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A randomized, double-blind, placebo-controlled trial of a botanical-based dietary supplement: Regulating postprandial blood glucose by reshaping gut microbiota and metabolism in prediabetic adults

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ABSTRACT: Prediabetes represents a critical and reversible window before the onset of type 2 diabetes, yet effective and patient-friendly dietary interventions are urgently needed. This randomized, double-blind, placebo-controlled trial investigated the efficacy of two botanical-based dietary supplements (formula A and formula B) in regulating postprandial blood glucose through gut microbiota modulation in prediabetic individuals. Sixty participants were assigned to formula A, Formula B, or placebo groups for an 8-week intervention. Both intervention groups exhibited significant reductions in fasting plasma glucose. Serum levels of carboxymethyllysine were also significantly decreased in both groups. Furthermore, Formula A demonstrated additional benefits in improving β -cell function. Continuous glucose monitoring revealed that both formulas significantly reduced the maximum postprandial glucose increment after breakfast, with formula B additionally shortening the time to peak. Serum metabolomic analysis indicated that Formula A primarily modulates lipid metabolism and microbe-host co-metabolism, whereas Formula B significantly perturbs amino acid and phospholipid pathways, particularly involving tryptophan-kynurenine metabolism. Gut microbiota analysis demonstrated that both interventions promoted the enrichment of beneficial gut microbes, such as *Bacteroides*. Moreover, the Formula B group showed a significant reduction in the abundance of potentially pathogenic genera, including *Escherichia*, *Citrobacter*, and *Collinsella*. Functional predictions indicated enrichment of propionate fermentation and glycan degradation pathways following both interventions. In conclusion, formula B intervention ameliorates prediabetic symptoms, which is associated with gut microbiota remodeling and altered metabolite profiles. These findings suggest that targeted botanical dietary supplements are promising dietary strategies for prediabetes management by simultaneously modulating gut microbiota and metabolic pathways.

Keywords: Prediabetes; Botanical-based dietary supplement; Postprandial glucose; Metabolome; Gut microbiota; Insulin resistance; Functional food.

1. Introduction

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Prediabetes—fasting glucose is 5.6 ~ 6.9 mmol/L or 2-h OGTT 7.8 ~ 11.0 mmol/L—heralds a reversible yet high-risk window before type 2 diabetes mellitus (T2DM) [2]. Modeling by the International Diabetes Federation suggests that >70% of the 635 million adults currently with impaired glucose regulation may convert to T2DM within a decade, doubling cardiovascular events and raising all-cause mortality 1.13-fold [3-5]. Lifestyle modification is the first-line intervention, but adherence falls below 25% after 12 months, while metformin causes gastrointestinal intolerance in approximately 30% of users [6-8]. Therefore, there is an urgent need for safer, more palatable, and patient-friendly dietary strategies or natural supplements that can improve adherence and glycemic control.

Insulin resistance, a hallmark of overt diabetes, emerges as early as the prediabetic stage and is mechanistically linked to chronic endotoxemia, which is driven by gut dysbiosis and impaired intestinal barrier integrity [9-10]. Pathologically, gut dysbiosis (e.g., an elevated *Firmicutes/Bacteroidetes* ratio) increases Gram-negative bacteria and lipopolysaccharide (LPS) production, while zonulin-mediated disruption of tight junctions enhances intestinal permeability. This facilitates systemic LPS translocation, activating the TLR4/NF- κ B pathway, promoting proinflammatory macrophage polarization, and triggering cytokine release (e.g., TNF- α , IL-6). These events ultimately impair insulin signaling (e.g., the IRS-1/PI3K/AKT axis), culminating in insulin resistance [11].

Botanical compounds hold great promise for metabolic health, but their translation into reliable dietary supplements faces several major hurdles: (1) poorly defined active compositions; (2) significant batch-to-batch variability; (3) limited generalizability from pre-clinical models to humans; and (4) a lack of rigorous human clinical trials [12-15].

To address these challenges, we developed two standardized botanical blends [Formula A (FA) and Formula B (FB)] using ingredients exclusively from China's "medicinal and edible homology" list and manufactured under strict Good Manufacturing Practices. This ensures not only safety and consistency (with chromatographic fingerprints showing < 5% batch variation) but also a regulatory status as dietary supplements (Generally Recognized as Safe or equivalent), making them accessible, convenient, and free from the adherence hurdles associated with pharmaceuticals. Here, we present the first randomized, double-blind, placebo-controlled trial evaluating these novel food-derived formulations.

The two formulas were designed based on principles of traditional dietary therapy. FA ("heat-induced fluid depletion" pattern) combines black tea polysaccharides (α -glucosidase/ α -amylase inhibition), resistant dextrin (endotoxin and cytokine reduction), mulberry leaf (PI3K-GLUT4 activation), *Cyclocarya paliurus* (*Faecalibacterium* enrichment), Rhizoma Polygonati Odorati (IRS1/PI3K/AKT amplification), cinnamon (restraint of hepatic gluconeogenesis), and sea buckthorn (IL-6/TNF- α suppression) [16-21]. FB ("qi-yin dual deficiency" pattern) merges *Pueraria* isoflavonoids (PI3K/AKT potentiation), ginsenoside Rb1/Rg1 (β -cell preservation), glycyrrhizin (AMPK activation), and sugar-cane polyphenols (SGLT1/GLUT2 antagonism) [22-25].

Using continuous glucose monitoring, untargeted metabolomics, and gut microbiome analysis, we aimed to: (1) evaluate the efficacy of these food-grade supplements in improving postprandial glucose regulation; (2) identify associated changes in the gut microbiota and host metabolism; and (3) elucidate the potential microbiota-metabolite pathways underlying their beneficial effects. By integrating multi-omics technologies with the principles of medicinal and edible homology, this study provides a scientific foundation for developing evidence-based, targeted functional foods for the dietary management of prediabetes.

2. Materials

2.1 Study design

This randomized, double-blind, placebo-controlled clinical trial was conducted at the Institute of Nutrition and Health, Qingdao University, between November 1, 2023, and May 30, 2024. The study enrolled 60 participants diagnosed with prediabetes from Weihai, China. Participants were randomly allocated in a 1:1:1 ratio to receive either FA group, FB group, or placebo, with an 8-week intervention period. All participants completed three scheduled clinical visits at baseline, week 4, and week 8. The study flowchart is presented in Fig. 1.

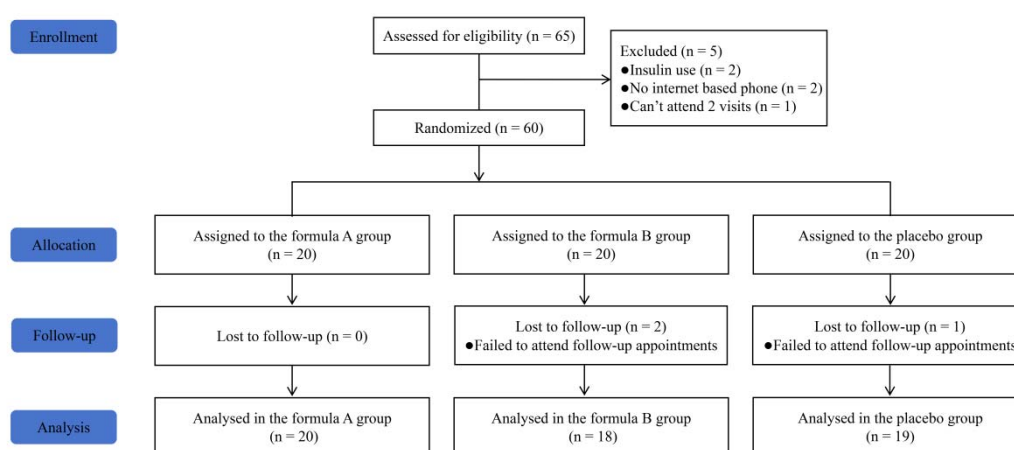


Fig. 1. Study flow diagram: Selection process of randomized controlled trials.

The study protocol received ethical approval from the Institutional Review Board of Qingdao University Medical School (Approval No. QDU-HC-2023201) and was registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR2300076388). The trial was conducted in full compliance with the Declaration of Helsinki principles, International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines, and relevant Chinese regulatory requirements. Written informed consent was obtained from all participants prior to initiation of any study procedures.

2.2 Participants

Patients with prediabetes (diagnosed within 12 months) aged between 30 and 60 years were eligible for participation if they met one of the following criteria: (1) a fasting plasma glucose level of 6.1 ~ 6.9 mmol/L, with a 2-hour post-load glucose level of < 7.8 mmol/L after a 75-g oral glucose tolerance test (OGTT); or (2)

a fasting plasma glucose level of < 7.0 mmol/L, with a 2-hour post-load glucose level of $7.8 \sim 11.0$ mmol/L after a 75-g OGTT.

Exclusion from the study was contingent upon meeting any of the following criteria: (1) a diagnosis of type 1 or type 2 diabetes mellitus; (2) renal impairment [eGFR (estimated Glomerular Filtration Rate) < 60 mL/min/1.73 m²] (3) active gastrointestinal disorders (e.g., inflammatory bowel disease or celiac disease); (4) current pregnancy or tobacco use; (5) significant lifestyle modifications (e.g., dietary or exercise changes) within the previous 3 months.

2.3 Study endpoints

The pre-specified primary endpoint of this trial was the between-group difference in the change in fasting plasma glucose (FPG) from baseline to week 8. Key secondary endpoints were assessed at week 4 and were predefined to elucidate the interventions' effects across complementary physiological domains. These included standard metabolic biomarkers such as 1,5-anhydroglucitol (1,5-AG), carboxymethyllysine (CML), fructosamine (FRU), glycated hemoglobin (HbA1c), insulin, β -cell function index (HOMA- β) and attenuated the insulin resistance index (HOMA-IR). We also assessed markers of intestinal permeability and systemic inflammation, including LPS, LPS-Binding Protein (LBP), zonulin, and C-reactive protein (CRP). Dynamic glycemic profiles were derived from continuous glucose monitoring (CGM) and comprised the glucose incremental area under the curve (AUC), glucose peak (GP), maximum glucose rise (Max GR), and time to peak (TP). Furthermore, serum metabolomic profiles and multi-omics signatures encompassing gut microbiota composition, were characterized to explore underlying mechanisms.

2.4 Sample size

Sample size determination was based on serum CML levels, an established biomarker of advanced glycation end products that correlate significantly with glucose intolerance and diabetic complications [26, 27]. Power calculations indicated that 18 participants per group would provide 80% power ($\alpha = 0.05$, two-tailed) to detect a $0.4 \mu\text{mol/L}$ difference in CML (20% reduction from baseline, SD = $0.4 \mu\text{mol/L}$) [28]. Accounting for a 15% attrition rate, we enrolled 60 eligible adults aged 30 ~ 60 years with prediabetes.

2.5 Randomization and blinding

After verifying participant eligibility according to the predefined inclusion criteria, independent statisticians at each medical center employed an online centralized randomization system to generate unique randomization codes for individual patients. The system allocated randomization sequences based on the order of patients' baseline visits and their corresponding medical centers. These sequences were kept strictly confidential for the duration of the study. Research personnel remained blinded to group assignments unless serious adverse events arose, which warranted unblinding.

The trial adhered to a double-blind protocol, meaning that both participants and investigators were unaware of treatment allocations until the conclusion of the study. Each placebo was meticulously matched to

the active supplement in terms of size, appearance, and taste. All investigational products were uniformly packaged and supplied to the participating centers. Upon study completion, the centralized randomization system disclosed each participants assigned number and group assignment. In cases of serious adverse events, the system administrator could promptly reveal the patients' group allocation to enable appropriate clinical management.

2.6 Product composition

All test products (including FA, FB, and placebo) were manufactured by Infinitus (China) Company Ltd. in strict compliance with GMP standards. FA was produced under batch number 230908, and FB under batch number 230911. FA contained black tea (Fuzhuan tea), resistant dextrin, *Cyclocarya paliurus* leaf extract, mulberry leaf extract, *Rhizoma Polygonati Odorati* (Fragrant Solomonseal Rhizome; Yuzhu) extract, cinnamon extract, and sea buckthorn fruit powder. FB included *Pueraria* root, loquat leaf, liquorice root, ginseng, sugarcane polyphenols. All active formulations and the placebo contained standardized amounts of erythritol and steviol glycosides in L-malic acid as flavor-masking excipients, while the placebo control consisted exclusively of this excipient base to maintain blinding integrity across study groups. The final products were prepared as oral solutions. The complete ingredient lists and concentration ranges for the FA and FB formulations are provided in Supplementary Table 1.

In this study, ultra-high performance liquid chromatography coupled with Quadrupole Time-of-Flight mass spectrometer was employed to identify the major constituents of the two formulations. A total of 36 compounds were characterized (Supplementary Table 2), including 19 flavonoids (e.g., rutin, quercetin, isovitexin, kuwanon C, and daidzin), 10 phenolic acids (e.g., gallic acid, catechin hydrate, epicatechin, chlorogenic acid, and neochlorogenic acid), 2 alkaloids (e.g., caffeine and gramine), and 5 other compounds.

2.7 Intervention and data collection

Participants were instructed to take the assigned product twice daily (once with breakfast and once with dinner), with each administration consisting of 3 bottles (30 mL). During the trial, 3 participants withdrew, and 57 completed the full 8-week intervention period. Postprandial blood glucose parameters were measured using a CGM (Sinocare i3) worn for 72 hours (3 consecutive days) at baseline and week 4; concurrent blood and stool specimens were obtained for downstream analyses. Fasting blood glucose was additionally measured at baseline and week 8.

Prior to intervention initiation, all participants underwent comprehensive baseline evaluations comprising: (1) demographic and anthropometric measurements (age, height, weight, blood pressure, and heart rate); (2) standardized lifestyle questionnaires assessing exercise frequency, intensity and duration; and (3) detailed dietary evaluation through three consecutive 24-hour dietary recalls (including two weekdays and one weekend day) coupled with food frequency questionnaires to establish habitual nutritional intake patterns. All assessments were conducted by trained personnel using calibrated equipment following standardized protocols.

During CGM, the onset and peak of postprandial glycemic responses were manually annotated for each participant through visual inspection, following the methodology established by Barua et al.^[29]. For each postprandial segment, five key parameters were derived: Starting Glucose (the baseline value at meal commencement); AUC (the positive area under the curve with baseline subtraction); GP (the maximum postprandial concentration); Max GR (the peak-to-baseline difference in mg/dL); and TP (the time in minutes from meal onset to glycemic peak). These metrics collectively enabled systematic evaluation of postprandial glucose excursions. Additionally, we recorded episodes of postprandial glucose crashes, defined as any glucose readings that fell below the pre-meal baseline levels.

2.8 Blood collection and analysis

After a 12-hour overnight fast, venous blood samples (10 mL per participant) were obtained at baseline and at the end of week 4. Collected samples were centrifuged at 3000 rpm for 15 minutes, and serum aliquots were promptly preserved at -80 °C pending subsequent analysis. Biochemical assessments encompassed the following categories: (1) glycemic indicators [1,5-AG, CML, FRU, HbA1c and insulin(INS)]; (2) markers of gut barrier integrity and systemic inflammation [LPS, LBP, zonulin, and CRP]; (3) hepatic function parameters [serum aminotransferases, alkaline phosphatase, bilirubin, and albumin]; and (4) lipid metabolism profiles [triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol]. Measurements were carried out using a Hitachi 3110 automated analyzer (Hitachi Instrument, Suzhou) in conjunction with commercial ELISA kits (Human 1,5-AG/CML/HbA1c/LPS/LBP ELISA Kits, Xiamen Huijia Biotechnology Co., Ltd.) in accordance with manufacturer instructions. All assays were performed in duplicate to enhance result reliability.

For untargeted metabolomic profiling, 50 µL of serum was combined with 250 µL of ice-cold acetonitrile under ambient temperature conditions, and then incubated on ice for 15 minutes. Subsequent to centrifugation at 15,000 rpm for 10 minutes at 4 °C, the supernatant was filtered via a 0.22 µm organic membrane and transferred into a sampling vial. A pooled quality control (QC) sample was created by combining equal 2 µL aliquots from all individual samples. A 1290 infinity ultra-performance liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) was used to achieve metabolic separation. To keep track of instrumental stability during liquid chromatography-mass spectrometry (LC-MS) analysis, the QC sample was injected repeatedly after every six experimental samples. An Agilent 6530B Q-TOF mass spectrometer, equipped with an iFunnel electrospray ionization source running in positive ion mode, was employed for MS data acquisition. Key source parameters were configured as follows: gas temperature 325 °C, drying gas flow 8 L/min, nebulizer pressure 35 psig, sheath gas temperature 350 °C, sheath gas flow 11 L/min, and fragmentor voltage 175 V. Full-scan mass spectra were collected across m/z 50 – 1700 at an acquisition rate of 2 spectra per second. Real-time mass calibration was maintained using reference ions at m/z 121.0509 and 922.0098. Data were all obtained in centroid mode, followed by conversion from the native (.d) format to mzXML and mgf (for QC obtained MSMS spectrogram) by MS-Convert. Subsequent data processing, including peak picking, alignment, gap filling, and metabolite annotation, was conducted with EasyMetaR development by

our group (<https://github.com/QSprobLab/EasyMetaR>). Orthogonal partial least squares-discriminant analysis (OPLS-DA) and peak table preprocessing were carried out using IP4M software^[30]. To identify differentially abundant metabolites, a variable importance in projection (VIP) threshold of over 1.0 from the OPLS-DA model was used. The comparisons included pre- versus post-intervention metabolic profiles within the FA and FB groups, and metabolic profiles between the intervention groups and the placebo group at the conclusion of the trial.

2.9 Stool collection and 16S rRNA amplicon sequencing

Instructions were given to participants to gather 5 ~ 10 g of fecal samples in 30-mL sterile screw-cap containers, with temporary storage in a cooling box or a –20 °C freezer. Within five hours, all samples were collected and subsequently placed in a –80 °C freezer for long-term storage. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene from the fecal microbiota were amplified and sequenced by Qingdao Biomarker Technologies Co., Ltd. Amplification was performed using barcoded primers: forward primer 5'-ACTCCTACGGGAGGCAGCA-3' and reverse primer 5'-GGACTACHVGGGTWTCTAAT-3'. The raw sequencing reads have been deposited in the NCBI Sequence Read Archive under accession number PRJNA1290893.

Bioinformatic processing of the gut microbiota data was performed using EasyAmplicon v1.14. Taxonomic assignment of representative amplicon sequence variants (ASVs) was conducted with the BLCA algorithm, referencing the NCBI 16S Microbial database, and processed via the `subset_db_acc.py` script from the BLCA package^[31]. Additional ecological and statistical analyses were carried out through the MicrobiomeAnalyst web platform^[32]. Microbial functional potential was inferred from 16S data using PICRUSt2 (v2.2.0-b) to predict KEGG ortholog abundances. Differential abundance analysis of taxa and functional pathways was evaluated using STAMP software.

2.10 Safety assessment

To assess safety, venous blood samples were collected after an overnight fast at baseline and post-intervention (4 weeks). Hematological parameters were measured using the Sysmex XT-4000i automated hematology analyzer, while clinical chemistry, hepatic, and renal function markers were assessed using the Hitachi 7180 automated biochemical analyzer. Participants were also asked to report any adverse events throughout the study, and their health status was monitored at each study visit to ensure the safety and tolerability of the intervention.

2.11 Statistical analysis

The primary statistical analyses were performed using GraphPad Prism (v9.3) and IBM SPSS Statistics (v23.0) for inferential tests. We conducted additional analyses (multiple imputation, correlation, visualization) in R (v4.2.1) using the following packages: `mice` (v3.17.0), `psych` (v2.5.4), `pheatmap` (v1.0.13), `dplyr` (v1.1.4), `ggplot2` (v3.5.1), and `ggrepel` (v0.9.6).

Study parameters were expressed as mean and standard deviation (SD). For variables with >5% missingness, random forest-based multiple imputation (10 iterations) was implemented using the mice R package, with sensitivity analyses confirming the robustness of the results. Within-group comparisons of normally distributed data were analyzed using paired t-tests. For between-group comparisons, one-way ANOVA was applied to data satisfying normality and homogeneity of variance assumptions, Welch's test was used for data with normal distribution but unequal variances, and the Kruskal–Wallis test was employed for non-normally distributed data. The Bonferroni method was used to adjust P-values for multiple comparisons and control the false discovery rate. Subgroup analyses were performed using the dplyr package. Relationships among glucose-related parameters, differentially abundant gut microbes, and serum metabolites were assessed using Spearman's rank correlation analysis via the psych package, with results visualized as a heatmap using pheatmap. Differential metabolite analysis and visualization were conducted using the dplyr, ggrepel, and ggplot2 packages. Beta-diversity differences were examined via principal coordinates analysis (PCoA) and statistically evaluated by permutational multivariate analysis of variance (PERMANOVA). Differential abundance testing between groups was performed using STAMP (Welch's t-test). Statistical significance was set at a two-tailed *P* value of < 0.05.

3. Results

3.1 Baseline characteristics of patients

Between November 1, 2023, and December 31, 2023, 65 individuals contacted the research team via telephone or email to express interest in participation. They were subsequently invited to attend a screening visit to determine their eligibility according to the established inclusion and exclusion criteria. Of these individuals, 5 (7.69%) were excluded and the reasons for this decision were recorded. The remaining 60 participants were randomly assigned to groups, and 57 (95.00%) completed the study. No significant differences in anthropometric or baseline characteristics were observed among the three groups (Table 1).

Table 1. Baseline characteristics of the participants.

Items	FA (n = 20)	FB (n = 18)	PL (n = 19)
Age (year)	56 ± 4	53 ± 5	56 ± 6
Number of males/females	11/9	11/7	7/12
BMI (kg/m ²)	25.8 ± 3.3	25.7 ± 3.8	27.1 ± 2.4
SBP (mmHg)	139.5 ± 22.7	149.2 ± 14.3	139.4 ± 13.8
DBP (mmHg)	87.3 ± 10.3	92.3 ± 11.3	88.1 ± 7.9
HR (times/min)	73 ± 11	74 ± 16	76 ± 8
FPG (mmol/L)	6.6 ± 0.4	6.5 ± 0.3	6.6 ± 0.4

FA, formula A group; FB, formula B group; PL, placebo group; BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; HR: heart rate; FPG: fasting plasma glucose; Data are presented as mean ± SD.

3.2 FA and FB improve fasting glycemia and key metabolic biomarkers

Comprehensive biomarker profiling was conducted to systematically evaluate the glycemic effects of the two plant-derived formulations (FA and FB) versus placebo. Critically, the trial met its primary endpoint: after the 8-week intervention, both FA and FB groups demonstrated significant reductions in FPG compared to the

placebo group (Fig. 2G; FA, $P < 0.001$; FB, $P < 0.01$). Specifically, the FA group exhibited a mean reduction in FPG of 6.3% (range: -4.8% to 15.7%), with 85% of participants showing a decrease from baseline. The FB group showed a mean reduction of 4.6% (range: -4.5% to 15.5%), with 61% of participants experiencing a decrease. In stark contrast, the placebo group displayed a mean increase in FPG of 2.9%, with 63% of participants showing an increase and 11% exhibiting no change.

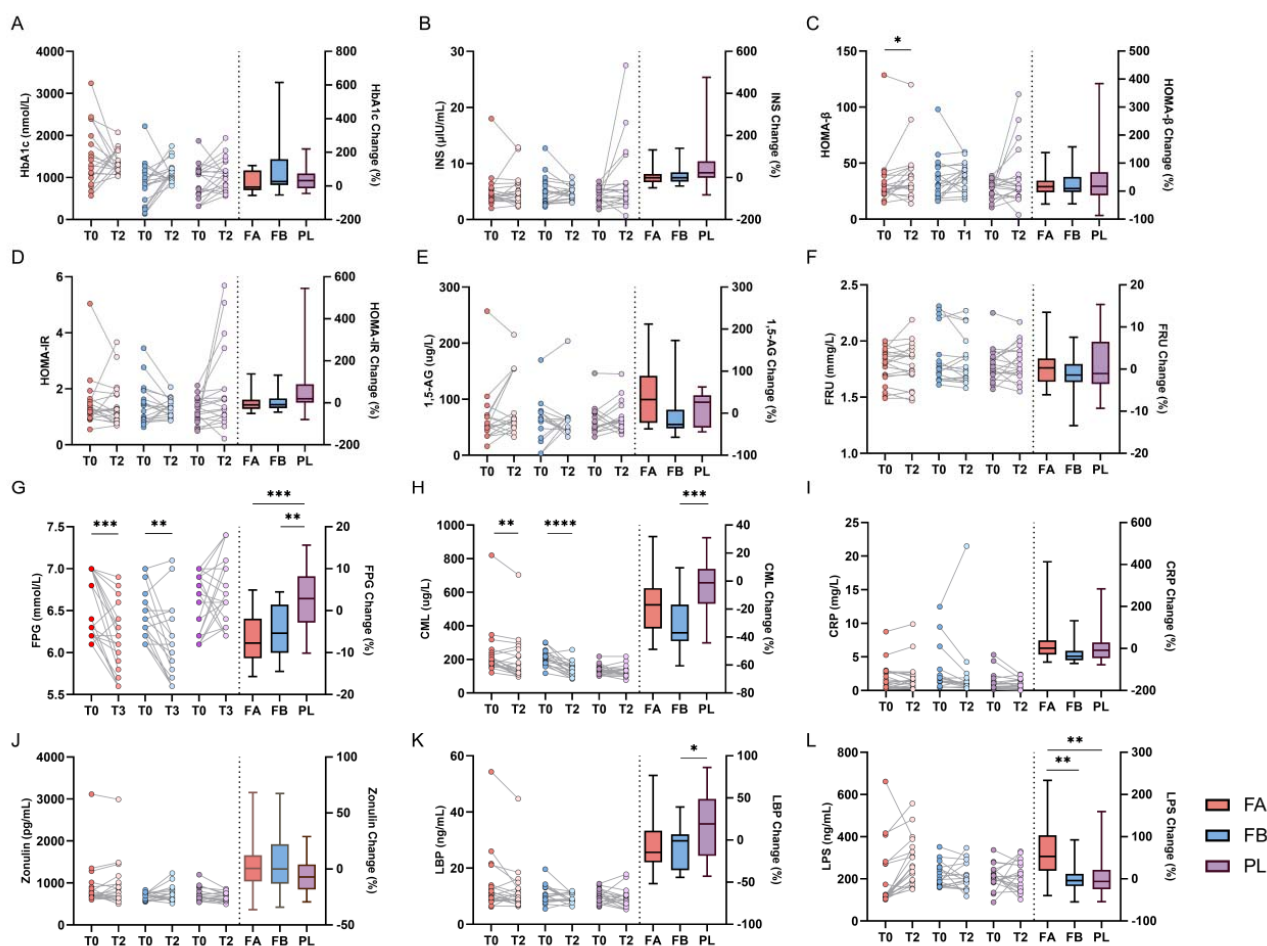


Fig. 2. Changes in parameters of glucose homeostasis, insulin sensitivity, and metabolic endotoxemia. (A) Serum level of glycated hemoglobin (HbA1c). (B) Serum insulin level (INS). (C) HOMA- β value: Homeostasis model assessment-beta. (D) HOMA-IR value: Homeostatic model assessment of insulin resistance. (E) Serum 1,5-anhydroglucitol level (1,5-AG). (F) Serum fructosamine level (FRU). (G) Fasting plasma glucose (FPG). (H) Serum carboxymethyllysine level (CML). (I) serum C-reactive protein level (CRP). (J) Serum zonulin level. (K) Serum lipopolysaccharide binding protein level (LBP). (L) Serum lipopolysaccharide (LPS). The change rates between the two groups were compared using the Mann-Whitney U test ($*P < 0.05$, $**P < 0.01$ and $***P < 0.001$). Change (%) = (week 4 – baseline) / baseline $\times 100\%$. Changes from baseline to weeks 4 and 8 were analyzed using paired t -tests ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$). HOMA- β was calculated as $20 \times \text{fasting insulin } (\mu\text{U/mL}) / \text{fasting glucose } (\text{mmol/L}) - 3.5$; HOMA-IR was calculated as $\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose } (\text{mmol/L}) / 22.5$. FA, formula A group ($n = 20$); FB, formula B group ($n = 18$); PL, placebo group ($n = 19$). T0, baseline; T2, week 4; T3, week 8.

Homeostasis model assessment indicated that FA supplementation significantly elevated HOMA- β from baseline (Fig. 2C; $P < 0.05$), whereas no significant within- or between-group differences were observed for HOMA-IR. Circulating CML concentrations were significantly reduced from baseline in both intervention arms (both $P < 0.01$); upon study completion, the FB group demonstrated a further significant reduction compared to placebo (Fig. 2H; $P < 0.0001$). No significant between-group differences were detected for HbA1c, INS, 1,5-AG, or FRU at the end of the intervention.

3.3 FB and FA differentially modulate markers of metabolic endotoxemia and gut barrier integrity

Given the link between metabolic endotoxemia (from intestinal barrier dysfunction) and insulin resistance [10], we quantified mucosal integrity and inflammation biomarkers. After 4 weeks of intervention, serum CRP and zonulin concentrations did not differ significantly among FA, FB and placebo groups. In contrast, LBP levels were significantly reduced in the FB group compared with placebo (Fig. 2K; $P < 0.05$), while the FA group exhibited a non-significant decreasing trend relative to placebo. Relative to baseline, LBP increased by a mean of 18.69% in the placebo group, whereas the FA and FB groups showed mean reductions of 6.33% and 8.44%, respectively. Conversely, LPS concentrations were significantly elevated in the FA group compared to both the placebo and FB groups (Fig. 2L; both $P < 0.001$).

3.4 Effect of FA and FB on postprandial glucose metabolism assessed by CGM

To rigorously quantify the glycemic impact of the two plant-derived formulations (FA and FB), 72-hour CGM was conducted before and after the intervention. Relative to baseline, a significant reduction in the time to peak postprandial glucose excursion after breakfast was demonstrated following FB supplement intake (Fig. 3G, $P < 0.01$), whereas no discernible alteration was observed following FA supplement intake. The 150-min postprandial incremental area under the curve, peak glucose concentration, and absolute glucose increment were not found to be modified by either FA or FB within groups.

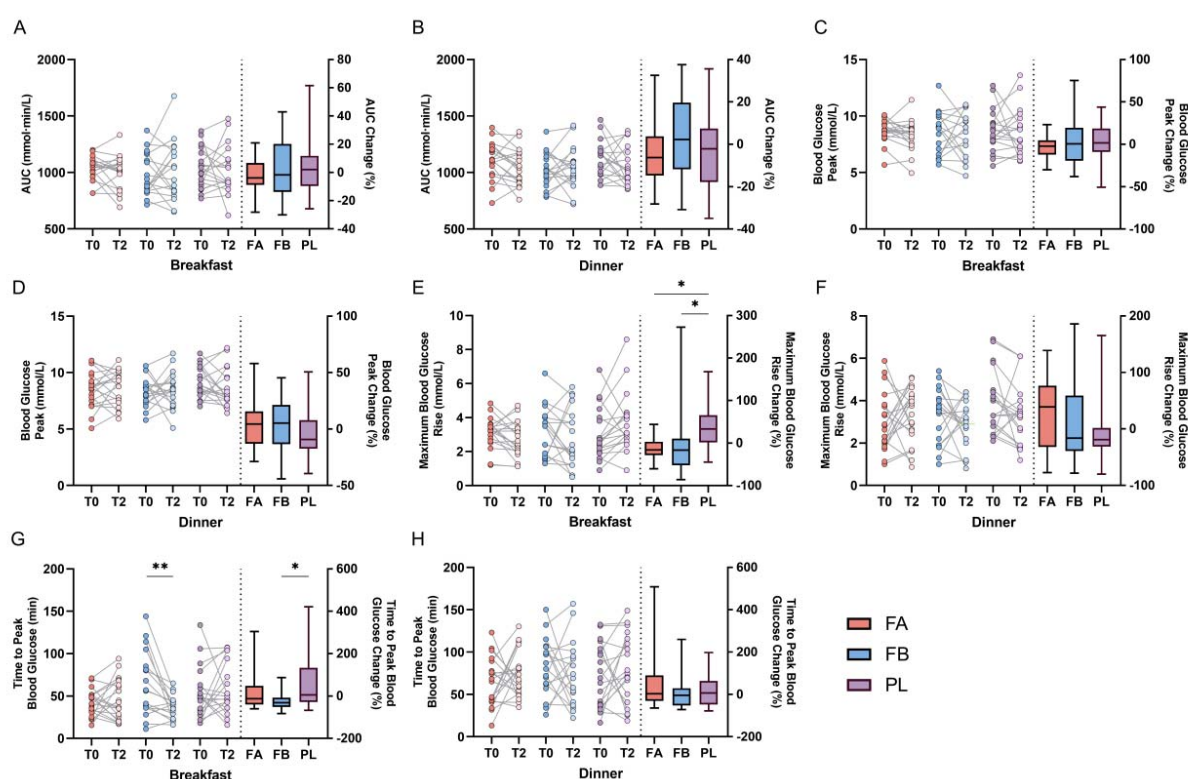


Fig. 3. Changes in CGM-monitored blood glucose-related metrics. (A) Area under curve of 150-minute post-breakfast blood glucose. (B) Area under curve of 150-minute post-dinner blood glucose. (C) Post-breakfast blood glucose peak. (D) Post-dinner blood glucose peak. (E) Maximum post-breakfast blood glucose increase. (F) Maximum post-dinner blood glucose increase. (G) Time to peak blood glucose post-breakfast. (H) Time to peak blood glucose post-dinner. Changes from baseline to week 4 were analyzed using paired t-tests (** $P < 0.01$). The change rates between the two groups were compared using the Mann-Whitney U test (* $P < 0.05$, ** $P < 0.01$). Change (%) = (week 4 – baseline) / baseline $\times 100\%$. FA, formula A group ($n = 20$); FB, formula B group ($n = 18$); PL, placebo group ($n = 19$); T0, baseline; T2, week 4.

Between-group comparisons further revealed that the maximal postprandial glucose surge was attenuated by both FA and FB relative to placebo (Fig. 3E, both $P < 0.05$). Additionally, the time-to-peak glucose excursion was uniquely shortened by FB compared with placebo (Fig. 3G, $P < 0.05$). Collectively, these findings indicate that a more rapid yet lower-amplitude glycemic response is elicited by FB, thereby underscoring its potential utility for postprandial glucose regulation.

3.5 Untargeted serum metabolomics reveals distinct host and microbial metabolic signatures induced by FA and FB supplementation and their link to glycemic traits

Untargeted metabolomics was performed on serum samples to systematically evaluate the metabolic effects of two phytochemical formulations. OPLS-DA models revealed significant metabolome shifts in both the FA and FB groups following supplementation (Supplementary Figure 1), and their robustness was verified through rigorous validation. Key potential biomarkers were identified based on variable importance in projection (VIP) scores >1 and statistical significance ($P < 0.05$) in the predictive models (Tables 2 and 3). To identify potential biomarkers, we first selected features with variable importance in projection (VIP) scores > 1.0 and $P < 0.05$ (Supplementary Tables 3 and 4). For a more definitive identification of differentially abundant metabolites, we performed volcano plot analysis with stringent criteria: $VIP > 1.0$, $|\log_2(\text{fold change})| > 2$, and statistical significance adjusted using the Benjamini-Hochberg false discovery rate (FDR) method (Fig. 4; Supplementary Tables 5-8).

Table 2. Serum metabolite differences and dynamic changes in FA and FB groups versus baseline.

Metabolite	FA vs.FABL		FB vs.FBBL	
	VIP	LFC	VIP	LFC
2-Hydroxybenzaldehyde	1.07	-6.74	1.64	-11.48
3-Hydroxy-cis-5-tetradecenoylcarnitine	1.39	2.63	1.04	0.72
3-Methylglutaryl carnitine	1.15	-3.37	1.08	-4.01
Cer(d18:0/18:0)	1.14	-12.05	1.43	-15.20
Cer(d18:0/23:0)	1.41	-7.21	1.52	-7.81
Decanoylcarnitine	1.45	1.61	1.22	0.47
gamma-Glutamyltryptophan	1.40	1.57	1.11	0.42
MG(0:0/16:1(9Z)/0:0)	1.07	-1.43	1.73	-2.60
Octadecanamide	1.47	1.78	1.23	0.45
Oleoylethanolamide	1.42	1.48	1.15	0.42
Linoleamide	1.47	1.69	1.12	0.41
N-Acetylglutamine	1.40	3.42	1.36	1.37
Palmitoylcarnitine	1.44	1.79	1.42	0.96
PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/20:0)	1.14	-1.57	1.49	-6.67
PE(16:1(9Z)/16:0)	1.48	-5.06	1.66	-8.79
SM(d18:0/16:1(9Z)(OH))	1.32	-8.27	1.46	-7.46
Tyrosyl-Leucine	1.19	-2.32	1.77	-3.10
Urobilin	1.04	-2.84	1.37	-4.16
2-Pentylthiophene	1.28	2.08	-	-
9-Hexadecenoylcarnitine	1.26	1.68	-	-
3-Hydroxybutyrylcarnitine	1.03	1.66	-	-
Dodecanoylcarnitine	1.27	1.17	-	-
Hexanoylcarnitine	1.30	1.64	-	-
L-Cysteine	1.30	2.09	-	-
N-Acetyl-L-methionine	1.12	-13.53	-	-

Metabolite	FA vs.FABL		FB vs.FBBL	
	VIP	LFC	VIP	LFC
Octanoylcarnitine	1.46	1.77	-	-
Pyruvate	1.21	1.88	-	-
Stearoylcarnitine	1.08	-13.60	-	-
L-Tryptophan	1.24	1.39	-	-
LysoPC(18:2(9Z,12Z)/0:0)	1.11	-2.23	-	-
N-(3-acetamidopropyl)pyrrolidin-2-one	1.31	1.30	-	-
PE(22:1(13Z)/14:0)	1.06	-12.34	-	-
Threonylvaline	1.31	1.51	-	-
4-Methylbenzaldehyde	-	-	1.54	-1.37
Cholestenone	-	-	1.50	1.05
D-Tryptophan	-	-	1.82	3.26
Ketoleucine	-	-	1.55	-2.45
3-Hydroxyhexadecanoylcarnitine	-	-	1.42	1.01
L-Kynurenine	-	-	1.72	-3.29
L-Threonine	-	-	1.58	-2.85
LysoPE(14:0/0:0)	-	-	1.40	1.09
LysoPE(15:0/0:0)	-	-	1.16	0.89
MG(20:5(5Z,8Z,11Z,14Z,17Z)/0:0/0:0)	-	-	1.20	0.78
N-Acetyl-L-tyrosine	-	-	1.07	0.82
Octopine	-	-	1.07	2.18
PC(O-16:0/18:0)	-	-	1.01	4.94
PC(O-16:1(9Z)/14:1(9Z))	-	-	1.32	4.01
PG(18:1(11Z)/18:0)	-	-	1.20	2.32

FA: formula A group post-intervention; FABL: formula A group baseline; FB: formula B group post-intervention; FBBL: formula B group baseline; LFC: log₂fold change.

Table 3. Serum metabolite variations in FA and FB groups versus placebo group at week 4.

Metabolite	FA vs.PL		FB vs.PL	
	VIP	LFC	VIP	LFC
2-Pentylthiophene	1.79	2.13	1.96	1.37
9-Hexadecenoylcarnitine	1.44	0.89	1.46	0.60
Octadecanamide	1.47	0.25	1.25	-
Oleoylethanolamide	1.43	0.27	1.59	-
Cer(d18:0/23:0)	1.75	11.91	2.14	10.26
Decanoylcarnitine	1.38	0.24	1.41	-
DG(18:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	1.18	10.76	1.38	-
gamma-Glutamyltryptophan	1.20	0.27	1.29	-
Ketoleucine	1.93	11.57	1.33	8.96
LysoPC(18:2(9Z,12Z)/0:0)	1.16	-1.10	1.01	-
Linoleamide	1.52	0.26	1.08	-
PC(14:1(9Z)/14:0)	1.43	2.73	1.83	2.23
PC(16:0/18:3(6Z,9Z,12Z))	1.15	7.48	1.67	7.06
PC(O-14:0/16:0)	1.78	7.85	1.86	3.67
PE(14:0/16:0)	1.20	4.03	1.67	-
PG(18:1(11Z)/18:0)	1.88	-3.91	2.21	-2.20
TG(16:1(9Z)/20:4(5Z,8Z,11Z,14Z)/O-18:0)	1.16	-18.60	1.14	-
Threonylvaline	2.13	4.06	1.89	3.04
D-Tryptophan	1.62	-1.17	-	-
L-Threonine	1.48	3.41	-	-
LysoPC(14:0/0:0)	1.51	-0.91	-	-
PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/20:0)	1.44	5.50	-	-
PC(O-16:1(9Z)/14:1(9Z))	1.22	-2.03	-	-
N-Acetylglutamine	1.11	0.45	-	-
N-Acetyl-L-methionine	1.53	-14.66	-	-
Stearoylcarnitine	1.59	-15.15	-	-
4-Methylbenzaldehyde	-	-	1.00	-
Octopine	-	-	1.31	1.75

Metabolite	FA vs.PL		FB vs.PL	
	VIP	LFC	VIP	LFC
Palmitoylcarnitine	-	-	1.87	0.48
Tyrosyl-Leucine	-	-	1.30	-

FA, formula A group; FB, formula B group; ; LFC: log2fold change; PL, placebo group.

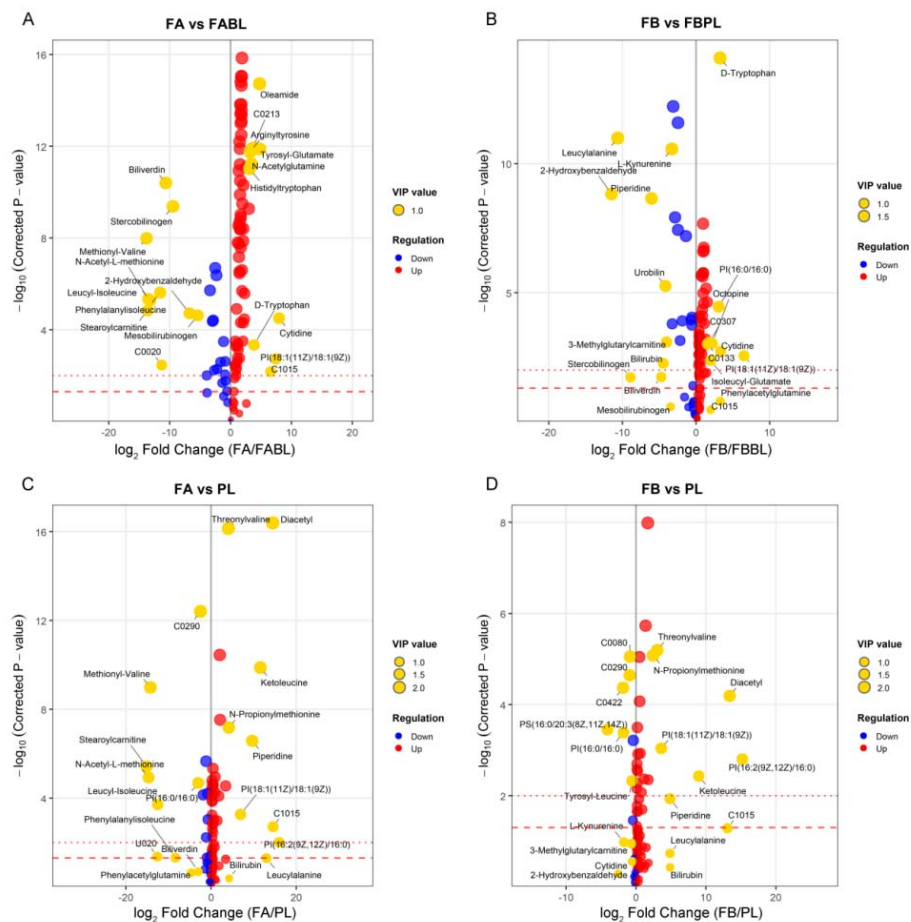


Fig. 4. Volcano plots illustrating differential serum metabolite profiles following FA and FB interventions. (A) Comparison of the FA group before and after intervention. Annotated metabolites: PI(20:4/18:1) (C1015), Dihydro-2(3H)-thiophenone (C0213), 5-Acetylamino-6-formylamino-3-methyluracil (C0020). (B) Comparison of the FB group before and after intervention. Annotated metabolites: PI(20:4/18:1) (C1015), 5b-Cholestane-3a,7a,12a,23S,25-pentol (C0307), 3-Hydroxy-9-hexadecenoylcarnitine (C0133). (C) Comparison between the FA group and the placebo group after intervention. Annotated metabolites: PI(20:4/18:1) (C1015), 5-Acetylamino-6-formylamino-3-methyluracil (U020), PS(18:0/20:4) (C0290). (D) Comparison between the FB group and the placebo group after intervention. Annotated metabolites: PI(20:4/18:1) (C1015), PS(16:0/20:4) (C0422), PS(18:0/20:4) (C0290), 1-Stearoylglycerophosphoserine (C0080). Metabolites with significantly altered levels are highlighted based on the thresholds of $|\log_2(\text{fold change})| > 1$ and a $P\text{-value} < 0.05$. Red dots denote significantly upregulated metabolites and blue dots denote significantly downregulated ones. FA: formula A group post-intervention ($n = 20$); FA_BL: formula A group baseline; FB: formula B group post-intervention ($n = 18$); FB_BL: formula B group baseline; PL: placebo group post-intervention ($n = 19$); PL_BL: placebo group baseline.

In the FA group, supplementation led to a marked reduction in several ceramide species (Cer(d18:0/18:0): VIP = 1.14, $\log_2\text{FC} = -12.05$; Cer(d18:0/23:0): VIP = 1.41, $\log_2\text{FC} = -7.21$), alongside significant increases in short- and medium-chain acylcarnitines (decanoylcarnitine: VIP = 1.45, $\log_2\text{FC} = 1.61$; palmitoylcarnitine: VIP = 1.44, $\log_2\text{FC} = 1.79$). Concurrently, substantial upregulation was observed in microbially modulated metabolites, including cytidine (VIP = 1.05, $\log_2\text{FC} = 7.96$), dihydro-2(3H)-thiophenone (VIP = 1.41, $\log_2\text{FC} = 3.65$), N-acetylglutamine (VIP = 1.40, $\log_2\text{FC} = 3.42$), and several phosphatidylinositols (PI(18:1/18:1): VIP = 0.83, $\log_2\text{FC} = 7.44$; PI(20:4/18:1): VIP = 0.74, $\log_2\text{FC} = 6.58$). In contrast, multiple dipeptides, including

methionyl-valine (VIP = 1.28, $\log_2\text{FC}$ = -13.80) and leucyl-isoleucine (VIP = 1.12, $\log_2\text{FC}$ = -13.19), were markedly downregulated.

The FB group exhibited a distinct metabolic signature, characterized by pronounced upregulation of gut microbiota-derived metabolites such as D-tryptophan (VIP = 1.82, $\log_2\text{FC}$ = 3.26) and phenylacetylglutamine (VIP = 0.48, $\log_2\text{FC}$ = 3.29). This was accompanied by significant phospholipid remodeling, reflected in elevated levels of specific phosphatidylcholine species (PC(24:0/16:0): VIP = 1.37, $\log_2\text{FC}$ = 17.20; PC(O-16:0/18:0): VIP = 1.01, $\log_2\text{FC}$ = 4.94). These changes occurred in conjunction with the downregulation of host-derived amino acid metabolites, including L-threonine (VIP = 1.58, $\log_2\text{FC}$ = -2.85), L-kynurenine (VIP = 1.72, $\log_2\text{FC}$ = -3.29), and leucylalanine (VIP = 1.73, $\log_2\text{FC}$ = -10.64).

Comparative analysis against the placebo group further underscored the intervention-specific nature of these effects. The FA group exhibited significantly lower levels of specific ceramides relative to placebo (Cer(d18:0/23:0): VIP = 1.75, log₂FC = -11.91), whereas the FB group showed elevated levels of microbially influenced metabolites and specific phospholipids (PC(O-16:0/18:0): VIP = 1.65, log₂FC = 7.20). Both interventions shared common features, including upregulation of PI(16:2/16:0) and diacetyl. However, differential effects were noted for metabolites such as ketoleucine, which was downregulated in within-group comparisons but significantly upregulated versus placebo (VIP = 1.93, log₂FC = 11.57).

Collectively, these findings demonstrate that FA and FB elicit distinct, formulation-specific metabolic responses through divergent mechanisms: FA primarily modulates lipid metabolism and microbial–host co-metabolism, whereas FB significantly perturbs amino acid and phospholipid pathways, particularly involving tryptophan–kynurenine metabolism. The consistent patterns observed across multiple comparisons confirm that these metabolic shifts are treatment-specific and not attributable to placebo effects or temporal variation.

Correlation analysis revealed significant associations between distinct serum metabolites and key glycemic parameters, including HbA1c, FPG, INS, advanced glycation end product (CML), and continuous glucose monitoring-derived postprandial metrics (Fig. 8)

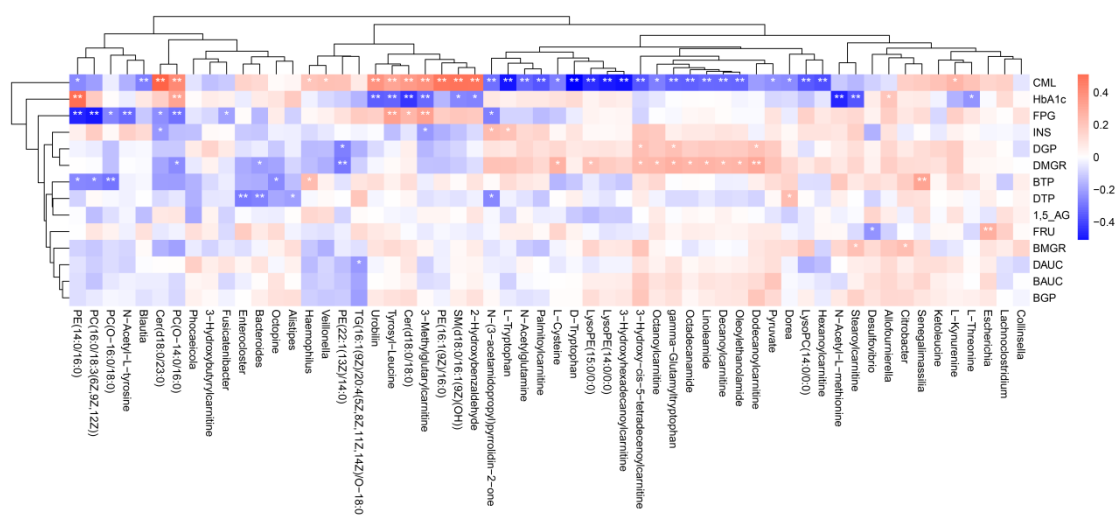


Fig. 8. Correlation heatmaps of differential microbial features and metabolites with specific glycemic parameters. BAUC (breakfast postprandial glucose area under the curve); DAUC (dinner postprandial glucose area under the curve); BMBS (breakfast maximum blood glucose spike); DMBS (dinner maximum blood glucose spike); BMBG (breakfast maximum blood glucose increment); DMBG (dinner maximum blood glucose increment); BTP (breakfast time to peak glucose); DTP

(dinner time to peak glucose). Colour gradients denote Pearson or Spearman correlation coefficients (r), with significance thresholds indicated as $*P < 0.05$, $**P < 0.01$.

Lipid metabolites showed prominent correlations with glycemic traits. Cer(d18:0/23:0) exhibited a strong positive correlation with CML ($r = 0.511$, $P < 0.0001$) while being negatively associated with FPG ($r = -0.224$, $P = 0.026$). LysoPE(14:0/0:0) demonstrated a strong negative correlation with CML ($r = -0.457$, $P < 0.0001$). Phospholipid species PC(O-14:0/16:0) showed positive correlations with both HbA1c ($r = 0.297$, $P = 0.003$) and CML ($r = 0.380$, $P < 0.0001$), alongside a negative correlation with FPG ($r = -0.281$, $P = 0.005$). PE(14:0/16:0) exhibited particularly strong correlations, with a positive association with HbA1c ($r = 0.474$, $P < 0.0001$) and a negative association with FPG ($r = -0.438$, $P < 0.0001$).

Acylcarnitines and microbial-host co-metabolites displayed complex correlation patterns. 3-Methylglutaryl carnitine showed negative correlations with HbA1c ($r = -0.298$, $P = 0.003$) and INS ($r = -0.205$, $P = 0.043$), but positive correlations with both CML ($r = 0.359$, $P < 0.0001$) and FPG ($r = 0.305$, $P = 0.002$). 3-Hydroxy-cis-5-tetradecenoyl carnitine showed positive correlations with DGP ($r = 0.210$, $P = 0.038$) and DMGR ($r = 0.226$, $P = 0.025$), and a negative correlation with CML ($r = -0.338$, $P = 0.001$). Gamma-Glutamyltryptophan was positively correlated with DGP ($r = 0.205$, $P = 0.043$) and DMGR ($r = 0.257$, $P = 0.010$), but negatively associated with CML ($r = -0.312$, $P = 0.002$).

Amino acid metabolites and related compounds also demonstrated significant associations. L-Tryptophan showed a strong negative correlation exclusively with CML ($r = -0.468$, $P < 0.0001$), whereas its metabolite L-Kynurenine demonstrated a positive correlation with CML ($r = 0.240$, $P = 0.017$). Urobilin was negatively correlated with HbA1c ($r = -0.321$, $P = 0.001$) while showing a positive association with CML ($r = 0.352$, $P < 0.0001$). Tyrosyl-Leucine exhibited a complex pattern, with negative correlation to HbA1c ($r = -0.311$, $P = 0.002$) but positive associations with both FPG ($r = 0.310$, $P = 0.002$) and CML ($r = 0.293$, $P = 0.003$).

These metabolite-glycemic trait correlations underscore the potential role of lipid remodeling, microbial-host co-metabolism, and amino acid metabolism in glucose regulation, suggesting plausible pathways through which gut microbiota-derived metabolites may influence postprandial glycemic homeostasis.

3.6 FA and FB induce formulation-specific restructuring of the gut microbiota and modulate taxa correlated with glycemic traits

Convergent evidence implicates dysbiotic gut microbiota in epithelial-barrier compromise and insulin-resistance induction; therefore, the capacity of plant-derived formulations FA and FB to re-establish microbial homeostasis was systematically investigated by profiling faecal microbiota in 57 participants (FA $n = 20$, FB $n = 18$, placebo $n = 19$) immediately before and after a 4-week intervention.

Alpha diversity indices (ACE, Chao1, Shannon, Simpson) did not differ among groups (Fig. 5A–D). PCoA using unweighted UniFrac distances showed clear separation among FA, FB, and placebo groups (Fig. 5E; $R^2 = 0.167$, $P < 0.05$). Further analysis via non-metric multidimensional scaling (NMDS) uncovered a remarkable temporal variation, which was particularly observed in the FA group (Fig. 5F; $R^2 = 0.060$, $P < 0.05$).

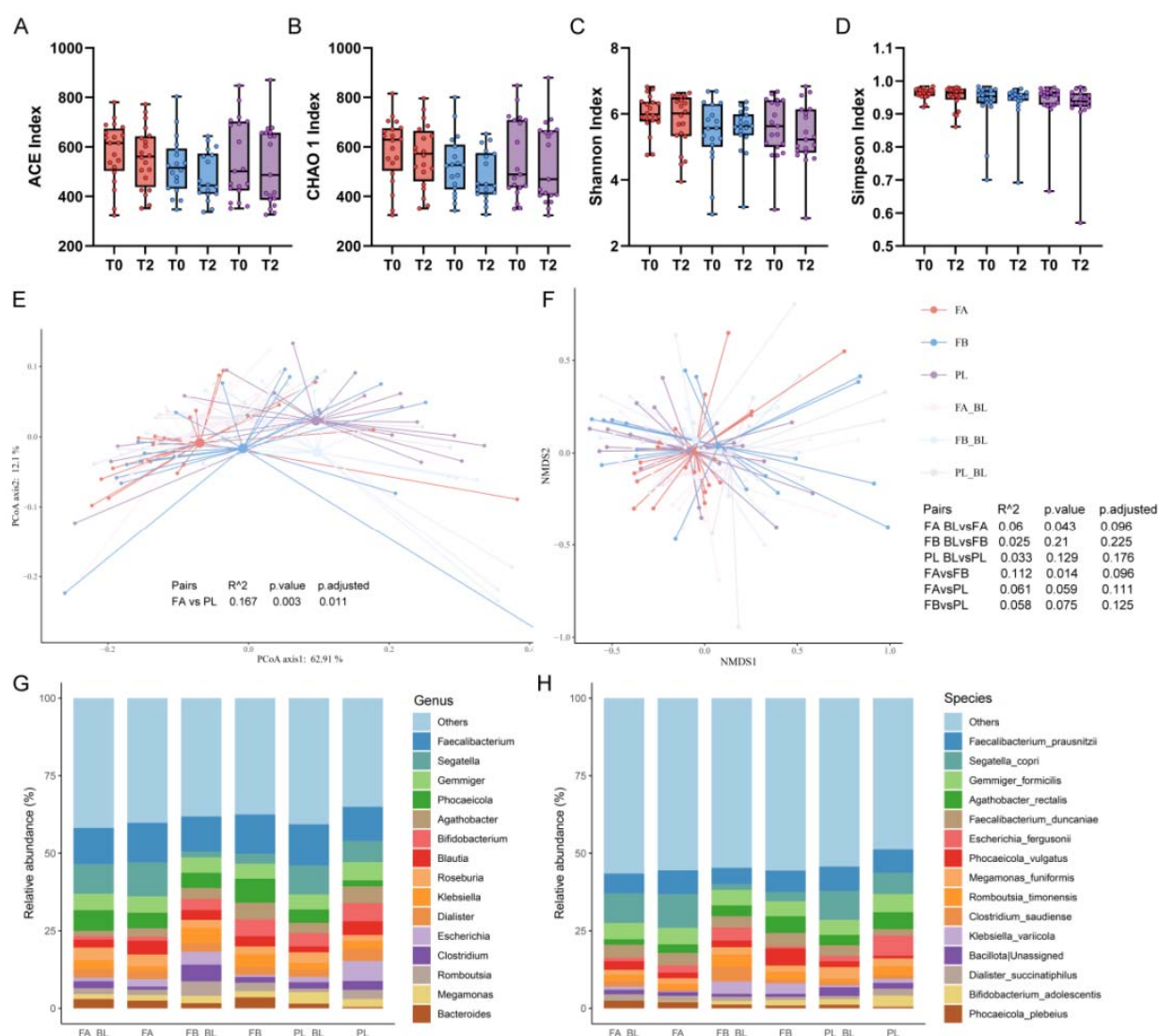


Fig. 5. Changes in microbiota diversity and composition. (A, B, C, D) α -Diversity as measured by the ACE, Chao1, Shannon, and Simpson indices. (E, F) Principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) were performed based on unweighted and weighted UniFrac distances. ((G, H) Relative abundance of bacterial taxa at genus and species levels. FA: Formula A group post-intervention (n = 20); FA_BL: Formula A group baseline; FB: formula B group post-intervention (n = 18); FB_BL: Formula B group baseline; PL: placebo group post-intervention (n = 19); PL_BL: placebo group baseline..

Given the limited species-level resolution of 16S rRNA gene sequencing targeting the V3–V4 hypervariable regions, all taxonomic classifications and subsequent analyses were restricted to the genus level.

Regarding within-group changes from baseline to post-intervention, divergent compositional shifts were observed following FA and FB supplementation compared with the placebo. FA intervention selectively enriched *Faecalibacterium* (+9.1%, $\log_2FC = 0.22$), *Agathobacter* (+59.7%, $\log_2FC = -0.97$), and *Blautia* (+74.1%), while it suppressed *Klebsiella* (-51.8%, $\log_2FC = -0.78$) and *Phocaeicola* (-24.9%, $\log_2FC = 1.34$). In contrast, FB intervention promoted the expansion of *Phocaeicola* (+58.3%, $\log_2FC = 0.46$), *Agathobacter* (+53.0%), and *Bifidobacterium* (+50.5%, $\log_2FC = -0.02$), and led to a minor reduction in *Klebsiella* (-14.2%, $\log_2FC = 0.19$). The placebo group was characterized by a contraction of *Faecalibacterium* (-17.5%) and *Phocaeicola* (-54.2%) (Fig. 5G, H).

STAMP analysis revealed formulation-specific restructuring of the gut microbiota at the genus level following the 4-week intervention (Fig. 6). The species-level analysis is provided in Supplementary Figure 2, and as such, inferences at this resolution require validation by more precise methods. Compared to placebo, both FA and FB significantly enriched beneficial taxa from the *Bacteroidetes* phylum, including *Phocaeicola* ($P < 0.05$) and *Bacteroides* ($P < 0.05$). The FB group demonstrated a broader suppressive effect, significantly reducing the abundance of *Escherichia* ($P < 0.001$), *Haemophilus* ($P = 0.040$), *Citrobacter* ($P = 0.030$), and *Lachnoclostridium* ($P = 0.027$), while also decreasing *Collinsella* ($P = 0.038$) and other taxa. In contrast, FA specifically downregulated *Enterocloster* ($P = 0.001$) and *Citrobacter* ($P = 0.028$). The placebo group exhibited a distinct pattern characterized by contraction of *Phocaeicola* ($P < 0.05$) and expansion of *Blautia* ($P < 0.01$) and *Dorea* ($P < 0.05$). These results demonstrate that both botanical formulations induce significant and distinct remodeling of gut microbial communities, with FB showing particularly pronounced suppression of potentially unfavorable genera.

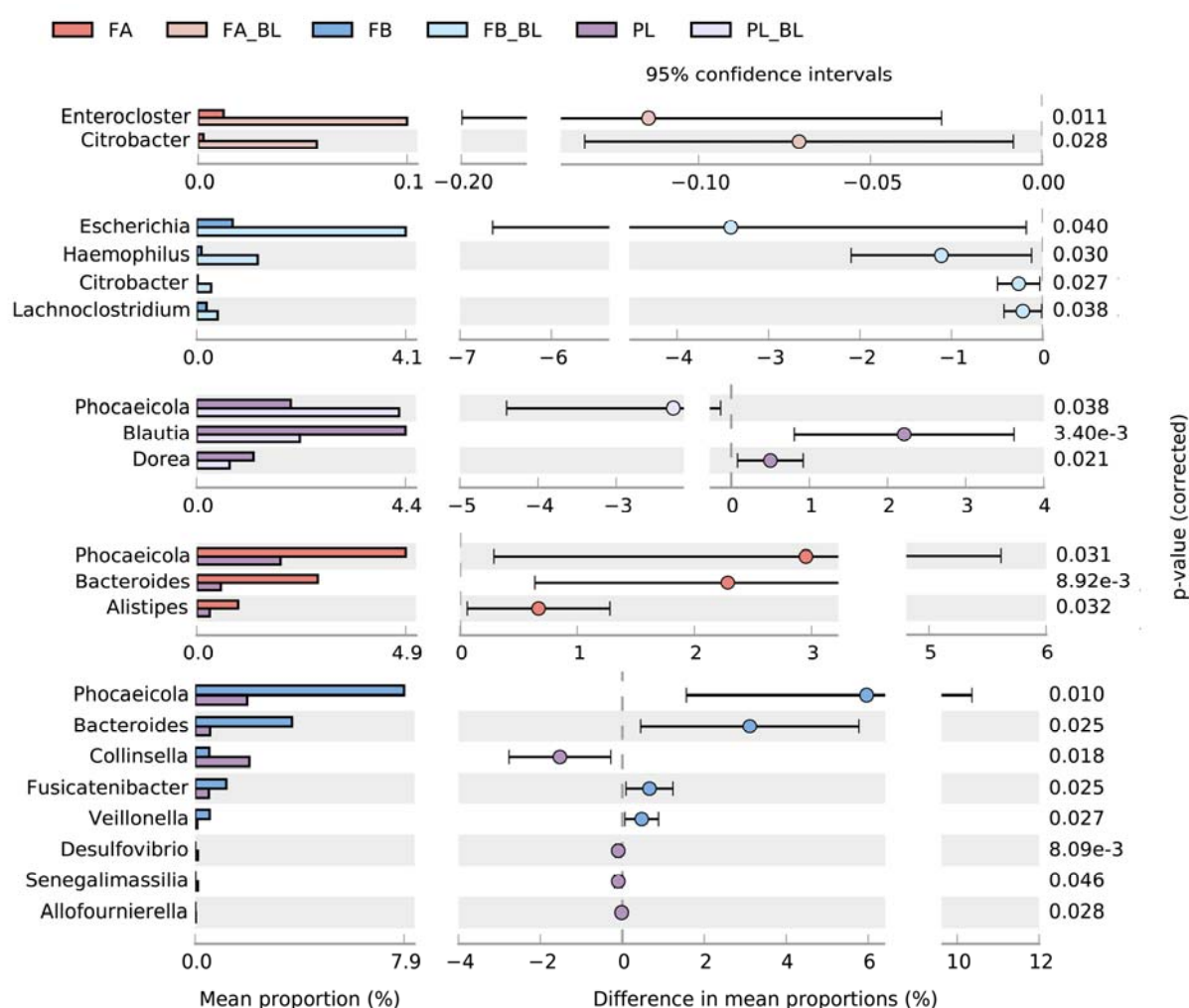


Fig. 6. Differentially abundant genera post-intervention identified by STAMP.

Correlation analysis at the genus level revealed significant associations with multiple glycemic parameters (Fig. 8). Given the inherent limitations of amplicon sequencing in achieving reliable species-level annotation, the corresponding correlation analysis at the species level is provided in Supplementary Figure. 3 and warrants further validation. The genus *Blautia* was negatively correlated with CML ($r = -0.254$, $P = 0.006$), as was the

genus *Dorea* ($r = -0.232$, $P = 0.013$). A significant negative correlation with FPG was observed for *Fusicatenibacter* ($r = -0.187$, $P = 0.047$).

Positive correlations were found between the genus *Citrobacter* and BMGR ($r = 0.211$, $P = 0.024$). The genus *Haemophilus* was positively correlated with BTP ($r = 0.218$, $P = 0.020$). A strong positive correlation was identified between *Senegalimassilia* and BTP ($r = 0.297$, $P = 0.001$). Furthermore, the genus *Bacteroides* was negatively correlated with DMGR ($r = -0.197$, $P = 0.036$) and DTP ($r = -0.248$, $P = 0.008$). The genus *Enterocloster* was negatively correlated with DTP ($r = -0.262$, $P = 0.005$).

Collectively, our findings demonstrate that FA and FB induce formulation-specific remodeling of the gut microbiome, wherein distinct bacterial taxa are associated with divergent effects on postprandial glucose homeostasis.

3.7 FA and FB reprogram the predicted gut microbial metabolic potential toward enhanced carbohydrate fermentation

To gain an initial insight into the potential mechanisms by which FA and FB modulate gut microbial function, we performed an *in silico* prediction of metagenomic functions using PICRUST2, followed by differential analysis with STAMP. Relative to the placebo, both FA and FB supplementation were predicted to significantly alter the microbial metabolic pathways (Fig. 7; $P < 0.05$).

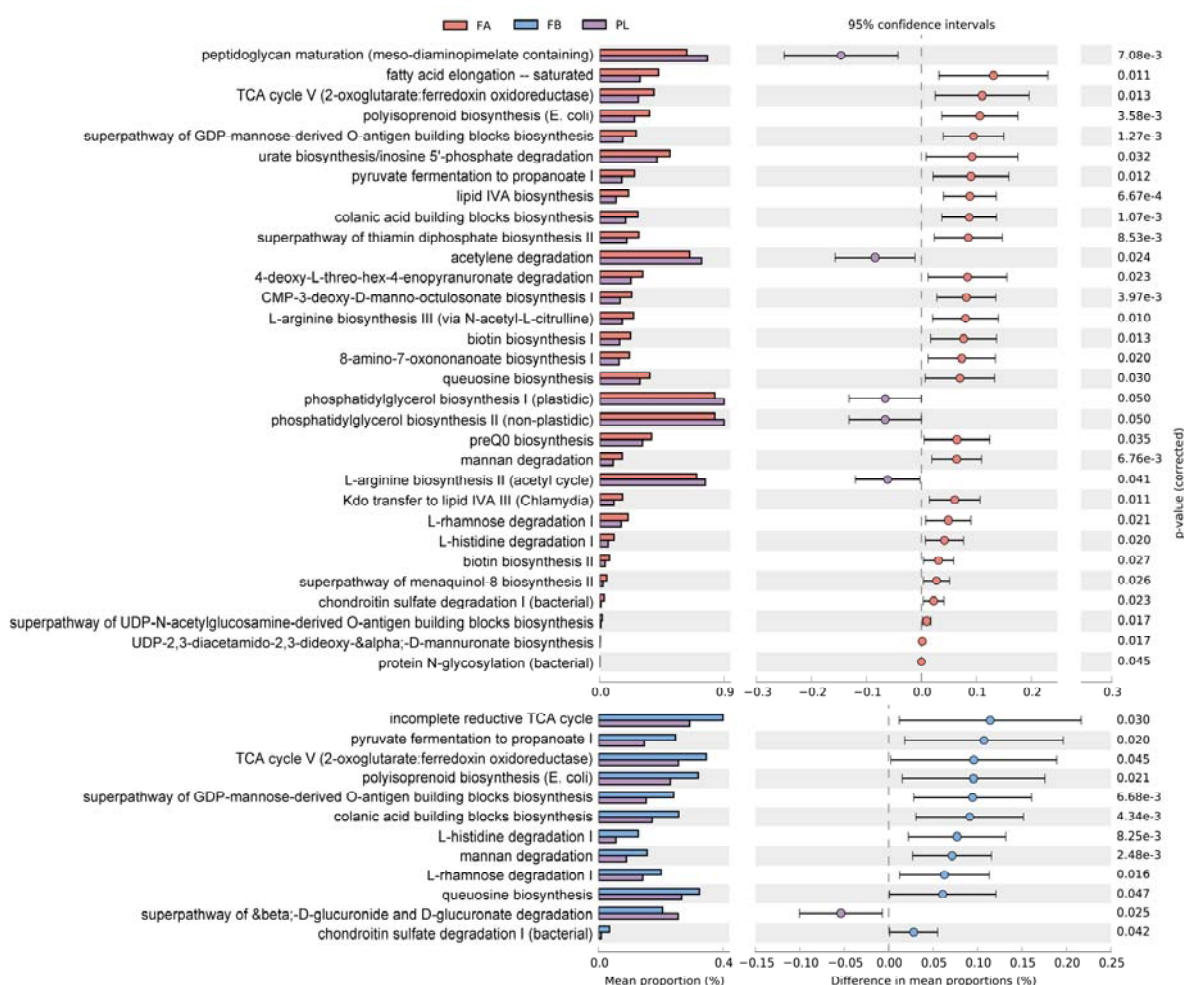


Fig.7. Functional prediction of gut microbiota based on PICRUST2.

The predictions suggested that FA selectively down-regulated bacterial cell-wall biogenesis pathways, including peptidoglycan maturation and phosphatidylglycerol biosynthesis I/II ($P = 0.007$ and $P = 0.050$, respectively). Concomitantly, nitrogen-metabolism pathways such as L-arginine biosynthesis II and acetylene degradation were also predicted to be suppressed ($P = 0.050$). In comparison, FB was predicted to predominantly weaken the super-pathway for GDP-mannose-derived O-antigen building-block synthesis ($P = 0.007$).

Conversely, the predictions indicated a convergent up-regulation of carbohydrate-catabolic and energy-generating pathways by both interventions. Mannan and L-rhamnose degradation I were predicted to be augmented by FA ($P = 0.007$ and $P = 0.021$), whereas FB was predicted to elevate chondroitin-sulfate degradation I, the β -D-glucuronide degradation super-pathway, L-rhamnose degradation I, and mannan degradation ($P = 0.042$, $P = 0.025$, $P = 0.016$, and $P = 0.002$, respectively). Parallel increases in the tricarboxylic acid (TCA) cycle V and pyruvate fermentation to propanoate I were predicted in both groups ($P = 0.013$, $P = 0.012$, $P = 0.045$, and $P = 0.020$), with FB additionally predicted to activate the incomplete reductive TCA cycle ($P = 0.030$). The predicted biosynthetic capacity also increased; vitamin and cofactor biosynthesis were elevated by FA, while queuosine and colanic-acid building-block synthesis were enhanced by FB.

Based on these predictive functional profiles, the coordinated augmentation of carbohydrate fermentation and energy metabolism pathways is posited to reflect an enhancement of microbial fermentative efficiency. The enrichment of propanoate fermentation and glycan-degradation pathways is further hypothesized to potentiate short-chain fatty acid (SCFA) generation. Collectively, these exploratory predictions suggest that FA and FB may reconfigure the gut microbial metabolic potential through partially non-overlapping mechanisms, with FA primarily affecting vitamin biosynthesis and cell-wall homeostasis, whereas FB preferentially amplifies carbohydrate-utilization pathways. These findings, derived from functional predictions, warrant further validation through direct metagenomic sequencing and metabolomic assays.

3.8 Subgroup analyses found no statistically significant evidence that baseline characteristics influenced the treatment effect

To elucidate whether baseline demographic or metabolic characteristics modulated intervention efficacy, pre-specified subgroup analyses were conducted, with grouping variables including sex, age, body-mass index (BMI), FPG, and HOMA-IR. While a numerical trend towards a greater reduction in FPG was observed in the FA arm compared to the FB arm (odds ratio 0.27; 95% CI 0.06–1.30; $P = 0.103$), particularly within the female and low-baseline-HOMA-IR strata, this difference was not statistically significant. Formal interaction tests for all subgroups were non-significant (all $P > 0.20$), indicating that heterogeneous treatment effects could not be established (Fig. 9).

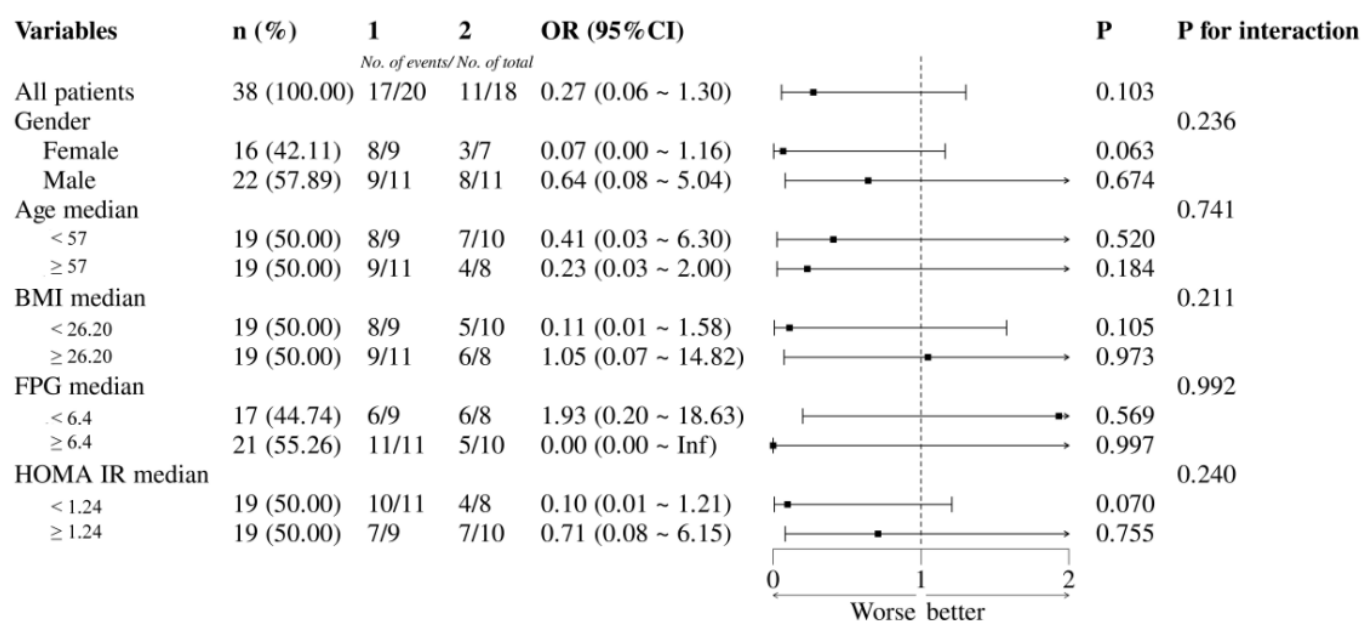


Fig.9. Forest Plot of Odds Ratios (FA vs. FB) for FPG Improvement Across Subgroups.

3.9 FA and FB demonstrate a favorable safety profile

All hematological, biochemical, hepatic, and renal function markers remained within clinical reference ranges at baseline and post-intervention (Table 4). After a 4-week intervention period, the FB group exhibited a significantly lower serum total bilirubin concentration compared with the placebo group ($P < 0.05$). In contrast, the serum alkaline phosphatase concentration in the FA group was significantly higher than that in the placebo group ($P < 0.01$). Notably, the serum alkaline phosphatase concentrations of all participants in the FA group remained within the normal physiological range after the intervention. All participants maintained good health throughout the study. Any alterations in these safety parameters were not clinically significant. Throughout the intervention period, participants reported no adverse events, which suggests that the two plant compound supplements are generally safe and well-tolerated.

Table 4. Safety parameters of participants.

Items	FA (n = 20)	FB (n = 18)	PL (n = 19)
TC (mmol/L)			
baseline	5.82 ± 1.08	5.53 ± 0.86	5.66 ± 0.84
week 4	5.69 ± 0.98	5.69 ± 0.72	5.69 ± 0.77
TG (mmol/L)			
baseline	1.89 ± 0.99	3.13 ± 3.23	1.74 ± 0.76
week 4	1.93 ± 0.76	2.65 ± 2.27	2.11 ± 1.11
HDL-C (mmol/L)			
baseline	1.33 ± 0.30	1.15 ± 0.30	1.33 ± 0.34
week 4	1.23 ± 0.26	1.17 ± 0.23	1.27 ± 0.31
LDL-C (mmol/L)			
baseline	3.18 ± 0.77	2.88 ± 0.40	3.17 ± 0.58
week 4	3.23 ± 0.78	3.13 ± 0.45	3.14 ± 0.55
TBIL (μmol/L)			
baseline	10.70 ± 3.12	11.51 ± 7.12	9.50 ± 4.01
week 4	9.07 ± 2.70	8.92 ± 5.48*	9.92 ± 4.03
DBIL (μmol/L)			
baseline	1.83 ± 0.63	2.51 ± 3.64	1.81 ± 1.58
week 4	2.13 ± 0.81	2.61 ± 2.40	2.04 ± 1.79

Items	FA (n = 20)	FB (n = 18)	PL (n = 19)
ALB (g/L)			
baseline	45.08 ± 1.97	44.70 ± 2.02	45.70 ± 2.69
week 4	45.78 ± 2.10	44.48 ± 2.67	45.99 ± 2.41
ALT (U/L)			
baseline	26.14 ± 16.62	23.55 ± 11.91	26.41 ± 19.20
week 4	26.63 ± 13.20	22.78 ± 11.78	18.99 ± 8.68
AST (U/L)			
baseline	25.93 ± 10.21	30.46 ± 36.24	24.12 ± 9.48
week 4	26.87 ± 8.99	29.03 ± 36.01	20.81 ± 6.00
ALP (U/L)			
baseline	76.69 ± 26.16	79.61 ± 29.16	76.94 ± 20.14
week 4	79.80 ± 24.65**	76.84 ± 45.82	72.13 ± 17.11

Data are presented as mean ± SD. The change rates between the two groups were compared using the Mann-Whitney U test, * $P < 0.05$ and ** $P < 0.01$ comparison with the placebo group. Change rate = (week 4 — baseline) / baseline × 100%. FA, formula A group (n = 20); FB, formula B group (n = 18); PL, placebo group (n = 19); ALB: albumin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DBIL: direct bilirubin; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TBIL: total bilirubin; TC: total cholesterol; TG: triglyceride.

4. Discussion

This study demonstrates that both FA and FB, two botanical dietary supplements, significantly improved fasting blood glucose and attenuated postprandial glycemic excursions. These effects appear to be mediated through modulation of the gut–insulin axis, as evidenced by integrated analyses of gut microbiota and serum metabolites. Specifically, both supplements reduced FPG and serum CML, while FB also significantly decreased LBP. FA was associated with enhanced HOMA- β , and both interventions mitigated the maximum postprandial glucose rise after breakfast—with FB additionally shortening the time to peak. Microbiota analyses revealed an enrichment of beneficial Bacteroidetes and a reduction in pathogenic genera such as *Escherichia* and *Collinsella* in the FB group. Functional predictions further suggested enhanced propionate fermentation and glycan degradation in both groups. Collectively, these findings position FA and FB as potential dietary prebiotics that improve glucose homeostasis through gut microbiota–host metabolic crosstalk.

A key finding was the significant reduction in both fasting plasma glucose and the advanced glycation end product CML following both interventions. Elevated CML levels, which are formed through non-enzymatic glycation, represent a recognized pathological hallmark of diabetes and are implicated in mitochondrial dysfunction and cellular injury via oxidative stress pathways [33, 34]. The observed reduction in serum CML from baseline in both the FA and FB groups is therefore of clinical importance. Although no significant change in HbA1c was detected, this finding aligns with the 8-week intervention period being too short to reflect long-term glycemic control, as meaningful changes in HbA1c typically require 8 to 12 weeks or more to manifest [35–38]. Nonetheless, the improvements in FPG and CML indicate a beneficial shift in early metabolic health. Additionally, FB supplementation significantly lowered LBP, a key transporter of endotoxin LPS that is known to promote inflammation and exacerbate insulin resistance [39]. In the FA group, the elevated LPS levels were not accompanied by changes in zonulin, a biomarker of intestinal barrier integrity [40],

suggesting that this increase may be attributable to factors such as dietary fat intake ^[41, 42], rather than impairment of gut barrier function.

Our multi-omics analyses reveal that FA and FB ameliorate prediabetes through distinct yet complementary mechanisms, converging on the gut-insulin axis. FA's efficacy is primarily mediated through a lipid-centric mechanism. The intervention led to a marked reduction in ceramides, lipotoxic molecules well-established as drivers of insulin resistance, inhibiting AKT signaling and activating inflammatory pathways ^[43]. This was coupled with an increase in short- and medium-chain acylcarnitines, suggesting enhanced mitochondrial fatty acid β -oxidation ^[44]. Notably, the increased level of oleoylethanolamide, a bioactive lipid previously demonstrated to improve glycemic control and alleviate insulin resistance in prediabetic individuals ^[45], likely represents a third arm of FA's beneficial action. Specifically, FA selectively enhanced *Bacteroidetes* taxa known for SCFA production, including *Phocaeicola*, *Bacteroides*, and *Alistipes* species ^[46-48], while suppressing potential pathogens such as *Enterocloster* and *Citrobacter*. The collective action of reducing lipotoxicity, promoting lipid oxidation, and fostering a beneficial microbial ecosystem underpins FA's ability to improve insulin sensitivity and β -cell function.

In contrast, FB's actions are characterized by a profound reprogramming of amino acid and microbial-host co-metabolism. The most salient changes included a pronounced increase in gut microbiota-derived D-tryptophan ^[49] and a decrease in the host-derived pro-inflammatory metabolite L-kynurenine, indicating a shift away from the kynurenine pathway ^[50]. This rewiring likely influences host glucose homeostasis through metabolites that can stimulate gut hormone secretion (e.g., GLP-1) via receptors such as MRGPRE ^[51]. FB also induced significant phospholipid remodeling and demonstrated the most robust suppression of potentially pathogenic genera, including *Escherichia*, *Citrobacter*, and the pro-inflammatory *Collinsella* ^[52, 53]. Predictions of microbial function indicated a strong enhancement of carbohydrate degradation pathways and propanoate fermentation, aligning with its capacity to enrich fiber-fermenting taxa.

This study demonstrates the strong potential of both FA and FB as safe and effective functional foods for improving metabolic health. Their excellent safety profile is evidenced by stable hepatic and renal function markers and the absence of adverse events. The summarized mechanistic diagram (Fig. 10) illustrates these distinct yet complementary pathways through which FA and FB exert their beneficial effects.

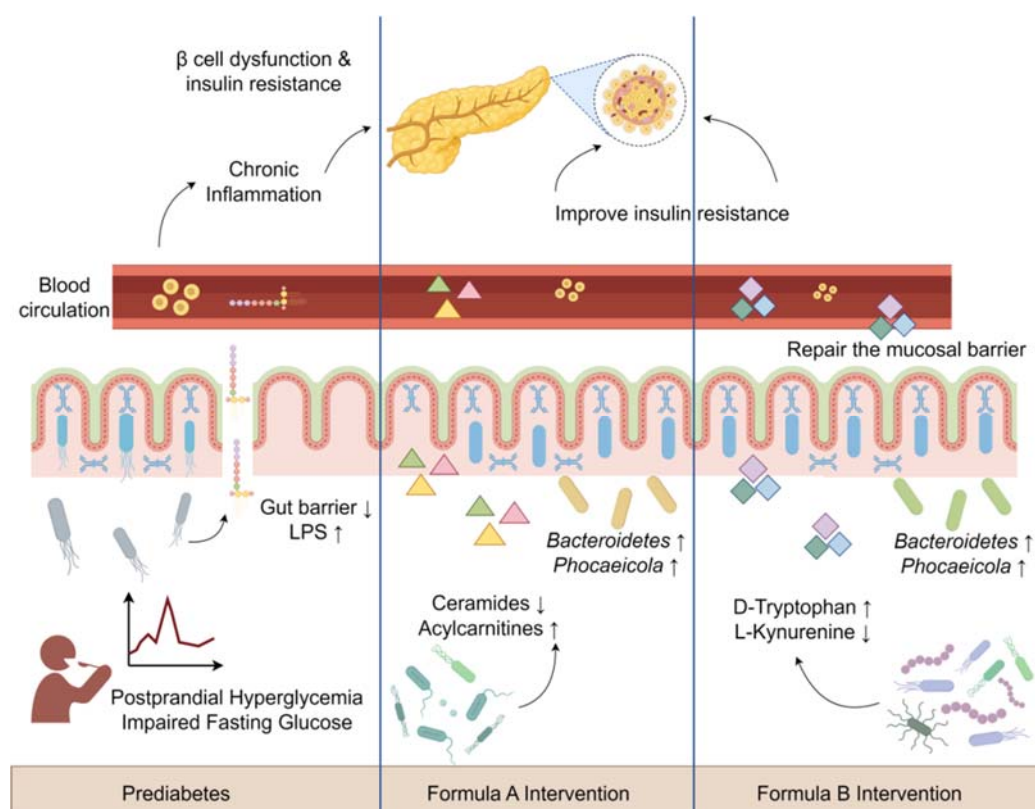


Fig.10. Proposed mechanisms underlying the improvement of prediabetic conditions by Formula A and Formula B interventions. FA primarily targets systemic inflammation and insulin resistance, while FB restores gut barrier integrity and modulates gut microbiota. Key microbial species and metabolites altered by each intervention are shown. Arrows (↑) indicate increase; blocked arrows (↓) indicate decrease.

Despite their mechanistic differences, both formulations converge on the gut-insulin axis by: (1) promoting the enrichment of beneficial gut microbes; (2) suppressing pathogenic or pro-inflammatory bacterial taxa; (3) altering host and microbial metabolite profiles in a manner that correlates with improved glycemic traits; and (4) enhancing microbial carbohydrate fermentation and SCFA production potential. This multi-targeted approach—simultaneously remodeling the gut microbiota, reducing inflammatory tone, and improving glucose metabolism—highlights an advantage of these multi-component blends over single-ingredient supplements.

This study has several limitations. The lack of active monitoring of diet and exercise during the intervention may introduce confounding factors. Furthermore, the use of only positive ion mode in metabolomics limits metabolite coverage, and the functional predictions from 16S rRNA data require validation through direct metagenomic sequencing.

5. Conclusion

In conclusion, this RCT demonstrates that the medicinal-edible homologous blends FA and FB are effective and safe dietary interventions for prediabetes management. Both supplements significantly improved glycemic control by modulating the gut-insulin axis, albeit through distinct pathways: FA primarily influenced lipid metabolism and β -cell function, while FB exerted stronger effects on amino acid metabolism and pathogen suppression. These findings underscore their potential as foundational strategies for personalized nutrition, warranting future studies to translate them into scalable functional foods.

Declaration of competing interest

The authors declare no competing interests.

Data availability

All data generated or analysed during this study are included in this published article. The sequence data generated from 16S rRNA gene sequencing of the fecal microbiota have been deposited in the NCBI Sequence Read Archive under accession number PRJNA1290893.

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