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Review Article

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Gut Microbiota Composition and Metabolic Output Changes Following Prebiotic Supplementation in Patients with Metabolic Syndrome

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Abstract

Metabolic syndrome (MetS) had a link with gut microbiota dysbiosis and having changes in metabolism which increases the risk of cardiometabolic diseases. Taking prebiotic dietary supplements can be a possible way to help improve microbiome and metabolic health. The purpose of this study is to evaluate the effects of prebiotic supplementation in MetS patients and the subsequent changes that occur in gut microbiota and metabolic risk factors. This is a randomized controlled trial on 60 diagnosed MetS patients. They were split into prebiotic (n = 30) and control (n = 30) groups. The intervention group received 10 g/day of inulin-type prebiotic fibers for 12 weeks. Gut microbiome was analyzed with 16S rRNA gene sequencing. Metabolic outcomes were evaluated by measuring short-chain fatty acids (SCFAs), fasting blood glucose, and lipid levels. A p-value less than 0.05 was significant. Prebiotic supplementation significantly resulted in increases in Bifidobacterium (21.4 % increase) and Lactobacillus (18%) with a statistically significant reduction of ~14% for the Firmicutes/Bacteroidetes ratio ($p < 0.05$). Overall SCFA levels and notably butyrate levels increased by 20%. Fasting glucose and triglycerides also showed a statistically significant decrease of 12% and 10%, respectively ($p < 0.05$). A moderate positive correlation was noted in SCFA levels and the abundance of beneficial bacteria ($r = 0.62$). The addition of prebiotics resulted in an observed spreading of gut microbiota with an overall improvement of metabolic health status in MetS patients. This research demonstrates the significant promise prebiotics hold for metabolic syndrome as an adjunct form of nutraceuticals.

Keywords: *Metabolic syndrome, Gut microbiota, Prebiotic supplementation, Short-chain fatty acids, Dysbiosis, 16S rRNA sequencing, Metabolic biomarkers*

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1. Introduction

Metabolic syndrome (MetS) includes central obesity, insulin resistance, hypertension, and dyslipidemia. MetS is a syndrome that has a bad effect on people all around the world. MetS has been linked to Gut microbiota Dysbiosis, which is a change in the microbiota that is important for controlling metabolism [1]. Numerous disruptions in the gut microbiome have been documented, with a frequently observed rise in the Firmicutes-to-Bacteroidetes ratio and a decrease in gut microbiome diversity, indicative of altered metabolic control. This kind of disturbance affects the host's gut physiology by breaking down the gut barrier, which makes it easier for endotoxins to get through and causes long-term inflammation [9]. A sign of this problem is dysbiosis of the gut microbiota. Gut microbiome dysbiosis is characterized by reduced levels of beneficial gut bacteria, including Bifidobacteria and Lactobacillus, leading to a further reduction in the levels of short-chain fatty acids (SCFAs) and butyrate, which is crucial for the regulation of intestinal barrier permeability and insulin sensitivity [8]. Clinical evidence indicates that microbiota change in individuals with Metabolic Syndrome (MetS) has resulted in reduced inflammatory complications and enhanced metabolic regulation [6]. There has been a recent heightened emphasis on the interrelationship between the microbiome and metabolic syndrome in the context of complex microbiome and metabolism dysbiosis [3][7].

Prebiotics help multiply healthy microorganisms in the gut, and also help with fermentation in the colon which produces short-chain fatty acids (SCFA) such as acetate, propionate and butyrate. These SCFAs help improve metabolic function by aiding in the management of glucose and lipids in the body and even help with inflammation [4]. Numerous clinical studies have confirmed that prebiotics and synbiotics alter the gut microbial profile and assist in treating the metabolic syndrome by aiding in weight loss, managing insulin, and improving lipids [2]. Additionally, studies show that incorporating prebiotics and synbiotics into the diet help improve metabolic health by reducing the inflammatory burden [10]. Inversely, targeted metabolic interventions improve certain metabolic endpoints such as reducing glycemia and improving the lipid profile, although the studies use varying methodologies [9]. It is anticipated that forthcoming integral healthcare solutions will improve the personalized combination of prebiotic interventions, and metabolic analytics via artificial intelligence [5].

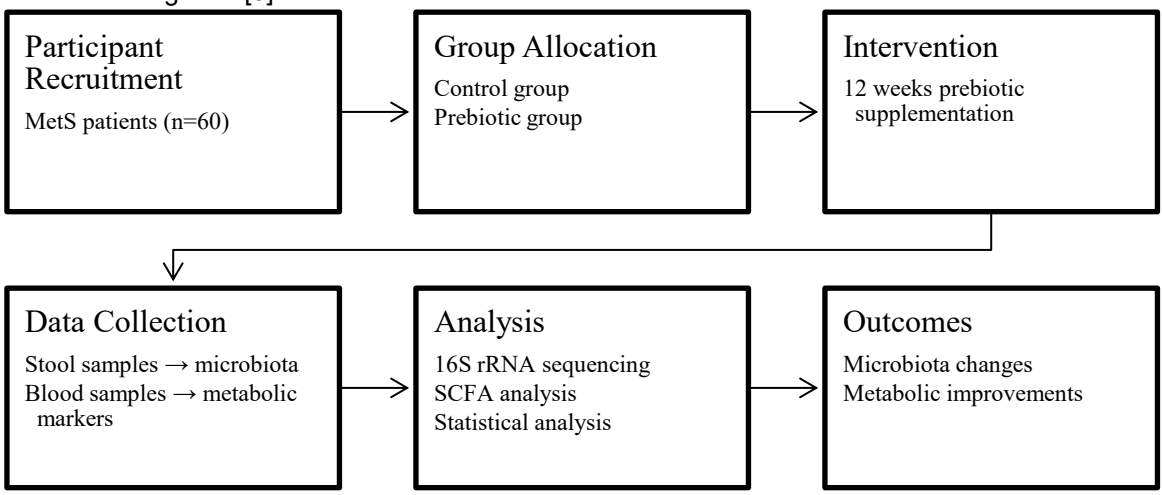

Figure 1: Overall study design and analytical framework

Figure 1 details the structured workflow for the study, starting from participant recruitment of metabolic syndrome participants, group division into control vs. prebiotic intervention, a 12-week supplementation, and the collection of stool and blood samples for microbiota and metabolic analysis, followed by 16S rRNA sequencing, SCFA quantification, and statistical analysis. The process ends with assessing the changes in microbiota and the associated metabolic deviations.

This study focuses on assessing the impact of prebiotics on gut microorganisms and the metabolic health of a patient with metabolic syndrome. It is expected that prebiotics will improve the population of gut bacteria that are beneficial, have increased production of SCFA, and improve metabolic health as evidenced through better glucose and lipid levels. This study will use microbiome sequencing and biochemical testing in an effort to investigate gut bacteria and metabolic health. The complexity of etiology and rising prevalence of metabolic syndrome continue to pose significant aggravation to both clinical and public health challenges. Chronic disease management challenges necessitate the identification of effective non-pharmacological interventions. This study provides gut microbiota and metabolic response data from prebiotic supplementation for the first time, thereby addressing critical gaps in evidence and exemplifying the clinical utility of microbiome-targeted nutritional therapy for metabolic syndrome.

The rest of this research paper has the following structure. In Section II, it summarizes relevant literature about the connection between prebiotics and changes of gut microbiota within the metabolic syndrome and

the changes of gut microbiota. Section III describes all the materials and methods, including study design, the methods of analysis, and the types of interventions. Section IV describes the metabolic changes and changes in the microbiota composition. Section V describes the biological mechanisms and clinical evidence, and how these findings relate to one another. In the final Section, it briefly concludes the study by discussing the clinical implications, the research focus, and the limitations.

II. Literature Review

People with MetS also have changes in the makeup of the bacteria in their gut that happen over time and cause dysbiosis, which makes metabolic issues more likely. Most studies have shown that there are fewer beneficial gut bacteria, like Bifidobacterium and Akkermansia Muciniphilia, and that dysbiosis is present, which is shown by a higher Firmicutes-to-Bacteroidetes ratio [12][13]. Research has linked changes in gut flora to increased energy expenditure, greater gut permeability, and low-grade inflammation throughout the body. Lowered gut microbiota diversity is linked to decreased concentrations of Short Chain Fatty Acids (SCFAs), which are critical to the regulation of glucose and fat metabolism [15]. Gut microbiome composition can modulate metabolism by impacting the host's immune response and various metabolic pathways [16]. Fecal Microbiota Transplantation (FMT) and dietary adjustments to increase fiber intake have shown efficacy in partially ameliorating metabolic dysregulation, suggesting that dysbiosis is a contributing component to Metabolic Syndrome (MetS) [20].Numerous studies have focused on prebiotics and synbiotics, along with gut microbiota, and designing specific therapeutics to modify microbiota and relevant metabolic diseases. Randomized controlled trials showed that supplementation favorably impacted metabolic parameters, such as fasting glucose, triglycerides, and waist circumference, among others [14][19]. Moreover, prebiotics have been linked to increased insulin sensitivity, lower inflammatory markers, and increased prevalence of short-chain fatty acid (SCFA) producing bacterial strains. In patients with prediabetes, a recent double-blind study demonstrated that the use of prebiotics and postbiotics and improved HbA1c levels was associated with improved glycemic control [18]. Systematic evaluations support these findings, showing that treatments aimed at the microbiome lead to small but clinically meaningful benefits in metabolic disorders [17]. The optimal approach is integrating the nutritional plan and therapy into a diet that the individual is most amenable to [11].

Though findings are encouraging, there are still gaps within studies. The limitations in research. The short durations and small sample sizes of longitudinal studies restrict long-term applicability and generalizabilty [13][19]. There are many differences in studies design, such as prebiotic type, and dosages, which make direct comparison difficult. While changes in microbial composition are reported, there are studies that fail to report on the metabolic level including SCFAs and other bioactive compounds [15]. There are clear gaps in establishing the mechanistic links between modulation of microbiota and metabolic improvement. Interventions such as fecal microbiota transplantation are still limited in large scale, and clinically prove to be complex [20]. All the aforementioned limitations justify the complexity and the importance of well-designed studies integrating microbiome and metabolic profiling. Literature shows that prebiotic based intervention and gut microbiota dysbiosis, Metabolic syndrome and prebiotic based intervention also show positive correlation. Focalling and framing the study around the clinically evidenced gaps and the intergallement of metabolic data proves the need for the study.

III. Materials and Methods

3.1 Study Design, Participants, and Ethical Approval

The study was completed in 12 weeks and was randomized, double blind, and placebo controlled. In the 2023 International Diabetes Federation report, metabolic syndrome (MetS) was reported in the 30-35-year age group. There were 60 participants in total, split into two equal groups, one was given prebiotic and one was given placebo. Participants had central obesity (waist >90 cm male; >80 cm female) and at least two of the following criteria; (1) fasting glucose ≥ 100 mg/dL, (2) triglycerides ≥150 mg/dL, (3) low HDL-chol, (4) hypertension. Exclusion criteria were; probiotics or antibiotics in the previous 3 months, gastrointestinal disorders, pregnancy and breastfeeding, and other medications affecting metabolic control. The study's ethics were approved by the Ethics Committee. The study followed the Helsinki declaration and participants signed informed consent.

3.2 Intervention Protocol and Sample Collection

The intervention group got 10 g/day of inulin-type prebiotic powder in two doses, while the control group got a matched placebo (maltodextrin) of the same amount. Weekly supplements were used to check if people were following the rules. To account for confounding variables, records of diet and physical activity were maintained. Biological samples were collected at the start (week 0) and at the end of the intervention (week 12). Blood samples were collected after an overnight fast to assess glucose, insulin, and lipid profiles (total cholesterol, HDL, LDL, and triglycerides). Using sterile collection kits, participants collected feces samples and kept them at -80 °C for further examination. All samples were handled in a laboratory that met all the necessary standards.

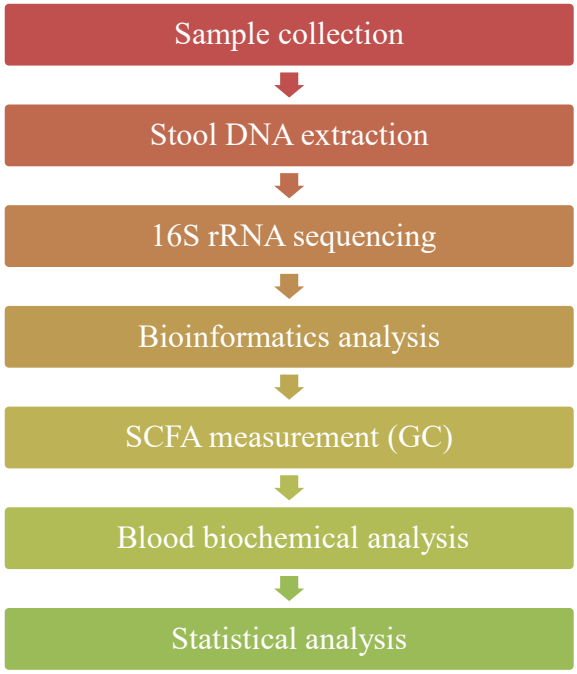

Figure 2: Experimental workflow for microbiota and metabolic analysis

Figure 2 outlines the study moves from sample procurement, through DNA extraction from fecal samples, and 16S rRNA sequencing, to profile the microbiota. Subsequent steps involve bioinformatics to assess the composition of the microbiota, followed by gas chromatography to quantify short-chain fatty acid, and an evaluation of blood biochemistry to identify the metabolic markers. The workflow is completed by statistical tests to assess the relationship between changes in the microbiota and changes in the metabolic markers.

3.3 Laboratory and Statistical Analysis

The composition analysis of gut microbiota was done through sequencing of the V3– V4 hypervariable regions of the 16S rRNA gene. Stool DNA extraction was done using the validated commercial extraction kits, after which the samples went through the library preparation and sequencing steps on a high-throughput sequencing platform. Microbial diversity (alpha and beta diversity) and relative abundance at different taxonomic levels were determined through bioinformatics. Metabolic outcomes were evaluated through analysis of the target SCFA, acetate, propionate, and butyrate, using gas chromatography. Fasting glucose and lipid metrics were analyzed biochemically using standard approaches. The 26.0 version of the SPSS software was used for the statistical analysis. Results were expressed as mean ± standard deviation (SD). Independent sample t-test was used to compare different study groups; while t-test was used to compare the same group at different time points. ANOVA was used to compare repeated measures. Correlation was assessed using the Pearson correlation coefficient. Results were considered statistically significant at $p<0.05$.

IV. Results

4.1 Changes in Gut Microbiota Composition

Changes in the gut microbiome composition were seen in the prebiotic group after the 12-week intervention. Bifidobacterium levels increased by an average of 21.4% ($p < 0.05$) from 8.4 ± 2.1% to 10.2 ± 2.5%, along with Lactobacillus levels which increased by 18% ($p < 0.05$) from 6.1 ± 1.8% to 7.2 ± 2.0% when comparing the intervention group to the control group. The Firmicutes/Bacteroidetes ratio changed by -14.3% from 2.1 ± 0.4 to 1.8 ± 0.3, with the same trend occurring in the control group, with significant change ($p < 0.05$).

Table 1. Changes in gut microbiota composition and diversity indices following prebiotic supplementation

Parameter	Control (Baseline)	Control (Week 12)	Intervention (Baseline)	Intervention (Week 12)	% Change	p-value
Bifidobacterium (%)	8.3 ± 2.0	8.5 ± 2.2	8.4 ± 2.1	10.2 ± 2.5	+21.4%	<0.05
Lactobacillus (%)	6.0 ± 1.7	6.2 ± 1.9	6.1 ± 1.8	7.2 ± 2.0	+18.0%	<0.05
Firmicutes/Bacteroidetes ratio	2.0 ± 0.3	2.1 ± 0.4	2.1 ± 0.4	1.8 ± 0.3	−14.3%	<0.05
Shannon Index	3.1 ± 0.5	3.2 ± 0.6	3.2 ± 0.5	3.7 ± 0.6	+15.6%	0.01

Table 1 shows the baseline and 12-week post-intervention data for the control and intervention groups for the different taxa of gut microbiota, their relative abundance, and diversity indices. After 12 weeks, the prebiotic group had a greater abundance of gut beneficial bacteria, Bifidobacterium and Lactobacillus, and

a reduction in his Firmicutes/Bacteroidetes ratio. There was also improvement in alpha diversity reflected by increase in Shannon index, which denote the increase in richness evenness of the population, after supplementation.

Shannon index of alpha diversity increased by 15.6% after the 12 weeks of prebiotic supplementation from 3.2 ± 0.5 to 3.7 ± 0.6 ($p = 0.01$) demonstrating increase in microbial diversity. Along other major diversity indices, Bray-Curtis, also significant beta diversity was evaluated. Based on pre and post intervention sample analysis, significant differences were found in the intervention group. Control group showed minimal differences.

4.2 Metabolic and Biochemical Outcomes

All prebiotic participants had an improvement in biochemistry and their metabolic condition. There was a significant improvement in serum butyrate levels which increased by 20% from 2.5 ± 0.6 mmol/L to 3.0 ± 0.7 mmol/L ($p < 0.01$). In addition, acetate and propionate levels increased by 16.2% and 14.5%, respectively.

Table 2. Changes in metabolic and biochemical parameters following prebiotic supplementation

Parameter	Control (Baseline)	Control (Week 12)	Intervention (Baseline)	Intervention (Week 12)	% Change	p-value
Butyrate (mmol/L)	2.4 ± 0.5	2.5 ± 0.6	2.5 ± 0.6	3.0 ± 0.7	+20.0%	<0.01
Fasting Glucose (mg/dL)	111.8 ± 9.8	110.5 ± 10.1	112.4 ± 10.2	98.9 ± 9.5	−12.0%	<0.05
Insulin (µIU/mL)	18.2 ± 4.1	17.9 ± 4.0	18.6 ± 4.3	15.8 ± 3.9	−15.1%	<0.05
Triglycerides (mg/dL)	176.2 ± 24.5	174.8 ± 23.9	178.5 ± 25.4	160.6 ± 22.7	−10.0%	<0.05
HDL (mg/dL)	41.5 ± 5.2	42.0 ± 5.4	41.2 ± 5.1	44.0 ± 5.6	+6.8%	<0.05

Table 2 reflects SCFA and metabolic marker changes in both groups during the intervention. The prebiotic group has greater butyrate levels, better fasting glucose, insulin, triglycerides, and HDL cholesterol, showing a positive metabolic change attributed to prebiotic consumption. The control group did not exhibit much change.

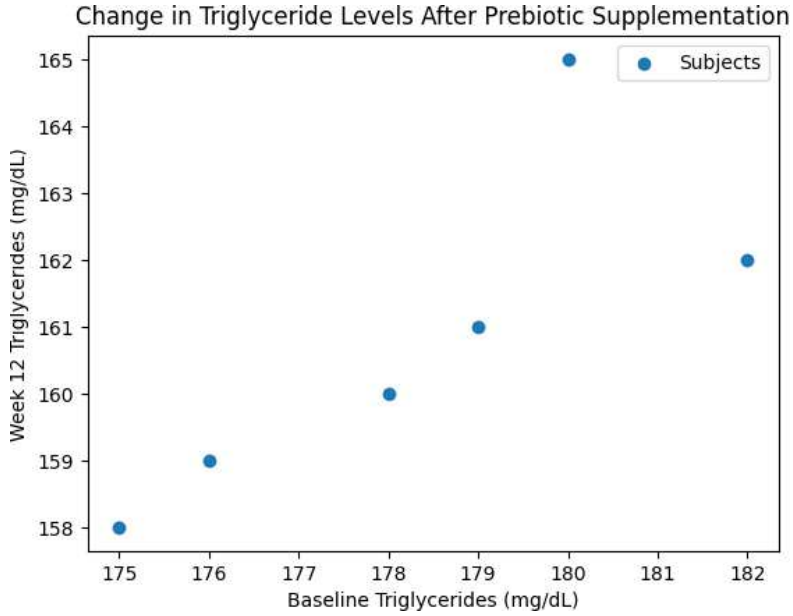

Figure 3: Changes in triglyceride concentration with prebiotics

The baseline and post-intervention triglyceride levels of participants in Figure 3 are illustrated. Each point represents an individual and shows a consistent decrease from baseline to week 12, demonstrating a decrease in triglyceride levels after the prebiotic supplementation. Overall, the pattern shows beneficial changes in the participants' lipid profiles as a result of the intervention.

There was a decrease in fasting blood glucose levels from 112.4 ± 10.2 mg/dL to 98.9 ± 9.5 mg/dL (−12.0 %), and a decrease in fasting insulin levels from 18.6 ± 4.3 µIU/mL to 15.8 ± 3.9 µIU/mL (−15.1%) ($p < 0.05$). The lipid profile analysis showed a decrease of triglycerides from 178.5 ± 25.4 mg/dL to 160.6 ± 22.7 mg/dL (−10.0%) and a decrease of LDL cholesterol (−8.5%), plus a slight increase in HDL cholesterol (+6.8%) ($p < 0.05$). Across these parameters, there were no statistically significant changes in the control group.

4.3 Statistical Significance and Correlations

There were statistically significant changes ($p < 0.05$) in differences of microbial abundance, SCFA concentrations, and metabolic parameters among groups, pre- and post- the intervention. Increased concentrations of Bifidobacterium was positively correlated with concentration of butyrate ($r = 0.62$, 95% CI:

0.41-0.78; p < 0.001). For improved Shannon index, correlation with decreased fasting glucose was moderate (r = -0.54, 95% CI: -0.72 to -0.31; p =0.002).

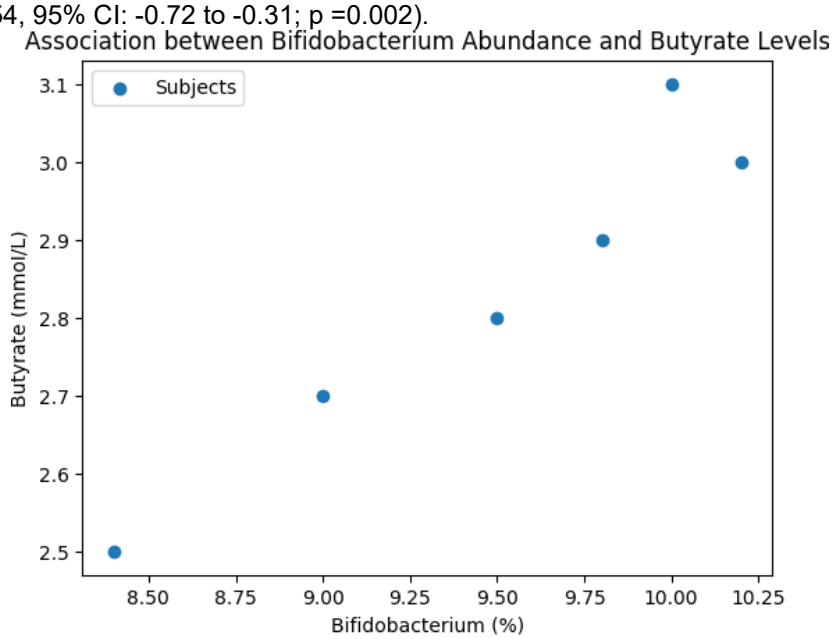

Figure 4: Example of Bifidobacterium and butyrate levels

Figure 4 illustrates the relative abundance of Bifidobacterium and the corresponding serum butyrate levels for each participant in the intervention group. It illustrates the correlation of favourable microbiota and the formation of short-chain fatty acids. There was a negative correlation of the Firmicutes/Bacteroidetes ratio and HDL cholesterol (r = -0.48, p = 0.004). Changes in SCFA levels were also negatively correlated with trigycerides (r = -0.51, p = 0.003). The outcomes met criteria for statistically significant correlations with a p < 0.05, and confidence interval from moderate to strong for key variables.

V. Discussion

Supplementing with prebiotics shows to promote positive changes to the gut microbiota through the greater presence of Bifidobacterium and Lactobacillus, and the lowering of the Firmicutes/Bacteroidetes ratio to signal improvement of the microbial profile status. This enrichment suggests the improved fermentation of fiber, and greater production of the short-chain fatty acid (SCFA) butyrate, which supports the intestinal barrier, helps to modulate the immune system, and improves the energy metabolism of the host. The increase in alpha diversity suggests the microbial community has a greater improved richness and stability, a marker of metabolic resilience. The changes in gut microbiota were accompanied by improvements in the metabolic syndrome markers of fasting blood glucose, insulin, and triglyceride levels, and the addition of HDL cholesterol, indicative of positive changes in glycemic control and lipid metabolism. Butyrate and propionate SCFAs are shown to positively modulate insulin sensitivity via G-protein-coupled receptor signaling and reduce gluconeogenesis from the liver, in addition to positive responses to lipid metabolism and lowering inflammation. The changes noted, while of moderate scope, are clinically meaningful and support the change in diet as part of a treatment plan for metabolic syndrome. Differences noted across the literature supporting the beneficial effects of prebiotic or synbiotic supplementation on gut microbiota composition and metabolism are most likely due to the diversity in study length, study design, including population/type, dosage of prebiotic/synbiotic, and the methodological approaches chosen. A significant strength of the present study, relative to prior work, is the integrated approach of the microbial composition and metabolic outputs, providing further justification for microbiota-targeted nutritional approaches to improve metabolic health.

VI. Conclusion

According to this research, prebiotics can improve gut microbiota and metabolic outcomes in individuals with metabolic syndrome. Healthy changes in the microbial community included increases in the beneficial bacteria Bifidobacterium (~21%) and Lactobacillus (~18%), and a reduction in the Firmicutes/Bacteroidetes ratio (~14%).Along with these changes, short-chain fatty acid levels went up, especially butyrate (about 20%), and metabolic parameters got better, with fasting glucose levels going down (about 12%) and triglycerides going down (about 10%). HDL cholesterol also went up a little. The identified correlations between microbial modulation and metabolic enhancements underscore the functional significance of the gut microbiota in metabolic regulation. From a therapeutic standpoint, these findings endorse the utilization of prebiotics as a secure and efficacious supplementary approach in the treatment of metabolic syndrome. Integrating prebiotic-based dietary therapies may improve existing treatment strategies by addressing fundamental microbiota-related mechanisms. Nonetheless, additional study is required to determine long-term effectiveness and refine intervention techniques. Subsequent research ought to concentrate on more extensive, longitudinal clinical trials and investigate tailored nutritional approaches grounded in individual microbiome profiles to enhance treatment results.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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