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Low Glycemic Index Scarlet Runner Bean-Based Multi-Grain Meal Replacement Powder Improves Metabolic Disorders and Gut Dysbiosis in T2DM Rats

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ABSTRACT: Low glycemic index (GI) diets play a pivotal role in the prevention and management of type 2 diabetes mellitus (T2DM). This study investigated the effects of a low GI multigrain meal replacement powder, primarily composed of scarlet runner beans (*Phaseolus coccineus* L.), on glycolipid metabolic disorders and gut dysbiosis in T2DM rats. A T2DM rat model was induced by a high-fat diet combined with streptozotocin, and rats were randomized into model control (MC), positive control (PC), low-, medium-, and high-dose intervention (LD/MD/HD) and normal control (NC) groups based on fasting blood glucose and body weight. After 8 weeks of intervention, the powder significantly ameliorated glycolipid dysmetabolism, attenuated inflammatory responses, and reduced histopathological damage in T2DM rats. Compared with MC, all intervention groups showed reduced fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and glucagon, along with increased insulin (INS) and glucagon-like peptide-1 (GLP-1) ($P < 0.05$). The HD group further reduced the serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and non-esterified fatty acids (NEFA), while increasing high-density lipoprotein cholesterol (HDL-C) ($P < 0.05$). The MD group also decreased serum TG and LDL-C and increased HDL-C ($P < 0.05$). Pro-inflammatory cytokines (IL-6, IL-1 β) were suppressed, whereas transforming growth factor- β (TGF- β) was upregulated in all intervention groups ($P < 0.05$). The intervention also restored gut microbial homeostasis, characterized by increased abundances of short-chain fatty acids (SCFA)-producing genera (*Intestinimonas*, *Lachnospiraceae_NK4A136_group*, *Ruminococcus*) and reduced levels of potentially pathogenic genera (*Allobaculum*, *Faecalibaculum*) ($P < 0.05$). Fecal SCFA concentrations increased in all intervention groups, with acetic acid significantly elevated in LD ($P < 0.05$). These findings suggest that this low GI meal replacement powder improves metabolic dysregulation, alleviates inflammation and tissue injury, and restores gut microbiota balance in T2DM, supporting its potential utility in T2DM management.

Keywords: Type 2 diabetes mellitus; Low glycemic index; Scarlet runner bean; Glycolipid metabolism; Gut microbiota.

1. Introduction

Diabetes mellitus has become a major global public health concern. Its prevalence among adults aged 20-79 years reached 11.1% (588.7 million) in 2021 and is projected to rise to 12.2% (852.5 million) by 2050

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[1]. Type 2 diabetes mellitus (T2DM), characterized by hyperglycemia, insulin resistance, and relative insulin deficiency, accounts for more than 90% of diabetes cases [2]. Current management strategies for T2DM encompass health education, physical activity, dietary therapy, glucose monitoring, and pharmacological interventions [3]. Notably, dietary therapy is considered a fundamental intervention and is recommended as a core strategy [4]. Substantial evidence demonstrates that dietary interventions facilitate safe and effective glycemic control in patients with T2DM [5, 6].

The concept of the glycemic index (GI) was first proposed in 1981 as a key parameter for evaluating food-induced glycemic responses [7]. Foods are categorized into three GI levels: low GI ($GI < 55$), medium GI ($55 \leq GI \leq 70$), and high GI ($GI > 70$) [8]. Substantial evidence indicates that low GI diets reduce fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and C-reactive protein in patients with T2DM [9, 10]. Li et al. demonstrated that a 12-week low GI dietary intervention improved glycemic control and body weight management [11]. Insulin resistance may compromise gut barrier integrity and microbial metabolism, reducing microbiota diversity [12]. Low GI diets may help restore intestinal homeostasis by alleviating insulin resistance. A 6-month low GI intervention not only improved glycemic parameters and insulin sensitivity but also increased the abundance of beneficial genera (e.g., *Bifidobacterium*, *Lactobacillus*) in patients with T2DM [13]. Thus, developing functional low GI foods for glycemic regulation remains a key priority in nutritional interventions.

The scarlet runner bean (*Phaseolus coccineus* L.), a perennial legume of the *Fabaceae* family, is among the most agronomically important *Phaseolus* species globally. Native to Mesoamerica, it is now extensively cultivated in temperate zones worldwide [14]. Introduced into China in the 1930s, it has become a specialty coarse grain crop in southwestern and southern regions (notably Yunnan, Sichuan, and Guizhou) with considerable economic potential [15]. Scarlet runner beans are rich in B vitamins, iron, zinc, and bioactive compounds, including total phenolics, flavonoids, and anthocyanins. Their extracts exhibit potent α -amylase and α -glucosidase inhibitory activities, both before and after boiling, suggesting potential roles in antioxidant defense and glycemic homeostasis [16–18]. While consumption of other *Phaseolus* species has been reported to ameliorate T2DM [19], no study has directly examined the antidiabetic effects of the scarlet runner bean. Thus, its potential as a functional food ingredient remains largely unexplored.

Meal replacements are increasingly adopted for weight management, glycemic regulation, and dietary convenience. Low GI meal replacement products (e.g., powders, shakes) designed to improve glucose metabolism in T2DM are under active development [20, 21]. According to the 2021 International Glycemic Index Tables, most coarse grains are classified as low GI foods [8]. Rich in resistant starch and dietary fiber, these grains delay carbohydrate digestion and stabilize postprandial glucose, making them ideal ingredients for low GI formulations. Among them, oats, which are rich in β -glucan, reduce postprandial glycemia and improve insulin resistance [22]. Quinoa, Job's tears, and adzuki beans have also demonstrated beneficial effects on glycolipid metabolism in T2DM [23–25]. Functional ingredients such as wolfberry (rich in polysaccharides, polyphenols, and carotenoids) and flaxseed (rich in α -linolenic acid, lignans, and fiber) have attracted

attention for metabolic disorder management. Previous studies have supported their efficacy, establishing their suitability for functional meal replacement formulations [26, 27].

In our prior work, we formulated a multigrain meal replacement powder (GI = 51.84) using scarlet runner bean as the primary ingredient, supplemented with oats, quinoa, Job's tears, adzuki beans, wolfberry, and flaxseed, optimized via response surface methodology [15]. Here, we investigated its effects on glycolipid metabolism and gut microbiota in T2DM rats, aiming to provide insights for the development of low GI functional foods and to expand the application of coarse grains.

2. Materials and methods

2.1 Preparation of meal replacement powder

Scarlet runner bean flour (*Phaseolus coccineus* L.) was obtained from Xianfeng Cooperative (Nanhua County, Yunnan, China). Oat, Job's tears, quinoa, and adzuki bean flours were provided by Jiangxi Cereal Source Food Co., Ltd. (Jiangxi, China); wolfberry powder by Hanzhong Natural Valley Biotechnology Co., Ltd. (Shanxi, China), and flaxseed powder by Qingdao Dehui Biotechnology Co., Ltd. (Shandong, China). Per 100.0 g dry weight, the formulation comprised 23.5 g scarlet runner bean flour, 13.5 g adzuki bean flour, 4.0 g wolfberry powder, 15.0 g oat flour, 15.0 g Job's tears flour, 15.0 g quinoa flour, and 14.0 g flaxseed powder. Following thorough mixing, the blend was sterilized in a hot-air circulating oven (110 °C, 2 h) and stored at 4 °C. The GI of the formulation was determined to be 51.84 [15]. The detailed nutritional composition of the low GI multigrain meal replacement powder is presented in Supplementary Table S1.

2.2 Animal experimental design

All experimental procedures were conducted in accordance with institutional guidelines and were approved by the Animal Ethics Committee of Southeast University (Approval No. 20240520002). Rats were group-housed by treatment (4-5 rats per cage), with no co-housing across different experimental groups, and were maintained under controlled environmental conditions (temperature 22 ± 2 °C, relative humidity $50 \pm 10\%$, 12-h light/dark cycle). Food and water were available ad libitum throughout the study, and no pair-feeding protocol was implemented.

2.2.1 Induction of T2DM rat model

The experimental timeline is shown in Fig. 1A. Seventy male Sprague-Dawley rats aged 4 weeks (body weight 100 ± 10 g) were acclimated for 7 days before the induction of T2DM. Subsequently, 10 rats were randomized into the normal control (NC) group and fed a standard diet. The remaining rats were fed a high-fat diet (HFD, 45% kcal from fat) for 4 weeks. After a 12-h fast, 12 rats from the modeling cohort were selected for a preliminary study. Three groups (4 rats per group) received intraperitoneal streptozotocin (30, 35, or 40 mg/kg in citrate buffer, pH 4.5). Random blood glucose levels were measured on days 3 and 7. Rats exhibiting blood glucose ≥ 16.7 mmol/L at both time points were considered diabetic [28]. Based on the optimal induction rate observed at 35 mg/kg, the remaining model rats received streptozotocin, while NC rats received an equivalent volume of citrate buffer.

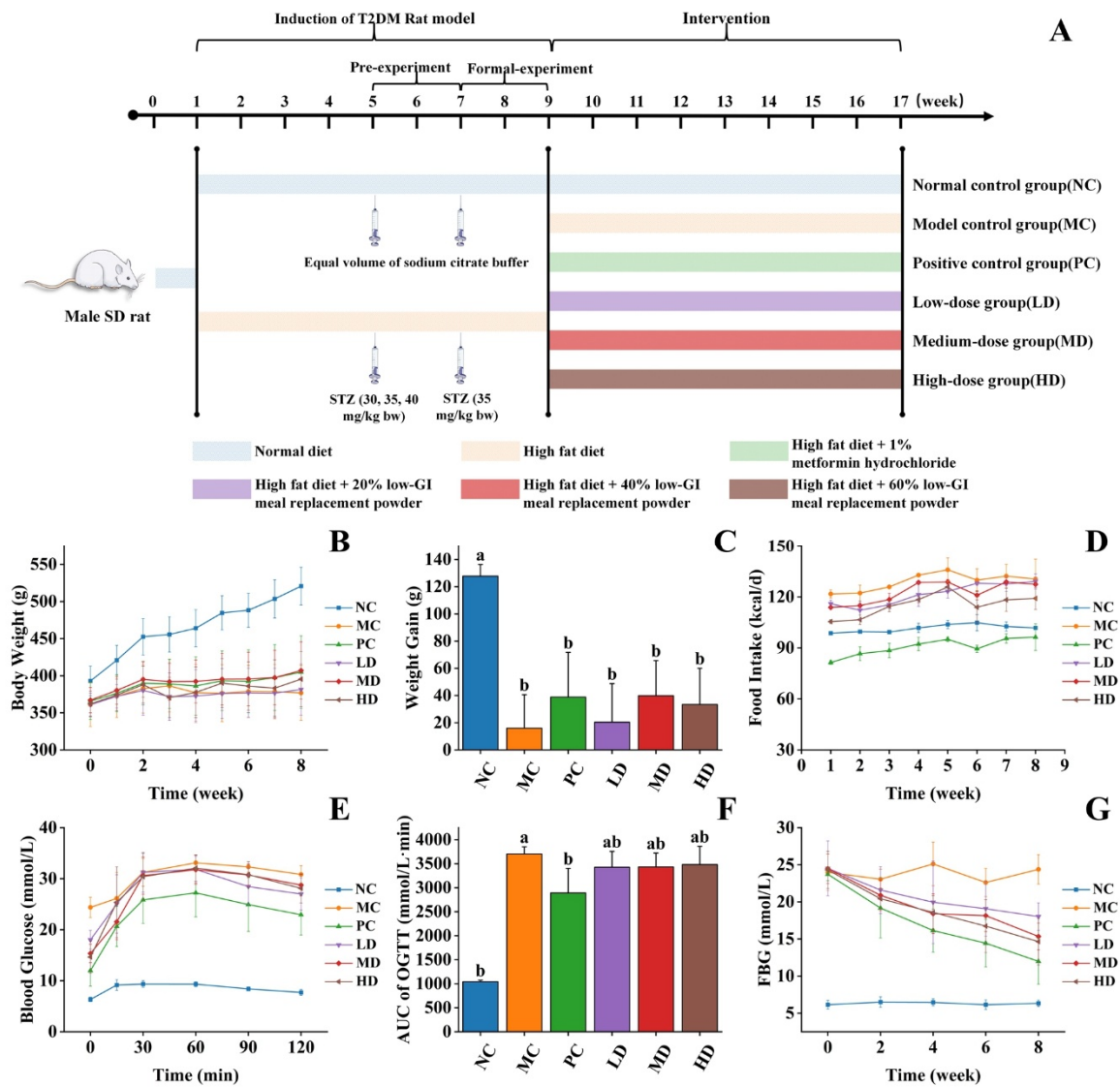


Fig. 1. The flowchart of animal experimental and effects of low GI MRP on body weight, food intake, OGTT and FBG. (A) The flowchart of animal experimental, (B) Body weight-time curve, (C) Weight gain, (D) Food intake-time curve, (E) Blood glucose-time curve of OGTT, (F) AUC of OGTT, (G) FBG-time curve. NC, normal control; MC, model control; PC, positive control; LD/MD/HD, low/medium/high- dose; OGTT, oral glucose tolerance test. $n = 9$ per group. Data are presented as mean $\pm$ SD. Different letters denote significant intergroup differences ($P < 0.05$).

2.2.2 Rat grouping and diets

Successfully modeled rats were randomized into five groups (9 rats per group) based on FBG and body weight for an 8-week intervention. Rats remained group-housed (4-5 rats per cage) by treatment under the same environmental conditions. Body weight was recorded weekly, FBG was measured biweekly, bedding was changed daily, and feed and water were provided ad libitum. The groups were as follows: (1) Model control (MC): HFD; (2) Positive control (PC): HFD containing 1% (w/w) metformin hydrochloride; (3) Low-dose (LD): HFD containing 20% (w/w) low GI meal replacement powder (MRP); (4) Medium-dose (MD): HFD containing 40% (w/w) MRP; (5) High-dose (HD): HFD containing 60% (w/w) MRP. Metformin hydrochloride powder was homogeneously incorporated into the base HFD at 1% (w/w), and metformin was administered continuously via this medicated diet, which PC rats consumed ad libitum throughout the 8-week intervention; no additional oral gavage or injections were used. Doses of the low GI MRP were determined based on its dietary fiber content (13.8%) and the Chinese Dietary Reference Intakes (2023) recommendation

for adult fiber intake (30 g/day), corresponding to approximately 5-, 10-, and 15-fold equivalents for the LD, MD and HD groups, respectively. Diets were formulated by nutrient-equivalent substitution to ensure isocaloric conditions and identical macronutrient energy distribution across groups. Detailed formulations of all diets are provided in Supplementary Table S2.

2.2.3 Oral glucose tolerance test

One week before sacrifice, an oral glucose tolerance test (OGTT) was performed after a 12-h fast. Rats received 50% glucose solution by oral gavage (2.5 g/kg). Tail-vein blood glucose levels were measured at 0, 30, 60, 90, and 120 min using a Roche glucometer. The area under the curve (AUC) was calculated using the trapezoidal rule:

$$\text{AUC} = \sum_{i=1}^{n-1} \frac{(C_i + C_{i+1})}{2} \times (T_{i+1} - T_i) \quad (1)$$

where C represents the blood glucose concentration (mmol/L) at each sampling time T (min), and n is the number of OGTT time points (n = 5).

2.2.4 Sample collection

Twenty-four hours before sacrifice, fresh fecal samples were collected into sterile tubes, snap-frozen in liquid nitrogen, and stored at -80 °C. After a 12-h fast at the endpoint, rats were anesthetized with isoflurane. Blood was collected from the femoral artery and centrifuged at 3500 rpm for 15 min at 4 °C. Serum samples were aliquoted and stored at -80 °C. The liver, kidneys, and pancreas were excised, rinsed, weighed, and fixed in 4% paraformaldehyde for histological analysis.

2.3 Biochemical measurements

2.3.1 Serum biochemical analysis

Serum levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-esterified fatty acids (NEFA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and serum creatinine (Scr) were measured using commercial kits. Enzyme-linked immunosorbent assay kits were used to measure serum concentrations of insulin (INS), HbA1c, glucagon, glucagon-like peptide-1 (GLP-1), tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β), interleukin-6 (IL-6), and interleukin-1 β (IL-1 β). The homeostasis model assessment of β -cell function (HOMA- β) was calculated as:

$$\text{HOMA} - \beta = \frac{20 \times \text{INS}}{\text{FBG} - 3.5} \quad (2)$$

2.3.2 Histopathology

Liver, kidney, and pancreas tissues fixed in 4% paraformaldehyde were embedded in paraffin, sectioned at 4 μm thickness, and stained with hematoxylin and eosin (H&E). Histopathological changes were examined under a light microscope (Olympus BX53, Japan), and digital images were captured using a NanoZoomer® S360 slide scanner (Hamamatsu Photonics, Japan).

2.3.3 Gut microbiota analysis

For microbiota analysis, fecal samples from five rats per group were selected. Total DNA was extracted from fecal samples using the TGuide S96 magnetic universal DNA kit (Tiangen Biotech, Beijing, China). The V3-V4 hypervariable region of bacterial 16S rRNA genes was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3'). PCR products were analyzed by 1.8% agarose gel electrophoresis (Bomei Fuxin Tech, Beijing, China) for size verification and integrity assessment, followed by quantification using ImageJ software. Purified amplicons were subjected to paired-end sequencing (2×250 bp) on the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA). Detailed analytical procedures are described in the Supplementary Materials.

2.3.4 Quantitative analysis of SCFAs

The same subset of fecal samples (5 rats per group) was used for quantitative short-chain fatty acids (SCFAs) analysis. SCFAs—including acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and hexanoic (caproic) acid—were quantified using gas chromatography-mass spectrometry following the method of Han et al. [29]. Samples were extracted with water, phosphoric acid, and ether, with 4-methylvaleric acid used as an internal standard. Standard curves were generated for quantification. Detailed analytical procedures are described in the Supplementary Materials.

2.4 Statistical analysis

Data distribution was tested for normality using the Shapiro-Wilk test. Normally distributed data are expressed as mean $\pm$ SD, and non-normally distributed data as median (interquartile range, IQR). Intergroup comparisons were performed using one-way ANOVA for normally distributed data with homogeneous variances or the Kruskal-Wallis H test for non-normal data. Post hoc tests were conducted using Duncan's multiple range test for homogeneous variances and Dunnett's T3 test for heterogeneous variances. All analyses were performed in IBM SPSS Statistics v26.0 (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effects of low GI MRP on body weight and food intake

Weekly monitoring revealed dynamic changes in body weight and energy intake. As shown in Fig. 1B, MC rats exhibited significantly lower body weight compared with NC ($P < 0.05$), whereas low GI MRP did not significantly alter body weight. Post-intervention weight gain (Fig. 1C) was lowest in MC (16.00 ± 24.62 g), but did not differ statistically from MRP-treated groups. In addition, as shown in Fig. 1D and Supplementary Table S2, food intake among the HFD-fed MC, LD, MD, and HD groups was broadly comparable over the 8-week intervention, consistent with the similar energy densities of their diets. The chow-fed NC group tended to have lower food intake than these HFD-fed groups, whereas the PC group consistently displayed the lowest food intake among all groups. The reduced food intake in the PC group is likely related to metformin-associated appetite suppression and gastrointestinal effects [30, 31].

3.2 Effects of low GI MRP on OGTT and FBG

OGTT was used to assess insulin sensitivity and glucose metabolism. MC rats showed a delayed glucose peak at 60 min (vs. 30 min in NC) and a significantly greater AUC ($P < 0.05$) (Fig. 1E-F). Low GI MRP exerted limited effects on OGTT profiles, with glucose peaks also at 60 min and no significant differences in peak values or AUC. After model induction, FBG was significantly elevated in all diabetic groups compared with NC. During the 8-week intervention, FBG was measured biweekly and progressively declined in both the PC and low GI MRP groups. At week 4, FBG levels in the PC, MD and HD groups were already significantly lower than in the MC group ($P < 0.05$), and by week 8, all intervention groups (PC, LD, MD, and HD) had significantly lower FBG levels compared with MC ($P < 0.05$) (Fig. 1G).

3.3 Effects of low GI MRP on glucose metabolism

As a systemic metabolic disorder, T2DM is characterized by impaired glucose homeostasis. As shown in Fig. 2A-B, compared with NC, MC exhibited significantly reduced INS and HOMA- β ($P < 0.05$), indicating severe β -cell dysfunction. These impairments were reversed in PC, MD, and HD groups. Additionally, MC displayed decreased GLP-1 and elevated glucagon and HbA1c relative to NC ($P < 0.05$) (Fig. 2C-E). Low GI MRP significantly increased GLP-1 and reduced HbA1c, with HD also suppressing glucagon levels ($P < 0.05$).

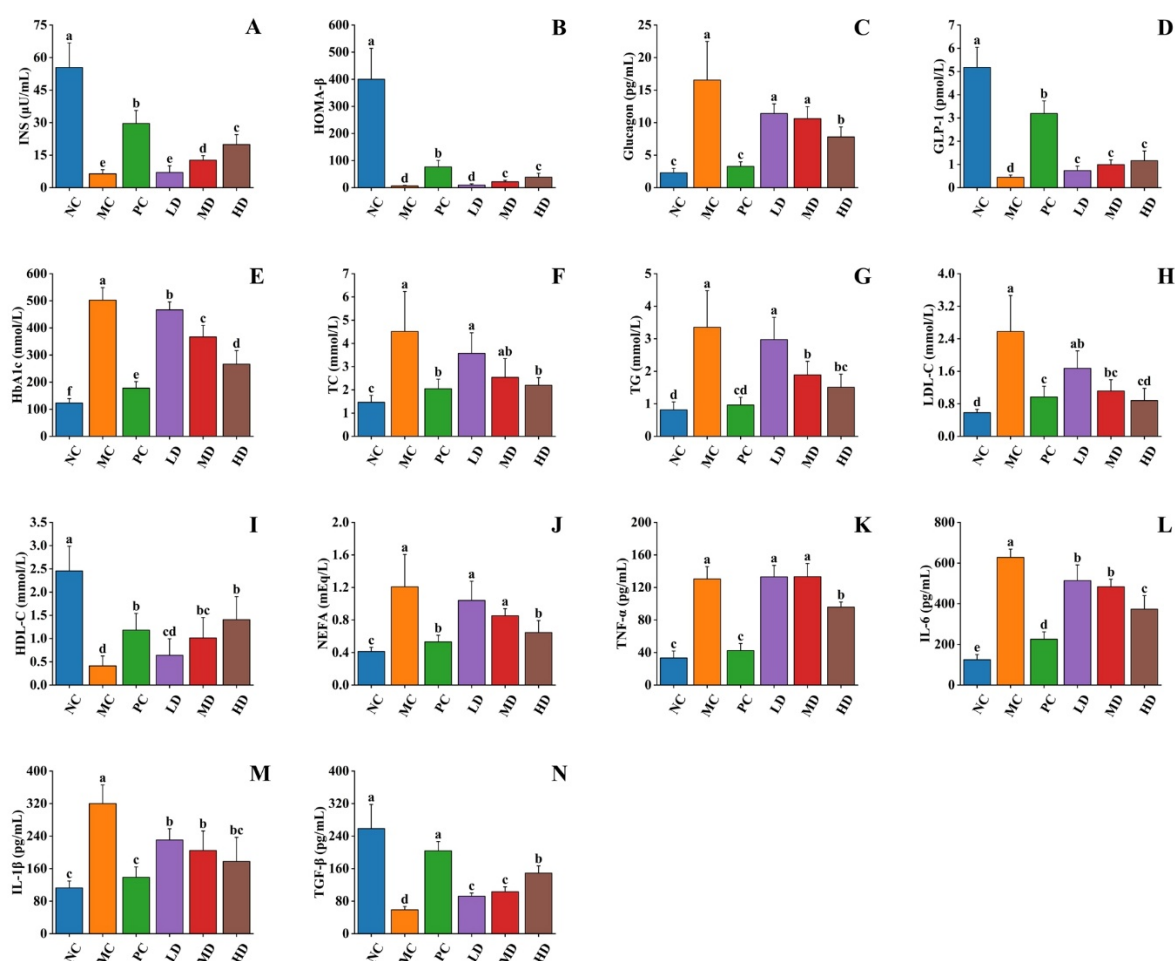


Fig. 2. Effects of low GI MRP on serum indices. (A) Insulin, INS, (B) HOMA- β , (C) Glucagon, (D) Glucagon-like peptide-1, GLP-1, (E) Glycosylated hemoglobin, HbA1c, (F) Total cholesterol, TC, (G) Triglyceride, TG, (H) Low-density lipoprotein cholesterol, LDL-C, (I) High-density lipoprotein cholesterol, HDL-C, (J) Non-esterified fatty acid, NEFA, (K) Transforming growth factor- α , TNF- α , (L) Interleukine-6, IL-6, (M) Interleukin-1 β , IL-1 β , (N) Transforming Growth Factor- β , TGF- β . NC, normal control; MC, model control; PC, positive control; LD/MD/HD, low/medium/high- dose. $n = 9$ per group. Data are presented as mean $\pm$ SD. Different letters denote significant intergroup differences ($P < 0.05$).

3.4 Effects of low GI MRP on lipid metabolism

Dyslipidemia is a critical factor in T2DM progression. As shown in Fig. 2F-J, MC rats developed significant dyslipidemia, characterized by elevated TC, TG, LDL-C, and NEFA, along with reduced HDL-C ($P < 0.05$). These abnormalities were markedly reversed by metformin and high-dose MRP intervention. Moreover, MD significantly reduced LDL-C and increased HDL-C compared with MC ($P < 0.05$).

3.5 Effects of low GI MRP on inflammation

As shown in Fig. 2K-N, compared with NC, T2DM rats displayed a pronounced inflammatory response, with elevated TNF- α , IL-6, and IL-1 β and decreased TGF- β ($P < 0.05$). Intervention with low GI MRP ameliorated this inflammation: PC and HD groups showed significant reductions in TNF- α , IL-6, and IL-1 β and elevated TGF- β ($P < 0.05$). In LD and MD, except for TNF- α , other inflammatory markers exhibited varying degrees of reversal relative to MC ($P < 0.05$).

3.6 Effects of low GI MRP on liver, kidney, and pancreas

As shown in Fig. 3A, compared with NC, MC rats exhibited hepatocellular swelling, pale cytoplasm, and lipid vacuolation, indicative of steatosis. Hepatic sinusoids were dilated and increased in number, accompanied by minor inflammatory infiltration. ALT and AST levels were significantly elevated in MC ($P < 0.05$) (Fig. 3B-C). Both metformin and low GI MRP improved hepatic injury, with HD showing a significant reduction in ALT and AST ($P < 0.05$).

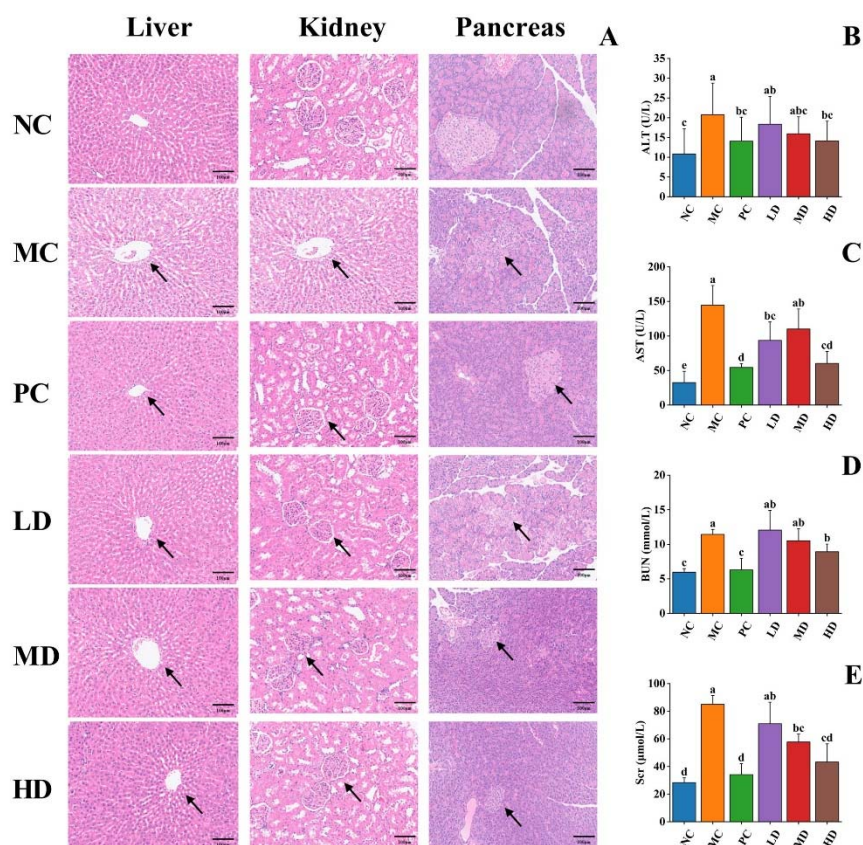


Fig. 3. Effects of low GI MRP on liver, kidney and pancreas. (A) H&E histology of liver, kidney, pancreas, (B) Alanine aminotransferase, ALT, (C) Aspartate aminotransferase, AST, (D) Blood urea nitrogen, BUN, (E) Serum Creatinine, Scr. NC, normal control; MC, model control; PC, positive control; LD/MD/HD, low/medium/high- dose. $n = 9$ per group. Data are presented as mean $\pm$ SD. Different letters denote significant intergroup differences ($P < 0.05$).

MC rats also developed mild renal injury, characterized by segmental mesangial hyperplasia, increased mesangial cells, indistinct capillary structures, tubular atrophy, narrowed lumens, and minor inflammatory infiltration (Fig. 3A). PC and all MRP groups showed marked improvement in renal pathology. As shown in Fig. 3D-E, HD significantly reduced BUN, while MD and HD significantly lowered Scr ($P < 0.05$).

Pancreatic histopathology (Fig. 3A) revealed that, compared with NC, MC rats exhibited irregular islet morphology, atrophy, reduced size or disappearance of islets, blurred boundaries, islet cell swelling/necrosis, and disordered acinar cells. PC, MD, and HD improved these pathological changes to varying degrees.

3.7 Effects of low GI MRP on gut microbiota

We performed 16S rDNA sequencing of fecal samples. After quality control, 1,720,926 sequences from 30 samples yielded 17,181 features for downstream analyses. Rarefaction curves reached a plateau, indicating sufficient sequencing depth (Fig. S1A), and rank–abundance curves suggested good evenness (Fig. S1B). Petal plots showed 83 shared OTUs across groups, with group-specific OTUs of 3226 (NC), 1163 (MC), 1072 (PC), 2256 (LD), 1164 (MD), and 1046 (HD) (Fig. S1C).

As shown in Fig. 4A-B, Principal coordinates analysis based on Bray-Curtis distances revealed clear group separations (PC1 = 20.02%, PC2 = 16.42%), with PERMANOVA showing 48% explained variance ($R^2 = 0.480$, $P = 0.001$). Weighted UniFrac yielded consistent patterns (PC1 = 36.61%, PC2 = 14.91%, $R^2 = 0.482$, $P = 0.001$). Nonmetric multidimensional scaling also corroborated these separations (Fig. S1C-D; Bray-Curtis: stress = 0.1365; Weighted UniFrac: stress = 0.1658). Moreover, as shown in Fig. 4C-F, MC exhibited significantly lower Shannon, Simpson, Chao1 and ACE indices than NC ($P < 0.05$), indicating reduced within-sample diversity and richness in T2DM. Relative to MC, LD increased all four indices ($P < 0.05$), whereas MD and HD displayed non-significant upward trends in Shannon and Simpson with minimal changes in Chao1 and ACE.

At the phylum level (Top 10), *Firmicutes*, *Actinobacteriota*, *Bacteroidota*, and *Desulfobacterota* were the dominant phyla, with a cumulative relative abundance > 90% (Fig. 4G). Compared with NC, MC showed higher *Actinobacteriota*, and lower *Campylobacterota* and *Patescibacteria*. In contrast, LD, MD, and HD showed elevated *Patescibacteria* abundance relative to MC (Fig. S2A-C; $P < 0.05$). At the genus level (Top 20), *Lactobacillus* and *unclassified_Muribaculaceae* were abundant across groups, and *Bifidobacterium* was enriched in all except NC (Fig. 4H).

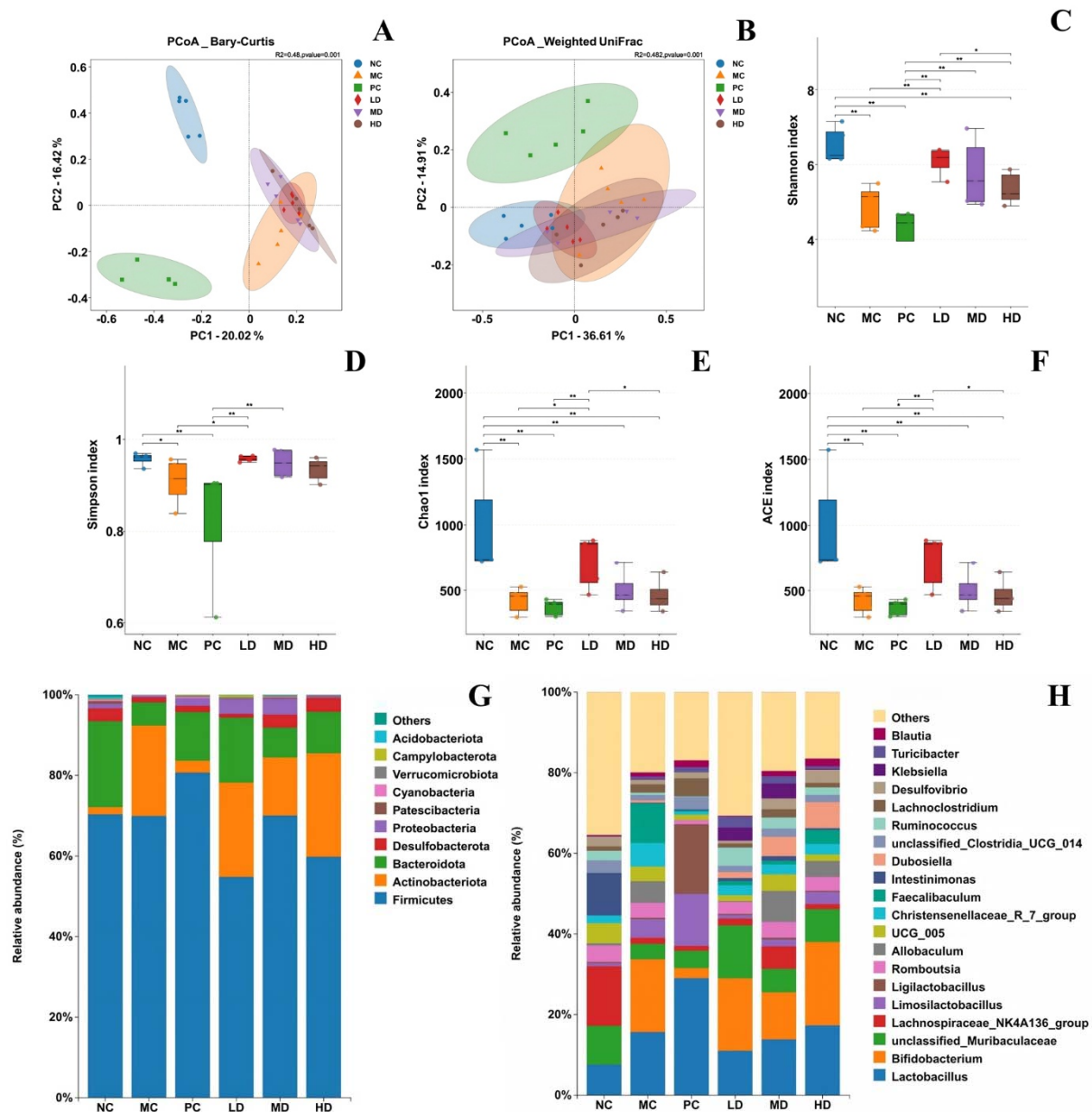


Fig. 4. Effects of low GI MRP on the diversity and composition of gut microbiota. (A) PCoA based on Bray-Curtis distances, (B) PCoA based on Weighted UniFrac distances, (C-F) α diversity indices (Shannon, Simpson, Chao1, ACE), (G) The relative abundance of the gut bacterial phylum, (H) The relative abundance of the gut bacterial genus. NC, normal control; MC, model control; PC, positive control; LD/MD/HD, low/medium/high- dose. $n = 5$ per group. * and ** denote significant difference ($P < 0.05$ and $P < 0.01$).

The linear discriminant analysis effect size cladogram identified 27 discriminative taxa across groups (Fig. 5A). In pairwise LDA bar plots (LDA score > 4.0 , $P < 0.05$), MC vs NC revealed enrichment of taxa such as *f_Atopobiaceae*, *g_Clostridium_sensu_stricto_1*, and *o_Clostridiales* in MC, with reductions in *c_Clostridia*, *o_Oscillospirales*, and *g_Lachnospiraceae_NK4A136_group* (Fig. 5B). Relative to MC, LD was enriched in *c_Gammaproteobacteria*, *p_Proteobacteria*, *g_Ruminococcus*, and showed reductions in *g_Allobaculum*, *f_Acidaminococcaceae*, and *o_Acidaminococcales* (Fig. 5C). MD showed enrichment of *g_Ruminococcus*, *g_Lachnospiraceae_NK4A136_group*, *g_Dubosiella*, whereas HD reduced *o_Clostridiales*, *g_Clostridium_sensu_stricto_1* and *f_Clostridiaceae* (Fig. S2D--). Furthermore, boxplots of six representative genera highlighted distinct alterations: compared with NC, MC displayed elevated abundances of *Allobaculum* and *Faecalibaculum*, but decreased *Intestinimonas*,

Lachnospiraceae_NK4A136_group, and *Ruminococcus*. The low GI MRP reversed these trends in LD and MD. Specifically, LD showed decreased *Allobaculum* but increased *Intestinimonas*, *Ruminococcus*, and *unclassified_Muribaculaceae*. MD exhibited decreased *Faecalibaculum* along with increased *Intestinimonas*, *Lachnospiraceae_NK4A136_group*, and *Ruminococcus* (Fig. 5D-I; $P < 0.05$).

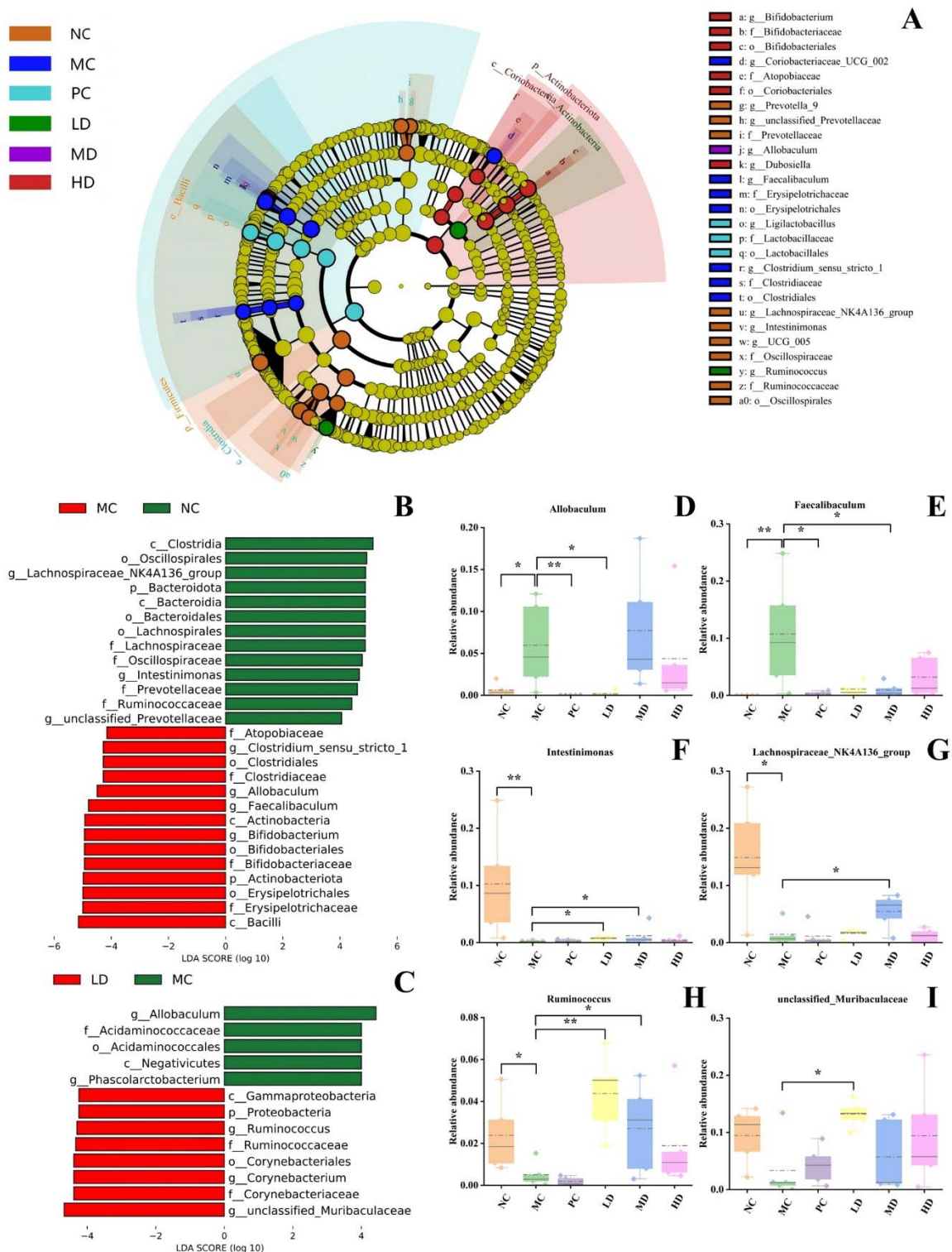


Fig. 5. Intergroup differential taxa analysis based on Lefse. (A) Cladogram generated from Lefse analysis, (B) LDA bar plot (LDA score > 4.0 , $P < 0.05$) comparing NC vs. MC, (C) LDA bar plot comparing LD vs. MC, (D-I) Representative boxplots of six genera illustrating relative abundance changes (*Allobaculum*, *Faecalibaculum*, *Intestinimonas*, *Lachnospiraceae_NK4A136_group*, *Ruminococcus*, *unclassified_Muribaculaceae*). NC, normal control; MC, model control; PC, positive control; LD/MD/HD, low/medium/high- dose. $n = 5$ per group. * and ** denote significant difference ($P < 0.05$ and $P < 0.01$).

3.8 Effects of low GI MRP on SCFA levels

Seven fecal SCFAs—acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and hexanoic acid—were quantified (Fig. 6). Compared with NC, all SCFAs were decreased in MC, with valeric acid and hexanoic acid significantly reduced ($P < 0.05$). PC rats showed increases in most SCFAs except butyric acid and hexanoic acid, with significant elevations in propionic acid and isobutyric acid ($P < 0.05$). In LD, acetic acid was significantly elevated ($P < 0.05$), and other SCFAs trended upward without significance. MD and HD generally showed non-significant increases in most SCFAs (except propionic acid and isobutyric acid).

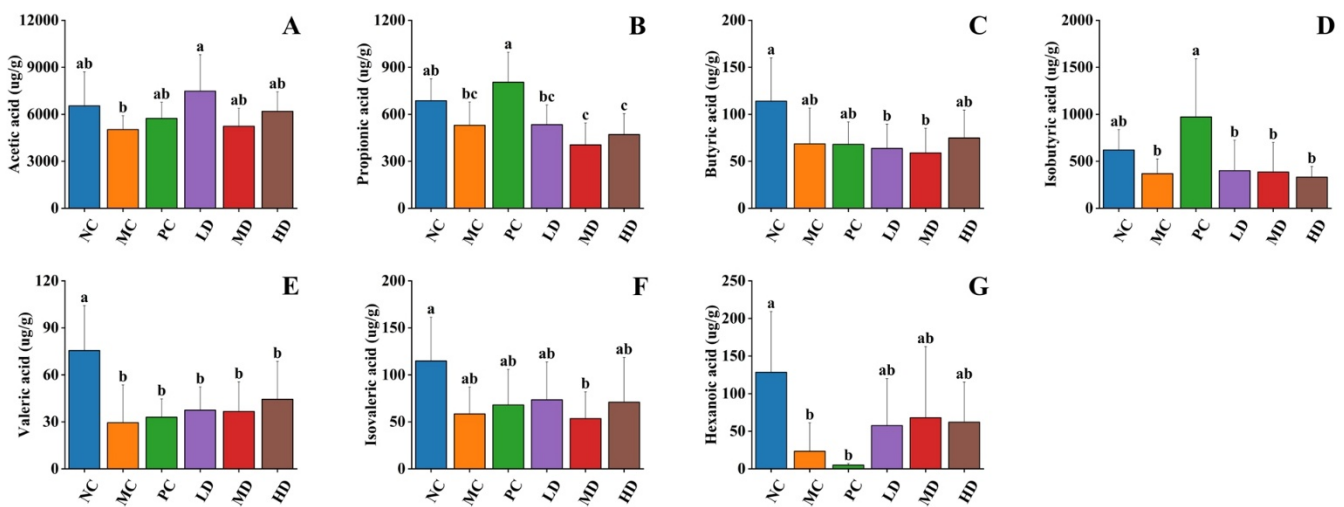


Fig. 6. Effects of low GI MRP on SCFAs levels. (A) Acetic acid, (B) Propionic acid, (D) Isobutyric acid, (D) Valeric acid, (E) Isovaleric acid, (F) Hexanoic acid. NC, normal control; MC, model control; PC, positive control; LD/MD/HD, low/medium/high- dose. $n = 5$ per group. Data are presented as mean $\pm$ SD. Different letters denote significant intergroup differences ($P < 0.05$).

3.9 Correlation analysis among gut microbiota, SCFAs and biochemical parameters

Finally, we established the correlation among the top 20 gut bacterial genera, SCFAs, and T2DM-related biochemical parameters. Spearman correlation analysis revealed that *Bifidobacterium*, *Faecalibaculum*, and *Allobaculum* were positively correlated with metabolic disorder and inflammatory markers (FBG, TC, TG, LDL-C, TNF- α , IL-6) but generally negatively correlated with SCFAs. In contrast, *Intestinimonas* and *Lachnospiraceae_NK4A136_group* exhibited positive correlations with GLP-1, TGF- β , and several SCFAs (butyric acid, valeric acid, isovaleric acid). *Ruminococcus* was also positively correlated with several SCFAs (Fig. 7A). Furthermore, propionic acid, butyric acid, isobutyric acid, and valeric acid were positively correlated with insulin, GLP-1, and TGF- β , but negatively with FBG, lipid parameters, and inflammatory cytokines, suggesting protective effects in metabolic regulation and inflammation attenuation (Fig. 7B).

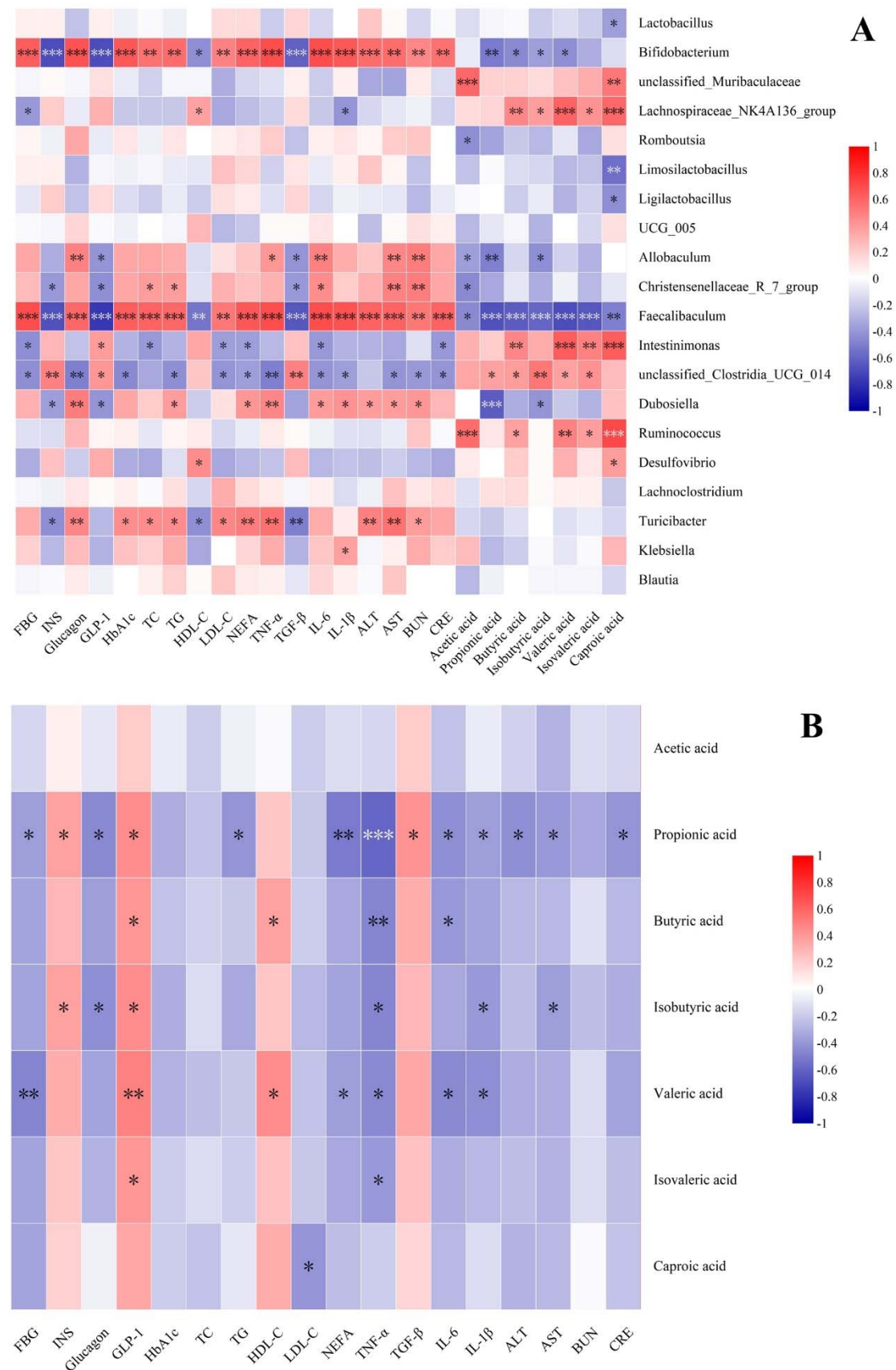


Fig. 7. The correlation analysis of gut microbiota, SCFAs and biochemical parameters. (A) Spearman correlation between top 20 gut bacterial genus and SCFAs/biochemical parameters (B) Spearman correlation between SCFAs and biochemical parameters. The color scale represents the Spearman r value, with red and blue indicating positive and negative correlations. *, ** and *** denote significant difference ($P < 0.05$, $P < 0.01$ and $P < 0.001$).

4. Discussion

In recent years, the development of low GI foods for patients with T2DM has become a key direction in nutritional interventions. Using HFD combined with STZ to induce T2DM in rats, we found that intervention with a low GI scarlet runner bean-based multigrain MRP reduced fasting and long-term glycemic indices, improved the lipid profile and NEFA, attenuated inflammatory responses, and alleviated histopathological injury in the liver, kidney, and pancreas. Concomitantly, the gut microbiota shifted toward putative SCFA-producing taxa and fecal SCFAs increased. Overall, these trends are concordant with prior clinical and population evidence on low GI dietary interventions, supporting the translational potential of the low GI MRP for T2DM management [11, 13, 32].

From a biochemical perspective, the low GI MRP markedly improved insulin resistance, as reflected by increases in INS and HOMA- β relative to the model group. GLP-1 is an enteroendocrine hormone that promotes β -cell insulin secretion and modulates postprandial glycemia by delaying gastric emptying and slowing carbohydrate absorption, while HbA1c is a key index of long-term glycemic control; both are closely linked to the development and progression of T2DM [33, 34]. In our study, the low GI MRP increased GLP-1 and lowered HbA1c, thereby ameliorating glucose dysregulation in T2DM rats. Elevated NEFA accelerates T2DM progression by impairing insulin signaling, activating pro-inflammatory pathways such as NF- κ B and JNK, and inducing lipotoxic β -cell dysfunction [35, 36]. Here, the high-dose MRP effectively reversed dyslipidemia by reducing NEFA, TC, TG, and LDL-C and increasing HDL-C in serum. Inflammation was likewise mitigated in the medium- and high-dose groups—TNF- α , IL-6, and IL-1 β decreased while TGF- β increased—consistent with improvements in hepatic/renal function and tissue pathology. Based on formulation principles and the coherence of our results, these benefits likely derive from the composite matrix of the low GI MRP, which is rich in soluble dietary fiber, resistant starch, and a legume/whole-grain base complemented by flaxseed and wolfberry; these components have previously been supported by high-quality evidence for optimizing glucose-lipid metabolism and alleviating inflammation [22–27].

An apparent discrepancy—clear improvements in multiple glycemic endpoints while the OGTT did not reach significance within the set timeframe—may be explained by features of the HFD + STZ model. Low-dose STZ compromises β -cell secretory capacity, and together with HFD-induced insulin resistance, the first-phase insulin response becomes the earliest and most vulnerable to impairment [28, 37]. In parallel, reductions in chronic low-grade inflammation and lipotoxicity (NEFA load), as well as restoration of insulin sensitivity, tend to accumulate over time and are more readily captured by “slow” indices such as FBG and HbA1c within a limited intervention window [38, 39]. It is therefore plausible that FBG and HbA1c improved earlier, whereas postprandial responses like OGTT required a longer duration or greater power to achieve significance, in line with prior reports [40, 41]. This interpretation is restricted to the model characteristics and measurement differences, future studies with longer timelines or larger sample sizes are warranted.

Diversity of the gut microbiota is typically lower in T2DM than in healthy controls [42], and the low GI MRP partially reversed this trend, consistent with prior evidence from low GI animal and human studies [13, 43].

Differential taxa in our study showed clear directionality. At the phylum level, the MRP at all doses counteracted the reduction of *Patescibacteria* observed in the model group. However, *Patescibacteria* is of very low abundance in the human gut, and conventional 16S primers have limited detection, current T2DM cohort data lack reproducible conclusions on its direction of change; only small studies in type 1 diabetes have suggested decreases [44, 45]. At the genus level, we observed an overall rise in SCFA-related taxa and a decline in potentially unfavorable taxa. First, *Intestinimonas* and *Lachnospiraceae_NK4A136_group* increased: the former can convert lysine/fructosyl-lysine into butyrate and has been linked to metabolic improvement in cohorts and animal interventions, while the latter is repeatedly reported as a butyrate-associated subgroup [46–48]. Second, *Ruminococcus* also increased, but its species-level heterogeneity warrants emphasis: *R. bromii* is a keystone in resistant-starch ecosystems and has been associated with improvements in tolerance and insulin sensitivity along with SCFA increases in human interventions [49], whereas *R. gnavus* is more often linked to metabolic syndrome, T2DM, and inflammation [50]. Third, *Allobaculum* and *Faecalibaculum* decreased. In HFD models, *Allobaculum* often rises with dyslipidemia and correlates positively with intestinal ANGPTL4 expression, although in some interventions it declines in parallel with reduced inflammation/obesity [51]. For *Faecalibaculum*, high-sugar diets can expand *F. rodentium* and displace Th17-inducing commensals, aggravating metabolism; yet in certain interaction contexts, reductions in fasting glucose have been reported [52, 53]. Fourth, the overall rise in fecal SCFAs (with acetate significantly elevated in LD) was directionally consistent with the recovery of acid-producing taxa. Building on this, our correlation analyses provided internal consistency across the microbiota–SCFA–biochemistry triad: on one hand, several SCFA-related genera correlated negatively with FBG, lipids, and inflammatory factors and positively with GLP-1, INS, and HOMA- β ; on the other, most SCFAs displayed concordant associations with biochemical indices.

An interesting feature of our data is that dose-response patterns were not fully concordant: low and medium doses were more sensitive for certain microbial measures and acetate, whereas medium and high doses more readily achieved significance in clinically relevant endpoints such as HbA1c, lipids, NEFA, and inflammation. This may reflect structure- and dose-specific responses to fiber and resistant starch as microbial fermentation substrates. Human dose-response trials indicate that distinct resistant-starch structures can differentially bias propionate versus butyrate production and do not necessarily conform to a linear “more is better” assumption [54]. Responses also vary markedly across individuals: supplementation with resistant starch 2 has yielded clear butyrate and taxa shifts in some participants, whereas others show low or no response [55]. Moreover, most SCFAs are rapidly absorbed in the colon; circulating rather than fecal SCFAs are more consistently associated with GLP-1 and insulin sensitivity. Thus, reliance on fecal SCFAs alone may underestimate their relationships with host endpoints [56, 57].

This study has several limitations. First, the 8-week intervention limits inference on long-term effects and causality. Second, the microbiota and SCFA analyses were performed in a subset of five rats per group, and animals were group-housed by treatment throughout the study. Although no cage contained animals from

different experimental groups, coprophagy and other cage-level influences may reduce inter-individual variation and introduce cage effects; because of the limited number of cages, we did not explicitly model cage as a random effect. Accordingly, the microbiota and SCFA findings should be regarded as exploratory and hypothesis-generating. Third, SCFAs were quantified only in feces, and circulating SCFAs and gut-barrier markers were not measured, which limits our ability to directly link gut microbial shifts to systemic SCFA exposure and intestinal barrier function. Fourth, we did not perform UPLC-based profiling of individual phenolic and other bioactive compounds in the low GI MRP, which restricts mechanistic attribution of the observed effects to specific constituents. Future work should extend the intervention duration, increase the sample size and number of cages for microbiota and SCFA analyses, explicitly account for cage-level effects or use metabolic cage housing where appropriate, and incorporate measurements of circulating SCFAs, gut-barrier indices and detailed chemical profiling of the MPR to strengthen the mechanistic and physiological interpretation of the gut microbiota–SCFA–host axis.

5. Conclusion

In conclusion, this study demonstrated that a low GI scarlet runner bean-based multigrain meal replacement powder exerts multifaceted benefits in T2DM rats. The intervention improved glycemic and lipid metabolism, reduced systemic inflammation, and alleviated hepatic, renal, and pancreatic injury, while simultaneously reshaping the gut microbiota and enhancing SCFA production. These findings suggest that the beneficial effects of the formulation may be mediated through a gut microbiota–SCFA–host metabolic axis. Taken together, our results provide mechanistic and preclinical evidence supporting the translational potential of this low GI functional food as an adjunctive strategy for the prevention and management of T2DM.

Conflict of interest

The authors report there are no competing interests to declare.

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Ethics approval statement

The animal study complied with all relevant ethical regulations and approved by the Institutional Animal Care and Use Committee of Southeast University (Approval No. 20240520002).

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