $p = 0.001$], in trials with β-glucan [MD = −0.66; 95%CI: (−1.27, −0.05), $p = 0.033$], psyllium [MD = −1.40; 95%CI: (−2.50, −0.31), $p = 0.012$], and glucomannan [MD = −1.82; 95%CI: (−3.25, −0.40), $p = 0.012$] or guar gum [MD = −0.91; 95%CI: (−1.67, −0.15), $p = 0.020$], and in trials with doses >8.3 g/day [MD = −1.29; 95%CI: (−1.97, −0.61), $p < 0.001$].

Additionally, the study heterogeneity also disappeared in subgroup analyses concerning fasting insulin. In parallel trials [MD = −4.93; 95%CI: (−9.84, −0.02), $p = 0.049$] or in trials with supplementation doses ≤10.2 g/day [MD = −1.49; 95%CI: (−2.62, −0.36), $p = 0.010$], the effect of viscous soluble dietary fiber supplementation was significant in T2DM subjects.

3.5. Sensitivity analysis

Sensitivity analysis was performed, excluding one or two studies at a time to observe their impact on the overall results. The results are shown in Supplementary Table 3. For HbA1c, studies of Abutair et al. (27) and Ziai et al. (39) were excluded, and the overall heterogeneity was changed (from $I^2 = 57\%$, $p = 0.004$ to $I^2 = 4\%$, $p = 0.40$), but the effect variable only increased by 0.11%. After eliminating the data from Abutair et al. study (27), the overall heterogeneity of fasting insulin changed (from $I^2 = 84\%$, $p < 0.00001$ to $I^2 = 0\%$, $p = 0.53$). The effect variables also changed [from MD = −3.64; 95% CI: (6.98, 0.30), $p < 0.00001$ to MD = 1.47; 95%CI: (−2.55, −0.39), $p = 0.008$], increasing by 2.17. After excluding the studies of Cugnet-Anceau et al. (24) and Ziai et al. (39), the overall heterogeneity of TC also changed. When Cugnet-Anceau et al. study (24) was removed, the overall heterogeneity of TC changed (from $I^2 = 19\%$, $p = 0.0.25$ to $I^2 = 0\%$, $p = 0.46$). Similarly, when Ziai et al. study was removed (39), the overall heterogeneity of TC was reduced to ($I^2 = 5\%$, $p = 0.40$). However, when these two studies were deleted separately, the overall effect variable of TC barely changed. For LDL-C, after removing the studies of Chen et al. (28) and Cugnet-Anceau et al. (24), the overall heterogeneity changed (from $I^2 = 37\%$, $p = 0.09$ to $I^2 = 0\%$, $p = 0.48$), but the change in the overall effect variable could be ignored. Similarly, after removing the study conducted by Ziai et al. (39),

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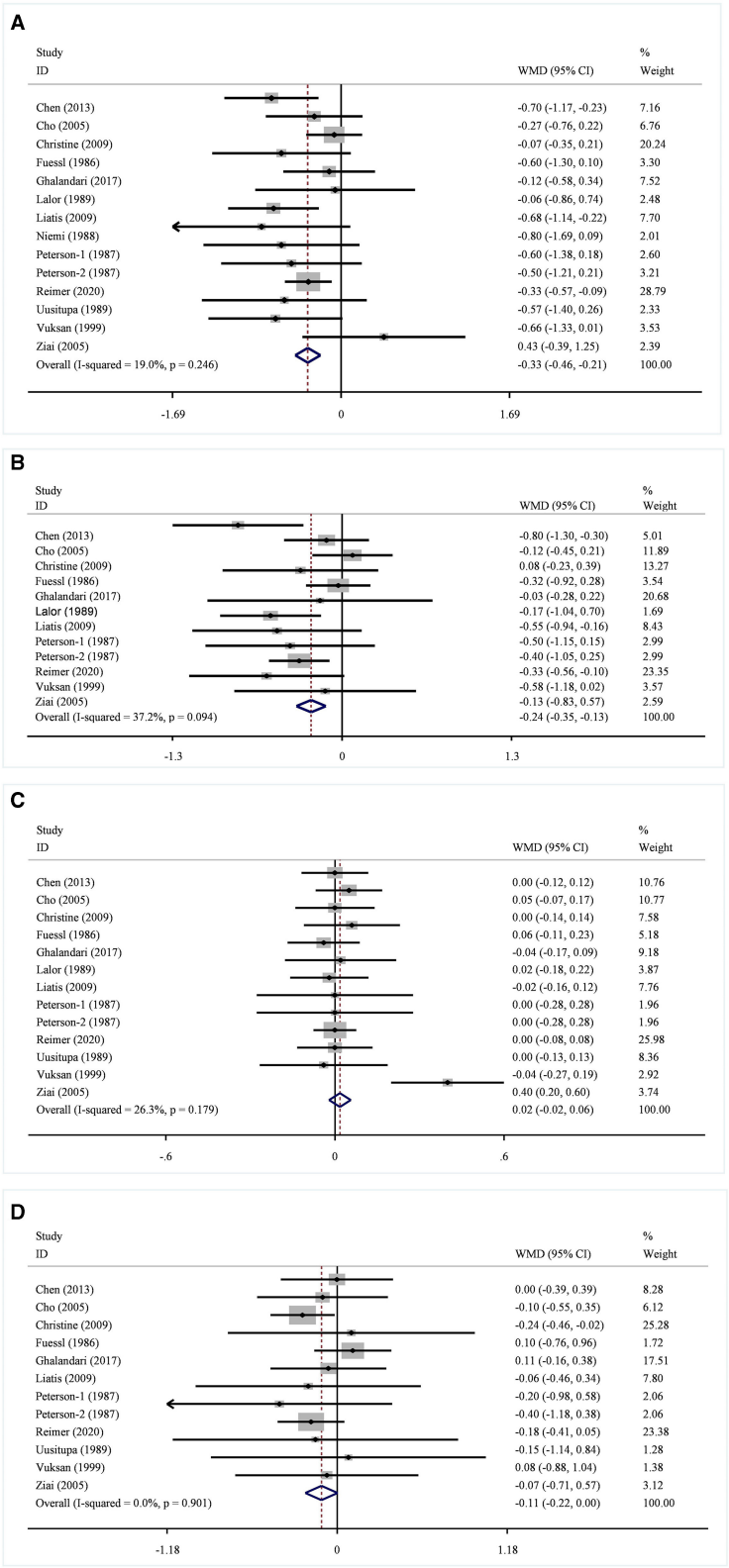

FIGURE 5
Effects of viscous soluble dietary fiber on TC **(A)**, LDL-C **(B)**, HDL-C **(C)**, and TG **(D)**. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; WMD, weighted mean difference.

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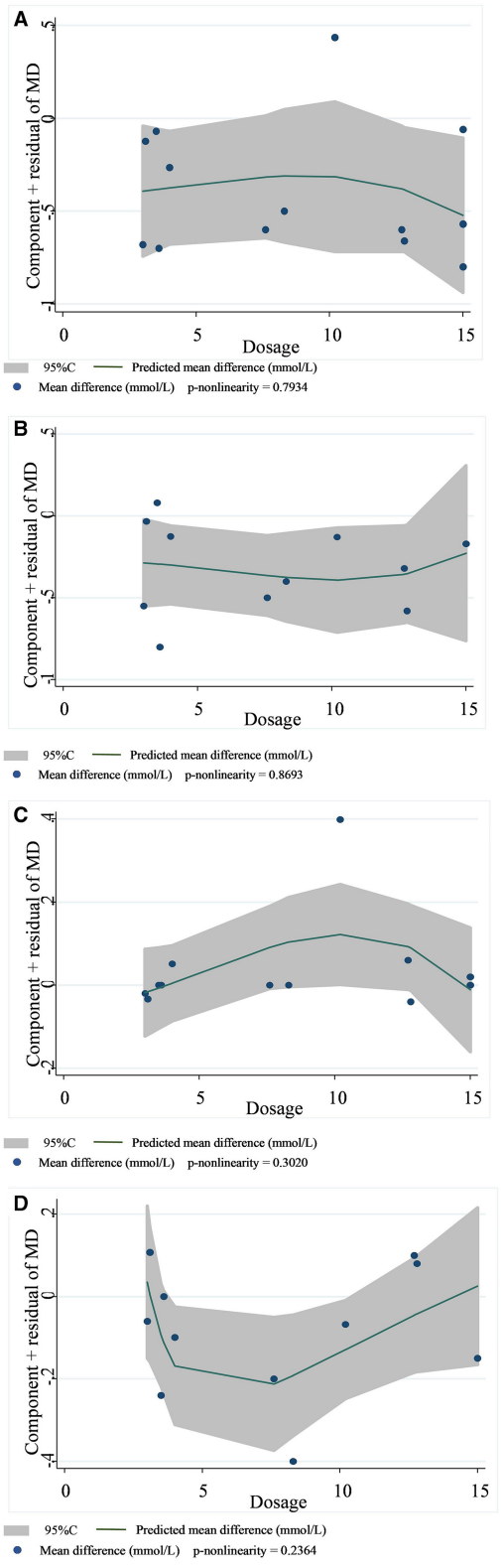

FIGURE 6
Non-linear dose–response relationships between viscous soluble dietary fiber (g) and the nonstandard mean difference (g/day) in TC **(A)**, LDL-C **(B)**, HDL-C **(C)**, and TG **(D)**. 95%CI is displayed in shaded areas. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

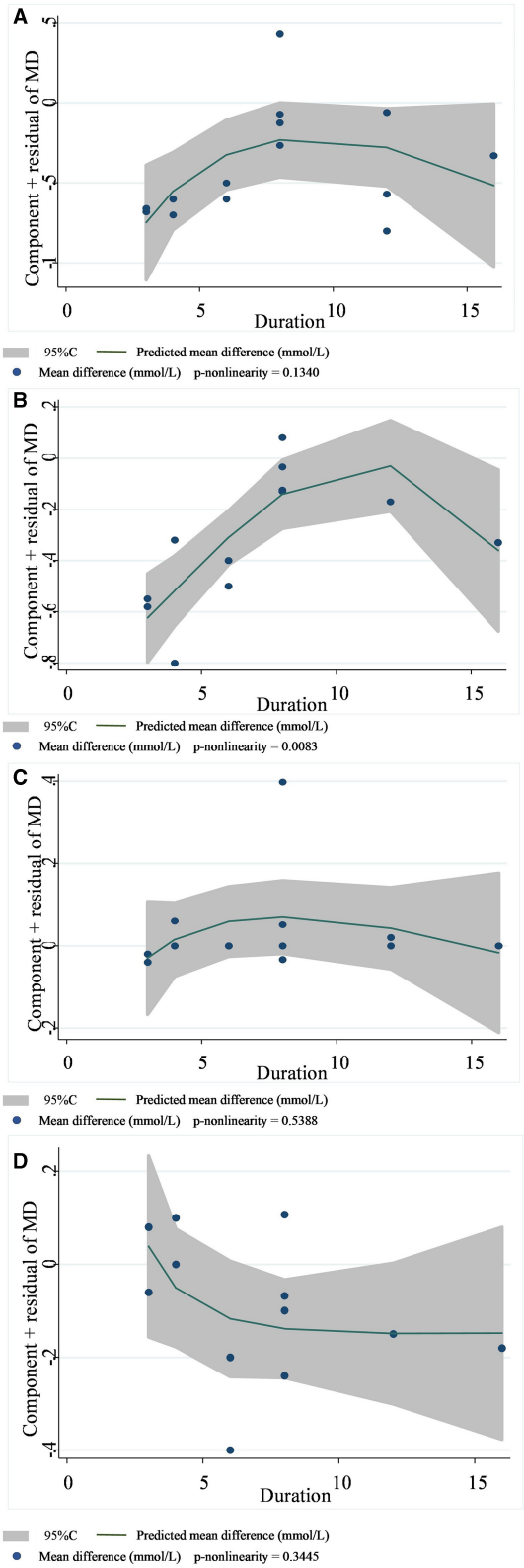

FIGURE 7
Non-linear dose–response relationships between supplementation duration (weeks) and the nonstandard mean difference (g/day) in TC **(A)**, LDL-C **(B)**, HDL-C **(C)**, and TG **(D)**. 95%CI is displayed in shaded areas. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride.

the overall heterogeneity of HDL-C was reduced (from $I^2 = 26\%$, $p = 0.18$ to $I^2 = 0\%$, $p = 1.00$), and the overall effect variable was almost negligible. For FBG and TG, there was no significant change in heterogeneity or population effect variables after deleting any studies.

3.6. Publication bias analysis

The publication bias of HbA1c, FBG, fasting insulin, TC, LDL-C, HDL-C, and TG was initially assessed by funnel plot, and no significant asymmetry was found (Supplementary Figure). In addition, the Egger test results showed that the *p*-values of HbA1c: $p = 0.236$; FBG: $p = 0.870$; fasting insulin: $p = 0.502$; TC: $p = 0.315$; LDL-C: $p = 0.190$; HDL-C: $p = 0.382$; and TG: $p = 0.652$ were all greater than 0.05, and there was no evidence of potential publication bias in the combined results.

4. Discussion

Although a large number of studies have reported the beneficial effects of dietary fiber in the human body, the effect was actually associated with the type of dietary fiber (41). This systematic review aimed to investigate the effect of sticky soluble dietary fiber on glucose and lipid metabolism in patients with T2DM. Seventeen RCTs were included, and we found that sticky soluble dietary fiber can significantly modulate the levels of HbA1c, FBG, and fasting insulin in patients with T2DM. The effect on the TC and LDL-C levels in patients was also significant. Furthermore, the analysis of the non-linear dose–response relationship revealed a correlation between the supplementation cycle and LDL-C levels.

Although previous meta-analyses have shown that some sticky soluble dietary fibers are not effective in controlling certain glycemic indices (17, 19, 21, 42, 43), we collected and included recent studies and found that viscous soluble dietary fiber supplementation had a significant effect on HbA1c, FBG, and fasting insulin levels in patients with T2DM. In our study, the average total effect of HbA1c was reduced by 0.47% in patients with T2DM. A study on the acute effect of soluble dietary fiber on postprandial blood glucose in T2DM showed that soluble dietary fiber supplementation could reduce postprandial blood glucose, and postprandial blood glucose level contributed to HbA1c (44). HbA1c accurately reflects long-term glycemic control. Studies have shown that a 1% reduction in HbA1c significantly reduces the risk of complications associated with T2DM, such as peripheral vascular disease, microvascular complications, myocardial infarction, and stroke (45). Another meta-analysis of a prospective cohort study revealed an association between HbA1c levels and cardiovascular risk in patients with diabetes, with increased cardiovascular risk associated with increased HbA1c levels (46). Moreover, a recent 6-year follow-up study suggested that early control of HbA1c levels in newly diagnosed T2DM patients is more conducive to long-term and lasting glycemic control than late control, and it can better reduce the incidence of diabetes complications, especially microvascular complications (47). This suggests the importance of controlling HbA1c levels for diabetic patients. In our subgroup analysis, the effect of viscous fibers on HbA1c was significant only when the duration of supplementation was longer than 6 weeks, which is consistent with a previous study that demonstrated a reduction in HbA1c levels at weeks 4 and 6 with guar gum supplementation (19).

Similarly, FBG is also an important indicator for evaluating the effectiveness of blood glucose control. Previous studies have shown that sticky soluble dietary fiber can significantly reduce FBG levels in patients with T2DM (48, 49). In addition, a meta-analysis showed that 12 g/day of psyllium fiber reduced FBG by an average of 37 mg/dL (2.06 mmol/L) in patients with T2DM (50). In our study, the overall effect of viscous soluble fiber on the reduction of FBG was 0.93 mmol/L, and the difference in values may be related to the lower dose of the fiber intake (the median dose included in this study was 10.2 g/day). It is important to note that in the subgroup analysis we performed, viscous soluble dietary fiber did not have a significant effect on the reduction of FBG when the dose was $\leq$8.3 g/day, and the inclusion of such studies in our meta-analysis may have led to an underestimate of its benefit. However, our results were inconsistent with a previous report where daily supplementation of 7.6–8.3 g of soluble dietary fiber could effectively control the blood sugar of patients with type 2 diabetes and improve insulin resistance (51); the inclusion of non-viscous fiber might be the underlying reason for the difference. In addition, the difference in values may also be due to the fact that this previous study (50) only used psyllium fiber, while there were a variety of viscous fibers involved in our study. In our subgroup analysis, the type of fiber imposed a significant impact on the reduction of FBG (Supplementary Table 2). The viscosity and the stickiness of the fiber may have contributed to such a difference (15). The chemical structure of different types of viscous fibers is different, resulting in differences in their viscosity and specific functions in the gastrointestinal tract. β-glucans are composed of glucose molecules connected by a β-glycosidic bond (7). The viscosity of β-glucans with the same molecular weight but different volumes is different, but there is no significant difference in the effect on blood glucose. The objective existence and high molecular weight of β-glucans are more important than their volume in regulating blood glucose; the viscosity of the digesta produced after consumption in response to amylase is responsible for gastric emptying and glucose absorption, rather than the initial concentration of the fiber solution (16). The mucopolysaccharide mixture of psyllium, which is composed of pentose, hexose, and uronic acids, cannot be fermented in the body; it maintains a persistent water-holding capacity and swelling effect in the intestine (7), inhibits glucose diffusion, α-amylase, and pancreatic lipase activities, lowers postprandial blood glucose and lipid levels, and binds to bile acids to reduce cholesterol (52). Guar gum and glucomannan are fermentable fibers. In addition to increasing the viscosity of small intestinal contents and affecting gastric emptying, they can also produce beneficial products through fermentation in the colon, mainly short-chain fatty acids (SCFAs) such as propionate, which can indirectly inhibit the biosynthesis of cholesterol and fatty acids (53, 54).

Patients with T2DM are often accompanied by insulin resistance, and the fasting insulin level is positively correlated with insulin resistance (55). A cohort study conducted in the Netherlands found a linear relationship between fasting insulin and the incidence of T2DM, with lower fasting insulin levels

associated with lower risk (56). Another study showed a stronger association, suggesting that people with high fasting insulin levels were more likely to develop T2DM (57). Our study found that increasing viscous dietary fiber intake improved fasting insulin levels in patients with T2DM, which is consistent with the findings of other studies (20, 42, 58). However, the results of another meta-analysis (49) showed that β-glucan did not improve fasting plasma insulin concentration in subjects with T2DM. Interestingly, the subgroup analysis results in this study showed that the effect of viscous dietary fiber was not significant, except for the dosage ≤10.2 g/day and for the β-glucan fiber type. However, only one study with a β-glucan fiber type was included in our meta-analysis. Therefore, more long-term and high-quality RCTs in this aspect are needed.

Previously, it was found that long-term supplementation of medium to high doses of sticky soluble fiber (psyllium and guar gum) improved metabolic indices in patients with metabolic syndrome. By the fourth month of intervention, the reduction in FBG and LDL-C ranged from 9.3 to 16.4% and from 4.3 to 4.4%, respectively. The beneficial effect was more obvious by the 6th month of intervention, with the reduction range of FBG increasing to 11.1–27.9%, that of LDL-C decreasing by 7.9–8.5%, and that of TC decreasing by 6.3–7.5%; by the 6th month, psyllium supplement improved TG concentration significantly (−13.3%) (59). The effect of improving the lipid profile of soluble dietary fiber was also demonstrated by another meta-analysis of guar gum, where guar gum significantly reduced TC and LDL-C, but the intervention did not change the TG or HDL-C levels (60). However, the above studies were not restricted to patients with T2DM. In our results, sticky fibers significantly reduced the TC and LDL-C levels in patients with T2DM, and the heterogeneity among these studies was low. Additionally, we performed a dose–response analysis and found a non-linear relationship between the intervention period of sticky soluble dietary fiber and LDL-C levels, suggesting that the length of the period affects the effect of sticky fiber on LDL-C. When the intervention period was <12 weeks, the reduction level of viscous soluble dietary fiber on LDL-C was better, with the extension of the intervention time, but when the intervention period was more than 12 weeks, the effect showed a reverse trend. Such a phenomenon is in contradiction with previous findings (59) that the LDL-C reduction effect is more significant when supplementing with viscous fiber for 6 months than when supplementing with it for 4 months. We think the possible reasons for this are as follows: many types of viscous fibers were involved in our study, and different fiber types may have affected the results. The longest study period in our study was 16 weeks; considering that Cicero et al. (59) studied up to 6 months, our existing results may change when studies with longer periods are included. Hence, we recommend longer RCTs to find regular changes in glycemic and lipid improvement during intervention with sticky soluble dietary fiber.

Due to the high heterogeneity between studies, subgroup analysis of HbA1c, FBG, and fasting insulin was performed. Except for the dose in the FBG index, the heterogeneity among the other subgroups changed significantly. According to the comprehensive results, all the factors for subgroup analysis may be the sources of heterogeneity. To further determine the source of heterogeneity, we also conducted a meta-regression analysis. Accordingly, the region and fiber type were confirmed as potential sources of heterogeneity in the regression of HbA1c. Furthermore, sensitivity analysis was conducted to evaluate the stability of the results to exclude studies that affected the heterogeneity. We found that removing the study conducted by Ziai et al. (39) significantly affected the heterogeneity of the studies. We reviewed the study but found no probable factors contributing to the heterogeneity, and the bias risk assessment tool acknowledged the high quality of this study. Finally, by comparing the original association results with the association results after removing the studies that significantly affected the heterogeneity, we found that the overall results before and after the removal did not change significantly. In other words, although these studies affected the overall heterogeneity of the study, our results were still stable.

Although some previous studies have suggested that sticky soluble dietary fiber is beneficial for glycemic and lipid control in patients with T2DM, this meta-analysis synthesized and quantified the effect of sticky soluble dietary fiber on adults with T2DM. Additionally, a dose–response analysis was performed to investigate the effect of supplemental dose and intervention period on the efficacy of sticky dietary fiber. Furthermore, the studies we included were across several ethnic and geographic groups, which enhanced the generalizability of the results. In this meta-analysis, the risk of bias was considered to be low, and the results were evaluated objectively, which provided some reliability for the final results and conclusions.

Results from the present meta-analysis suggested that viscous soluble dietary fiber can be used as a dietary supplement for the management of T2DM. However, it should be noted that the high viscosity of viscous fiber can lead to excessive viscosity during swallowing and reduce the palatability of food. Hence, the development of palatable viscous fiber foods is still a challenge. At present, the commonly used food processing method involves adding viscous fibers to proteins, starches, or beverages or adding acidic fruit films to stimulate saliva secretion (16). Fiber intake can also have adverse effects, such as bloating, diarrhea, or constipation, so it is recommended to gradually increase the dose during intake to establish gastrointestinal tolerance (61, 62).

It should be admitted that this meta-analysis has several limitations. Firstly, our study did not separate male and female patients with diabetes, so we could not observe the differences in the control of sticky soluble dietary fiber in blood glucose and blood lipid between these two groups. Secondly, the longest study period among the included studies was only 16 weeks, since only a few studies with a longer period met the inclusion criteria, and only one article had an intervention period of 52 weeks (36). To maintain consistency in the intervention period, we chose results from the first 16 weeks of the study. Longer intervention cycles should be considered and higher quality RCTs should be conducted to better obtain the long-term efficacy of sticky soluble dietary fiber. Besides, the number of studies on some types of dietary fiber was significantly limited. For example, only 2 studies on glucomannan and β-glucan and only 1 study on Cassia tora were included in this study, whereas 6 to 7 studies on guar gum and psyllium were included. More studies on these fiber types should be conducted in subsequent research. In addition, the type of medication during the trial may also impact the effect of the viscous fiber intervention. However, due to the limited information provided by each trial, we did not conduct a more detailed analysis to identify the potential

impact of the medication. Finally, the included studies were highly heterogeneous. Therefore, more RCTs with a large number of participants and more reasonable designs are required.

5. Conclusion

This meta-analysis confirmed that the supplementation of viscous soluble dietary fiber has potential benefits for the control of blood glucose and lipids in patients with T2DM. In addition, the recommended supplemental dose is from 8.3 g to 10.2 g/day, and the recommended duration of supplemental treatment is more than 6 weeks.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing—original draft. TY: Writing—original draft, Data curation, Investigation. XC: Writing—original draft, Data curation, Investigation. HX: Writing—review and editing. SW: Writing—review and editing. GS: Writing—review and editing. LC: Writing—review and editing. WL: Funding acquisition, Methodology, Writing—original draft, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1253312/full#supplementary-material

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The associations between dietary fibers intake and systemic immune and inflammatory biomarkers, a multi-cycle study of NHANES 2015–2020

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Background: In recent years, there has been considerable growth in abnormal inflammatory reactions and immune system dysfunction, which are implicated in chronic inflammatory illnesses and a variety of other conditions. Dietary fibers have emerged as potential regulators of the human immune and inflammatory response. Therefore, this study aims to investigate the associations between dietary fibers intake and systemic immune and inflammatory biomarkers.

Methods: This cross-sectional study used data from the National Health and Nutrition Examination Survey (2015–2020). Dietary fibers intake was defined as the mean of two 24-h dietary recall interviews. The systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), neutrophil-to-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), red blood cell distribution width-to-albumin ratio (RA), ferritin, high-sensitivity C-reactive protein (hs-CRP), and white blood cell (WBC) count were measured to evaluate systemic immune and inflammatory states of the body. The statistical software packages R and EmpowerStats were used to examine the associations between dietary fibers intake and systemic immune and inflammatory biomarkers.

Results: Overall, 14,392 participants were included in this study. After adjusting for age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activity, body mass index, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index, dietary fibers intake was inversely associated with SII ($\beta = -2.19885$, 95% CI: −3.21476 to −1.18294, $p = 0.000248$), SIRI ($\beta = -0.00642$, 95% CI: −0.01021 to −0.00263, $p = 0.001738$), NLR ($\beta = -0.00803$, 95% CI: −0.01179 to −0.00427, $p = 0.000284$), RA ($\beta = -0.00266$, 95% CI: −0.00401 to −0.00131, $p = 0.000644$), ferritin ($\beta = -0.73086$, 95% CI: −1.31385 to −0.14787, $p = 0.020716$), hs-CRP ($\beta = -0.04629$, 95% CI: −0.0743 to −0.01829, $p = 0.002119$), WBC ($\beta = -0.01624$, 95% CI: −0.02685 to −0.00563, $p = 0.004066$), neutrophils ($\beta = -0.01346$, 95% CI: −0.01929 to −0.00764, $p = 0.000064$). An inverse association between dietary fibers and PLR was observed in the middle ($\beta = -3.11979$, 95% CI: −5.74119 to −0.4984, $p = 0.028014$) and the highest tertile ($\beta = -4.48801$, 95% CI: −7.92369 to −1.05234, $p = 0.016881$) and the trend test ($\beta_{trend} = -2.2626$, 95% CI: −3.9648 to

−0.5604, P_{trend} = 0.0150). The observed associations between dietary fibers intake and SII, SIRI, NLR, RA, ferritin, hs-CRP, WBC, and neutrophils remained robust and consistent in the sensitivity analysis. No significant interaction by race was found.

Conclusion: Dietary fibers intake is associated with the improvement of the parameters of the immune response and inflammatory biomarkers, supporting recommendations to increase dietary fibers intake for enhanced immune health.

KEYWORDS

dietary fiber, National Health and Nutrition Examination Survey, systemic immune-inflammation index, systemic inflammation response index, red blood cell distribution width-to-albumin ratio

Introduction

In recent decades, there has been a significant increase in abnormal inflammatory responses and immune system dysfunction, contributing to the development of chronic inflammatory disorders, as well as conditions such as cancer and diabetes (1–3). Therefore, the identification of potential regulators of inflammation and the immune system holds great significance in preventing and treating these diseases. It is well-established that changes in dietary factors play a crucial role in regulating immune function and inflammatory biomarkers (4). Both preclinical and clinical studies provide compelling evidence that a dietary shift from traditional diets abundant in plant-based foods to ultra-processed foods renders individuals susceptible to various chronic and debilitating inflammatory diseases (5, 6). Consequently, the influence of dietary nutrients on immune and inflammatory responses has emerged as an attractive and vital area of research. This study will specifically focus on one such dietary component: dietary fibers.

Dietary fibers are complex dietary components found mainly in grains, vegetables, and fruits that consist of three or more monomeric units (7, 8). These fibers are indigestible in the intestinal tract, but they play a unique and important role in the human body. Higher dietary fibers intake has been reported to improve immune responses and certain inflammatory disorders (8). *In vitro* and *in vivo* experiments have identified that dietary fibers impact immune cells through gut microbiota and may help prevent inflammatory conditions (9). More specifically, clinical studies suggest that dietary fibers act as protective factors against asthma (10), metabolic syndrome (11, 12), and radiation-induced gastrointestinal toxicity (13). Beyond diseases, a variety of immune and inflammatory biomarkers such as C-reactive protein, fibrinogen (14), tumor necrosis factor-α, and interleukin-10 (15) are associated with dietary fibers intake.

The systemic immune-inflammation index (SII) was first proposed by Hu et al. (16) as a prognostic predictor for hepatocellular carcinoma patients (16). However, the clinical interest in SII has grown significantly due to its ability to reflect systemic inflammation and immunity. Previous studies have established strong associations between SII and various diseases, including cancer (17), diabetes (18), hepatic steatosis (19), kidney injury (20), and cardiovascular risk (21). Similarly, the systemic inflammation response index (SIRI) was initially developed to predict the prognosis of pancreatic cancer, with higher levels of SIRI being linked to unfavorable prognostic outcomes (22). The neutrophil-to-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are calculated based on blood cell count and have been widely recognized as potential indicators for early diagnosis and prognosis monitoring in inflammatory diseases and cancers (23). Additionally, Red blood cell distribution width-to-albumin ratio (RA) has emerged as a novel inflammatory biomarker, showing associations with conditions such as stroke (24), diabetic ketoacidosis (25), acute respiratory distress syndrome (26), and all-cause mortality in cancer patients (27). Ferritin and high-sensitivity C-reactive protein (hs-CRP) are classical inflammatory biomarkers extensively used in routine clinical practice and inflammatory research.

Consequently, it has been established with certainty that these biomarkers can serve as reliable indicators of the immune and inflammatory condition of the human body, and they are correlated with various diseases that pose a threat to health. However, few studies have delved into whether these biomarkers can be modulated by dietary fibers. This study aimed to analyze the association between dietary fibers intake and systemic immunity and inflammation using data from the National Health and Nutrition Examination Survey (NHANES) survey conducted from 2015–2020.

Materials and methods

Study population

The NHANES is an epidemiological program developed to assess the health and nutritional conditions of adults and children in the United States. Conducted by the National Center for Health Statistics, a subdivision of the Centers for Disease Control and Prevention, NHANES combines interviews on demographic, socioeconomic, dietary, and health-related queries, physical examinations incorporating medical, dental, physiological measurements, and laboratory tests by highly qualified medical personnel. NHANES sample constitutes a representation of the noninstitutionalized civilian population in the United States, comprising the 50 states and the District of Columbia. From 1999 onwards, the sample design has employed a multi-year, stratified, clustered four-stage sampling approach, with data release in 2-year cycles.

This study included NHANES data from 2015–2020. A total of 20,520 participants remained after excluding those younger than 20. We further excluded those lacking systemic immune-inflammation index (SII) or dietary fibers intake data, leaving 14,392 participants for the association analysis. In order to perform a sensitivity analysis with

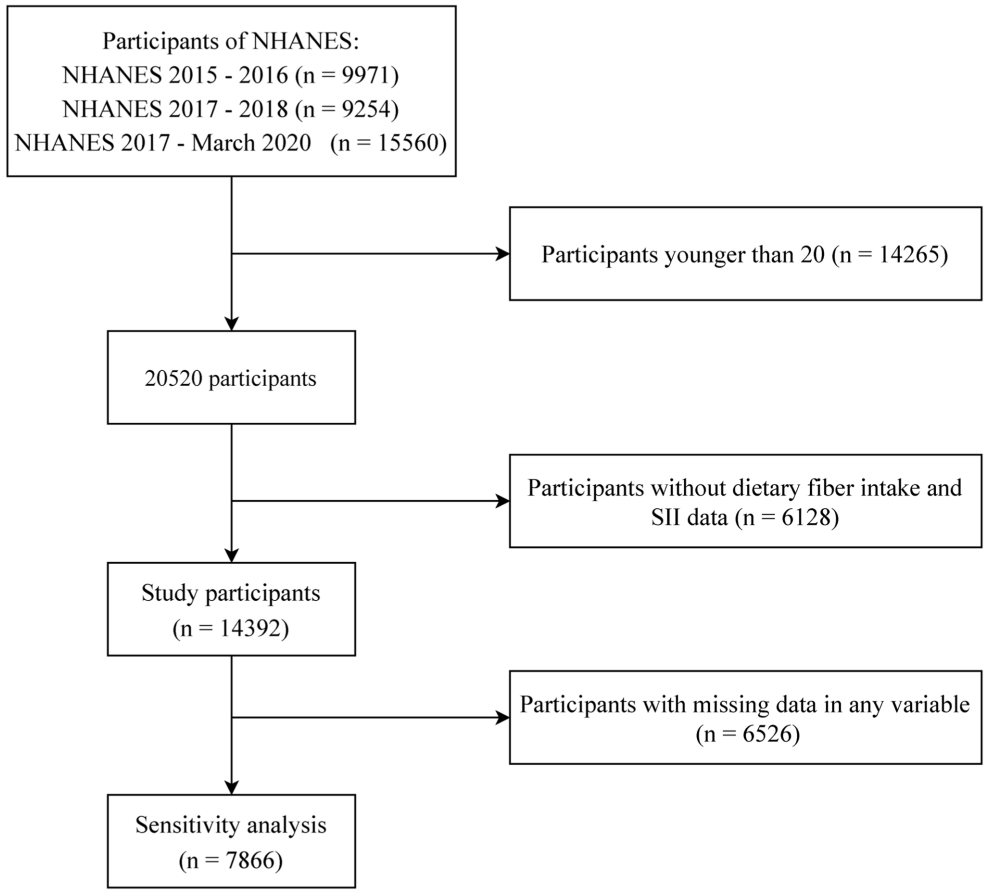

FIGURE 1
Participant screening flowchart based on age, dietary fibers intake, systemic immune/inflammatory biomarkers, and sensitivity analyses.

complete cases, 6,526 participants with incomplete data in any kind of variable were excluded. A flowchart presents the process of selecting participants (Figure 1).

Measurement of dietary fibers intake

Dietary intake data was collected through two 24-h dietary recalls conducted 3–10 days apart during the Mobile Examination Center component of NHANES. The recalls were jointly processed by NHANES, the United States Department of Agriculture, and the United States Department of Health and Human Services. Average daily dietary fibers intake was calculated using the two 24-h of intake data. Full documentation of the dietary assessment methods is available in the NHANES dietary interviewer procedures manuals (28, 29).

Measurement of primary and secondary outcomes

The primary outcome was the SII, calculated as: platelet counts × neutrophil count/lymphocyte count (16). SIRI, NLR, PLR, RA, ferritin, hs-CRP, and six kinds of white blood cell (WBC) count are the secondary outcomes of this study. The formulas for SIRI, NLR, PLR and RA are presented as follows: SIRI = neutrophil count × monocyte/lymphocyte count. (22), NLR = neutrophil counts/lymphocyte counts, PLR = platelet counts/lymphocyte counts, and RA = red blood cell distribution width (%)/albumin (mg/dl) (30). Ferritin and hs-CRP are well-acknowledged acute inflammation indicators obtained using blood specimen tests. NHANES provides standardized protocols for measuring these biomarkers, available on the NHANES website[1] (31).

Selection of covariates

Sociodemographic characteristics included age, gender (male and female), race (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), and family monthly poverty level index (≤1.3, 1.5–1.85, >1.85) were collected. Lifestyle characteristics included alcohol consumption (never, mild, moderate, and heavy), smoking status (never, former, and current), and vigorous recreational activity (Yes and

1 https://www.cdc.gov/nchs/nhanes/index.htm

No) were obtained. Never drinkers were ascertained by the questionnaire: "Ever had a drink of any kind of alcohol?" Furthermore, participants who had >4 drinks per day, 3–4 drinks per day, and up to 2 drinks per day were classified as heavy, moderate, and mild drinkers, respectively. Participants who smoked less than 100 cigarettes in life were considered as never smoking and the others were divided into former and current smokers according to the question "Do you now smoke cigarettes?" Metabolic characteristics included body mass index (BMI), hyperlipidemia, hypertension, and diabetes. An adult with a BMI below 18.5 kg/m2 is considered underweight, 18.5 to 24.9 is considered normal weight, 25 to 29.9 is considered overweight, and 30 or above is considered obesity. Hyperlipidemia was defined by high-density lipoprotein cholesterol <1.0 mmoL/L in men, < 1.3 mmoL/L in women, or triglycerides ≥1.8 mmoL/L regardless of gender. Hypertension was defined as systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 80 mmHg on ≥3 occasions. Moreover, participants who take an anti-hypertensive agent or who answered "yes" to the questions: "Are you now taking prescribed medicine for high blood pressure?" and "Ever told you had high blood pressure?" were also defined as having hypertension. Diabetes was defined as a positive response to the question "Doctor told you have diabetes?." Additionally, participants who achieved one or more of the following conditions were diagnosed with diabetes: glycohemoglobin ≥6.5%, fasting glucose ≥7 mmol/L, two-hour glucose of oral glucose tolerance test, or serum glucose ≥11.1 mmol/L. The dietary inflammatory index (DII) is a scoring algorithm developed through comprehensive analysis of scientific literature on the inflammatory properties of dietary components. The DII was used to categorize participants' dietary patterns as either pro-inflammatory or anti-inflammatory (32).

Statistical analyses

Dummy variables were used to denote missing covariate values. Continuous variables were presented as survey-weighted mean (95% confidence interval (CI)) and categorical variables were expressed as survey-weighted percentage (95% CI). The weighted $\chi 2$ test (categorical variable) and weighted linear regression model (continuous variable) compared tertiles of dietary fibers intake. A univariate and multivariate weighted linear regression model and/or weighted binary logistic regression model were used to examine the associations between dietary fibers intake and SII, as well as other outcomes. A total of three statistical models were constructed in each regression analysis. Model I was the non-adjusted model with no covariates adjusted. Model II was the minimally adjusted model with age and gender adjusted. Model III was a fully adjusted with age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, BMI, hyperlipidemia, hypertension, diabetes and DII adjusted. The fully adjusted model took into account demographic factors, lifestyle factors, dietary factors, and metabolic factors. Covariates were selected by referring to cross-sectional studies related to our prespecified outcome indicators (33–36). Sensitivity analysis was conducted by excluding the participants with incomplete data in covariates. Taking the biochemical markers between human races into consideration (37), a subgroup analyses between races were performed using a stratified logistic regression model and a interaction test for effect modification for different races were followed by the likelihood-ratio test. Data analysis was performed with the statistical software packages R[2] and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA). All statistical tests were two-sided, and a p value <0.05 was considered statistically significant.

Results

Baseline characteristics

Table 1 shows baseline of the 14,392 participants by dietary fiber intake tertiles (low: 0–11.65 g/d, n = 4,790; middle: 11.7–18.45 g/d, n = 4,801; high: 18.5–89.55 g/d, n = 4,801). Participants with higher dietary fibers intake had lower levels of SII, SIRI, NLR, RA, hs-CRP, WBC, neutrophils and basophils. Furthermore, these individuals also exhibited exhibited a higher proportion of male and Mexican American participants, greater affluence, lower prevalence of obesity, and healthier lifestyle reflected by increased engagement in rigorous recreational activities, decreased usage of cigarettes and alcohol, and a higher percentage of adherence to an anti-inflammatory diet.

Associations between dietary fibers intake and SII, SIRI, NLR, and PLR

Dietary fibers intake shows significant inverse associations with SII, SIRI, NLR in all 3 models (Table 2). The effect size (β) and 95% confidence interval (CI) for SII in the fully-adjusted model are −2.19885 (−3.21476, −1.18294) and the highest tertile significantly associated with decreased SII (β = −43.29833, 95% CI: −67.46845 to −19.12821, p = 0.001073). The p for trend across dietary fibers intake categories reaches statistical significance (β_{trend} = −21.5411, 95% CI: −33.0049 to −10.0772, P_{trend} = 0.0011).

The β and 95% CI for SIRI in the fully-adjusted model are −0.00642 (−0.01021, −0.00263) and the highest tertile significantly associated with decreased SIRI (β = −0.12477, 95% CI: −0.20495 to −0.04459, p = 0.003611). The p for trend across dietary fibers intake categories reaches statistical significance (β_{trend} = −0.0621, 95% CI: −0.1000 to −0.0242, P_{trend} = 0.0035).

The β and 95% CI for NLR in the fully-adjusted model are −0.00803 (−0.01179, −0.00427) and the highest tertile significantly associated with decreased NLR (β = −0.18596, 95% CI: −0.26639 to −0.10553, p = 0.000067). The p for trend across dietary fibers intake categories reaches statistical significance (β_{trend} = −0.0930, 95% CI: −0.1312 to −0.0547, P_{trend} = 0.0001).

The β and 95% CI for PLR in the fully-adjusted model are −0.13014 (−0.29189, 0.03161) and the highest tertile significantly associated with decreased PLR (β = −4.48801, 95% CI: −7.92369 to −1.05234, p = 0.016881). The P for trend across dietary fibers intake categories reaches statistical significance (β_{trend} = −2.2626, 95% CI: −3.9648 to −0.5604, P_{trend} = 0.0150).

2 http://www.R-project.org

TABLE 1 Survey-weighted baseline characteristics by dietary fibers intake level in the study population.

Weighted variates		Low-DF (0–11.65 g/d) *N* = 4,790	Middle-DF (11.7–18.45 g/d) *N* = 4,801	High-DF (18.5–89.55 g/d) *N* = 4,801	Survey-weighted *p* value
SII [mean (95% CI), 1,000 cells/μL]		542.883 (527.361, 558.406)	526.415 (510.436, 542.394)	496.221 (482.761, 509.682)	<0.0001
SIRI [mean (95% CI), 1,000 cells/μL]		1.341 (1.299, 1.383)	1.320 (1.267, 1.373)	1.257 (1.206, 1.308)	0.03
NLR [mean (95% CI), ratio]		2.206 (2.155, 2.258)	2.161 (2.102, 2.220)	2.104 (2.049, 2.159)	0.012
PLR [mean (95% CI), ratio]		122.383 (120.215, 124.550)	120.189 (117.829, 122.548)	119.709 (117.444, 121.974)	0.208
RA [mean (95% CI), g%/dL]		3.367 (3.338, 3.396)	3.309 (3.281, 3.337)	3.212 (3.188, 3.235)	<0.0001
Ferritin [mean (95% CI), ug/L]		128.157 (122.460, 133.854)	141.200 (134.674, 147.726)	139.984 (127.380, 152.588)	0.007
hs-CRP [mean (95% CI), mg/L]		4.625 (4.201, 5.048)	4.128 (3.822, 4.434)	3.103 (2.801, 3.405)	<0.0001
WBC [mean (95% CI), 1,000 cells/μL]		7.639 (7.443, 7.835)	7.498 (7.341, 7.655)	7.159 (7.040, 7.279)	<0.0001
Neutrophils [mean (95% CI), 1,000 cells/μL]		4.471 (4.371, 4.571)	4.402 (4.290, 4.513)	4.153 (4.062, 4.245)	<0.0001
Lymphocyte [mean (95% CI), 1,000 cells/μL]		2.318 (2.181, 2.456)	2.247 (2.172, 2.322)	2.178 (2.130, 2.226)	0.063
Monocyte [mean (95% CI), 1,000 cells/μL]		0.596 (0.585, 0.607)	0.596 (0.584, 0.607)	0.584 (0.570, 0.597)	0.279
Eosinophils [mean (95% CI), 1,000 cells/μL]		0.204 (0.197, 0.211)	0.204 (0.195, 0.213)	0.197 (0.189, 0.205)	0.496
Basophils [mean (95% CI), 1,000 cells/μL]		0.059 (0.056, 0.062)	0.057 (0.054, 0.060)	0.052 (0.049, 0.055)	0.005
Age [mean (95% CI), years]		47.434 (46.436, 48.432)	49.106 (48.208, 50.004)	48.701 (47.677, 49.726)	0.008
Sex [percentage (95% CI)]					<0.0001
	male	39.157 (36.689, 41.683)	46.828 (44.678, 48.989)	56.959 (54.471, 59.413)	
	female	60.843 (58.317, 63.311)	53.172 (51.011, 55.322)	43.041 (40.587, 45.529)	
Race [percentage (95% CI)]					<0.0001
	Non-Hispanic White	62.833 (59.071, 66.446)	66.185 (61.848, 70.265)	62.027 (57.716, 66.156)	
	Mexican American	5.813 (4.629, 7.276)	8.206 (6.167, 10.842)	11.669 (9.226, 14.654)	
	Non-Hispanic Black	16.158 (13.702, 18.957)	10.854 (8.737, 13.409)	6.565 (5.312, 8.089)	
	Other Hispanic	6.751 (5.386, 8.431)	5.521 (4.359, 6.971)	8.267 (7.003, 9.735)	
	Other Race – Including Multi-Racial	8.445 (7.430, 9.583)	9.234 (7.834, 10.855)	11.472 (9.424, 13.897)	
FMMPLL [percentage (95% CI)]					<0.0001
	<= 1.3	26.278 (24.156, 28.515)	20.069 (17.950, 22.369)	17.362 (15.396, 19.521)	
	>1.3, <= 1.85	13.273 (11.677, 15.050)	11.045 (9.634, 12.633)	10.105 (8.987, 11.344)	
	>1.85	53.820 (51.438, 56.184)	62.243 (59.207, 65.186)	66.511 (64.082, 68.855)	
	Not obtained	6.630 (5.603, 7.829)	6.644 (5.434, 8.099)	6.022 (4.914, 7.362)	
Diabetes [percentage (95% CI)]					0.171
	NO	83.600 (81.727, 85.316)	82.065 (80.292, 83.711)	83.725 (82.097, 85.232)	
	YES	15.744 (14.036, 17.618)	16.905 (15.313, 18.627)	15.081 (13.641, 16.644)	
	NA	0.655 (0.422, 1.016)	1.030 (0.634, 1.669)	1.193 (0.865, 1.644)	
Hyperlipidemia [percentage (95% CI)]					0.586
	NO	31.425 (29.068, 33.881)	30.583 (28.185, 33.092)	33.367 (30.609, 36.244)	
	YES	68.575 (66.119, 70.932)	69.415 (66.907, 71.814)	66.633 (63.756, 69.391)	
	NA	0.000 (0.000, 0.000)	0.001 (0.000, 0.009)	0.000 (0.000, 0.000)	
Hypertension [percentage (95% CI)]					0.458
	NO	63.988 (61.280, 66.610)	62.096 (59.801, 64.339)	63.497 (61.038, 65.887)	
	YES	36.012 (33.390, 38.720)	37.904 (35.661, 40.199)	36.503 (34.113, 38.962)	
BMI level [percentage (95% CI)]					0.004
	Not obtained	0.486 (0.337, 0.699)	0.630 (0.446, 0.889)	0.562 (0.316, 0.999)	
	< 18.5	1.527 (1.003, 2.320)	1.285 (0.882, 1.870)	0.851 (0.585, 1.235)	
	>= 18.5, <= 24.9	23.590 (21.320, 26.022)	23.686 (21.233, 26.328)	26.292 (23.733, 29.022)	
	>= 25, <= 29.9	28.935 (26.200, 31.832)	31.595 (29.270, 34.015)	33.729 (31.111, 36.452)	
	>= 30	45.462 (42.885, 48.064)	42.804 (40.205, 45.443)	38.565 (35.530, 41.692)	
VRA [percentage (95% CI)]					<0.0001
	NO	76.953 (74.506, 79.230)	72.588 (70.399, 74.673)	61.764 (58.315, 65.099)	
	YES	23.047 (20.770, 25.494)	27.412 (25.327, 29.601)	38.236 (34.901, 41.685)	
Smoking status [percentage (95% CI)]					<0.0001
	NA	0.045 (0.012, 0.172)	0.024 (0.003, 0.173)	0.077 (0.011, 0.511)	
	never	51.986 (48.822, 55.135)	59.600 (56.379, 62.741)	61.553 (59.628, 63.443)	
	former	23.339 (21.417, 25.378)	25.067 (22.860, 27.411)	27.631 (25.813, 29.526)	
	current	24.630 (22.153, 27.286)	15.309 (13.453, 17.370)	10.739 (9.395, 12.250)	

(Continued)

TABLE 1 (Continued)

Weighted variates		Low-DF (0–11.65 g/d) *N* = 4,790	Middle-DF (11.7–18.45 g/d) *N* = 4,801	High-DF (18.5–89.55 g/d) *N* = 4,801	Survey-weighted *p* value
Alcohol consumption [percentage (95% CI)]					0.023
	NA	14.551 (12.761, 16.544)	12.547 (11.446, 13.737)	12.228 (10.711, 13.925)	
	never	7.957 (6.975, 9.064)	8.430 (7.058, 10.040)	9.283 (7.841, 10.959)	
	former	4.334 (3.496, 5.360)	4.792 (3.674, 6.228)	4.379 (3.515, 5.443)	
	midl	47.414 (44.605, 50.240)	51.943 (49.015, 54.858)	49.138 (45.656, 52.629)	
	moderate	16.076 (13.938, 18.471)	15.984 (13.996, 18.195)	15.503 (13.581, 17.641)	
	heavy	9.668 (8.280, 11.260)	6.304 (5.037, 7.864)	9.469 (8.024, 11.143)	
DII [percentage (95% CI)]					<0.0001
	Pro-inflammatory diet	96.616 (95.686, 97.351)	84.194 (82.127, 86.063)	49.016 (46.087, 51.952)	
	Anti-inflammatory diet	3.384 (2.649, 4.314)	15.806 (13.937, 17.873)	50.984 (48.048, 53.913)	

SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-lymphocyte ratio; RA, red blood cell distribution width-to-albumin ratio; hs-CRP, high-sensitivity C-Reactive Protein; WBC, white blood cell; FMMPLL, family monthly poverty level index; VRA, vigorous recreational activities; BMI, body mass index, DII, dietary inflammatory index, CI, confidence interval, DF, dietary fiber.

TABLE 2 Survey-weighted univariate and multivariate regression analyses of associations between dietary fibers intake and SII, SIRI, NLR, and PLR.

Exposure	Non-adjusted model, β (95%CI) P	Minimally-adjusted model, β (95%CI) P	Fully-adjusted model, β (95%CI) P
SII			
Dietary fiber	−2.48688 (−3.32054, −1.65322) <0.000001	−2.21499 (−3.03924, −1.39074) 0.000003	−2.19885 (−3.21476, −1.18294) 0.000248
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−16.46863 (−35.98249, 3.04523) 0.104012	−15.81623 (−34.70707, 3.07462) 0.106952	−16.55926 (−36.44972, 3.3312) 0.098115
High	−46.66205 (−65.77292, −27.55118) 0.000014	−42.19161 (−60.33388, −24.04934) 0.000033	−43.29833 (−67.46845, −19.12821) 0.001073
P trend	−23.4625 (−32.9424, −13.9825) <0.0001	−21.1946 (−30.2038, −12.1854) <0.0001	−21.5411 (−33.0049, −10.0772) 0.0011
SIRI			
Dietary fiber	−0.00474 (−0.00753, −0.00195) 0.001555	−0.00713 (−0.00997, −0.0043) 0.000009	−0.00642 (−0.01021, −0.00263) 0.001738
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.02079 (−0.08507, 0.04349) 0.528897	−0.05021 (−0.11587, 0.01545) 0.140109	−0.04873 (−0.11394, 0.01648) 0.13556
High	−0.0838 (−0.14515, −0.02245) 0.009862	−0.13215 (−0.19208, −0.07221) 0.000072	−0.12477 (−0.20495, −0.04459) 0.003611
P trend	−0.0423 (−0.0730, −0.0116) 0.0093	−0.0664 (−0.0964, −0.0364) 0.0001	−0.0621 (−0.1000, −0.0242) 0.0035
NLR			
Dietary fiber	−0.00448 (−0.00757, −0.00139) 0.006316	−0.00601 (−0.00903, −0.00299) 0.000278	−0.00803 (−0.01179, −0.00427) 0.000284
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.0451 (−0.11806, 0.02786) 0.231031	−0.07218 (−0.14502, 0.00065) 0.057609	−0.09348 (−0.16845, −0.0185) 0.016393
High	−0.1024 (−0.16757, −0.03723) 0.003281	−0.13784 (−0.19768, −0.078) 0.000038	−0.18596 (−0.26639, −0.10553) 0.000067
P trend	−0.0513 (−0.0839, −0.0187) 0.0032	−0.0689 (−0.0988, −0.0389) <0.0001	−0.0930 (−0.1312, −0.0547) 0.0001
PLR			
Dietary fiber	−0.0566 (−0.19744, 0.08424) 0.434314	−0.00215 (−0.13867, 0.13436) 0.975435	−0.13014 (−0.29189, 0.03161) 0.126899
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−2.19396 (−5.08296, 0.69503) 0.142554	−2.17535 (−4.98881, 0.6381) 0.135829	−3.11979 (−5.74119, −0.4984) 0.028014
High	−2.6734 (−5.90449, 0.55769) 0.110802	−1.91749 (−5.12276, 1.28778) 0.246431	−4.48801 (−7.92369, −1.05234) 0.016881
P trend	−1.3203 (−2.9431, 0.3025) 0.1166	−0.9360 (−2.5470, 0.6751) 0.2600	−2.2626 (−3.9648, −0.5604) 0.0150

SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-lymphocyte ratio; OR, odds ratio; CI, confidence interval.

Non-adjusted model: no covariates were adjusted. Minimally-adjusted model: age and gender were adjusted. Fully-adjusted model: age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, body mass index level, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index.

TABLE 3 Survey-weighted univariate and multivariate regression analyses of the association between dietary fibers intake and RA.

Exposure	Non-adjusted model, β (95%CI) P	Minimally-adjusted model, β (95%CI) P	Fully-adjusted model, β (95%CI) P
RA			
Dietary fiber	−0.00744 (−0.00903, −0.00586) <0.000001	−0.00576 (−0.00734, −0.00418) <0.000001	−0.00266 (−0.00401, −0.00131) 0.000644
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.05793 (−0.08976, −0.02611) 0.000773	−0.05149 (−0.08072, −0.02227) 0.001122	−0.02287 (−0.05325, 0.00751) 0.133181
High	−0.15514 (−0.1925, −0.11778) <0.000001	−0.12805 (−0.16465, −0.09145) <0.000001	−0.07064 (−0.10227, −0.03901) 0.000097
P trend	−0.0780 (−0.0968, −0.0591) <0.0001	−0.0643 (−0.0827, −0.0459) <0.0001	−0.0351 (−0.0503, −0.0199) 0.0001

RA, red blood cell distribution width-to-albumin ratio.
Non-adjusted model: no covariates were adjusted. Minimally-adjusted model: age and gender were adjusted. Fully-adjusted model: age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, body mass index level, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index.

TABLE 4 Survey-weighted univariate and multivariate regression analyses of the associations between dietary fibers intake and ferritin and hs-CRP.

Exposure	Non-adjusted model, β (95%CI) P	Minimally-adjusted model, β (95%CI) P	Fully-adjusted model, β (95%CI) P
Ferritin			
Dietary fiber	0.29962 (−0.26961, 0.86886) 0.306821	−0.85335 (−1.32295, −0.38376) 0.000798	−0.73086 (−1.31385, −0.14787) 0.020716
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	13.04284 (4.70996, 21.37572) 0.003393	0.92668 (−6.62744, 8.4808) 0.810956	3.11558 (−3.64433, 9.87549) 0.374636
High	11.82705 (−2.60333, 26.25744) 0.114127	−9.73583 (−20.30168, 0.83002) 0.076817	−5.24483 (−18.5334, 8.04373) 0.446152
P trend	5.8988 (−1.3422, 13.1397) 0.1162	−4.8841 (−10.1972, 0.4290) 0.0774	−2.3954 (−8.8288, 4.0381) 0.4718
hs-CRP			
Dietary fiber	−0.08331 (−0.10509, −0.06152) <0.000001	−0.07581 (−0.09749, −0.05413) <0.000001	−0.04629 (−0.0743, −0.01829) 0.002119
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.49671 (−0.96947, −0.02395) 0.044393	−0.44696 (−0.91731, 0.0234) 0.068297	−0.23148 (−0.70177, 0.23881) 0.320052
High	−1.52145 (−2.04615, −0.99675) <0.000001	−1.38093 (−1.90503, −0.85683) 0.000004	−0.8598 (−1.49918, −0.22043) 0.010218
P trend	−0.7661 (−1.0272, −0.5049) <0.0001	−0.6954 (−0.9563, −0.4345) <0.0001	−0.4261 (−0.7299, −0.1224) 0.0105

hs-CRP, high-sensitivity C-Reactive Protein; OR, odds ratio; CI, confidence interval.
Non-adjusted model: no covariates were adjusted. Minimally-adjusted model: age and gender were adjusted. Fully-adjusted model: age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, body mass index level, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index.

Associations between dietary fibers intake and RA

Dietary fibers intake presents significant inverse associations with RA (β = −0.00266, 95% CI: −0.00401 to −0.00131, $p = 0.000644$). The β and 95% CI for the highest tertile is −0.07064 (−0.010227, −0.03901) in the fully-adjusted model. A significant negative trend is observed across dietary fiber intake categories ($\beta_{trend} = -0.0351$, 95% CI: −0.0503 to −0.0199, $P_{trend} = 0.0001$). The β and corresponding 95% CI for all the statistical models are presented in Table 3.

Associations between dietary fibers intake and ferritin and hs-CRP

An inverse association is observed between dietary fibers intake and ferritin (β = −0.73086, 95% CI: −1.31385 to −0.14787, $p = 0.020716$). However, when we stratified dietary fibers into tertiles, statistical significance was not attained in any tertile or across tertiles (Table 4). Dietary fibers intake also shows an inverse correlation with hs-CRP (β = −0.04629, 95% CI: −0.0743 to −0.01829, $p = 0.002119$), with the highest tertile significantly associated with decreased hs-CRP (β = −0.8598, 95% CI: −1.49918 to −0.22043, $p = 0.010218$) and the P for trend across dietary fibers intake categories reaches statistical significance ($\beta_{trend} = -0.4261$, 95% CI: −0.7299 to −0.1224, $P_{trend} = 0.0105$).

Associations between dietary fibers intake and white blood cell

Significant inverse correlations are observed between dietary fibers intake and WBC (β = −0.01624, 95% CI: −0.02685 to −0.00563, $p = 0.004066$; $\beta_{trend} = -0.1268$, 95% CI: −0.2277 to −0.0258, $P_{trend} = 0.0209$), and neutrophils (β = −0.01346, 95% CI: −0.01929 to −0.00764, $p < 0.000064$; $\beta_{trend} = -0.1047$, 95% CI:

−0.1641 to −0.0453, P_{trend} = 0.0019) (Table 5). However, no significant associations are seen with lymphocytes, monocytes, eosinophils or basophils.

Sensitivity analysis of complete cases for SII

Sensitivity analysis continues to show an inverse association between dietary fibers intake and SII (Table 6). The β and 95% CI are −2.0099 (−3.08293, −0.93687) for the non-adjusted model, −1.7928 (−2.85182, −0.73377) for the minimally-adjusted model, and − 1.59067 (−3.09644, −0.08491) for fully-adjusted model. The highest tertile (β = −34.10908, 95% CI: −65.05815 to −3.16001, p = 0.03861) is significantly associated with decreased SII in fully-adjusted model. The P for trend across dietary fibers intake categories reaches statistical significance (β_{trend} = −17.2185, 95% CI: −32.7649 to −1.6721, P_{trend} = 0.0375). Additionally, robust inverse associations are also observed between dietary fibers intake and SIRI, NLR, RA, ferritin, hs-CRP, WBC, and neutrophils (Supplementary Table S1).

Interaction effect of race on the associations between dietary fibers intake and outcomes

Interaction tests showed no significant difference in the associations between dietary fibers intake and systemic immune and inflammatory biomarkers by race (Table 7). The $P_{interaction}$ for race and SII, SIRI, NLR, PLR, RA, ferritin, hs-CRP, and six kinds of WBC count were 0.9941, 0.9085, 0.9054, 0.0495, 0.6856, 0.476, 0.1873, 0.3227, 0.1548, 0.2794, 0.2081, 0.659 and 0.6209.

Discussion

This study conducted a comprehensive cross-sectional investigation using data from the NHANES 2015–2020 survey, which represents the U.S. population, to explore the association between dietary fibers intake and systemic immune and inflammatory biomarkers. The results of our study indicate that dietary fibers intake is inversely associated with SII, SIRI, NLR, RA, hs-CRP, WBC, and neutrophils. Furthermore, the sensitivity analysis confirmed the robustness of these findings. To the best of our knowledge, this is the initial investigation to evaluate such associations within a nationally representative sample.

SII, SIRI, NLR, and RA are potent biomarkers of the body's immune and inflammatory state and have demonstrated predictive value for a wide range of diseases. The role of dietary factors as potential regulators of these biomarkers is evident in the literature review. In a case–control study involving 527 participants, dietary inflammation levels in women with polycystic ovary syndrome showed a positive correlation with SII, NLR, and PLR (38). Similarly, in a cross-sectional study with 1,050 participant, dietary inflammation level was positively associated with SIRI in individuals with mild cognitive impairment (39). Another study revealed a negative correlation between dietary antioxidant capacity and NLR in cancer patients (40). Additionally, a retrospective study found that dietary omega- 6 to omega- 3 fatty acids was associated with reduced PLR level in men with chronic coronary syndrome. (41). Our study unveiled an inverse association between dietary fibers and SII, SIRI, NLR, and RA, suggesting that a high-fiber diet may help regulate these biomarkers and potentially benefit the immune system.

Ferritin, initially identified as a reactant of acute inflammation caused by infectious agents, has subsequently been linked to acute and chronic inflammatory conditions precipitated by non-infectious sources. Moreover, it has been been demonstrated to play a pivotal role in the pathogenesis of various inflammatory and autoimmune diseases (42, 43). The rapid elevation in serum ferritin levels at the onset of viral or bacterial infections renders it a sensitive biomarker with clinical utility (44). However, it takes up to 5 weeks for ferritin levels to decrease (45). Elevated ferritin levels have been shown to be associated with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis, in which ferritin predicts disease severity or contributes to disease development (46–48). *In vitro* experiments have shown that Low Phytate Peas containing dietary fibers can affect hepatic ferritin concentrations (49). However, the relationship between dietary fibers intake and ferritin is controversial in clinical and cross-sectional studies. A prospective, randomized, placebo-controlled clinical trial conducted in China on end-stage renal disease patients treated with dietary fibers (composed of galactomannan, resistant dextrin, fructooligosaccharide, and starch) or potato starch for 8 weeks showed that the patients in the dietary fibers group had higher serum ferritin levels (50). Another randomized, double-blind, placebo-controlled with 32 female athletes demonstrated that daily synbiotic supplement along with Fe supplementation increased serum ferritin levels (51). However, some studies have come to the opposite conclusion. In a crossover-design clinical trial, healthy participants who took a high-fiber snack for 6 weeks and maintained it with a low-fiber snack for 6 weeks had lower ferritin levels compared to the control group (52). A French epidemiological survey study that included 4,358 subjects also found a negative association between dietary fibers intake and serum ferritin levels (53). In addition, vegans with high-fiber diets have been found to have low ferritin levels in several dietary investigations (54). Although the fully adjusted model indicated that dietary fibers intake was negatively associated with ferritin levels, there was no statistical difference in the analysis of the trend test, making the relationship between dietary fibers intake and ferritin unstable in our study. Hs-CRP is a biomarker of systemic inflammation in the body, in addition to being regarded as an indicator of acute inflammation, it is associated with many chronic diseases, including coronary heart disease (55), metabolic syndrome (56), diabetes mellitus (57), and cancer (58). Evidence of an inverse correlation between the dietary fibers intake and hs-CRP concentrations has emerged from multiple cohort studies conducted on the American population. Two cross-sectional analyses of NHANES data from 1999–2000 included 3,920 and 4,900 participants, respectively (59, 60). Concurrently, a longitudinal cohort study involving 524 healthy adults (61) and a small clinical trial have been conducted (62). A parallel dietary intervention trial has demonstrated that incorporating high-fiber wholegrain rye foods with added fermented rye bran led to a reduction in hs-CRP levels among Chinese adults (63). However, no association between dietary fibers intake and hs-CRP was seen among postmenopausal women in a cross-sectional study of 1958 participants (64). Our current cross-sectional study, which boasts the largest sample size to date, aligns with prior research findings.

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TABLE 5 Survey-weighted univariate and multivariate regression analyses of the association between dietary fibers intake and WBC.

Exposure	Non-adjusted model, β (95%CI) P	Minimally-adjusted model, β (95%CI) P	Fully-adjusted model, β (95%CI) P
WBC			
Dietary fiber	−0.02843 (−0.0357, −0.02116) <0.000001	−0.02688 (−0.03436, −0.0194) <0.000001	−0.01624 (−0.02685, −0.00563) 0.004066
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.14077 (−0.37434, 0.0928) 0.242777	−0.11933 (−0.35091, 0.11225) 0.31729	−0.04567 (−0.24459, 0.15325) 0.656584
High	−0.4794 (−0.67069, −0.28811) 0.000009	−0.442 (−0.63081, −0.25319) 0.000029	−0.25703 (−0.46003, −0.05402) 0.02016
P trend	−0.2416 (−0.3357, −0.1475) <0.0001	−0.2229 (−0.3158, −0.1300) <0.0001	−0.1268 (−0.2277, −0.0258) 0.0209
Neutrophils			
Dietary fiber	−0.01934 (−0.02451, −0.01418) <0.000001	−0.01832 (−0.02361, −0.01303) <0.000001	−0.01346 (−0.01929, −0.00764) 0.000064
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.06914 (−0.20712, 0.06883) 0.33046	−0.05651 (−0.19298, 0.07996) 0.420792	−0.02977 (−0.15053, 0.09098) 0.633115
High	−0.31726 (−0.42947, −0.20506) <0.000001	−0.29314 (−0.40377, −0.18251) 0.000004	−0.21267 (−0.33229, −0.09305) 0.001835
P trend	−0.1603 (−0.2158, −0.1049) <0.0001	−0.1483 (−0.2030, −0.0935) <0.0001	−0.1047 (−0.1641, −0.0453) 0.0019
Lymphocyte			
Dietary fiber	−0.0074 (−0.01113, −0.00367) 0.00028	−0.0059 (−0.00947, −0.00234) 0.002064	−0.00183 (−0.00863, 0.00497) 0.58431
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.07136 (−0.22374, 0.08101) 0.362818	−0.05303 (−0.20219, 0.09613) 0.489059	−0.01814 (−0.1611, 0.12483) 0.795617
High	−0.14025 (−0.28676, 0.00625) 0.06612	−0.10871 (−0.24628, 0.02885) 0.127573	−0.03531 (−0.18774, 0.11712) 0.636733
P trend	−0.0701 (−0.1422, 0.0020) 0.0620	−0.0544 (−0.1221, 0.0133) 0.1214	−0.0177 (−0.0903, 0.0550) 0.6376
Monocyte			
Dietary fiber	−0.00108 (−0.00173, −0.00042) 0.002248	−0.00173 (−0.00242, −0.00104) 0.000009	−0.00093 (−0.00188, 0.00003) 0.056673
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.00065 (−0.01447, 0.01316) 0.926339	−0.00676 (−0.02073, 0.0072) 0.346969	−0.00069 (−0.01311, 0.01172) 0.913595
High	−0.0124 (−0.02897, 0.00417) 0.148336	−0.02452 (−0.04134, −0.0077) 0.006171	−0.00926 (−0.02949, 0.01096) 0.377849
P trend	−0.0063 (−0.0146, 0.0020) 0.1439	−0.0124 (−0.0208, −0.0039) 0.0060	−0.0045 (−0.0146, 0.0055) 0.3817
Eosinophils			
Dietary fiber	−0.00039 (−0.00089, 0.0001) 0.121687	−0.00067 (−0.0012, −0.00015) 0.015107	0.00008 (−0.00052, 0.00067) 0.802277
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.00043 (−0.0093, 0.00843) 0.923931	−0.00328 (−0.01214, 0.00557) 0.470761	0.00131 (−0.00756, 0.01019) 0.7741
High	−0.00699 (−0.01861, 0.00463) 0.243849	−0.01232 (−0.02431, −0.00033) 0.049358	0.00017 (−0.01265, 0.01299) 0.979598
P trend	−0.0036 (−0.0094, 0.0023) 0.2422	−0.0062 (−0.0123, −0.0001) 0.0501	0.0001 (−0.0062, 0.0064) 0.9729
Basophils			
Dietary fiber	−0.00031 (−0.00045, −0.00016) 0.000122	−0.0003 (−0.00045, −0.00016) 0.000155	−0.00009 (−0.00029, 0.0001) 0.359748
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−0.00232 (−0.00581, 0.00118) 0.199226	−0.0027 (−0.00613, 0.00073) 0.129011	−0.00089 (−0.00449, 0.00272) 0.633727
High	−0.00668 (−0.01058, −0.00279) 0.001442	−0.00683 (−0.0107, −0.00297) 0.001089	−0.00347 (−0.00797, 0.00102) 0.142744
P trend	−0.0034 (−0.0053, −0.0014) 0.0014	−0.0034 (−0.0054, −0.0015) 0.0010	−0.0017 (−0.0040, 0.0005) 0.1449

WBC, white blood cell; OR, odds ratio; CI, confidence interval.

Non-adjusted model: no covariates were adjusted. Minimally-adjusted model: age and gender were adjusted. Fully-adjusted model: age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, body mass index level, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index.

TABLE 6 Sensitivity analysis for the association between dietary fibers intake and SII.

Exposure	Non-adjusted model, β (95%CI) P	Minimally-adjusted model, β (95%CI) P	Fully-adjusted model, β (95%CI) P
SII			
Dietary fiber	−2.0099 (−3.08293, −0.93687) 0.000554	−1.7928 (−2.85182, −0.73377) 0.00166	−1.59067 (−3.09644, −0.08491) 0.038831
Dietary fiber tertiles			
Low	Ref	Ref	Ref
Middle	−26.80641 (−50.98476, −2.62806) 0.034268	−25.94692 (−49.43197, −2.46188) 0.035053	−23.67319 (−48.36226, 1.01589) 0.069626
High	−38.6293 (−64.27285, −12.98576) 0.004689	−36.1454 (−60.8432, −11.4476) 0.005986	−34.10908 (−65.05815, −3.16001) 0.03861
P trend	−19.1824 (−31.9955, −6.3693) 0.0049	−17.9224 (−30.2670, −5.5778) 0.0063	−17.2185 (−32.7649, −1.6721) 0.0375

SII, systemic immune-inflammation index; OR, odds ratio; CI, confidence interval.
Non-adjusted model: no covariates were adjusted. Minimally-adjusted model: age and gender were adjusted. Fully-adjusted model: age, gender, race, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, body mass index level, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index.

TABLE 7 Associations between dietary fibers and outcomes in different races.

Outcome	Non-Hispanic white	Mexican american	Non-hispanic black	Other hispanic	Other race – including multi-racial	Survey-weighted P interaction
SII	−2.1584 (−3.5905, −0.7263) 0.0073	−2.1199 (−3.6506, −0.5891) 0.0127	−2.0635 (−3.5913, −0.5356) 0.0147	−2.1489 (−3.6529, −0.6448) 0.0104	−2.5560 (−4.3696, −0.7424) 0.0114	0.9941
SIRI	−0.0059 (−0.0111, −0.0008) 0.0350	−0.0059 (−0.0114, −0.0004) 0.0463	−0.0083 (−0.0143, −0.0023) 0.0123	−0.0073 (−0.0111, −0.0036) 0.0009	−0.0074 (−0.0121, −0.0028) 0.0046	0.9085
NLR	−0.0078 (−0.0135, −0.0020) 0.0143	−0.0082 (−0.0145, −0.0019) 0.0185	−0.0091 (−0.0144, −0.0038) 0.0028	−0.0067 (−0.0122, −0.0012) 0.0265	−0.0093 (−0.0144, −0.0043) 0.0015	0.9054
PLR	−0.0117 (−0.2605, 0.2371) 0.9275	−0.2484 (−0.4497, −0.0472) 0.0242	−0.3982 (−0.6300, −0.1663) 0.0028	−0.2636 (−0.5567, 0.0295) 0.0918	−0.3085 (−0.5458, −0.0712) 0.0183	0.0495
RA	−0.0027 (−0.0042, −0.0011) 0.0024	−0.0032 (−0.0060, −0.0003) 0.0381	−0.0040 (−0.0073, −0.0006) 0.0296	−0.0034 (−0.0055, −0.0013) 0.0048	−0.0008 (−0.0047, 0.0030) 0.6714	0.6856
Ferritin	−0.9309 (−1.5520, −0.3098) 0.0074	−0.5142 (−1.5663, 0.5379) 0.3481	0.0393 (−0.7400, 0.8185) 0.9222	−0.8746 (−2.0068, 0.2575) 0.1436	−0.5149 (−2.1876, 1.1579) 0.5522	0.476
hs-CRP	−0.0484 (−0.0839, −0.0129) 0.0137	−0.0734 (−0.1036, −0.0431) 0.0001	−0.0278 (−0.0698, 0.0142) 0.2072	−0.0294 (−0.0679, 0.0091) 0.1483	−0.0350 (−0.0762, 0.0063) 0.1106	0.1873
WBC	−0.0213 (−0.0346, −0.0080) 0.0047	−0.0072 (−0.0214, 0.0069) 0.3261	−0.0149 (−0.0303, 0.0004) 0.0696	−0.0064 (−0.0197, 0.0068) 0.3535	−0.0088 (−0.0231, 0.0054) 0.2355	0.3227
Neutrophils	−0.0169 (−0.0244, −0.0093) 0.0002	−0.0085 (−0.0197, 0.0028) 0.1539	−0.0048 (−0.0143, 0.0047) 0.3326	−0.0088 (−0.0178, 0.0001) 0.0664	−0.0107 (−0.0198, −0.0017) 0.0299	0.1548
Lymphocyte	−0.0033 (−0.0116, 0.0050) 0.4405	0.0017 (−0.0049, 0.0082) 0.6217	−0.0086 (−0.0241, 0.0070) 0.2917	0.0040 (−0.0033, 0.0113) 0.2923	0.0018 (−0.0041, 0.0077) 0.5622	0.2794
Monocyte	−0.0008 (−0.0021, 0.0004) 0.1913	−0.0005 (−0.0019, 0.0008) 0.4331	−0.0015 (−0.0026, −0.0003) 0.0231	−0.0017 (−0.0028, −0.0006) 0.0058	−0.0008 (−0.0017, 0.0001) 0.0887	0.2081
Eosinophils	−0.0001 (−0.0008, 0.0007) 0.8063	−0.0000 (−0.0007, 0.0006) 0.8913	−0.0000 (−0.0009, 0.0009) 0.9955	−0.0001 (−0.0026, 0.0024) 0.9360	0.0011 (−0.0004, 0.0026) 0.1590	0.659
Basophils	−0.0001 (−0.0003, 0.0002) 0.6171	−0.0002 (−0.0005, 0.0002) 0.3254	0.0001 (−0.0003, 0.0004) 0.7134	−0.0003 (−0.0007, 0.0000) 0.0945	−0.0001 (−0.0004, 0.0003) 0.6843	0.6209

SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-lymphocyte ratio; RA, red blood cell distribution width-to-albumin ratio; hs-CRP, high-sensitivity C-Reactive Protein; WBC, white blood cell.
Aage, gender, family monthly poverty level index, alcohol consumption, smoking status, vigorous recreational activities, body mass index level, hyperlipidemia, hypertension, diabetes, and dietary inflammatory index were adjusted.

Dietary fibers constitute essential components of human nutrition. The Institute of Medicine stipulates a daily recommended intake of 30.8 g for males aged 31–50 and 25 g for females aged 31–50 (65). However, the European Food Safety Authority advocates for a higher intake range of 25–38 g/day to mitigate risks associated with type 2 diabetes, cardiovascular disease, colorectal cancer, overweight, and obesity (66). It is evident that a significant portion of participants in this study fail to meet the recommended dietary fibers intake.

Dietary fibers have demonstrated both direct or indirect protective effects on the immune system in *in vivo*, *in vitro* and population-based study. Despite their lack of digestion or absorption in the intestinal tract, dietary fibers are regarded as vital fuel sources for gut microbiota

(67). The gut microbiota, such as *Clostridium*, *Bacteroides*, *Bifidobacterium*, *Prevotella*, and *Ruminococcus* (3), can ferment dietary fibers and produce a variety of metabolites associated with immune system and inflammation, the most pivotal of which are short-chain fatty acids (SCFAs) (68). Cellular experiments have illustrated that SCFAs can function as inhibitors of histone deacetylases and as ligands for G-protein-coupled receptors and aryl hydrocarbon receptors, impacting various physiological processes including immunophysiology (69–72). Previous studies have demonstrated the ability of SCFAs to affect immune niches in the lungs, intestines, and other organs of the host. Lung dendritic cells in propionate-treated mice displayed high phagocytic capacity but impaired promotion of T helper type 2 cell effector function, owing to SCFA-induced alterations in bone marrow hematopoiesis leading to increased macrophage and dendritic cell precursors (72). Lung Type 2 innate lymphoid cells (ILC2s)-driven airway hyperreactivity and inflammation were ameliorated by systemic or intranasal SCFA butyrate administration in mice, likely through histone deacetylase inhibition suppressing ILC2 proliferation, GATA3 expression, and cytokine production; similar SCFA butyrate effects were confirmed in human ILC2s (73). For intestinal immunity, *in vitro* SCFA treatment of human intestinal epithelial cells enhances the epithelial barrier and dampens immune responses *via* increased IL-10RA (74), while SCFA binding to GPR43 on colonocytes stimulates potassium (K+) efflux and hyperpolarization, activating the NLRP3 inflammasome and protecting intestinal epithelial integrity (75). Beyond SCFAs production, recent studies suggests that dietary fibers have direct effect on the epithelial cells and immune cells in the gastrointestinal tract. *In vitro* studies show dietary fibers can directly attenuate inflammatory cytokine production from dendritic cells co-cultured with intestinal epithelial supernatants, dependent on fiber interactions with Toll-like receptors. Specific fibers differentially modulate T cell responses and regulatory T cell cytokines. β-Glucan protects intestinal epithelial barrier integrity during Salmonella infection by preserving tight junctions and limiting invasion. Additionally, some fibers elicit cytokine secretion from intestinal epithelial cells through MyD88/TLR4 signaling (76–78). These findings demonstrate dietary fiber interactions with intestinal immune and epithelial cells regulate inflammatory responses and barrier function *via* pattern recognition receptor pathways. In addition to *in vivo* and *in vitro* evidence, prospective cohort studies have indicated that the early consumption of dietary fibers may assist in decreasing the chances of allergies and asthma in adulthood (79). Likewise, high fiber maternal diets during pregnancy are linked to lower risk of allergic diseases like rhinitis and eczema in offspring (80). A cross-section study based on NHANES data conducted in adults indicates that high-fiber diet may reduce the serum CRP level and decrease odds of having asthma (10).

This study had several advantages. It pioneers the identification of the association between dietary fiber intake and systemic immune and inflammatory states, with the SII serving as the primary indicator. Leveraging a substantial and representative sample from NHANES, the study employs a comprehensive array of indicators to gauge systemic immune and inflammatory status. Nevertheless, the study had some limitations. A cross-sectional study design is incapable of determining the causality and is unable to remove the insidious residual confusing results from unmeasured or unidentified confounding factors. Despite our adjustments to DII, the confounding effects of the anti-inflammatory component of the diet such as vitamins, flavonoids, and other substances could not be completely eliminated. The study's reliance on dietary fiber intake data from just two 24-h dietary reviews introduces a potential limitation, as dietary preferences naturally fluctuate from day to day, potentially impacting the precision of the assessment. Furthermore, short-term dietary assessments are not considered to be an accurate representation of a participant's true dietary intake and the recall bias in dietary questionnaire was difficult to evaluate.

The current study has the following implications for future research. Our findings provide evidence for a negative correlation between dietary fibers intake and systemic immunity and inflammation biomarkers, which highlights the potential therapeutic role of dietary fibers in immune and inflammatory diseases. Therefore, well-designed randomized controlled trials or prospective cohort studies with long-term follow-up are warranted to further evaluate dietary fiber intake as an intervention or exposure, respectively. The relationship between ferritin and dietary fibers remains a matter of debate, and further exploration of their association in populations with varying disease states is essential to elucidate the nature of their relationship.

Conclusion

Dietary fibers intake is inversely associated with systemic immune and inflammatory biomarkers in the human body. The associations persisted in the sensitivity analysis. Thus, dietary fibers should be recommended to promote immune health.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by National Health and Nutrition Examination Survey. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XQ designed the study. XQ, CF, YL, and YJ collected the data. XQ, YL, MC, XC, and JJ analyzed the data and drafted the manuscript. JJ revised and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1242115/full#supplementary-material

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Unraveling the gut health puzzle: exploring the mechanisms of butyrate and the potential of High-Amylose Maize Starch Butyrate (HAMSB) in alleviating colorectal disturbances

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Colorectal disturbances encompass a variety of disorders that impact the colon and rectum, such as colitis and colon cancer. Butyrate, a short-chain fatty acid, plays a pivotal role in supporting gut health by nourishing colonocytes, promoting barrier function, modulating inflammation, and fostering a balanced microbiome. Increasing colorectal butyrate concentration may serve as a critical strategy to improve colon function and reduce the risk of colorectal disturbances. Butyrylated high-amylose maize starch (HAMSB) is an edible ingredient that efficiently delivers butyrate to the colon. HAMSB is developed by esterifying a high-amylose starch backbone with butyric anhydride. With a degree of substitution of 0.25, each hydroxy group of HAMSB is substituted by a butyryl group in every four D-glucopyranosyl units. In humans, the digestibility of HAMSB is 68% (w/w), and 60% butyrate molecules attached to the starch backbone is absorbed by the colon. One clinical trial yielded two publications, which showed that HAMSB significantly reduced rectal O^6-methyl-guanine adducts and epithelial proliferation induced by the high protein diet. Fecal microbial profiles were assessed in three clinical trials, showing that HAMSB supplementation was consistently linked to increased abundance of *Parabacteroides distasonis*. In animal studies, HAMSB was effective in reducing the risk of diet- or AOM-induced colon cancer by reducing genetic damage, but the mechanisms differed. HAMSB functioned through affecting cecal ammonia levels by modulating colon pH in diet-induced cancer, while it ameliorated chemical-induced colon cancer through downregulating miR19b and miR92a expressions and subsequently activating the caspase-dependent apoptosis. Furthermore, animal studies showed that HAMSB improved colitis via regulating the gut immune modulation by inhibiting histone deacetylase and activating G protein-coupled receptors, but its role in bacteria-induced colon colitis requires further investigation. In conclusion, HAMSB is a food ingredient that may deliver butyrate to the colon to support colon health. Further clinical trials are warranted to validate earlier findings and determine the minimum effective dose of HAMSB.

KEYWORDS

resistant starch, butyrate, colorectal cancer, colitis, microbiota, gut health, digestive health

1 Background

Colorectal disturbances encompass a variety of disorders that negatively impact the colon and rectum, including but not limited to colitis and colorectal cancer. An inflamed colon is a hallmark phenotype of colitis, which is a persistent gastrointestinal illness (1). Several types of colitis have been identified including ulcerative, microscopic ischemic, pseudomembranous, infectious, and neutropenic colitis, with ulcerative colitis (UC) being the most common type (2). In Europe, the annual expenses associated with ulcerative colitis, both direct and indirect, are estimated to range from €12.5 billion to €29.1 billion (3). In the United States, the estimated expenses are between US$8.1 billion and US$14.9 billion annually (3). Colitis is a risk for colorectal cancer (CRC), although the degree of association depends on disease duration and extent (4). CRC is the third most common cancer globally, and the second leading cause of cancer mortality in the United States (5). The main risk factors shared by colitis and colorectal cancer include age, being overweight or obese, a sedentary lifestyle, and unhealthy diet (6). It is well established that the consumption of a westernized diet, characterized by enriched red meat, is one of the most ubiquitous environmental factors causing UC and colorectal cancer (7).

Fibers, on the contrary, are beneficial dietary compounds that showed effects in preventing colorectal disturbances. Studies have shown that participants with a higher dietary fiber intake may have a lower risk of developing colorectal adenoma and distal colon cancers (8). Dietary fibers cannot be digested by amylase and brush border enzymes; instead, they enter the colon and be subsequently fermented by the gut microbiota. Short-chain fatty acids (SCFAs) are organic acids with fewer than six carbons, typically products of fiber fermentation. Acetate, propionate, and butyrate are the major types of SCFAs that are gaining increasing research interest. Butyrate, in particular, has attracted considerable attention as a major source of energy for colonocytes and due to its effects in modulating various health outcomes, including gut health (9), immune health (10), metabolic health (11), and cognitive and mood health (12).

Typically, starch granules are composed of amylose and amylopectin, which are two distinct types of glucose polymer. Amylose is a linear long polysaccharide consisting of α-D-glucose units that are linked through α(1→4) glycosidic bonds (13). Amylopectin, with a branched structure, has both α(1→4) and α(1→6) glycosidic bonds and a branch point occurring at every 25 to 30 glucose residues (13). Compared to amylopectin, amylose is less easily digested due to having fewer intramolecular hydrogen bonds for enzymes to target and a rougher surface area that blocks hydrolysis enzymes access (14). Other properties that contribute to the low-digestibility of amylose include its self-interactions during retrogradation, a native semicrystalline structure, and its capability of forming an enzyme-resistant inclusion complex with other nutrients, such as lipids, in the food matrix (15).

Derived from a special cultivar of corn, high-amylose maize starch (HAMS) contains a high portion of amylose, with levels typically ranging from 50 to 90% (16). HAMS is a type 2 resistant starch and a dietary fiber. It has been demonstrated that HAMS can escape the digestion at the small intestine and enter the colon, where it is metabolized to deliver SCFAs due to the microbial activities (17). However, In some individuals, the production of SCFAs by consuming resistant starch may be hindered as they are unable to ferment certain types of resistant starch (18). To consistently deliver the beneficial SCFAs to the colon in individuals with various fermentation challenges, chemical modification to add SCFAs to starch backbone has been shown to be an effective strategy (19). Acylated starch with specific SCFAs renders an efficient vehicle to directly deliver those SCFAs to the colon. The current work aims to review the biological characteristics of a SCFA-modified starch, butyrylated high-amylose maize starch (HAMSB), and its potentially beneficial effects in modulating colorectal disturbances.

2 *De novo* production, absorption, and distribution of SCFAs

SCFAs are found in natural food sources such as ruminant milks, plant oil and animal fats (20, 21), but these volatile fatty acids are primarily produced in the gut through the anaerobic fermentation of fibers that are indigestible by the small intestine. The fermentation of amino acids also leads to the production of SCFAs, but it is accompanied with the generation of other compounds including branched-chain and aromatic amino acids, ammonia, amines, hydrogen sulfide, and phenols and indoles (22). Carbohydrate-Active enzymes (CAZymes) play a vital role in constructing and disassembling intricate carbohydrates and glycoconjugates (23), which serves as the first step of producing SCFAs. Due to their essential functions, CAZymes typically operate with a high degree of specificity, leading to different pathways of SCFAs production. This can be exemplified by the widespread presence of acetate production pathways among microbiota, compared to the limited distribution of propionate production pathways that are presented in only a few bacterial genera (24, 25). Specifically, butyrate is produced via the butyryl-CoA:acetate CoA-transferase pathway or the butyrate kinase pathway through the glycolysis of various substrates including acetate, lactate, amino acids and multiple carbohydrates (21) (Figure 1). Species such as *Akkermansia municiphilla* has been identified as a critical propionate producer, whereas *Faecalibacterium prausnitizii* and *Rominococcus bromii* are the key microbiota for butyrate production via fermenting resistant starch (25). The variation in the quantity and types of CAZyme genes expressed by different microorganisms suggests that the selective consumption of dietary fibers determines which bacterial groups are favored in the gut, affecting the balance of bacterial species and strains in the colon (26). Using equations for fermentation, the estimated daily SCFA production is about 200–600 mM based on the assumption that 20–60 g carbohydrates were fermented per day (27). Therefore, fermenting 1 g fiber may produce 10 mM SCFAs. In the United States, the average dietary fiber intake is around 16.2 g (28), indicating that the SCFA production among the United States population is at the lower end. However, it is important to note that the approximations of SCFA production in the intestine are predicated on investigations by using animal studies, which may not necessarily mirror the authentic circumstances in humans.

In the colon, where the microbial biomass is at its highest, SCFAs can accumulate to a concentration of 50–150 mM (21). Although SCFAs can be absorbed by the small intestine, colon remains to be the major site of SCFA production and absorption (29, 30). The absorption rate of SCFAs in the human rectum and descending and transverse colon is at a rate of 6.1–12.6 μmol/cm^2 per hour (31) in a SCFA

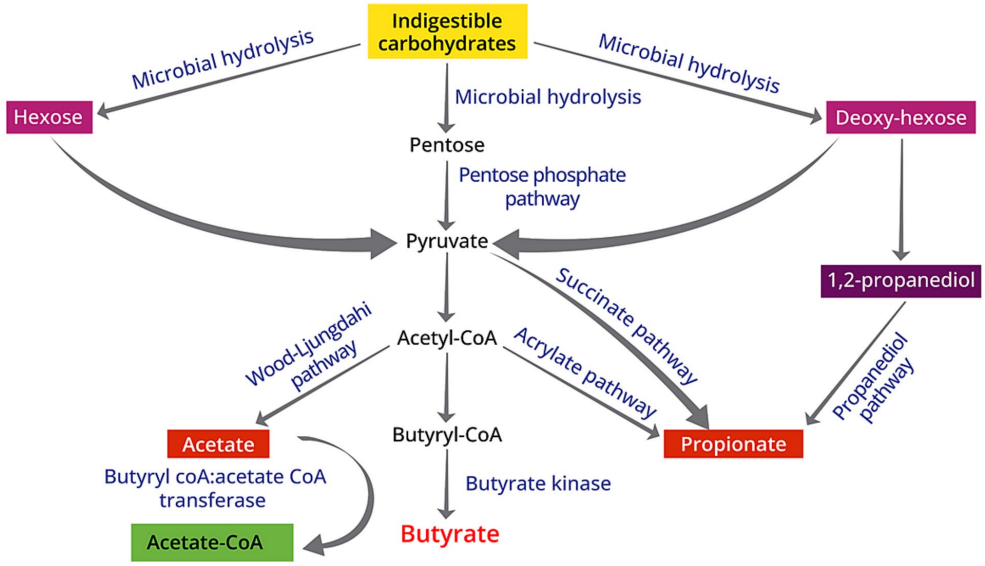

FIGURE 1
Schematic diagram of biosynthesis of SCFAs from indigestible carbohydrates due to the gut microbiota activities: (1) acetate is produced via the Wood–Ljungdahl pathway; (2) butyrate originates from the butyryl-CoA:acetate CoA-transferase pathway or the butyrate kinase pathway; (3) propionate from the acrylate pathway, succinate pathway, or propanediol pathway.

concentration-dependent manner (29). Factors that influence the absorption rate of SCFAs include the epithelial permeability to SCFAs, blood flow through the absorption surface, and the substrate composition (32, 33). A higher SCFA absorption rate is associated with increased chain length, which indicates that butyrate has the highest absorption rate among the major SCFAs (33). Approximately 60% of colonic SCFA absorption is attributed to nonionic diffusion (29), whereas the rest of SCFAs are absorbed by certain transporters in the ionized forms (21). Different SCFA transporters are selectively expressed at different segment of intestine. In the small intestine, monocarboxylate transporter (MCT)1, sodium-coupled MCT (SMCT)2, and SLC17A7 are expressed, while MCT1, SMCT2, SMCT1, and SLC26A3 are mainly expressed in the colon (34). Among these transporters, MCT1, SMCT1, and SLC26A3 have affinities for all three major SCFAs, whereas SMCT2 exclusively transports butyrate (35). The mechanisms underlying basolateral transport of SCFAs still remain unknown. The quantitative assessment suggests that the human colon exhibits the potential to assimilate a maximum of 540 kcal per day through the uptake of SCFAs (29).

The spatial variation of total SCFA concentrations in the colon was observed, showing that SCFA concentrations decrease from 70 to 140 mM in the proximal colon to 20–70 mM in the distal colon (30). The molar ratio of acetate, propionate, and butyrate is approximately 3:1:1 in the colon and stool (27, 36). The prevailing hypothesis is that almost all of the SCFAs assimilated by the colon traverse the portal vein via the colon capillaries and ultimately arrive at the liver, albeit with variable concentrations of SCFAs in the human portal vein (37). The evaluations suggest that among adults with normal liver function, the peripheral blood contains SCFAs at approximately 173 to 220 μmol/L for acetate, 4 to 7 μmol/L for propionate, and 8 to 12 μmol/L for butyrate (38). This observation indicates a significant decrease in the concentration of SCFAs in peripheral blood compared to their levels in the intestinal tract (36). The rate of SCFAs being released to the circulating system from the gut amounted to about 34.9 μmol/kg body weight per hour, which was equivalent to the rate of hepatic SCFA uptake (38), indicating that the gut and the liver are the major sites where SCFA metabolism occurs. As acetate was scarcely taken up by the liver, the molar ratio of circulating acetate, propionate, and butyrate is 9:1:1 (38), which remains stable regardless the change of body weight (38, 39).

3 Mechanisms by which butyrate benefits colorectal health

3.1 Energy source for colonocytes

Notably, the gastrointestinal milieu is principally characterized by an anaerobic milieu, affording an ecologically favorable niche for the thriving of anaerobic commensals (40, 41). Within the intricate landscape of the gut microbiome, there exists a nuanced cohabitation of both aerobic and anaerobic commensal microorganisms; however, it is noteworthy that the preponderance of the gut microbiota, constituting a staggering 99%, is comprised of anaerobic microbes (40).

It has been well-established that the gut anaerobes cannot use long-chain fatty acids for energy source. SCFAs, particularly butyrate, are important fuel for colonic epithelium (27). In the colon, butyrate can be oxidized through β-oxidation and the tricarboxylic acid cycle by the gut microbiota, partially forming ketone bodies (42, 43). Consequently, the existence of bacteria proficient in butyrate production contributes substantively to the preservation of an anaerobic milieu within the gastrointestinal tract (41), which further prevents the colonization of opportunistic aerobic pathogens, such as *Salmonella* and *E. coli* (44). This makes the colon differ from the small intestine, which does not possess the capability of oxidizing butyrate and generate ketone bodies (21).

The colonocytes have a relatively higher affinity for butyrate (43, 45), followed by ketone bodies, amino acids, and glucose, ordered from higher to lower affinity (21). Colonocytes exhibit a stronger preference for butyrate as a source of fuel in the distal colon compared to the proximal colon (43). Evidently, SCFAs impose a trophic effect on the colonic mucosa, considering that mucosal atrophy occurs after a few days of bowel rest (46). Colonocytes from patients diagnosed with ulcerative colitis exhibit a distinct defect in butyrate oxidation (47, 48). Additional investigations have reported that impaired butyrate oxidation by colonocytes could potentially induce the colorectal disturbances (49, 50).

3.2 Histone deacetylase inhibitor

Histone acetylation, a well-characterized approach for posttranslational histone modification, is one of the fundamental regulators of gene expression by remodeling chromatin into a state that is open and transcriptionally competent (51). This process is tightly regulated by a series of enzymes including acetyltransferases and histone deacetylases (HDACs) (52). Accumulating scientific evidence has revealed that HDAC inhibition can mitigate intestinal inflammation and inflammation-mediated carcinogenesis by suppressing the expression of proinflammatory cytokines at the site of inflammation, in conjunction with inducing specific alterations in the cellular composition of the lamina propria (53).

Apart from serving a vital source of energy for the colonocytes, butyrate possesses the capability to modulate signaling pathways through acting as an inhibitor of class I and class II HDACs (54). *In vitro* investigations showed that butyrate was found to be the most potent HDAC inhibitor among all the SCFAs (55). However, The repression of HDAC activity only impacts the expression of a small proportion, approximately 2%, of genes in mammals (56). Mechanistic investigation shows that promoters regulating genes that respond to butyrate possess specific binding sites known as butyrate response elements, the biological activity of butyrate is frequently facilitated via the interaction of Sp1/Sp3 transcription factors with these binding sites, as observed with the $p21^{Waf1/Cip1}$ gene (56).

By inhibiting the HDAC activities, butyrate treatment affected histone decrotonylation in the intestine crypt and colon (57), and decreased malignant transformation and increased apoptosis of precancerous colonic cells (55, 58) by regulating p-21 mediated cyclin B1 expression (58). Propionate and valerate were able to induce growth arrest and differentiation in human colon carcinoma cells, but the magnitude of their effects was lower compared with butyrate (58). It has been on debate that butyrate may act as a double sword on colon health as inhibiting HDAC may affect the growth of both normal and cancerous colonocytes. However, Donohoe et al. showed that butyrate exerted opposing effects on normal cells and cancerous cells in the colon, based on their findings that the inhibition of aerobic glycolysis hindered the capability of butyrate to block normal cell proliferation, whereas the normal cells were unaffected (59). By inhibiting HDAC I, butyrate restored the activity of FoxP3 and then promoted the differentiation of naïve CD4+ T cells to maintain an optimal ratio of T helper 17 cell (Th17)/regulatory T cell (Treg) or T helper 1 cell (Th1)/Th17 (60, 61), which leads to decreased intestinal inflammation and ameliorated colon disturbances (60–62).

3.3 G protein-coupled receptors

Two decades ago, two orphan G protein-coupled receptors (GPR), GPR41 and GPR43, were identified as receptors for SCFAs (63). Later, it was shown that both receptors expressed in human colon epithelial cells and might mediate the SCFA-induced phasic and tonic contractions in colonic circular muscle, suggesting that the physiological effects that SCFAs impose on colon might be attributable to the activation of GPR41 and GPR43 (64). GPR109A was originally identified in an effort of exploring proteins that were differentially expressed in macrophages with different stimulations (65), but following research revealed its critical role as a receptor for butyrate, although the affinity is low (66). GPR41 has the highest affinity for propionate and butyrate, whereas GPR43 exhibits high affinity for all SCFAs, particularly propionate and acetate (63). GPR41, GPR43 and GPR109A are frequently lost in patients with colon cancer, animal cancer models, and colon cancer cells (66–68). Nevertheless, Kim et al., reported that only the knockout of GPR43, not GPR41, promoted colon carcinogenesis (69), which led the research within colorectal cancer to primarily focus on GPR43 (70).

From a mechanistic perspective, the targeting of GPR43 by propionate and butyrate resulted in a G0/G1 cell cycle arrest, accompanied by a decrease in S and G2/mitotic phases, which was achieved through the down-regulation of CDK1, CDK2, cyclin D3, and proliferating cell nuclear antigen. This process was concomitantly associated with an increase in p21, independent of p53. Additionally, propionate exhibited an ability to induce caspase 3/6/7/8 cleavage and decrease the anti-apoptotic enzyme Bcl-2. Notably, the expressions of cyclin D1, B1, 3, and CDK1 have been associated with the promotion of colon cancers (70). The activation of GPR109A signaling by butyrate has been shown to exert anti-inflammatory effects on colonic antigen-presenting cells (71, 72), which leads to the differentiation of regulatory T cells and T cells that produce IL-10, while also stimulating the production of IL-18. This subsequently alleviated colonic inflammation and colorectal cancer development (71, 72). In addition, butyrate-activated GPR109A reduced the levels of Bcl-2, Bcl-xL, and cyclin D1, while upregulating the death receptor pathway independent of HDAC inhibition. These efforts collectively promoted the apoptosis of cancer cells (66).

3.4 Peroxisome proliferator-activated receptor-γ

PPARs belong to a family of ligand-activated transcription factors and have three isoforms: PPAR-α, PPAR-γ, and PPAR-δ. It has been shown that butyrate treatment significantly enhanced the mRNA and protein expressions of PPAR-γ in Caco-2 cells in a dose- and time-dependent manner, which led to rapid cell differentiation (73). Similar with HT-29 cells, butyrate treatment significantly increased differentiation and inhibited cell growth by activating PPAR-γ, subsequently reduced colonic paracellular permeability and prevented colon inflammation (74). Notably, in Caco-2 cells, only butyrate treatment activated PPAR-γ; incubation with propionate and valerate did not affect PPAR-γ expression (73). However, it is currently unclear whether this selectivity is cell specific. Sodium butyrate induced autophagy both in HT-29 cells and HCT-116 cells by activating PPAR-γ, and a prolonged incubation significantly promoted cell death, particularly in HCT-116 cells (75). The variability of responses

exhibited by colon cancer cells to butyrate treatment could be attributed to the dosage, incubation period, and distinctive sensitivity to differentiation of different cells that is determined by differential engagement of autophagy, caspases, and PPAR-γ signaling pathways.

In animals, the PPAR-γ signaling pathway triggered by butyrate is a homeostatic mechanism that impedes the aberrant proliferation of potentially pathogenic *Escherichia* and *Salmonella* by limiting the availability of respiratory electron acceptors to Enterobacteriaceae within the colonic lumen (76). There is a lack of research on how butyrate functions through activating PPAR- γ in humans. However, by using human colon organoids, researchers found that butyrate was capable of restoring the disrupted colonic PPAR-γ gene expression caused by hypertension (77).

In summary, butyrate is capable of manipulating the intestinal permeability, cellular growth and proliferation, as well as the gastrointestinal immune system via providing energy for colonocytes, inhibiting the HDACs, inducing the G protein-coupled receptors, and activating the PPAR-γ signaling pathways.

4 Butyrylated high-amylose maize starch: development and functions

4.1 The synthesis of HAMSB

HAMSB synthesis typically involves an organocatalytic reaction. To elaborate, a mixture of butyric acid, tartaric acid, and oven-dried corn starch is prepared at a ratio of 245:7.4:4 (w/w) and heated to 120°C in a thermostatized oil bath. Notably, tartaric acid functions as a catalyst in this process. Throughout the reaction, careful measures are implemented to ensure that distilled water washings are not initiated until the solid product has adequately cooled to prevent any potential partial gelatinization of the recovered starch esters. The degree of organocatalytic butyrylation undergoes an increase within the initial 2 h and remains at 40% acylation between 2 and 7 h. Within 2.5 h of reaction, a D.S. of 1.54 was achieved (78). Starch acetate with a DS ranging from 0.01 to 0.2 has received approval from the Food and Drug Administration (FDA) for use in food, enhancing attributes such as binding, thickening, stability, and texturizing (79). In contrast, HAMSB represents a relatively novel ingredient that has not yet secured registration with the FDA for a Generally Recognized as Safe (GRAS) status. In Australia where most studies regarding HAMSB were performed, HAMSB has not been submitted for approval for use in foods. The specific modification process determines whether it necessitates a Novel Food application with Food Standards Australia New Zealand (FSANZ). Currently, HAMSB is not registered with The Pharmaceuticals and Medical Devices Agency (PMDA) or Japan's Specifications and Standards for Food Additives (JSFA) as a food ingredient.

4.2 Butyrylated high-amylose maize starch: a vehicle for butyrate delivery

The backbone of HAMSB contains about 72% amylose, which is substantially higher than the regular maize starch that typically contains 25% amylose (80). The esterification of the backbone with butyric anhydride leads to the generation of HAMSB, a SCFA-modified starch that is partly resistant to digestion in the small intestine. The degree of substitution (DS) reflects the number of hydroxy groups per each monomeric unit derivatized by a substituent (81). The DS of HAMSB is 0.25, meaning that a hydroxy group is substituted by a butyryl group in every four D-glucopyranosyl units (Figure 2). The concentration of butyrate in HAMSB is around 10% (w/w). Compared with animals fed a purified or low-amylose starch diet, animals with HAMSB supplementation exhibited significantly increased levels of acetate, propionate, and butyrate in the cecum (82–87), and a trend of increased SCFA concentrations in the distal colon (82–85). HAMS induces the production of SCFAs, but intriguingly, *in vivo* HAMSB supplementation caused a significantly higher SCFA pool in the colon (82, 85, 88–91) and circulating system (85, 88), compared with HAMS supplementation. In humans, the starch digestibility of HAMSB was around 68% (w/w), while 73% of the esterified SCFAs were indigestible in the small intestine (92), and 15.8% of was recovered in the feces when HAMSB was ingested (93). This indicates that approximately 60% butyrate molecules attached to the backbone were absorbed at the level of colon (Figure 3). However, the form of supplementation may affect the digestibility of attached butyrate molecules. For example, HAMSB released a higher amount of esterified butyrate to the colon when it was applied in milk, compared with bakery (92, 94). As SCFAs are absorbed from the human gastrointestinal tract in a concentration-dependent manner (29), increasing their concentrations within the colon through the consumption of acylated starches may yield a greater uptake compared with the consumption of comparable quantities of unacylated HAMS.

Presently, diverse delivery vehicles exist for conveying butyrate to the colon. Sodium butyrate is conventionally synthesized through an acid–base reaction, forming a salt characterized by a high melting point. Each sodium butyrate molecule yields 87 g of butyric acid. In its salt form, sodium butyrate readily dissolves in water, liberating butyrate, and ostensibly, complete butyrate release is anticipated upon dissolution. However, sodium butyrate is accompanied by an offensive odor, deemed undesirable for human consumption. Consequently, to ensure a gradual release in the intestines, sodium butyrate is commonly encapsulated within a lipid matrix coating to mitigate the unpleasant odor. Tributyrin, a precursor to butyric acid, exhibits a gradual release of butyric acid in the colon. Functioning as a triacylglyceride (TAG), tributyrin necessitates the action of lipase to release the butyrate attached to the glycerol. Despite each tributyrin molecule containing three butyrate entities, the assured release of all these moieties is not guaranteed. Lipase displays regioselectivity. While they have a degree of promiscuity irrespective of chain length and saturation/unsaturation, each enzyme can exhibit preferential or even exclusive hydrolysis of specific types of fatty acid esters (95). The reliance of tributyrin on lipase for butyrate release introduces a potential competition with other TAGs for lipase activity (96), causing the release of butyrate from tributyrin relatively inefficient. Although tributyrin is generally not coated due to its non-volatile nature at room temperature, its increased vapor pressure upon heating necessitates the use of inert silica dioxide as a carrier to preserve the intact molecule during delivery to the colon (97), concurrently masking its astringent taste. In contrast to sodium butyrate and tributyrin, High-Amylose Maize Starch Butyrate (HAMSB) represents a more natural conduit for delivering butyrate to the colon, with butyrate molecules affixed to edible starch. Furthermore, HAMSB exhibits mild odor and taste, rendering it seamlessly incorporable into various consumables such as custard, protein powder, milk, flavored

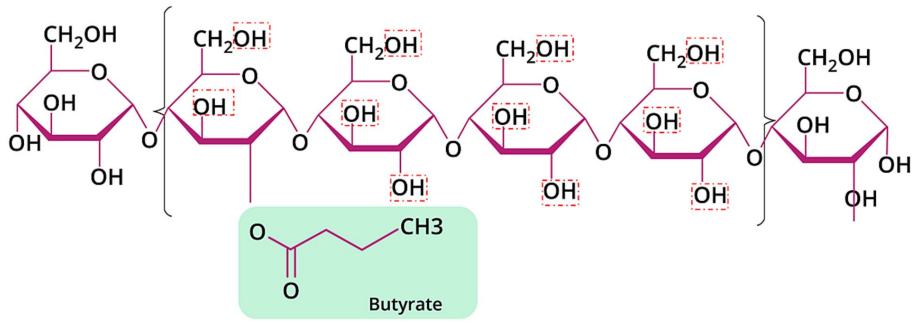

FIGURE 2
Chemical structure of HAMSB with DS of 0.25. The red dashed boxes signified the hydroxyl groups that can be substituted by butyric acid.

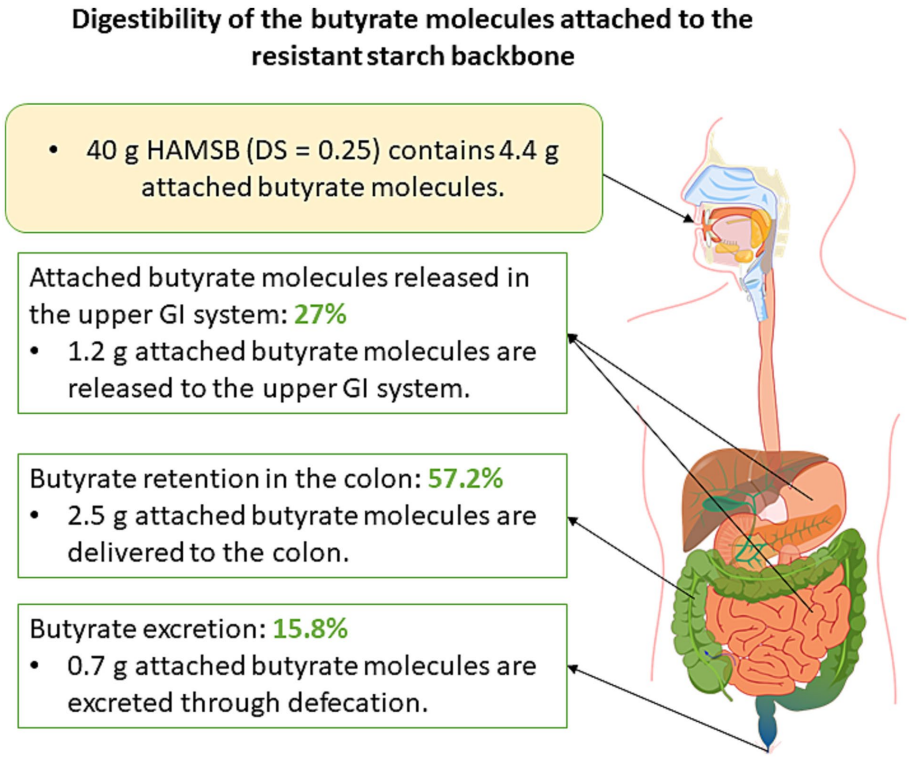

FIGURE 3
Schematic diagram that illustrates the digestibility of attached butyrate molecules. Forty-gram HAMSB was used as an example in this diagram as this was the dosage of HAMSB used in clinical trials.

milk, and orange juice without compromising flavor profiles (92–94, 98, 99). Consequently, HAMSB emerges as an advantageous candidate for butyrate delivery through integration into food and beverages.

5 Butyrylated high-amylose maize starch alleviates colorectal disturbances–animal and human studies

The effects of HAMSB in modulating colorectal disturbances and colon health-related biomarkers were reported by three clinical trials and 10 animal studies. Among the animal studies, eight studies focused on colon cancer and three on colitis, using various disease models (82, 84–87, 89, 90, 100–103) (Table 1). A human study explored the role of HAMSB in reducing colon cancer-related biomarkers and generated two publications (98, 99). All the clinical trials reported how HAMSB affected the gut microbial profile (93, 94, 99) (Table 2).

5.1 Butyrylated high-amylose maize starch changes microbial composition

Animal and human studies that examined the effects of HAMSB in modulating the gut microbial composition consistently reported a

TABLE 1 Characteristics of the animal studies that investigated the role of HAMSB in modulating colorectal disturbances.

First author, year	Animal, animal model	Control group (backbone)	HAMSB: dosage, duration	Key findings
Bajka et al. (2006) (89)	Rat, high protein diet-induced CRC	HAMS (RS backbone)	10% diet, 10 days	↑ cecal digesta weight, ↑ cecal and distal colon acetate, propionate and butyrate concentrations. ↑ portal plasma propionate and butyrate concentrations. ↓ high protein-induced colonocyte genetic damage. ↓ cecal, proximal and distal colon pH. Affected cecal ammonia.
Clarke et al. (2008) (84)	Rat, AOM-induced CRC	LAMS; LAMS +3% tributyrin; HAMS (N/R).	10% diet, 4 weeks	HAMSB ↑ cecal tissue and digesta weight. HAMSB ↑ cecal, proximal, and distal colon butyrate; HAMSB ↑ portal plasma butyrate. HAMS, HAMSB ↓ tumor incidence compared with LAMS, HAMSB ↓ tumor number compared with LAMS. Cecal butyrate pools and concentrations were significantly and negatively correlated with the number of large bowel tumors.
Abell et al. (2011) (82)	Rat, AOM-induced CRC	HAMS (N/R)	10% diet, 31 weeks	↑ distal colon butyrate, did not change acetate, propionate concentrations. Did not change distal colon pH. Colon cancer incidence, tumor number and surface area were similar. ↑ *Lactobacillus gasseri*, *Phascolarctobacterium* and *Parabacteroides distasonis*.
Clarke et al. (2012) (85)	Rat, AOM-induced CRC	HAMS (N/R)	10% diet, 4 weeks	↑ SCFAs in large bowel digesta and plasma. ↑ apoptotic rates in the proliferate zone of distal colon (↑caspase-3), cellular proliferation did not change.
Conlon et al. (2012) (86)	Rat, Western diet-induced CRC	HAMS (Hi-Maize® 260)	28% diet, 11 weeks	↑ cecal tissue and digesta weight, ↑ cecal SCFA pool and portal vein propionate and butyrate ↓ western diet-induced weight and fat gain ↓ cecal and colon ammonia and phenols concentrations ↓ colonocyte genetic damage. ↑ *Ung*, *Gmnn*, *Cebpa* mRNA, ↓*Rere* mRNA.
Furusawa et al. (2013) (100)	Mouse, genetic modification-induced colitis	HAMS (N/R)	15% diet, 4 weeks	↓ colitis Induced Treg cells independent of TLR-MyD88 pathway ↑ histone H3 acetylation in the promoter and conserved non-coding sequence regions of the Foxp3 locus.
Toden et al. (2014) (87)	Rat, AOM-induced colon cancer	LAMS (AIN-93G)	5, 10, 20, 40%, 4 weeks	↑ Gut total SCFA, acetate and butyrate pools; ↑ hepatic portal venous plasma total SCFA, acetate, butyrate pools, ↓cecal ammonia pools. ↑ distal colonic epithelial apoptotic index, mucus thickness. ↓ Genetic damage dose-dependently; ↑ apoptotic rates, not affect colonocyte proliferation.
Le Leu et al. (2016) (102)	Rat, AOM-induced CRC	LAMS (AIN-93G)	20% diet, 4 weeks	↓ AOM-induced O_6MeG adducts, especially in the lower third of the crypts. Crypt column height did not change. ↑ apoptotic rates
Nielsen et al. (2019) (99)	Rat, high protein diet-induced CRC	HAMS (Hi-Maize® 260)	10% diet, 4 weeks	↓ cecal acetate, not affect propionate, ↑ cecal butyrate, ↓ branched-chain fatty acids, ↑ fecal output. ↓ Diversity, ↑ Proteobacteria Sutterella, Proteobacteria Bilophila, Parabacteroides. ↓ miR19b and miR92a, ↓ O_6MeG formation (not statistically significant).
Isobe et al. (2019) (101)	Mouse, DSS-induced colitis	HAMS (N/R)	15% diet, 4 weeks	↓ the translocation of luminal bacteria to the liver. ↑ IgA production in the colonic lamina propria by ↑ the T-cell independent response, which was mediated by GPR41 and GRP109a/HCA2, and the inhibition of HDAC. ↑ colonic barrier function; ↓ systemic bacterial dissemination under inflammatory conditions.
Yap et al. (2021) (103)	Mouse, *Citrobacter rodentium* infection-induced colitis	HAMS (N/R)	15% diet, 3 weeks	Did not change infection-induced weight loss. ↑ epithelial damage of distal colon, ↓ neutrophils at lamina propria.

AOM, Azoxymethane; DSS, dextran sulfate sodium; GPR: G protein-coupled receptor; HAMS, high-amylose maize starch; HAMSB, Butyrylated high-amylose maize starch; HDAC, histone deacetylase; O_6MeG, O_6-methyl guanine; N/R, not reported; SCFA: short-chain fatty acid.

TABLE 2 Characteristics of the human clinical studies that investigated the role of HAMSB in colorectal disturbances.

First author, year	Number of subjects	Dietary groups and dosages	Duration	Key findings
Clarke et al. (2011) (94)	16	-Control: HAMS 20 g/d or 40 g/d; -Intervention: HAMSB 20 g/d or 40 g/d.	2 weeks[a]	1. Free and esterified butyrate concentrations were highest in HAMSB40, and were overall higher in the HAMSB groups. 2. ~57.2% of ingested esterified butyrate was released in the colon when the subjects consumed HAMSB at 40 g/d. 3. ↑ *Parabacteroides distasonis* at both dosages.
West et al. (2013) (93)	23	-Control: Low-amylose starch, 40 g/d; -Intervention: HAMSB, 40 g/d.	4 weeks	1. Saliva IgA, lysozyme, lactoferrin did not change. 2. ↑ plasma IL-10 and TNFα, – IL-1RA, IL-6, IL-8, or granulocyte macrophage-colony-stimulating factor (GM-CSF). 3. ↑ *Parabacteroides distasonis*, *Faecalibacterium prausnitzii* 4. ↑ Fecal output, fecal acetate did not change, ↑ fecal propionate, free/bound/total butyrate.
Humphreys et al. (2014) (98)	23	-Control: HRM, 300 g/d; -Intervention: HRM, 300 g/d + HAMSB, 40 g/d	4 weeks[a]	1. ↑ fecal SCFAs (acetate, butyrate, propionate) 2. ↓ miR 17, miR19a, miR20a, miR19b, miR92a; 3. ↓ cdkn1a, ↑ pten, bcl2l11 mRNA levels, PCNA (all NS) 4. A significant effect of treatment order: HRM + HAMSB first group had significantly less proliferation compared with the HRM first group.
Le Leu et al. (2015) (99)	23	-Control: H, 300 g/d; -Intervention: HRM, 300 g/d + HAMSB, 40 g/d	4 weeks[a]	1. ↓ HRM-induced rectal O^6MeG adducts and epithelial proliferation; 2. ↑ total fecal SCFA, acetate, butylrate, propionate, and ammonia excretion, – N-nitroso compounds; 3. ↑ *Parabacteroides distasonis* and *Ruminococcus bromii*, ↓ *Ruminococcus torques*, *Ruminococcus gnavus*, and *Escherichia coli*.

[a]Cross-over study. HAMS: high-amylose maize starch; HAMSB: Butyrylated high-amylose maize starch; HRM, high red-meat diet; NS: non-significant.

significantly increased relative abundance of *Parabacteroides distasonis* in the HAMSB-supplemented group, compared with that without HAMSB supplementation (82, 90, 93, 94, 99). Interestingly, the treatment of acetylated-HAMS (HAMSA) or a combination of HAMSA and HAMSB also showed an increased abundance of *P. distasonis* (103, 104). Nevertheless, this species was not selectively improved by supplementing butyrate alone (105), suggesting that the starch backbone might play a role. The bacterial strain *P. distasonis* serves as the reference organism for the taxonomic category of Parabacteroides, a class of anaerobic, gram-negative bacteria that are frequently present in the gastrointestinal tracts of various species (106). Recent studies showed that *P. distasonis* were lower in patients with certain diseases, including multiple sclerosis (107) and colorectal cancer (108), but the causality remains unknown. There have been reports indicating that *P. distasonis* may exhibit probiotic properties capable of promoting digestive health in humans, as demonstrated by *in vitro* and *in vivo* studies (106). Nonetheless, divergent experimental data have also been presented, which suggest the potential for pathogenic effects in diverse disease models. Such observations indicate that *P. distasonis* may exhibit a dichotomous role contingent upon the context of its interaction with the host, including factors such as the host's susceptibility to immune suppression and impaired bacterial clearance, as well as the promotion of hyperinflammatory responses. Additionally, strain-to-strain variations may play a role in accounting for potential differences in its pathogenicity (106).

Among humans with HAMSB supplementation, other commensal bacteria including *Faecalibacterium prausnitzii* (93) and *Ruminococcus bromii* (99) were found increased, while certain bacterial species including *Ruminococcus torques*, *Ruminococcus gnavus*, and *Escherichia coli* were reduced (99), but the results were inconsistent. *F. prausnitzii* has been consistently identified as a principal butyrate producer (109) and shown to mitigate the severity of inflammation by producing metabolites that enhance the mucosal barrier function and decrease the intestinal permeability (110). *R. bromii* is a pivotal species that plays a crucial role in the process of breaking down resistant starch within the human colon (111). The increased *F. prausnitzii* and *R. bromii* may be attributed to the consumption of the backbone itself. In animals, HAMSB treatment significantly enhanced genus *Bacteroides* (91, 112–114) and *Blautia* (91, 113). However, caution is warranted for data interpretation as the animal studies used heterogenous disease models.

5.2 Butyrylated high-amylose maize starch reduces the risks for colorectal cancer

High consumption of red meat (115) and western dietary patterns (116) are associated with increased risks of CRC. The occurrence of the O^6-methyldeoxyguanosine (O^6-MedG) lesion, which is recognized as an indicator of exposure to numerous N-nitroso compounds, is frequently detected in tumor DNA isolated from colon tissue (117). Two publications generated by one study showed that HAMSB significantly reduced rectal O^6-MedG and epithelial proliferation induced by the high red meat diet (300 g lean beef per day), potentially by inhibiting microRNA (miR) 17, 19a, 20a, 10b, and 92a, and modulate the genes in cell cycle control. Notably, rectal miR17-92 cluster miRNAs have been found elevated in CRC (118, 119) and are linked with invasion and metastasis of colon cancer cells (120) and a higher risk of cancer-related death (119). Using diet-induced CRC models, researchers consistently reported beneficial effects of HAMSB supplementation in alleviating colonocyte DNA damage (86, 89, 90) and reducing O^6-MedG formation, which were associated with decreased miR19b and 92a (90) that might be modulated by histone hyperacetylation (121). However, it needs to

be mentioned that in the United States, the total red meat consumption is around 0.74 servings per day in women and 1.03 servings per day in men (122), a dosage that is substantially lower than the amount of red meat given to the subjects in the trials. Therefore, in future studies investigating the relationship between diet and the development of colorectal cancer, it is advisable to utilize a reduced amount of red meat to better reflect its impact on public health.

Azoxymethane (AOM) is the most commonly utilized carcinogen to simulate the progression of sporadic CRC (123), which represents the 90–95% of CRC cases (124). HAMSB was found to be effective in reducing AOM-induced CRC risk in four animal studies (82, 84, 85, 87, 102), where elevated apoptotic rates were consistently observed (85, 87, 102) with a higher caspase-3 expression (85). Caspases are fundamental regulators of programmed cell death, with caspase-3 being a frequently activated death protease that facilitates the targeted cleavage of numerous essential cellular proteins (125), and can be induced by histone deacetylase inhibitors including butyrate (126). Therefore, it is possible that HAMSB, acting as a HDAC inhibitor, mitigated AOM-induced colon carcinogenesis by promoting caspase-3 associated apoptosis. Intriguingly, while HAMSB showed anti-CRC effects in animals, tributyrin exhibited no impact on colon tumor development (84). Notably, at the concentration of tributyrin included in the LAMS diet in this study (3%), hepatic portal plasma butyrate concentrations were comparable to those achieved through the ingestion of the HAMS diet and were than those achieved through the consumption of the HAMSB diet. The data suggest that HAMSB could be a more efficient carrier for delivering butyrate compared to tributyrin.

Most studies that quantified colon metabolites reported a reduced level of cecal ammonia in the animals supplemented with HAMSB (86, 90). Ammonia is recognized as a carcinogenic agent that can induce colon mucosal cell damage (127, 128) by improving the colonic pH (129). HAMSB treatment led to a lower cecal and distal pH (88, 89), which may contribute to eliminating ammonia and preventing colonic carcinogenesis.

5.3 Butyrylated high-amylose maize starch and colon colitis

The role of HAMSB in modulating colitis was examined by three studies using different animal models. Researchers found that HAMSB was beneficial in mitigating genetic modification induced colitis (100) and dextran sulfate sodium (DSS)-induced colitis (101) through activating innate and adaptive immune responses (100, 101). In specific, HAMSB favored the differentiation of naïve T cells into regulatory T (Treg) cells through the stimulation of histone H3 acetylation within both the promoter and conserved non-coding sequence regions of the Foxp3 locus in the Rag1 knockout mice that received the adaptive transfer of $CD4^{+}CD45RB^{hi}$ T cells (naïve T cells) (100). In the mice injected with DSS, HAMSB intake significantly promoted IgA production in the colonic lamina propria by conditioning dendritic cells and intestinal epithelial cells (101). This effect was mediated by GPR41 and GPR109a activation as well as epigenetic modification (101).

However, in the study conducted by Yap et al., HAMSB did not ameliorate colitis induced by *Citrobacter rodentium* infection (103). *C. rodentium* is a Gram-negative species of bacteria in rodents that shares several pathogenic mechanisms with *E. coli*, making it a valid model to investigate common human intestinal diseases (130). However, the finding needs to be validated with more studies as this result was in contradiction with the *in vitro* data where butyrate significantly inhibited the growth of *C. rodentium* in a dose-dependent manner (103).

5.4 Butyrylated high-amylose maize starch improves mucosal barrier

Mucosal barrier is a semipermeable structure that functions through the combined effects of multiple extracellular and cellular processes to establish physical and chemical defenses against toxins and pathogens. In the context of an intact epithelium, tight junction barrier function represents the principal factor governing mucosal permeability (131).

In mice with DSS-induced colitis, HAMSB supplementation substantially enhanced colonic barrier function and inhibited the translocation of luminal bacteria to the liver by reducing systemic bacterial dissemination (101). Feeding the depressed mice with HAMSB that was produced by utilizing HAMS as backbone, Tian et al. reported elevated mRNA levels of *claudin* and *occludin* (114), which are crucial tight junction proteins that regulate intestinal permeability. In a model of type I diabetes, dietary HAMSB significantly enhanced the colonic *occludin* mRNA expression and decreased lipoprotein saccharide concentration in the peripheral blood (112). Although these studies shed light on the mechanism by which HAMSB improved colon health, they only detected the biomarkers of the mucosal barrier; the dual sugar absorption test should be employed as the gold standard test for intestinal permeability to validate the effects of HAMSB in modulating the epithelial barrier function.

Overall, HAMSB was effective in reducing the risk of diet- or AOM-induced colon cancer through different mechanisms. HAMSB alleviated diet-induced cancer by affecting cecal ammonia levels whereas ameliorated AOM-induced colon cancer by inducing cancer apoptosis through downregulating miR19b and miR92a. HAMSB mitigated genetic modification-induced colitis by playing a role as HDAC inhibitor, while alleviated DSS-induced colitis through conditioning dendritic cells and epithelial cells and subsequently improving IgA release (Figure 4).

6 Discussion and future direction

The current work reviews the function of HAMSB, an edible ingredient that efficiently delivers butyrate to the colon. We also reviewed the research that examined the role of HAMSB in supporting colon health. Together these studies paint a positive picture for HAMSB in alleviating colorectal disturbances including CRC, colitis, and gut dysbiosis. Further studies are warranted to validate the function of HAMSB in modulating pathogenic bacteria infection-induced colon diseases.

In humans, approximately two-thirds of the HAMSB were digested in the small intestine (92), which was higher than what was reported in an animal *study*, where half raw acylated starches escaped the digestion in the upper GI tract of the colectomized rats (132). The discrepancy might be due to the high temperature during cooking, as it was reported that cooking decreased the indigestibility of HAMS in the small intestine from 64 to 28% (133). Importantly, the digestibility

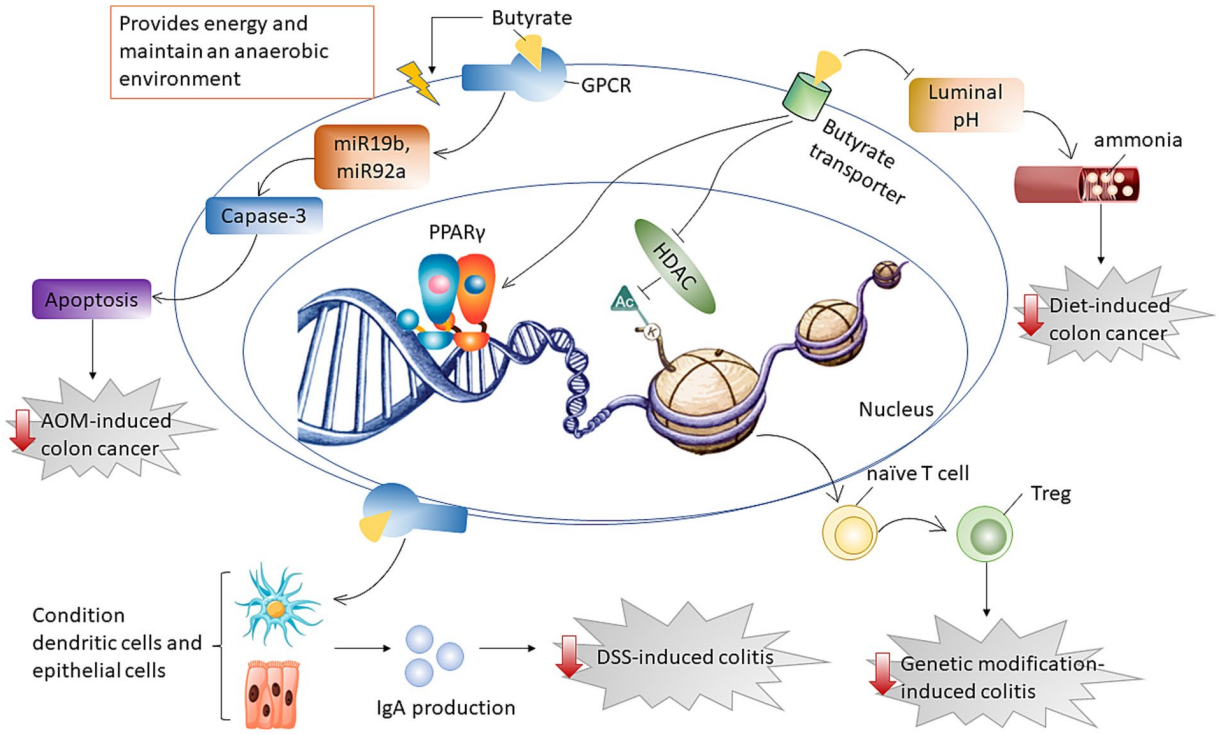

FIGURE 4
Diagram that illustrates the mechanism by which butyrate supports gut health. Butyrate delivered by HAMSB was effective in alleviating diet-induced cancer by affecting cecal ammonia levels whereas ameliorating AOM-induced colon cancer by inducing cancer apoptosis through downregulating miR19b and miR92a. Butyrate mitigated genetic modification-induced colitis by inhibiting HDAC for epigenetic modification, while alleviated DSS-induced colitis through improving IgA release that was generated from conditioning dendritic and epithelial cells.

of starchy foods is influenced by multiple factors such as food matrix, moisture, storage conditions, and processing methods (134). Hence, it would be valuable to investigate the impact of cooking methods on the structure and digestibility of HAMSB with more studies to understand its application in food and beverages.

Although colon can absorb SCFAs at a rapid rate and high amount (30, 31), around 15% butyrate were excreted with a supplementation at 4 g/d (93). This indicates that a lower dose of butyrate supplementation at around 3.4 g/d might be optimal. Notably, individual variance may exist in the capability of absorbing SCFAs, as recent studies have identified polymorphisms in several SCFA transporters including MCT1 and MCT2 genes (135, 136). As mentioned in Section 2, the fermentation of 1 g fiber may correspond to the production of 10 mM SCFAs. Therefore, consuming 16.2 g dietary fiber may generate 162 mM SCFAs in the colon. By assuming a colon capacity of 1.45 L [1.4 L for healthy female and 1.5 L for healthy male (137)], the daily butyrate production is around 4.14 g based on the average United States fiber intake of 16.2 g/d (28) (Figure 5). In the United States, the daily value (DV) of dietary fiber is designated at 28 g, meaning that the United States population is recommended to consume at least 28 g/d dietary fiber on most days. Such fiber deficit may result in a gap of butyrate production of 3 g/d (Figure 5). Typical butyrate supplements in the market deliver butyrate at a daily dosage of 150–300 mg, which may not cover the demand and an increase in dosage of supplementation should be considered (138), preferably at 3–3.4 g/d based on our calculation. Nevertheless, this does not indicate that any changes of health outcomes resulted from fiber deficit is causally associated with colorectal butyrate production. Future prospective cohort studies and clinical trials are warranted to identify the causal relationship between the butyrate deficit, the dosage gap, and potential negative health outcomes.

There has been debates about whether the circulating SCFAs or colon SCFAs confer greater health benefits. Acetate, propionate, and butyrate exhibit considerable agonistic activity on G protein-coupled receptors and PPAR-γ when compared to other SCFAs (i.e., branched SCFAs), with an EC_{50} of around 0.5 mM (63, 139–141). The activation of these receptors necessitates millimolar concentrations of SCFAs, indicating a low potency in comparison to other G protein-coupled receptor ligands such as the CCL chemokine, which activates the chemokine receptor CCR2 with an EC_{50} of around 1 nM (142). As a result, the activation of GPR41, GPR43, and PPAR-γ may be limited to specific areas within the human body (143), such as in the gut lumen where SCFA concentrations are greater than 20 mM (21, 30). As the most potent HDAC inhibitor, the IC_{50} of butyrate is around 30–90 μM (55, 144), a concentration that is unachievable in the circulating system even with HAMSB supplementation that conferred butyrate at a dosage of 4 g per day (145). Therefore, it suggests that the colon is the primary site where SCFAs perform physiological, biological, and immunological modulations. Delivering the SCFAs to the colon efficiently is critical to enable SCFAs to function properly.

The studies list in the present work have several limitations. First, two animal studies used Hi-Maize® 260 instead of the resistant starch backbone as positive control (86, 90). Hi-Maize® 260 is physically modified by using the resistant starch backbone as a base starch (146). Compared with this starch backbone, Hi-Maize® 260 has a comparable concentration of amylose but an increased level of RS, which may

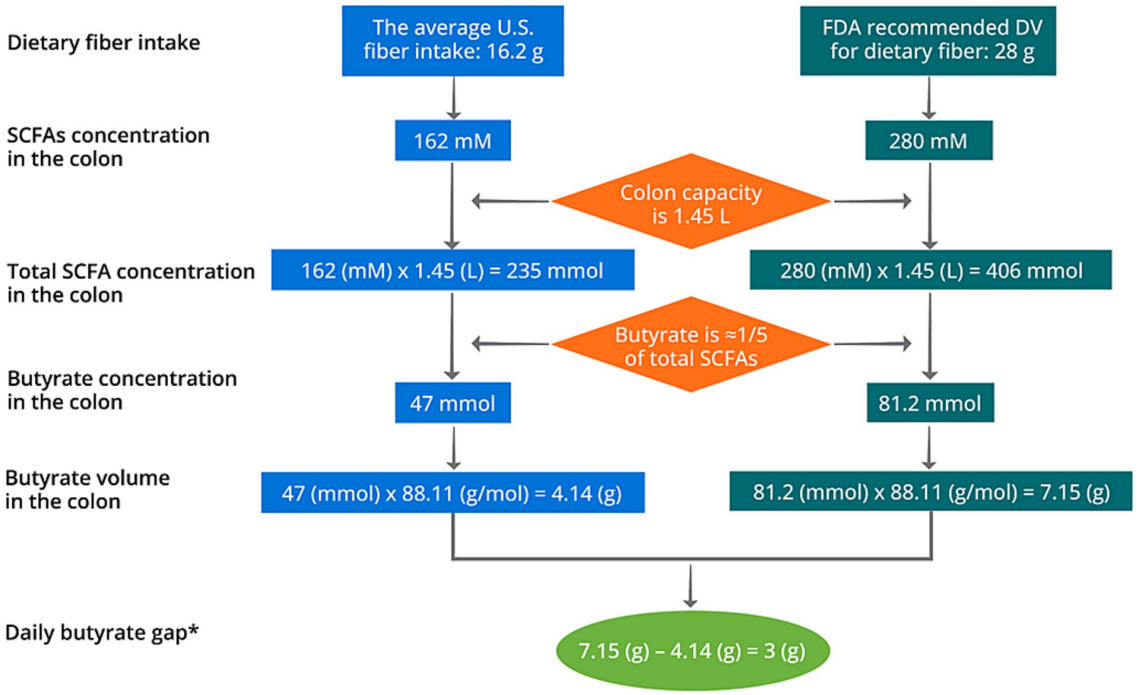

FIGURE 5
Schematic diagram of the calculation of daily butyrate gap in the United States. *The daily butyrate gap solely represents the short of butyrate production based on fiber deficit. It does not indicate that the health outcomes generated from fiber deficit is a result from the gap of colorectal butyrate production.

elicit a more potent apoptotic response to AOM in the colon of rats (146). Thus, using Hi-Maize® 260 as control may decrease the effect size and increase the possibilities of observing null results. This suggests that HAMSB might have more compelling effects in alleviating colorectal disturbances than what were reported by the existing studies. Another limitation stems from the fact that HAMSB supplementation enhanced other SCFAs including acetate and propionate in the colon and circulating system (86, 89), which renders challenges to investigate the health benefits that are incurred by butyrate alone. However, such limitation does not defeat the conclusion that HAMSB as an intact dietary compound can improve colon health.

In addition, all the clinical trials that evaluated health-related endpoints used HAMSB at a dosage ≥40 g/d, which requires the subjects to take multiple servings of food to reach the designated amount (92). The animal studies provided HAMSB at a range of 10–28% diet, which is equivalent to 181–507 g/d HAMSB (18.1–50.7 g/d butyrate) in humans by assuming that four pounds of food is consumed each day. Since the physiological range of oral butyrate supplementation is 1–10 g/d (138), these animal studies lack physiological relevance. Future research should focus on exploring the minimum effective dose of HAMSB or its dose–response effects. It's important to note that the number of studies investigating the effectiveness of HAMSB in alleviating colorectal disturbances is limited, and the majority of these studies are conducted on animals, which generated a logical leap generated from extrapolating the results from animal studies to humans. Rodents exhibit a larger body surface area and weight relative to humans, thereby manifesting an augmented metabolic capacity. In toxicology studies, administration of dosages denoted as "human equivalent doses" is a customary practice. Specifically, these doses are calibrated to be 12.3 and 6.2 times the equivalent human dose when administered to mice and rats, respectively (147). While murine have adapted to an enlarged colon and cecum capacity, allowing them to extract additional nutrients from a comparatively higher proportion of indigestible food components in their diet compared to humans (148), they may exhibit intolerance to components flowing excessively intact from the small intestine into the colon. Thus, the appropriateness of such dosages for animals is contingent only when the test component is absorbed in the small intestine, and are ineffective when the components' functionality is dependent on the intestinal bacteria within the hosts. Consequently, it would be premature to consider HAMSB as a standalone solution for addressing colorectal disturbances. Instead, the main emphasis should be on adopting a healthier diet and lifestyle. Further clinical trials are necessary to establish and validate the potential effects of HAMSB in promoting colon health.

In conclusion, HAMSB is an edible ingredient that can efficiently deliver butyrate to the colon. Existing clinical trials and animal studies suggest that HAMSB supplementation at a dosage equal or larger than 40 g/d may mitigate dysbiosis, fortify mucosal barrier, and reduce the risks for colorectal cancer and colitis. Therefore, it serves as a promising dietary strategy to support gut health. Future studies are warranted to validate such findings with additional clinical trials and a lower dosage of HAMSB.

Author contributions

JC: Writing – original draft, Writing – review & editing. JZ: Writing – review & editing.

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Conflict of interest

JC and JZ were employed by Ingredion Incorporated.

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Role of dietary fiber and lifestyle modification in gut health and sleep quality

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Dietary fiber has an immense role in the gut microbiome by modulating juvenile growth, immune system maturation, glucose, and lipid metabolism. Lifestyle changes might disrupt gut microbiota symbiosis, leading to various chronic diseases with underlying inflammatory conditions, obesity, and its associated pathologies. An interventional study of 16 weeks examined the impact of psyllium husk fiber with and without lifestyle modification on gut health and sleep quality in people with central obesity (men = 60 and women = 60), those aged from 40 to 60 years, those having WC ≥ 90 cm (men) and WC ≥ 80 cm (women), and no history of any chronic disease or regular medication. The participants were subgrouped into three intervention groups, namely, the psyllium husk fiber (PSH) group, the lifestyle modification (LSM) group, and the LSM&PSH group and control group with equal gender bifurcation (men = 15 and women = 15). A 24-h dietary recall, gastrointestinal tract (GIT) symptoms, and sleep quality analysis data were collected on validated questionnaires. The analyses of variance and covariance were used for baseline and post-intervention, respectively. Student's *t*-test was applied for pre- and post-intervention changes on the variable of interest. The intervention effect on GIT health was highly significant ($P < 0.001$). The mean GIT scores of the LSM, PSH, and LSM&PSH groups were 2.99 ± 0.14, 2.49 ± 0.14, and 2.71 ± 0.14, respectively, compared to the mean GIT scores of the control group. No significant ($P = 0.205$) effect of either intervention was observed on sleep quality. The study concluded that psyllium husk fiber significantly improved the GIT symptoms, while no significant effect of the intervention was observed on sleep quality analysis.

KEYWORDS

sleep analysis, gastrointestinal tract, PSQI, GIT score, psyllium husk fiber, lifestyle modification, dietary fiber

1 Introduction

Diet and nutrition are significant aspects in the promotion and maintaining of good health throughout one's life; their function as predictors of chronic non-communicable diseases is well-recognized, and they thus hold a major place in preventive medicine (1). The World Health Organization (2) reports that non-communicable diseases (NCDs) cause 38 million deaths annually. By 2020, the proportion of non-communicable diseases (NCDs) is expected to increase, contributing to 75% of all fatalities globally. Of these deaths, 71% will be attributable to ischemic heart disease (IHD), 75% to stroke, and 70% to diabetes in developing nations (3).

There is growing recognition of the role of diet and other environmental factors in modulating the composition and metabolic activity of the human gut microbiota, which in turn can impact health (4). Along the GI tract's length, there are variations in microbe quantity, kind, and function. The majority, however, are concentrated in the large intestine, where they support fecal bulk and ferment undigested food items, especially carbohydrates and fiber (5). There are accumulating symptoms that indicate that abnormalities in gut microbial populations are linked to diseases, especially inflammatory bowel disease (IBD) (6), and may serve as contributing factors. Some bioactive substances, such as vitamins, are useful, while others are poisonous and are produced by microorganisms in the gut (7). Along the intestine, host immune systems, including a mucus barrier, help prevent potentially hazardous germs from causing tissue damage. By competing for nutrition and colonization sites, a diverse and robust community of good gut bacteria helps keep dangerous bacteria at bay. It has been demonstrated that dietary fibers significantly affect the gut microbiota's functionality and composition, which has positive implications on health due to their structural, physical, and chemical properties such as viscosity, water binding and bulking ability, and fermentability (8). To sustain microbial richness with more apparent (additive or synergetic) impacts on the immunological status and metabolic health, mixing various fibers that stimulate a multitude of different bacterial species may be helpful (9). High-fiber diets benefit the host's health by influencing glucose and cholesterol metabolism, among other things. Important pathways include nutrition absorption control and SCFA synthesis (10). Ingestion of live beneficial bacteria (probiotics) may also contribute to health maintenance (8).

Short-chain fatty acids (SCFA), which are produced by large bowel bacteria from the fermentation of fiber and protein, are some of the most prevalent and physiologically significant products. Colorectal tissues and bacteria rely on SCFA for energy, as they are essential for the proper functioning of cellular mechanisms that ensure tissue integrity (11, 12). SCFA can enter the blood and influence immunological function and inflammation in the lungs and other tissues (13). Numerous additional products, such as *Bifidobacterium*, which produce specific vitamins in the large intestine, are noteworthy for their impact on health (e.g., K, B_{12}, biotin, folate, and thiamine) (14). On the whole, the effect of non-dietary lifestyle factors on the gut microbiota has been neglected. As risk factors for colorectal cancer, smoking and a lack of exercise can have a major impact on the large intestine (and potentially the microbiome) (15). Obesity-related changes in microbial communities may be influenced by exercise (or, conversely, its absence). The diversity of gut microbial communities in professional athletes is a result of exercise and food (16).

All living things require sleep to maintain good mental health, facilitate learning, and remove metabolic waste from the brain (17). Homeostatic and circadian processes control sleep behavior; the latter seems to be influenced by the genetic makeup of the gut microbiota (18, 19). Epidemiological evidence has suggested that poor sleep health is associated with adverse outcomes such as cardiovascular diseases (20, 21), metabolic syndrome (MetS), and mental illnesses (22, 23) and plays a vital role in the development of MetS (24). Generally, sleep health has two main dimensions: duration and quality (25, 26). Sleep length and quality may overlap to some extent, but there are qualitative variations between both. In addition, previous research has demonstrated that associations between sleep length and sleep quality are weak (27), implying that the two distinct sleep estimation areas may have different health consequences (28, 29). Previous studies have shown that the average self-reported sleep duration has decreased from over 8 h in the 1960s to 6.5 h in 2012 (30, 31).

Physiochemical properties, including solubility, viscosity, and fermentability, control how dietary fiber behaves in the human gastrointestinal tract (32). Additionally, the quantity and type of fiber residue that is not digested in the small intestine and enters the colon affect the degree of fermentation (33). Psyllium, which is soluble and poorly fermentable, is fermented by gut microbes down the length of the colon to create SCFA. Resistance starch (RS) ferments more intimately in the colon because it is more fermentable and less soluble (or insoluble, depending on the type of RS) (34). Certain microorganisms in the gut specialize in the breakdown and fermentation of particular fermentable fibers (35).

Dietary fiber promotes fecal SCFAs, particularly butyrate, which was followed by improvements in glucose homeostasis (36). It is the principal source of energy for colonic epithelial cells to sustain their growth and integrity (37). Butyrate contributes to host health through having anti-inflammatory and antioxidant properties that provide benefits (38) and prevent diseases such as colorectal cancer (39, 40), diabetes, and obesity (41). Butyrate concentration mainly depends on the quantity and quality of dietary fiber reaching the colon (42). Research has shown that higher butyrate concentrations in human feces are associated with greater fiber intake (43). Foods rich in dietary fiber, such as nuts, fruit, vegetables, and cereal, are also linked to a greater abundance of SCFA producers in the human gut microbiota (44).

Optimal dietary fiber consumption, whether from foods or supplements, helps with weight loss and has positive consequences (45, 46). Most fibers reduce plasma total and low-density lipoprotein cholesterol (47). Intake of a high dietary fiber diet or wholegrain cereals lowers the risk of heart diseases (48, 49). Dietary fiber shows a significant effect as a laxative, helping reduce blood cholesterol and blood glucose levels (50). Dietary fiber is primarily used for controlling diarrhea and constipation. Different cereals and vegetables, such as cereals, gum guar, psyllium husk, and oat, are used as soluble and insoluble fibers; however, fibers from legumes, fruits, and vegetables are preferred in various metabolic syndromes (51).

Among the dietary fibers, psyllium (*Plantago ovata*) husk fiber is water soluble and derived from psyllium seed, promoting the intestinal flora. It is globally used as the best source of dietary fiber, either as functional food or supplements. Psyllium is a highly water-soluble fiber source and readily fermentable, which thereby causes less abdominal bloating (52). Psyllium husk fiber increases insulin sensitivity in a healthy individual, hinders glucose absorption, decreases postprandial glucose concentrations in the blood (53), and is an effective supplement for decreasing CVD (54) and blood pressure (55). Psyllium is a common fiber supplement widely used due to its affordability and well-tolerated than other fiber source supplements (56). It improves the blood lipid profile and acts as a bowl regulator (57). Studies suggest a

strong association between psyllium husk fiber and inflammation. Increased intake of psyllium fiber further exerts an appreciable effect on risk factors for developing cancers, especially breast cancer (58, 59).

2 Materials and method

2.1 Inclusion and exclusion criteria

School teachers aged 40–60 years (men and women) with central obesity (where central obesity for Asians is defined as men having ≥ 90 cm waist circumference (WC) and for women ≥ 80 cm WC) (60–62), with no history of any chronic disease such as hypertension, diabetes, cardiovascular diseases, consumption of any regular medication, food allergies, smoking, or physical disabilities impairing the food intake and mobility, qualified the inclusion criteria. Pregnant or lactating female school teachers were also excluded during screening. Subjects with an allergy to psyllium husk fiber, a history of drug abuse, or any psychological or emotional disorder that might prevent the completion of the study were also excluded. Intervention flow chart is summarized in Figure 1.

2.2 Recruitment of the subject

Out of 206 screened school teachers, 185 were eligible based on waist circumference (men > 90 cm and women > 80 cm). In terms of predicting cardiovascular and metabolic risk, WC and BMI have a substantial correlation. BMI is simple to calculate, but it does not differentiate between lean and fat masses (63). Among the 185 eligible school teachers, 33 were excluded from the study. In total, 22 school teachers (eight men and 13 women) refused a blood sample (due to syringe needle allergy/phobia), and 11 (six men and five women) were not willing to use psyllium husk regularly due to some myths and personal reasons. In total, 76 men and 76 women school teachers (considering that 10% dropped out for many reasons) were enrolled in the study.

2.3 Study design

A group of 120 school teachers (60 men and 60 women) was divided into four subgroups for 16 weeks of an interventional study. One group was kept as control, while the other three were assigned interventions. One group was assigned the intervention of lifestyle modification (LSM), another group was assigned the intervention of 5 g of psyllium husk fiber (PSH) twice daily, and the third group was assigned the combination of LSM and PSH. Each group consisted of 30 subjects, equally divided by gender (15 men and 15 women). Maximum homogeneity in terms of the geographical, social, and financial conditions among and within groups was maintained (summarized in Table 1).

2.4 Intervention

An informed consent form was signed by each subject, explaining the data privacy and the subject's obligations. School teachers were actively involved in a group discussion about their current food behavior based on locally available food. They were probed about the importance of situation-specific nutrients and the development of a particular diet. A guided tool was developed from the Pakistan Dietary Guidelines for Better Nutrition 2018, covering the nutritional recommendations for subjects (64).

A written and informed consent explaining the study's importance and the subject's obligation was signed under the approval of the ethical committee from the enrolled school teachers.

2.5 Abdominal and epigastric health symptoms

A Dutch-developed English version of the questionnaire (65) assessed abdomen and epigastric health. The questionnaire depicts the health symptoms of the gastrointestinal tract throughout the last 4 weeks, rated from 0 to 6, where 0 represents no complaints and six refers to the severity of symptoms. In a physical demonstration, the questionnaire explained the severity of GI symptoms on a scale of 0–6. The subject was asked about their symptoms and probed for their severity on a defined scale of 0–6. Pre- and post-intervention questionnaires about GI symptoms were filled out during face-to-face seating, and the subjects shared their past 1-month experiences. The subjects' symptoms were assessed using the wide, general phrase stomach discomfort. GI health is discomfort or pain in the lower or upper abdomen, depending on abdominal and epigastric sensations. Epigastric pain was classified as an intense burning or gnawing pain in the mid-epigastric region, frequently associated with other upper GI symptoms and perhaps caused by back radiation. Pain in the retrosternal region (heartburn) is caused by stomach acid, which frequently progresses to the neck and worsens with bending over, that is frequently associated with eating fatty foods, chocolate, or even restrictive clothes. Regurgitation, or a spontaneous reflux of stomach acid or contents into the esophagus and occasionally into the mouth, can grow into a pitiful condition, particularly in the morning or after consuming a large amount of oily meal. Belching was once thought to be the audible escape of air from the stomach via the mouth. Additionally, bloating, or abdominal distension, was defined as a condition in which the belly feels full and constricted, not just after eating, and was frequently interpreted as an abnormal amount of intestinal gas (66).

2.6 Sleep quality analysis

The Pittsburgh Sleep Quality Index (PSQI) questionnaire was used to assess the preceding month's sleep quality of school teachers. The Pittsburgh Sleep Quality Index (PSQI) is a self-report questionnaire that evaluates sleep quality and disruptions over 1 month. There are seven "component" scores generated

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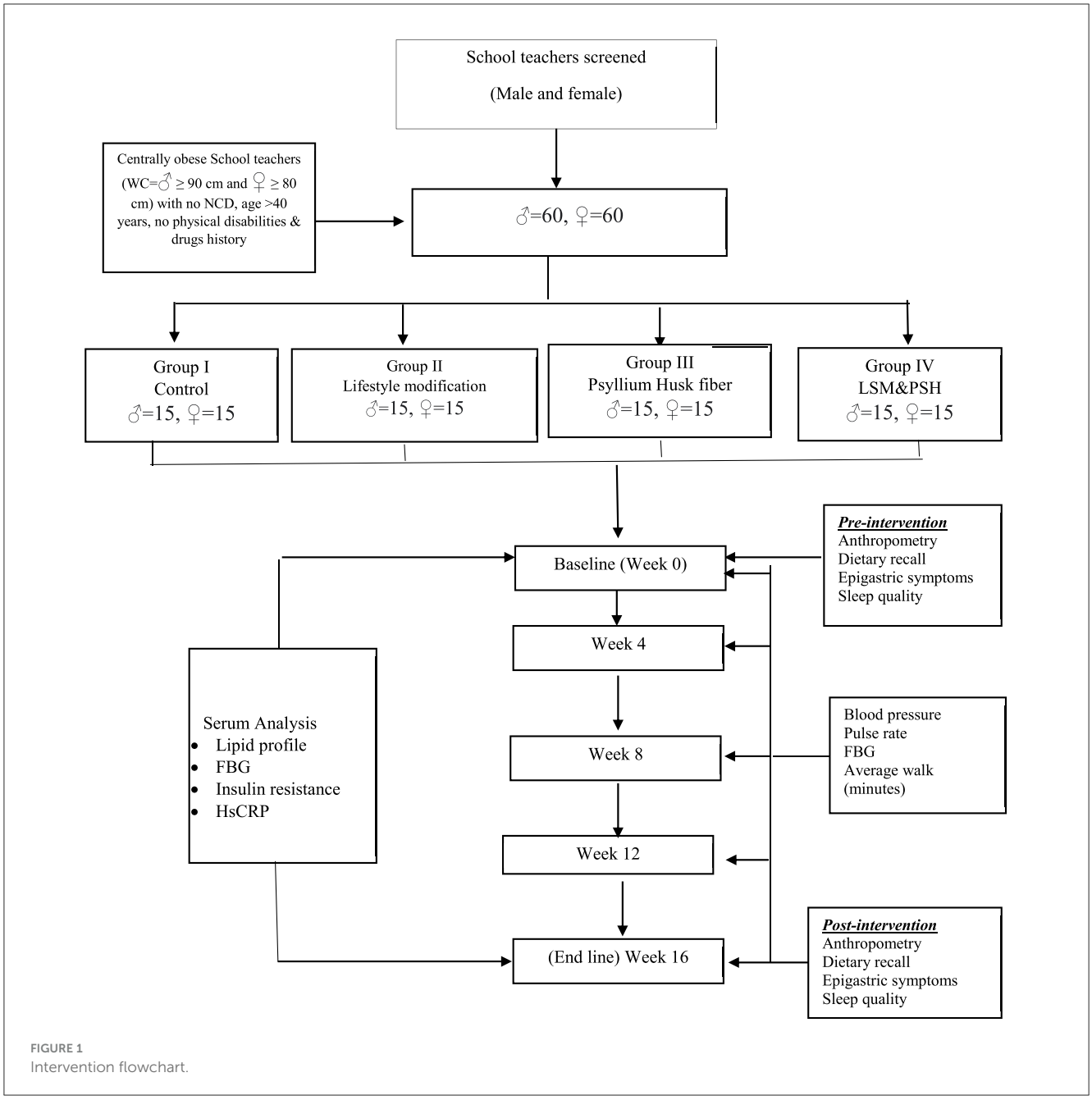

FIGURE 1
Intervention flowchart.

by 19 individual items: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disruptions, usage of sleeping medication, and daytime dysfunction. The sum of the scores for these seven components results in a single global score (67). All questions were based on a subjective sleep quality rating scale ranging from 0 to 3 (a score of 0 indicates that it has not happened in the last month, 1 indicates that it has happened less than once a week, 2 indicates that it has happened once or twice a week, and 3 indicates that it has happened three or more times a week). The component scores are added together to generate a global score (range 0–21). Higher scores suggest poorer sleep quality. Subjects were asked about their sleep quality, sleep duration, sleep efficiency, sleep disruption, sleep medicine, and sleep-related daily dysfunction using the PSQI questionnaire scale at both the beginning and end of the study.

2.7 Preparation and consumption of psyllium husk fiber

The finest quality psyllium husk fiber from Sinhala Herbs (23, Industrial Estate, Neemuch, Madhya Pradesh, India) was procured. The subjects were instructed regarding proper preparation and consumption. Based on previous studies (68, 69), 5 g of psyllium husk fiber in a zip-lock bag was provided to the subjects in the PSH and LSM&PSH groups. In a practical demonstration, the subjects were briefed about preparation and consumption. Psyllium husk fiber was immersed in half a glass of warm water/milk and waited for 10–15 min. The psyllium husk fiber absorbed sufficient water and swelled to the maximum to form a gel. The subject consumed the swollen gel psyllium husk fiber with one glass of warm water to clear the epigastric tract of any fiber debris and to avoid choking.

TABLE 1 Study groups assignment

Group ($n = 120$)	Role	Intervention	Compliance
Group I ($n = 30$)	Control	No intervention	NA
Group II ($n = 30$)	Lifestyle modification (LSM)	(Diet, physical activity, and behavior modification). 1. Minimum 30 min walks 2. Inclusion of fruits and vegetables (mini 5 servings/day) 3. Low salt and refined sugar intake	Appendix III a Appendix III b Appendix IV
Group III ($n = 30$)	Psyllium husk fiber (PSH)	30 min before breakfast and dinner, consume 5 g of psyllium husk fiber (in swollen form) twice daily.	Demonstration of the preparation of psyllium husk fiber. Reminder texts and calls. Collection of empty zip lock PSH bags.
Group IV ($n = 30$)	The combined group of LSM&PSH	a. Diet, physical activity, and behavior modification 1. Minimum 30 min walks 2. Inclusion of fruits and vegetables (mini 5 servings/day) 3. Low salt and refined sugar intake b. 30 min before breakfast and dinner, consume 5 g of psyllium husk fiber (in swollen form) twice daily.	Appendix III a Appendix III b Appendix IV Demonstration of the preparation of psyllium husk fiber. Reminder texts and calls. Collection of empty zip lock PSH bags.

The subjects were advised to consume 5 g of psyllium husk fiber twice daily, 30 min before breakfast and 30 min before dinner.

2.8 Lifestyle modification

2.8.1 Physical activity

The subjects in the lifestyle modification and combined lifestyle modification groups, along with psyllium husk fiber, integrated walking into their daily lives. Based on the WHO guidelines, a minimum walk of 150–300 min of moderate-intensity aerobic physical activity or 75–150 min of vigorous-intensity aerobic physical activity throughout the week (70) was suggested. The subject's average weekly data was recorded on the diary page (Appendix A).

2.8.2 Dietary modification

A 24-h dietary recall for any 3 random days was collected with the support of portion size estimation using standard household measures like the cup, bowl, and spoon (data reported somewhere else). The five-step multiple-pass method was used for a 24-h dietary recall, including the quick list of food consumed on any random day, the forgotten food list, the time and occasion of food consumed, the cooking method (fried, boiled, roasted, and steamed), and the amount of food consumed. The subjects were interviewed in a relaxed, conducive environment about their meal intake and portion size and probed for food taken in complex forms. Portion sizes consumed were entered in gram weights, and the nutrient composition of the food consumed was calculated using NutriSurvey (Nutrisurvey for Windows. Copyright 2007. Dr. Juergen Erhardt, SEAMEO-TROPMED RCCN, Indonesia) (71). Total calories, protein, carbohydrates, fats, dietary fiber, vitamins A, E, B1, B2, B6, folic acid, vitamin C, sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, polyunsaturated fatty acid (PUFA), and cholesterol were calculated.

2.8.3 Behavior change toward healthy and nutritious food

The 5A tool (Appendix B) was used to assess the dietary behavior of the subjects and align with the dietary guidelines of Pakistan (Appendix C) for food exchange choices and compliance. For subject awareness, a standard format (Appendix D) of dietary messages adopted from the dietary guidelines of Pakistan was developed and shared in groups. Subjects were oriented in one-to-one and group discussions and during follow-up visits.

2.9 Intervention compliance

Weekly and monthly follow-up visits were planned to their respective schools for efficient compliance with the intervention protocols. Each teacher's progress was noted, and bottlenecks were sorted out for compliance. An average walk-in minutes of 4 weeks was asked and reconfirmed with the Android or iOS health software record available on certain school teachers' smartphones. The count of empty sachets confirmed regular consumption of psyllium husk fiber at the end of each week/month. All the subjects were in close liaison via text/WhatsApp (group messages). The reminder messages were sent to the subjects in intervention groups before breakfast (06:00 a.m.) and dinner (05:00–06:00 p.m.).

2.10 Statistical analysis

For the baseline, an ANOVA was used to compare the means of the four groups. Student's *t*-test was used to determine the mean difference between pre- and post-interventions. The post-intervention effect was analyzed using ANCOVA after adjustment for age, gender, and baseline.

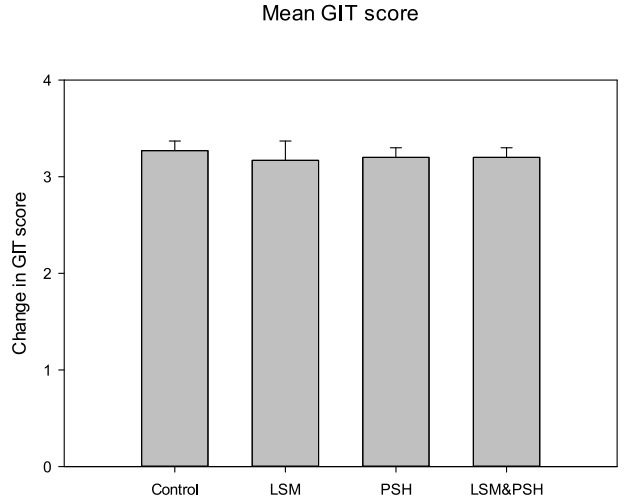

FIGURE 2
GIT mean symptom score of the study subjects at baseline. A one-way analysis of variance was employed for the study of differences between variables ($P = 0.05$). PSH, psyllium husk fiber; LSM, lifestyle modification; GIT, gastrointestinal tract.

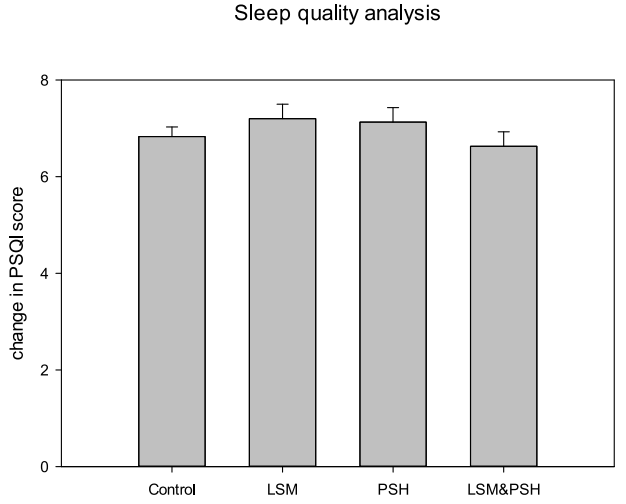

FIGURE 3
Sleep quality analysis based on the mean PSQI score. A one-way ANOVA was performed for the comparisons ($P = 0.05$). PSQI, Pittsburgh Sleep Quality Index; PSH, psyllium husk fiber; LSM, lifestyle modification.

2.11 Ethical approval

Ethical committee approval was sorted out for the school teachers who participated in this interventional study voluntarily under the ethical committee approval HN-HREC-2020-0012, dated 26 August 2020, and signed the consent form.

3 Results

3.1 Pre-existing abdominal and epigastric health conditions

Figure 2 explains the abdominal and epigastric health symptoms on a widely used and validated questionnaire to evaluate GI symptoms. Gastrointestinal health (GIT) was evaluated on a Likert scale (0–6), where 0 represents no symptoms and 6 reflects unbearable conditions (65). Group-wise mean GIT score variance was non-significant ($P = 0.985$). The mean GI score of symptoms perceived in the control group was 3.27 ± 0.5, the LSM mean GIT score was 3.17 ± 1.6, the PSH mean score was 3.2 ± 0.9, and the LSM&PSH group had a mean GIT score of 3.2 ± 0.7 at baseline. The mean GIT score of symptoms was non-significant ($P = 0.86$) between men and women; the mean GI scores of men and women were 3.2 ± 0.9 and 3.23 ± 1.07 at baseline, respectively (Table A1).

3.2 Sleep quality assessment at the baseline

Figure 3 shows the Pittsburgh Sleep Quality Index (PQSI), an established questionnaire for sleep quality analysis. The PSQI is composed of 19 items that produce a global score for sleep quality, and the study participants were evaluated at baseline on the following seven components: sleep quality, sleep latency, sleep length, sleep efficiency during habitual sleep, sleep disturbance, usage of sleeping medicine, and daytime dysfunction (72).

As measured by the PSQI score, the quality of sleep did not differ significantly ($P = 0.661$). The LSM group had the highest PSQI score at baseline, while the LSM&PSH group had the lowest.

A significant difference ($P < 0.05$) was observed between men and women sleep quality at baseline. Men have poor sleep quality, with a mean PSQI score of 7.2 ± 1.05, compared to women, with a mean PSQI score of 6.6 ± 1.32 (Table A2).

3.3 Post-intervention mean GIT health status-group-wise trend

Figure 4 shows the gastrointestinal tract (GIT) health after the intervention in the control and intervention groups. Both psyllium husk fiber alone and in combination with lifestyle changes had a substantial influence on GIT health. The psyllium husk fiber group showed the maximum effect ($P < 0.05$) with a mean of -0.7 ± 0.1 (-22%), and the mean GIT score of the LSM&PSH group was -0.5 ± 0.1 (-16%). In intervention groups, the LSM group had a minimal effect (-6%) compared to the PSH and LSM&PSH groups. However, the GIT score increased by 9% in the control group.

A significant effect has been observed in both genders of GIT health. Women showed the highest response in relieving GIT scores compared to men. The mean GIT score of women was -0.3 ± 0.1 (-9%), and the post-intervention mean GIT score of men was -0.2 ± 0.1 (-7%) (Table A3).

3.4 Mean adjusted changes in epigastric and abdominal health

Table 2 explains the post-intervention effect of treatment on GIT health. After adjusting for age, gender, and baseline, a significant effect was observed in the intervention groups.

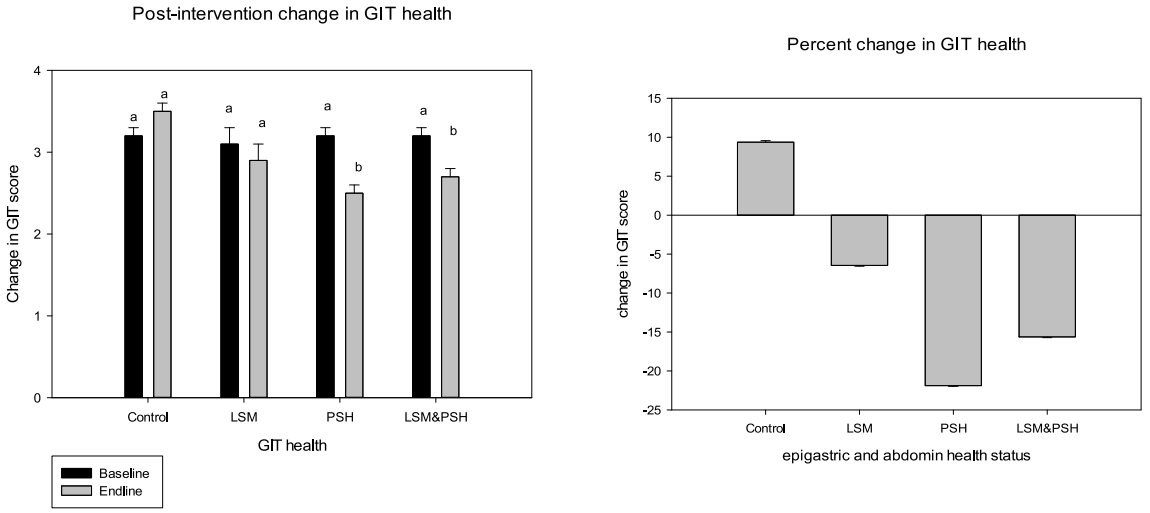

FIGURE 4
Post-intervention GIT health status. A paired sample *t*-test was employed to evaluate the difference between the pre- and post-intervention ($P = 0.05$). GIT, gastrointestinal tract; LSM, lifestyle modification; PSH, psyllium husk fiber. Result between similar alphabets remains non significant.

The PSH group showed maximum relief (−22%) in GIT symptoms. Similarly, the LSM&PSH group showed a −16% improvement and the LSM group showed a 6% improvement in GIT symptoms.

3.5 Post-intervention sleep quality analysis: the group-wise trend

Figure 5 shows the effect of the intervention on sleep quality based on the Pittsburgh Sleep Quality Index (PSQI) score, which ranges from 0 to 21. A non-significant change was observed in the sleep quality of the subjects; however, an improvement was observed in the LSM group compared to the other groups, with a mean difference in the PSQI score of −0.7 ± 0.4 (−12%). The PSQI score was reduced by 7% in the LSM&PSH group, while the PSH group's PSQI score remained unchanged. Gender-wise sleep quality analysis showed a non-significant change in the sleep quality of men (4%) but a significant change in that of women, which thereby demonstrated a decrease of 15% PSQI score (Table A4).

3.6 Effect of intervention on sleep quality (mean PSQI score)

Table 3 explains the effect of the intervention on sleep quality between the groups based on the PSQI score. The intervention's effect, when compared to the control group and after controlling for age, gender, and baseline, was not statistically significant ($P = 0.205$). PSQI scores increased in the control group and remained the same in the PSH group, while they decreased in the LSM and LSM&PSH groups. The maximum effect was observed in the LSM group (−12%), followed by the LSM&PSH group (−7%).

4 Discussion

Post-intervention findings showed that consumption of 10 g of psyllium husk fiber twice a day, 30 min before breakfast, and dinner in soaked form, improved the GIT symptoms, with the most significant improvement observed in the PSH group, followed by the combined group of LSM&PSH. However, gender-wise data showed that maximum improvement was observed in the female group compared to the male group of the study. Based on the PSQI score, the study revealed that the effects of either psyllium husk fiber alone or combined with lifestyle modification were non-significant. However, an improvement has been observed in the LSM group.

Jalanka et al. (73) assessed the role of psyllium husk fiber in the wellbeing of GIT health by examining the role of psyllium husk fiber on the fecal microbiota, which plays a crucial role in gut physiology. In a short study, he shared that psyllium has a small but significant effect on the microbial composition of healthy adults while having a more significant effect on the microbial composition of constipated subjects.

Dietary fiber plays a significant role in lowering the risk of colorectal cancer. The mechanism involved the dilution of fecal carcinogens, quicker gut transit time, bonding of carcinogenic bile acids, and alteration in the microbiota composition and microbial metabolites such as short-chain fatty acid production (74). Bovenschen et al. (65) reported the same GIT symptoms based on this questionnaire. They reported that the severity score of GI symptoms (type and severity) at the end line compared with the baseline and difference (post-base) showed the trend in GIT health (severity or improvement).

Marlett et al. (75) studied the psyllium husk fiber (15 g/day) effect on a stool alone. They reported that psyllium considerably improved the apparent viscosity, stool wetness, and wet and dry stool weight of an aqueous stool extract. Compared to other study subjects who consumed other dietary fiber and control, the subjects

TABLE 2 Post-intervention means GIT health status.

Change in variables	Adjusted mean ± SEM (95% CI) ($n = 30$				*P*-value
	Control	LSM	PSH	LSM&PSH	
GIT	3.50 ± 0.14 (3.79; 3.21)a	2.99 ± 0.14 (3.27; 2.70)ab	2.49 ± 0.14 (2.78; 2.20)b	2.71 ± 0.14 (3.0; 2.42)ab	<0.001

The following values are assigned to covariates appearing in the model: age = 45.91, gender = 1.50, GIT baseline score = 3.2167 ($P < 0.05$). GIT, gastrointestinal tract; PSH, psyllium husk fiber; LSM, lifestyle modification. R-squared = 0.437 (adjusted R-squared = 0.407). Design: intercept + age + gender + GIT baseline score + study group. Similar alphabet in the row shows non significant relation.

TABLE 3 Adjusted mean PSQI score.

Change in variables	Adjusted mean ± SEM (95% CI) ($n = 30$)				*p*-value
	Control	LSM	PSH	LSM&PSH	
PSQI	7.04 ± 0.32 (7.68; 6.41)a	6.16 ± 0.32 (6.8; 5.53)b	6.84 ± 0.32 (7.48; 6.20)ab	6.40 ± 0.32 (7.05; 5.76)ab	0.205

The following values are assigned to covariates appearing in the model: age = 45.91, gender = 1.50, GIT baseline score = 6.9333 ($P < 0.05$). GIT, gastrointestinal tract; LSM, lifestyle modification; PSH, psyllium husk fiber. *R*-squared = 0.342 (adjusted *R*-squared = 0.307), design: intercept + gender + age + PSQI baseline score + study group. Similar alphabet in the row shows non significant relation.

with psyllium husk fiber showed a significant improvement in gut health and open defecation.

Similarly, Marteau et al. (76) reported that the improved digestibility of psyllium husk fiber and its fecal bulking effect improve gut transit time and gas excretion. The positively impact-producing short-chain fatty acid concentration in stool provides the best medium for intestinal flora growth. Desai et al. (77) studied the actual benefits of dietary fiber in the context of irritable bowel syndrome. They related its influence to the microbiota and the maintenance of mucosal integrity.

PSQI is widely used to assess an individual's sleep quality without medication. Subjects with a score of more than five are considered to have poor sleep quality, while less than five PSQI scores reflect good quality sleep (67). Katagiri et al. (78) evaluated sleep quality using the PSQI questionnaire. According to their findings, poor sleep quality is substantially associated with consuming more sweets and beverages and fewer fruits and vegetables. Epidemiological studies suggest a bidirectional relationship between sleep and overall dietary patterns. Most notably, dietary fiber, whole grains, fruits, and vegetables are associated with longer sleep duration, better sleep quality, and fewer insomnia episodes (79).

Sleep disturbances have been linked to hypertension, stroke, and obesity via increased ghrelin and decreased leptin levels, impaired glucose tolerance, anxiety and depression, increased evening cortisol production, and higher inflammatory markers (80, 81). Inadequate sleep delays the circadian melatonin phase while also causing the circadian waking time phase to begin sooner. Sex differences revealed that women, not men, maintained weight during adequate sleep, whereas poor sleep impaired dietary control and caused weight gain in women. Liang et al. (82) studied the dietary approaches to stop hypertension (DASH) and their association with sleep quality. An inverse relationship was found between the DASH score and poor sleep-related daytime dysfunction. The fiber DASH component was most notably associated with better sleep quality and inversely related to sleep-related daytime dysfunction.

Grandner et al. (83) assessed the national data from the US adult survey to quantitatively demonstrate the relation between dietary fiber intake and sleep quality. Subjects with decreased intake (13.2 ± 10.1 g) have less sleep duration of <5 h, while subjects who consumed 14.2 ± 8.7 g and 15.9 ± 10.9 g have 5–6 h and 9+ h of sleep, respectively. High-fiber foods like fruits, vegetables, and cereals encourage the production of short-chain fatty acids (SCFA) in the human gut microbiota (44). These gut micro-biotas enhance sleep (84) and modulate the host circadian clock (85), which in turn maintains mammalian homeostasis and rhythmic physiology such as sleep-wake cycle, eating, and fasting (86). Contraction with the literature augments the need for further studies to correlate the other factors influencing sleep quality and suggest modifications in the PSQI questionnaire in light of modern technological interference in sleep quality components.

The association of specific diets such as high energy intake or different food nutrient intakes at different levels of carbohydrates, fats, and protein on sleep quality and duration is unclear. Previous studies have shown that increased consumption of energy intake induces insufficient sleep and could induce body weight gain (87, 88), while others reported that less energy intake had a profound effect on insomnia (89).

5 Summary and conclusion

A 16-week interventional study was conducted to assess the effect of lifestyle modification with and without psyllium husk fiber on the abdomen, epigastric symptoms, and sleep quality. In total, 120 school teachers with equal gender bifurcation were divided into four subgroups ($n = 30$), a control group, and an intervention group. The enrolled subjects were oriented about the intervention execution and compliance, encouraged to participate in one-to-one and group discussions, and signed informed consent.

In multiple seating, GIT health status, and sleep quality analysis were recorded using validated questionnaires. GIT health symptoms improved post-intervention in the PSH and combined LSM&PSH groups. GIT health symptoms improved by 22% in the PSH group and 16% in the LSM&PSH group; however, a non-significant 6% improvement was noted in the LSM group. Gender-wise data showed a significant improvement in GIT health in both

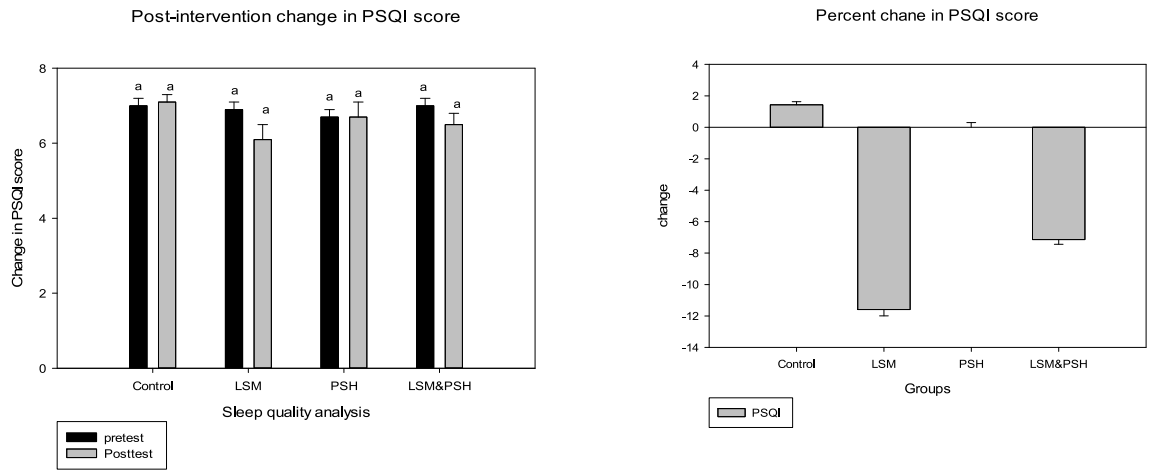

FIGURE 5
Post-intervention trend based on the mean PSQI score. A paired sample *t*-test was employed to evaluate the difference between the pre- and post-intervention ($P = 0.05$). PSQI, Pittsburgh Sleep Quality Index; LSM, lifestyle modification; PSH, psyllium husk fiber. Similar alphabet represent the non significant relation among the group.

genders. However, women improved more than men (9 vs. 7%). Based on the PSQI score, the intervention has a non-significant effect on sleep quality, yet the LSM group showed the highest effect on sleep quality compared to the PSH and the LSM&PSH groups. Gender-wise PSQI analysis showed a non-significant effect of the intervention on the sleep quality of men but a highly significant effect on the sleep quality of women.

The study concluded that psyllium husk fiber significantly affects the abdomen and epigastric health. At the same time, lifestyle modification is more potent in enhancing the subjects' sleep quality. Further studies are suggested to include the technological effect on sleep quality by modifying the PSQI questionnaire.

6 Limitations

Adherence to the protocol intervention and myths about psyllium husk fiber were challenging at inception. Dietary counseling, sparing time for a dedicated walk, and reporting by subjects in intervention groups on a regular basis pose a challenge for acceptance. Individual follow-up, protocol compliance, and reporting require additional effort along with the intervention. Dietary behavior modification for the subject and in support of the family increase the budget of households. Lack of technological awareness and usage for measuring physical activity added to the extra burden of proper reporting on daily diaries, which required in-depth training and continuous follow-up to minimize bias.

7 Strength

This is a detailed study in the region, particularly in the teacher community. Through individual and group counseling, awareness about the lifestyle modification and the role of psyllium husk fiber, apart from anti-constipation, develops the social desirability and willingness of the subjects. Including school teachers as an influential segment increased the generalizability of the study. Regular follow-up and subject's capacity building on reporting formats and adherence to the lifestyle modification and consumption of psyllium husk fiber protocol improve the study quality, increase the subjects' interests in learning and outcomes, reshape the work-life balance, develop dietary modification behaviors, and promote more health and nutrition concerns.

8 Recommendations

To sustain the intervention achievements, adherence to lifestyle modification and establishing a strong work-life balance are required. Further studies are suggested to include <40-year-old adults, pre-diabetic, and hypertensive subjects from the general population. The health sector and practitioners are involved in disseminating the right dietary approaches and compliance. Mass-level nutrition education is a preventive tool for non-communicable diseases. The inclusion of objective sleep assessment methods, such as actigraphy, has to be included in future studies for a comprehensive evaluation of sleep quality.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical Committee of the Department of Human Nutrition, University of Agriculture Peshawar HN-HREC-2020-0012. The studies were

conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

AB: Investigation, Methodology, Writing – original draft, Writing – review & editing. MS: Formal analysis, Data curation, Writing – review & editing. FA: Funding acquisition, Formal analysis, Writing – review & editing. EI: Data curation, Visualization, Writing – review & editing. HA: Software, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1324793/full#supplementary-material

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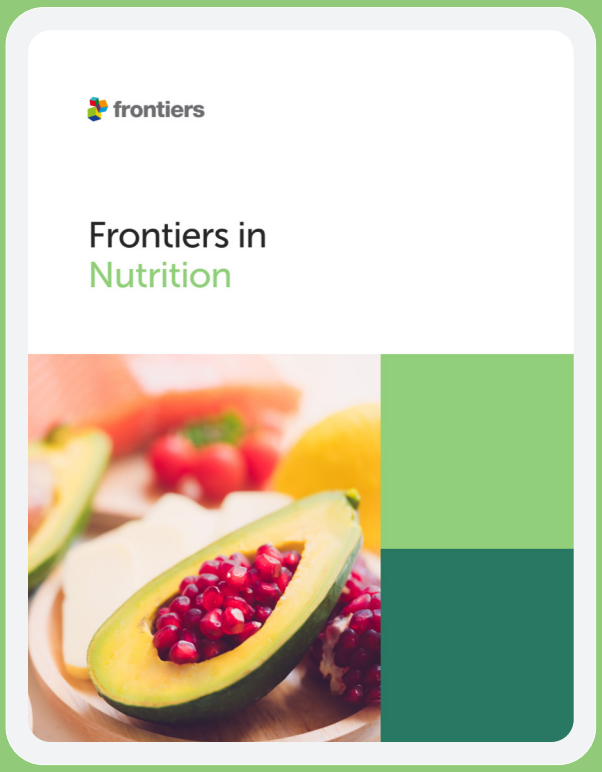

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