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INVESTIGATING THE EFFECTS OF GUT MICROBIOTA MODULATION ON NON—ALCOHOLIC FATTY LIVER IN ADULTS

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is driven by lifestyle factors and limited by existing treatment options. Gut microbiota modulation, focusing on restoring microbial balance, offers a promising alternative by reducing inflammation and improving lipid metabolism. Therefore, this randomized controlled trial assessed the impact of gut microbiota modulation on NAFLD progression over six months in 80 adult participants. Interventions included probiotics, prebiotics, synbiotics, and dietary changes, with a control group. Liver function, lipid profiles, insulin sensitivity, and gut microbiota composition were evaluated. Results showed significant improvements in liver function and lipid profiles in the intervention groups, especially with synbiotics, which exhibited the lowest ALT (29.6 ± 4.3 U/L) and AST (27.0 ± 4.1 U/L) levels. Lipid profiles were most favorable in the dietary and synbiotics groups, with the lowest total cholesterol (158 ± 11 and 160 ± 12 mg/dL, respectively) and LDL levels. Gut microbiota analysis revealed higher diversity in the synbiotics group (Shannon Index: 3.8 ± 0.5) and increased levels of Lactobacillus and Bifidobacterium compared to the control. Insulin sensitivity, measured by HOMA-IR, was also notably better in the synbiotics group (1.5 ± 0.3) versus the control (2.5 ± 0.6). Our study concluded that gut microbiota modulation, especially with synbiotics improved liver health, lipid profiles, and insulin sensitivity in NAFLD patients. Synbiotics showed the most benefit among interventions, suggesting their potential as an effective addition to standard care. Future studies should further explore these benefits with larger samples and extended follow-up to establish synbiotics' role in long-term NAFLD management.

Keywords: Dysbiosis, Inflammation, Insulin Sensitivity, Lipid Metabolism, Synbiotics.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver condition marked by excessive fat accumulation in liver cells in individuals who consume little or no alcohol, making it the most common liver disease globally, with an estimated prevalence of 25-30% among adults (Loomba et al., 2021). The prevalence of NAFLD is rising, especially in regions with high rates of obesity and metabolic syndrome, such as North America and Asia, largely due to modern lifestyle factors, including calorie-dense diets and physical inactivity (Gupta et al., 2012). NAFLD's progression to cirrhosis is particularly concerning, as it compromises liver function and increases mortality risks due to liver failure and HCC, which has a poor prognosis upon diagnosis (Stål et al., 2015). Moreover, patients with NASH are at a higher risk of cardiovascular disease (CVD), which is often the leading cause of death in NAFLD cases (Targher et al., 2021). Current treatment guidelines recommend lifestyle modification; however, the long-term adherence to these changes remains challenging, and pharmacological options are limited, highlighting the need for innovative therapies (WHO, 2003). NAFLD develops from a complex interplay of metabolic dysfunctions, primarily involving lipid accumulation, insulin resistance, and chronic inflammation, which collectively contribute to liver damage and disease progression. Hepatic steatosis, or liver fat buildup, results from an imbalance between lipid intake and breakdown, often worsened by insulin resistance, which disrupts fat metabolism and advances NAFLD (Musso et al., 2019). Chronic inflammation adds to liver damage, with oxidative stress and inflammatory signals leading to fibrosis and possibly cirrhosis in severe cases.

Current treatment options for NAFLD are limited, primarily relying on lifestyle modifications such as diet and physical activity, which are often challenging for patients to sustain long-term. Although some pharmaceuticals like pioglitazone and vitamin E have shown efficacy in treating non-alcoholic steatohepatitis (NASH), their limited applicability and side effects render them suboptimal for widespread use (Yin et al., 2023). This gap in effective therapies has spurred research into alternative approaches, particularly targeting the gut-liver axis and microbiome modulation, which may provide adjunctive or standalone benefits in managing NAFLD. The gut-liver axis, highlighting the bidirectional relationship between the gut and liver via the portal vein, plays a crucial role in regulating liver metabolism, immunity, and inflammation, influencing NAFLD's pathophysiology. Dysbiosis, or microbial imbalance, can disrupt liver function, with mechanisms such as endotoxemia—where bacterial lipopolysaccharides (LPS) enter circulation, triggering liver inflammation (Guerville and Boudry, 2016) and alterations in bile acid metabolism impacting liver fat accumulation. Furthermore, short-chain fatty acids (SCFAs) produced by gut bacteria may protect liver function, although dysbiosis can negatively affect their production (Markowiak-Kopeć and Śliżewska, 2016).

Emerging evidence indicates that gut dysbiosis, characterized by an imbalance in gut microbiota composition, plays a significant role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), with specific bacterial species, microbial metabolites, and intestinal barrier integrity being critical to liver health. Studies reveal that patients with NAFLD

typically exhibit reduced gut microbiota diversity and increased abundance of bacterial families like *Proteobacteria* and *Enterobacteriaceae*, which are linked to higher lipopolysaccharide (LPS) production and systemic inflammation. Dysregulated bile acid metabolism due to altered gut bacteria can contribute to hepatic lipid accumulation and inflammation, further worsening NAFLD (Gottlieb and Canbay, 2019). Additionally, gut dysbiosis can lead to increased intestinal permeability, or "leaky gut," allowing bacterial endotoxins to enter the bloodstream and induce hepatic inflammation and insulin resistance—key factors in NAFLD development (Portincasa et al., 2021). Specific microbial imbalances, such as lower levels of *Akkermansia muciniphila*, correlate with heightened inflammation and lipid accumulation in NAFLD patients. These findings highlight the potential of microbiome-targeted therapies, including probiotics, prebiotics, synbiotics, dietary interventions, and fecal microbiota transplantation (FMT), in reshaping microbial communities to support liver health. Probiotics, like *Lactobacillus* and *Bifidobacterium*, can enhance gut barrier function and reduce endotoxemia, while prebiotics such as inulin stimulate beneficial bacteria and boost short-chain fatty acid (SCFA) production, which is linked to improved lipid metabolism and reduced hepatic inflammation (Hu et al., 2023).

Synbiotics, which combine probiotics and prebiotics, aim to create a balanced microbial environment to further benefit liver health. Dietary changes, including increased fiber and reduced simple sugars, also positively affect gut microbiota composition and contribute to metabolic improvements relevant to NAFLD management (Leung et al., 2016). Fecal microbiota transplantation (FMT), which involves transferring fecal matter from a healthy donor to a patient, has shown promise in resetting the gut microbiome and reducing NAFLD-associated dysbiosis and inflammation (Tkach et al., 2022). Given the strong link between gut dysbiosis and NAFLD, gut microbiota modulation emerges as a compelling adjunct or alternative therapy. By addressing microbial imbalances, these interventions aim to decrease liver inflammation, enhance insulin sensitivity, and limit lipid accumulation, potentially improving the effectiveness of current NAFLD treatments.

The primary aim of this research is to investigate how gut microbiota modulation affects the progression of NAFLD in adults, specifically its influence on lipid accumulation, inflammation, and insulin resistance. This study is significant for advancing NAFLD treatment options through gut-liver axis-based interventions, offering novel, non-invasive strategies to overcome the limitations of current therapies. By enhancing our understanding of microbiota modulation in NAFLD progression, this research seeks to provide valuable insights into microbiome-centered therapeutic approaches as adjunct or alternative treatments for this prevalent liver disease.

RESEARCH METHODOLOGY

Study design

The study employed a randomized controlled trial (RCT) design to investigate the effects of gut microbiota modulation on the progression of non-alcoholic fatty liver disease (NAFLD)

in adults. The trial lasted for six months and was conducted in an outpatient clinic setting, allowing for regular monitoring of participants while providing a controlled environment for intervention implementation. Participants were randomly assigned to either the intervention group, which received targeted microbiome modulation strategies (such as probiotics, prebiotics, synbiotics, and dietary changes), or a control group receiving standard care focused on lifestyle modifications.

Inclusion criteria

Adults aged 18-65 with a diagnosis of non-alcoholic fatty liver disease (NAFLD), confirmed through imaging and liver function tests, were included in the study. Participants had to have no history of significant alcohol consumption, defined as fewer than 14 drinks per week, and must not have secondary causes of liver disease, such as viral hepatitis or autoimmune liver disease.

Exclusion criteria

Individuals were excluded from the study if they had been on antibiotics, probiotics, or prebiotics in the past three months, as these could influence gut microbiota composition. Additionally, patients with comorbidities that could adversely affect liver health, such as significant cardiovascular disease or active malignancy, were not eligible. Pregnant or breastfeeding women were also excluded to ensure the safety of both the mother and child.

Participants demographic

A total of 80 respondents were selected. Participant demographics were collected, including age, gender, and medical history. Medical history focused on relevant comorbidities, such as diabetes, hypertension, and cardiovascular disease, obtained through structured questionnaires and medical records.

Intervention

Probiotics

Specific strains, including *Lactobacillus* and *Bifidobacterium*, were selected based on their documented effects on liver health and gut microbiota balance. Participants received a daily dosage appropriate for each strain, tailored to maximize therapeutic benefits.

Prebiotics

Dietary fibers i.e. inulin were incorporated into the intervention to promote the growth of beneficial gut bacteria, enhancing the efficacy of probiotics.

Synbiotics

A combination of selected probiotics and prebiotics was administered to provide synergistic benefits, aiming to optimize gut microbiota composition.

Dietary interventions

Participants in the intervention group received guidance on implementing dietary changes, focusing on increasing fiber intake and reducing consumption of simple sugars to support gut health and metabolic improvements.

Control group

The control group received standard care, focusing on general lifestyle advice to promote healthy habits, such as dietary recommendations and physical activity, without incorporating specific microbiota modulation strategies.

Parametric evaluation

Liver function tests (LFTs)

To assess liver function, venous blood samples were collected from participants using standard venipuncture techniques. The biochemical analysis of these samples was conducted using an automated biochemical analyzer to measure liver enzyme levels, specifically Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). ALT is a marker that reflects hepatocellular injury, while AST indicates overall liver function and potential injury. The results were reported in units per liter (U/L), and the AST/ALT ratio was calculated to assist in differentiating the causes of liver disease, distinguishing between alcoholic and non-alcoholic etiologies.

Lipid profiles

Fasting venous blood samples were collected to evaluate the lipid profiles of participants. These samples underwent biochemical analysis using lipid analyzers or automated clinical chemistry analyzers to assess total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels. The LDL levels were calculated using the Friedewald formula, which is expressed as:

LDL = Total cholesterol- HDL – (Triglycerides/5)

All lipid values were reported in mg/dL. This comprehensive lipid profile assessment helps in understanding the lipid metabolism and cardiovascular risk associated with NAFLD.

Insulin sensitivity

To evaluate insulin sensitivity, fasting blood samples were obtained to measure glucose and insulin levels. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the formula:

HOMA-IR = Fasting insulin (mU/L) × Fasting glucose (mmol/L)/22.5

Higher HOMA-IR values indicate greater insulin resistance, which is a key factor in the pathogenesis of NAFLD.

Gut microbiota composition

Stool samples were collected from participants using sterile containers and were stored at -80°C for preservation. The microbial DNA was extracted from these samples using commercially available kits. Following DNA extraction, the 16S rRNA gene was amplified and sequenced to determine the microbial composition. Bioinformatics analysis was then employed to analyze the sequencing data, allowing for the assessment of microbial diversity and specific bacterial populations.

Statistical analysis

The data analysis plan for this study involved employing a one-way ANOVA to evaluate differences among group means. Subsequently, post-hoc comparisons were conducted using the Least Significant Difference (LSD) method at a significance level of $p < 0.05$ to determine specific mean differences between the intervention and control groups.

Ethical considerations

This study adhered to ethical guidelines by obtaining approval from an institutional review board (IRB) before initiation. Informed consent was secured from all participants, detailing the study's purpose, procedures, risks, and benefits. Participants were assured of confidentiality and the option to withdraw at any time without consequences, ensuring their rights and welfare were protected throughout the research.

RESULTS

Demographic analysis

A total of 80 participants were included in the study, with a balanced representation of gender, as 50% (n=40) were male and 50% (n=40) were female (Table 1). The mean age of the participants was 45.3 years (± 10.5), indicating a middle-aged cohort with moderate variability. In terms of medical history, 25% (n=20) of the participants reported diabetes, 37.5% (n=30) had hypertension, and 18.75% (n=15) had a history of cardiovascular disease. An additional 18.75% (n=15) reported no relevant comorbidities.

Liver profile

The study assessed the effects of various interventions (probiotics, prebiotics, synbiotics, dietary interventions) and a control group on liver enzymes—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—and the AST/ALT ratio (Table 2). Participants in the synbiotics group had the lowest mean ALT levels at 29.6 ± 4.3 U/L, significantly different from the dietary intervention group (36.2 ± 5.0 U/L) and the control group (40.7 ± 5.6 U/L). The probiotics group (32.5 ± 5.1 U/L) and prebiotics group (34.8 ± 4.7 U/L) had intermediate ALT levels, with no statistically significant differences between them. The synbiotics group also showed the lowest AST levels at 27.0 ± 4.1 U/L, differing significantly from the dietary intervention (32.9 ± 4.5 U/L) and control groups (36.4 ± 5.0 U/L). The probiotics (28.4 ± 4.3 U/L) and prebiotics (30.1 ± 4.9 U/L) groups had lower AST levels than the control but did not differ significantly from each other. Moreover, the AST/ALT ratio was slightly higher in the synbiotics (0.91 ± 0.06) and dietary intervention groups (0.91 ± 0.04), whereas the control group had a ratio of 0.89 ± 0.05. The probiotic and prebiotic groups also had an AST/ALT ratio close to the control, at 0.87 ± 0.05 and 0.88 ± 0.04, respectively, with no significant differences between these groups.

Table 1. Demographic description of the participants of study

Demographic variables	Category	Frequency	Percentage
Total respondents		80	100
Age (Years)	Mean (±SD)	45.3 (± 10.5)	
Gender	Male	40	50
	Female	40	50
Medical history	Diabetes	20	25.00
	Hypertension	30	37.50
	Cardiovascular disease	15	18.75
	No relevant comorbidities	15	18.75

Table 2. Liver profile as impacted by gut microbiota modulation

Intervention	ALT (Mean ± SD, U/L)	AST (Mean ± SD, U/L)	AST/ALT Ratio (Mean ± SD)
Probiotics	32.5 ± 5.1^{a}	28.4 ± 4.3^{a}	0.87 ± 0.05^{b}
Prebiotics	34.8 ± 4.7^{ab}	30.1 ± 4.9^{b}	0.88 ± 0.04^{b}

Synbiotics	29.6 ± 4.3[b]	27.0 ± 4.1[a]	0.91 ± 0.06[ab]
Dietary Interventions	36.2 ± 5.0[bc]	32.9 ± 4.5[bc]	0.91 ± 0.04[b]
Control Group	40.7 ± 5.6[c]	36.4 ± 5.0[c]	0.89 ± 0.05[b]

Lipid profile

The lipid profile of participants showed notable changes across different gut microbiota modulation interventions (Table 3). The dietary intervention group exhibited the lowest mean total cholesterol at 158 ± 11 mg/dL, followed by the synbiotics group (160 ± 12 mg/dL). Participants in the control group had the highest total cholesterol (180 ± 10 mg/dL), with the probiotics and prebiotics groups showing intermediate levels (170 ± 12 mg/dL and 165 ± 15 mg/dL, respectively). Triglyceride levels were also lowest in the dietary intervention group (115 ± 16 mg/dL) and synbiotics group (120 ± 18 mg/dL), with the control group displaying the highest triglycerides at 140 ± 18 mg/dL. The probiotics and prebiotics groups had triglyceride levels of 130 ± 20 mg/dL and 125 ± 19 mg/dL, respectively. Dietary intervention participants had the lowest LDL levels at 88 ± 7 mg/dL, while synbiotics participants had similar LDL levels (90 ± 8 mg/dL). In contrast, the control group showed the highest LDL levels (110 ± 8 mg/dL), with probiotics and prebiotics groups in the mid-range at 100 ± 10 mg/dL and 95 ± 9 mg/dL, respectively. The dietary intervention group had the highest HDL levels at 54 ± 6 mg/dL, and the synbiotics group also showed a favorable HDL level (52 ± 4 mg/dL). The control group had the lowest HDL levels (45 ± 5 mg/dL), while probiotics (48 ± 6 mg/dL) and prebiotics (50 ± 5 mg/dL) groups fell in between. The lowest LDL/HDL ratio was observed in the dietary intervention group (1.63 ± 0.18), with the synbiotics group following closely at 1.73 ± 0.20. The control group had the highest LDL/HDL ratio (2.44 ± 0.2), while the probiotics (2.08 ± 0.25) and prebiotics (1.90 ± 0.22) groups showed intermediate ratios.

Table 3. Lipid profile of participants as impacted by gut microbiota modulation

Intervention	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	LDL/HDL Ratio
Probiotics	170 ± 12	130 ± 20	100 ± 10	48 ± 6	2.08 ± 0.25
Prebiotics	165 ± 15	125 ± 19	95 ± 9	50 ± 5	1.90 ± 0.22
Synbiotics	160 ± 12	120 ± 18	90 ± 8	52 ± 4	1.73 ± 0.20
Dietary Interventions	158 ± 11	115 ± 16	88 ± 7	54 ± 6	1.63 ± 0.18

Control Group	180 ± 10	140 ± 18	110 ± 8	45 ± 5	2.44 ± 0.2

Gut microbiota composition and insulin sensitivity

The effects of various gut microbiota modulation interventions on gut microbiota composition and insulin sensitivity were evaluated using the Shannon Diversity Index, levels of Lactobacillus and Bifidobacterium, and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). The synbiotics group exhibited the highest microbial diversity, with a mean Shannon Diversity Index of 3.8 ± 0.5, followed by the probiotics group at 3.5 ± 0.4. The control group had the lowest diversity (2.7 ± 0.3), while the prebiotics and dietary intervention groups had intermediate diversity scores of 3.2 ± 0.3 and 3.1 ± 0.4, respectively. Lactobacillus levels were highest in the synbiotics group, with a mean of 6.5 ± 0.4 Log CFU/g, indicating a significant increase compared to all other groups. The probiotics group also showed relatively high Lactobacillus levels at 6.0 ± 0.5 Log CFU/g, while the control group had the lowest levels at 4.0 ± 0.4 Log CFU/g. Similarly, Bifidobacterium levels were highest in the synbiotics group (6.0 ± 0.3 Log CFU/g) and lowest in the control group (3.5 ± 0.4 Log CFU/g). Probiotics and prebiotics groups also had increased Bifidobacterium levels at 5.5 ± 0.4 and 5.3 ± 0.5 Log CFU/g, respectively, while the dietary intervention group had slightly lower levels at 5.0 ± 0.4 Log CFU/g. The HOMA-IR values were lowest in the synbiotics group (1.5 ± 0.3), indicating improved insulin sensitivity, followed by the dietary intervention (1.7 ± 0.4) and prebiotics groups (1.8 ± 0.4). The probiotics group had a higher HOMA-IR value of 2.1 ± 0.5, while the control group had the highest HOMA-IR (2.5 ± 0.6), suggesting poorer insulin sensitivity.

Table 4. Gut microbiota composition and insulin sensitivity of participants as impacted by gut microbiota modulation

Intervention	Shannon Diversity Index Mean ± SD	Lactobacillus (Log CFU/g) Mean ± SD	Bifidobacterium (Log CFU/g) Mean ± SD	HOMA-IR Mean ± SD
Probiotics	3.5 ± 0.4	6.0 ± 0.5	5.5 ± 0.4	2.1± 0.5
Prebiotics	3.2 ± 0.3	5.8 ± 0.6	5.3 ± 0.5	1.8± 0.4
Synbiotics	3.8 ± 0.5	6.5 ± 0.4	6.0 ± 0.3	1.5± 0.3
Dietary Interventions	3.1 ± 0.4	5.5 ± 0.5	5.0 ± 0.4	1.7± 0.4
Control Group	2.7 ± 0.3	4.0 ± 0.4	3.5 ± 0.4	2.5± 0.6

Discussion

The demographic profile, characterized by an even gender split and a middle-aged cohort with notable rates of hypertension, diabetes, and cardiovascular disease, reflects a population at elevated risk for non-communicable diseases (NCDs), consistent with global trends.

Middle age often marks an increase in NCD prevalence due to lifestyle factors like diet, inactivity, and stress, underscoring the need for targeted prevention strategies (Budreviciute et al., 2020). Having a balanced representation of both genders in this study helps us understand how health impacts can differ between men and women. Men and women often face different patterns of risk for chronic diseases, shaped by a mix of biological and social factors. By including both genders equally, we gain valuable insights that can lead to more tailored and effective health interventions for everyone. (Hernandez et al., 2022). This age group serves as a critical target for interventions due to the compounding effects of lifestyle behaviors, which, if unaddressed, can lead to metabolic and cardiovascular complications by midlife (Vodovotz et al., 2020). Given the high rates of comorbidities observed, preventive measures such as dietary adjustments, increased physical activity, and lifestyle interventions are essential, with recent studies suggesting that gut microbiota modulation may further improve metabolic and insulin responses (Juárez-Fernández et al., 2020).

The findings from this study highlighted the role that gut microbiota modulation, particularly through synbiotics, plays in liver health. Elevated liver enzymes such as ALT and AST are recognized markers of liver injury and metabolic dysfunction (McGill, 2016). The pronounced reduction in these enzymes in the synbiotics group suggests a protective effect on liver function, likely due to enhanced gut-liver axis interactions. The gut-liver axis refers to the dynamic communication between the gut microbiota and the liver, where beneficial microbial populations can influence liver metabolism and immune responses (Tripathi et al., 2022). Research indicates that synbiotics, which combine probiotics and prebiotics, promote a more diverse and active gut microbiome, potentially improving gut barrier integrity and reducing systemic inflammation. This is particularly relevant in conditions such as non-alcoholic fatty liver disease (NAFLD), where an imbalance in gut microbiota (dysbiosis) has been implicated in liver injury. The ability of synbiotics to enhance the production of short-chain fatty acids (SCFAs) further contributes to their hepatoprotective effects, as SCFAs are known to exert anti-inflammatory properties (Pant et al., 2023). In contrast, the intermediate liver enzyme levels observed in the probiotics and prebiotics groups indicate that while these interventions may confer some benefits, they do not achieve the same level of liver protection as synbiotics. This distinction emphasizes the synergistic effects of combining probiotics with prebiotics, which may lead to more significant improvements in liver health.

The analysis of lipid profiles across different intervention groups reveals compelling evidence for the efficacy of dietary interventions and synbiotics in managing lipid levels, which are critical indicators of cardiovascular health. The dietary intervention group demonstrated the most favorable lipid profile, characterized by the lowest total cholesterol and LDL levels, as well as the highest HDL levels. These findings align with the growing body of literature emphasizing the positive impact of dietary modifications—particularly those rich in fiber, healthy fats, and low in saturated fats—on lipid metabolism and cardiovascular risk (Sacks et al., 2017). The significant reductions in triglycerides and total cholesterol in both the dietary intervention and synbiotics groups suggest that these approaches may effectively reduce the risk of dyslipidemia, a key contributor to cardiovascular disease (Flaig et al., 2032). The LDL/HDL ratio serves as a crucial indicator of

cardiovascular risk, with lower values associated with reduced risk of heart disease. The notable reduction in this ratio among participants in the dietary intervention and synbiotics groups highlights the potential of these interventions to improve lipid profiles and overall heart health (Zheng et al., 2021). In contrast, the control group exhibited elevated total cholesterol, triglycerides, and LDL levels, alongside reduced HDL levels, underscoring the importance of active dietary and lifestyle management in preventing dyslipidemia. The intermediate lipid levels seen in the probiotics and prebiotics groups indicate that while these interventions may offer some benefits, they are less effective compared to comprehensive dietary changes or synbiotic supplementation.

The findings from this analysis of gut microbiota modulation interventions underscore the importance of microbial diversity and specific bacterial populations in enhancing insulin sensitivity and overall metabolic health. The synbiotics group exhibited the highest Shannon Diversity Index, reflecting a richer and more diverse gut microbiota composition, which is often associated with improved metabolic outcomes (Sergeev et al., 2020). Increased microbial diversity is linked to enhanced resilience of the gut microbiome, potentially leading to better metabolic regulation and reduced risk of insulin resistance (Fassarella et al., 2021). The significant elevation of Lactobacillus and Bifidobacterium levels in the synbiotics group further supports the positive impact of these interventions on gut health. Both of these bacterial genera are known for their beneficial effects on gut health, including modulation of inflammation and enhancement of the gut barrier function (Hiippala et al., 2018). The marked differences in levels among groups indicate that synbiotics can effectively promote the growth of these beneficial microbes, which may contribute to improved insulin sensitivity as evidenced by the lower HOMA-IR values observed in this group.

The HOMA-IR values highlight the relationship between gut microbiota composition and insulin sensitivity. The synbiotics group, with the lowest HOMA-IR, indicates a notable improvement in insulin sensitivity, supporting the notion that interventions promoting beneficial gut bacteria can play a significant role in metabolic health (Rajkumar et al., 2015). Conversely, the control group exhibited the highest HOMA-IR, suggesting poorer insulin sensitivity, likely due to a less favorable gut microbiota composition. While probiotics and prebiotics showed some positive effects, their impact on HOMA-IR values was not as pronounced as that of the synbiotics, indicating the potential synergistic effects of combining these two types of interventions. This suggests that while both probiotics and prebiotics contribute to gut health, their combined use in synbiotics may offer superior benefits for enhancing insulin sensitivity and supporting metabolic health.

Conclusion

In summary, this study provides compelling evidence for the positive impact of various gut microbiota modulation interventions on metabolic health markers in the participant cohort. Among the interventions, synbiotics demonstrated the most pronounced effects, significantly reducing liver enzyme levels (ALT and AST) and improving insulin sensitivity, as indicated by the lower HOMA-IR values. Additionally, the dietary intervention group exhibited a

notably favorable lipid profile, characterized by lower levels of total cholesterol, triglycerides, and LDL, alongside higher HDL levels. These results suggest that incorporating synbiotics and dietary modifications could be a promising strategy for enhancing metabolic health and mitigating risk factors related to insulin resistance and dyslipidemia.

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