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A synbiotic supplement reduces body fat and increases GLP-1 secretion in a pilot clinical study

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ABSTRACT

Obesity is closely associated with gut microbiota and their metabolites. We developed a multi-component synbiotic supplement that includes lychee polyphenols, *Bifidobacterium longum*, *Bifidobacterium animalis*, methionine, and other supportive ingredients such as prebiotics and postbiotics. A 5-week pilot clinical trial was conducted to evaluate its effects on body composition, metabolic markers, gut microbiota-derived metabolites, and GLP-1 secretion. The results showed significant reductions in body weight, fat mass, visceral fat, HbA1c, insulin resistance, and blood lipids. Metabolite analysis revealed increases in short-chain fatty acids and tryptophan metabolites, including acetic acid, isobutyric acid, isovaleric acid, serotonin, and indolelactic acid. GLP-1 secretion increased in both humans and mice, with a notable rise in *Akkermansia muciniphila* observed in mice given the synbiotic. These findings suggest that this synbiotic supplementation could serve as a potential approach for improving metabolic health and reducing obesity.

1. Introduction

Obesity is a major global health issue and a key risk factor for numerous chronic diseases, including insulin resistance, type 2 diabetes, metabolic dysfunction-associated steatotic liver disease (MASLD), cardiovascular diseases, and some cancers (GBD 2021 Adult BMI Collaborators., 2025; Hildebrand & Pfeifer, 2025). Recently, increasing evidence has highlighted the important role of gut microbiota as a vital regulator of host health, especially in metabolic regulation (Amabebe, Robert, Agbalalah, & Orubu, 2020; Y. Fan & Pedersen, 2021; K. Hou et al., 2022; Xu et al., 2022). It indicates that influencing gut microbiota may be a potential strategy to prevent and treat obesity. The probiotics *Bifidobacterium* species have been extensively studied for their beneficial effects on metabolic disorders, gastrointestinal conditions, immune modulation, and the respiratory system (Bocchio et al., 2025; Schellekens et al., 2021). Administration of *Bifidobacterium longum* has been shown to reduce body weight gain, decrease fat mass, alleviate hyperlipidemia, and improve blood sugar control in mouse models of high-fat diet-induced obesity (In Kim et al., 2019; Kou et al., 2023; Yun, Shin, Ma, & Kim, 2024). Clinical studies have further supported these benefits (Ben Othman et al., 2023; Chaiyasut et al., 2021; Kilic Yildirim, Dinleyici, Vandenplas, & Dinleyici, 2022; Sergeev, Aljutaily, Walton, & Huarte, 2020), suggesting that *B. longum* supplementation could be a promising approach to alleviating obesity. In addition to live bacteria, the culture supernatant of *B. longum* has also shown potential to improve obesity (Hossain et al., 2020; Rahman, Lee, Park, & Kim, 2023),

Abbreviations: AA, cetic acid; APO-A1, apolipoprotein A1; APO-B, apolipoprotein B; CV, coefficient of variation; DPP-4, dipeptidyl peptidase-4; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; FDR, false discovery rate; FFAR2, free fatty acid receptor 2; GLP-1, glucagon-like peptide-1; Glucose-AC, glucose-ante cibum; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; IAA, indoleacetic acid; iBA, isobutyric acid; ILA, indolelactic acid; IPA, indolepropionic acid; IQR, interquartile range; iVA, isovaleric acid; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LDL-C, low-density lipoprotein cholesterol; SCFAs, short-chain fatty acids; T-cholesterol, total cholesterol; TRP, tryptophan; MASLD, metabolic dysfunction-associated steatotic liver disease; MRI-PDFF, magnetic resonance imaging-estimated proton density fat fraction; MRM, multiple reaction monitoring; MS, mass spectrometry; MTA, 5′-methylthioadenosine; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; QC, quality control.

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indicating that its derived components, such as microbial metabolites, may contribute to these anti-obesity effects. Furthermore, in obese mouse models, *B. longum* and its fermented milk have been found to modify gut microbiota, notably increasing levels of *Akkermansia*, a next-generation probiotic that supports metabolic health (Jiang et al., 2022; Kim, Kim, et al., 2022; Lyu et al., 2024). These findings suggest that microbiota changes driven by *B. longum* supplementation may play a role in combating obesity.

Along with *B. longum*, another widely studied probiotic, *Bifidobacterium animalis* subsp. *lactis*, has gained significant attention for its positive effects on host metabolism and gut health. Multiple preclinical studies show that supplementing with *B. animalis* subsp. *lactis* can reduce obesity and improve glucose tolerance in rodent models (Mao et al., 2022; Stenman et al., 2014; Zhang et al., 2024). Notably, a previous study demonstrated that *B. animalis* subsp. *lactis* enhances mitochondrial function in adipose tissue, helping prevent weight gain in diet-induced obese mice (Huo et al., 2020). Additionally, *B. animalis* subsp. *lactis* increased the abundance of short-chain fatty acid (SCFA)-producing bacteria in human microbiota-associated rats, indicating a broader influence on gut microbial composition and metabolite production (Mao et al., 2022). Importantly, these preclinical results are supported by human studies; in a double-blind, randomized, placebo-controlled trial, daily intake of fermented milk containing *B. animalis* subsp. *lactis* significantly decreased visceral fat in overweight Japanese adults (Takahashi et al., 2016).

A growing body of research highlights the important roles of microbial-derived metabolites, such as short-chain fatty acids (SCFAs) and tryptophan metabolites, in regulating host metabolism (K. Hou et al., 2022). SCFAs, produced by the fermentation of dietary fibers by gut microorganisms, regulate metabolism, inflammation, gut barrier integrity, and gut hormone secretion (den Besten et al., 2015; Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016; Larraufie et al., 2018; Miyamoto et al., 2025). Prebiotics such as indigestible maltodextrin, fructooligosaccharides, isomalto-oligosaccharide, and rice bran concentrate have been shown to selectively promote the growth of beneficial bacteria, including *Bifidobacterium* and other SCFA-producing microbes, thereby enhancing SCFA production and contributing to metabolic improvements (Astina & Sapwarobol, 2019; Borewicz et al., 2024; Mahalak et al., 2024; Mo et al., 2025). SCFAs can stimulate intestinal L cells to release glucagon-like peptide-1 (GLP-1), an incretin that promotes insulin secretion, inhibits glucagon release, delays gastric emptying, and suppresses appetite (Christiansen et al., 2018; Drucker, 2018; Larraufie et al., 2018). Furthermore, gut microbes are vital for tryptophan metabolism, resulting in the production of indole and its derivatives, which are involved in metabolism, immune regulation, and gastrointestinal function (Min et al., 2024; G. Wang et al., 2024; Xue et al., 2023). Dysregulation of SCFAs and tryptophan-indole pathways has been linked to obesity and other metabolic disorders, underscoring the importance of microbe-derived metabolites in host health (Ecklu-Mensah et al., 2023; Y. Hou, Li, & Ying, 2023).

Polyphenols extracted from lychee have been shown to improve obesity-related parameters, including fat mass, blood glucose, and circulating lipids, in both obese mouse models and humans (Bahijri et al., 2018; Kim, Lee, et al., 2022; Lyu et al., 2024). Lychee polyphenols increase the abundance of *Bifidobacterium* species in the guts of obese mice fed a high-fat diet (Lyu et al., 2024). Additionally, supplementing with lychee polyphenols alleviates MASLD, as measured by magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF), along with a reshaping of the gut microbiota, including an increase in *Akkermansia* (Jinato et al., 2022). This suggests that modulating microbiota with lychee polyphenols may contribute to improving metabolic disorders. Notably, a recent study by Lyu et al. showed that among the *Bifidobacterium* species enriched by lychee polyphenols, *B. longum* can convert methionine into 5′-methylthioadenosine (MTA) (Lyu et al., 2024), a metabolite known to alleviate obesity, MASLD, inflammation, and tumorigenesis (Andreu-Pérez et al., 2010; Hevia et al., 2004; Lyu et al., 2024). This implies a mechanistic link: lychee polyphenols promote the growth of *B. longum*, which in turn utilizes methionine to produce MTA (Lyu et al., 2024). These findings support the idea that combining lychee polyphenols, *B. longum*, and MTA (or its precursor methionine) could be a new strategy to regulate host metabolism and improve obesity-related outcomes.

In this study, we conducted a pilot clinical trial and a supporting mouse experiment to assess the effects of a synbiotic supplement that combines *B. longum*, lychee polyphenols, and methionine, along with other supportive ingredients, including *B. animalis* subsp. *lactis*, prebiotics, and postbiotics on body composition, metabolic parameters, microbially derived metabolites, and GLP-1 secretion. Our goal was to clarify the impact of this synbiotic formulation on obesity and host metabolic regulation, thereby providing mechanistic insights and a potential synbiotic-based strategy for improving metabolic health.

2. Material and Method

2.1. Pilot clinical trial design and participants

The clinical pilot study was conducted at Leeuwenhoek Precision Health Clinic (Taipei, Taiwan) from January to July 2024. The study protocol was approved by the Institutional Review Board of Development Center for Biotechnology (IRB-AP-11305) and is accessible on ClinicalTrials.gov (https://clinicaltrials.gov/study/NCT06363864?term=LL-IRB-2401&rank=1). The key recruitment criteria included adults over 18 years old and weighing more than 50 kg. Key exclusion criteria involved recent use (within one month) of antibiotics, immunosuppressants, or chemotherapy agents.

Participants were recruited from individuals who visited the Leeuwenhoek Precision Health Clinic for routine health exams and planned to use the synbiotic supplement FLORA LE LIGHT™ (Leeuwenhoek Laboratories Co. Ltd., Taipei, Taiwan). This supplement contains indigestible maltodextrin, fructooligosaccharides, oligonol® (a polyphenolic extract from lychee fruit and green tea), *Bifidobacterium animalis* subsp. *lactis* CP-9, *Bifidobacterium longum* subsp. *infantis* BLI-02, DL-methionine, isomalto-oligosaccharide, totipro® postbiotics, and rice bran concentrate. All participants provided written informed consent before enrollment and received personalized dietary instructions from dietitians before starting the study. Fifty-eight participants were enrolled, and all completed the synbiotic supplementation, taking two capsules twice daily for five consecutive weeks.

Participants' body weight, fat mass, body fat percentage, skeletal muscle mass, and visceral fat levels were measured using an InBody 570 Body Composition Analyzer (InBody, Seoul, Korea) at baseline and after the five-week supplementation. To collect fasting blood samples, all participants were required to fast for at least eight hours on the day of the assessment. Previous studies show plasma GLP-1 levels increase after food consumption (Brynes, Frost, Edwards, Ghatei, & Bloom, 1998; Lee et al., 2015; Vilsbøll et al., 2003). Among these participants, 21 provided fasting and postprandial blood samples for plasma GLP-1 measurement. To measure plasma GLP-1, a dipeptidyl peptidase-4 (DPP-4) inhibitor (DPP4-M, Sigma-Aldrich, USA) was added to the blood collection tubes. For assessing postprandial GLP-1 responses, participants were given a liquid nutritional supplement: Protison Cancer Nutrition Shake (oatmeal flavor, 237 mL, 372 kcal, protein 23%, fat 25%, carbohydrate 50%, dietary fiber 2%, SMAD Biotechnology Co. Ltd., Taipei, Taiwan). The postprandial blood sample was taken 30 min after consuming the liquid nutritional supplement. Plasma was separated from whole blood by centrifugation at 1800 xg for 15 min at 4 °C. Plasma samples were collected and immediately stored at −80 °C for further analysis.

2.2. Biochemistry measurements

Fasting blood samples were collected from participants at baseline and after 5 weeks of the synbiotic supplementation for biochemical

analysis. The following parameters were measured by the Taipei Institute of Pathology in Taipei, Taiwan: glucose-ante cibum (glucose-AC), glycated hemoglobin A1c (HbA1c), insulin, total cholesterol (T-cholesterol), triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL—C), apolipoprotein A1 (APO-A1), apolipoprotein B (APO-B), homocysteine, and high-sensitivity C-reactive protein (hsCRP). The ApoB/ApoA1 ratio was calculated by dividing the plasma ApoB concentration (mg/dL) by the ApoA1 concentration (mg/dL). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as follows: $HOMA-IR = \frac{Glucose-AC\ (mg/dL) \times Fasting\ Insulin\ (\mu U/mL)}{405}$ (Matthews et al., 1985).

2.3. Sample preparation for targeted metabolite analysis

A comprehensive list of analytical standards and isotopically labeled internal standards used in targeted metabolomics assays is provided in Table S1. To prepare serum samples for SCFAs analysis, a 50 μL serum aliquot was combined with 120 μL of internal standard solution, followed by the addition of 125 μL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1-EDC, Tokyo Chemical Industry, Tokyo, Japan) and 125 μL of 2-nitrophenylhydrazine (2-NPH, Tokyo Chemical Industry, Tokyo, Japan). The mixture was vortexed and incubated at 45 °C for 20 min for derivatization. The reaction was then quenched by placing the samples on ice for 10 min. After centrifugation at 15,000 rpm for 10 min at 4 °C, the supernatants were collected for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Calibration standards were processed using the same derivatization protocol as the samples.

For analyzing tryptophan and its derivatives, 50 μL of serum was mixed with 200 μL of LC-MS grade methanol (Duksan Pure Chemicals, Ansan, Korea) containing internal standards to precipitate proteins. The mixture was vortexed and centrifuged at 15,000 rpm for 10 min at 4 °C. Then, 100 μL of the supernatant was combined with 100 μL of deionized water containing additional internal standards. After vortexing, the samples were directly injected into the LC-MS/MS system. Calibration curves for quantification were prepared over a concentration range of 1 to 5000 ppb in 50% methanol with internal standards.

2.4. Targeted metabolite analysis by mass spectrometry

For SCFAs analysis, chromatographic separation was carried out using an ACQUITY UPLC CSH C18 column (100 × 2.1 mm, 1.7 μm, Waters, Milford, MA, USA) maintained at 40 °C. The mobile phases consisted of (solvent A) water with 5 mM ammonium acetate (Nippon Gene, Toyama, Japan) and (solvent B) 95% acetonitrile (Duksan Pure Chemicals, Ansan, Korea) with 5 mM ammonium acetate. The gradient elution program was as follows: 0–0.5 min, 20% B; 0.5–2.5 min, linear increase to 26% B; 2.5–5.5 min, hold at 26% B; 5.5–9.0 min, ramp to 50% B; 9.0–10.0 min, ramp to 99% B; 10.0–11.5 min, hold at 99% B; 11.5–11.6 min, return to 20% B; 11.6–15.0 min, re-equilibration at 20% B. The flow rate was set at 0.35 mL/min, and 1 μL of each sample was injected. Detection employed a SCIEX QTRAP® 5500 mass spectrometer (Sciex, Marlborough, MA, USA) with a negative electrospray ionization (ESI) source, operating in multiple reaction monitoring (MRM) mode. Monitored ion transitions (m/z) included: acetic acid (194 → 164), propionic acid (208 → 178), butyric acid and isobutyric acid (222 → 192), and valeric acid and isovaleric acid (236 → 206). Data processing and quantification were conducted using SCIEX OS software (Sciex, Marlborough, MA, USA), and the determined concentrations were expressed in micromolar (μM).

For tryptophan-related metabolites analysis, chromatographic separation was performed on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm, Waters, Milford, MA, USA). The mobile phases consisted of (A) water with 0.1% formic acid (Honeywell Fluka, Seelze, Germany) and (B) acetonitrile with 0.1% formic acid. The LC gradient was set as follows: 0–1 min, 2% B; 1–13 min, linear increase to 55% B; 13–14 min, ramp to 99% B; 14–15.5 min, hold at 99% B; 15.5–15.6 min, return to 2% B; 15.6–19 min, re-equilibration at 2% B. The flow rate was 0.35 mL/min with a 1 μL injection volume. Mass spectrometry was performed using the same SCIEX QTRAP® 5500 system in positive ESI mode and MRM acquisition. The monitored transitions (m/z) were: tryptophan (205.2 → 188.0), serotonin (177.1 → 160.1), indole-3-acetic acid (176.0 → 130.1), indole-3-propionic acid (190.1 → 130.1), and indole-3-lactic acid (206.1 → 118.0). Metabolite concentrations were calculated based on standard curves and normalized to internal standard levels, with the determined concentration expressed in micromolar (μM).

2.5. Sample preparation for untargeted metabolomics analysis

Briefly, a 100 μL plasma sample was extracted with 800 μL of ice-cold methanol containing an internal standard. Following extraction, the supernatant was dried under nitrogen and reconstituted with 200 μL of methanol. Prior to UHPLC-Orbitrap analysis, the reconstituted sample was filtered through a 0.22-μm filter and stored at −20 °C.

2.6. Untargeted metabolomics analysis by mass spectrometry

Metabolomics analysis was conducted utilizing a Dionex Ultimate 3000 UHPLC system equipped with quaternary pumps, coupled to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer system (Thermo Fisher Scientific, Waltham, MA, USA). Separation procedures were carried out on an ACQUITY Premier HSS T3 column (2.1 × 100 mm, 1.8 μm) employing a mobile phase composed of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.3 mL per minute. The injection volume was set at 5 μL. The liquid chromatography gradient profile was as follows: 0–1.5 min, 1% B; 1.5–9.5 min, 1–35% B; 9.5–11.5 min, 35–80% B; 11.5–13.5 min, 80–100% B; 13.5–16.5 min, 100% B; 16.5–20.0 min, 1% B. The autosampler temperature was maintained at 10 °C, while the column oven was maintained at 40 °C (Poland, Schrimpe-Rutledge, Sherrod, Flynn, & McLean, 2019).

For untargeted metabolomics, mass spectrometry (MS) was performed with these parameters: sheath gas flow at 50 arbitrary units (au), auxiliary gas flow at 12.5 au, capillary temperature at 325 °C, S-lens RF level at 50%, and spray voltage of 3.5 kV (positive) and 3.3 kV (negative). MS spectra were collected in positive ion mode using top-5 data-dependent acquisition, covering a scan range of 80–1200 m/z at a resolution of 70,000. The ddMS2 spectra were recorded at 17,500 resolution with a 1.5 m/z isolation window, stepped normalized collision energies of 15, 30, and 45, and a 15-s dynamic exclusion. The AGC targets were 3×10^6 for full MS and 5×10^5 for ddMS2.

2.7. Metabolomics data processing

Compound Discoverer 3.3.0 (Thermo Fisher Scientific, Waltham, MA, USA) processed raw metabolomics data by performing peak extraction, retention time alignment, metabolite identification, gap status and background subtraction. Key parameters for peak extraction and retention time alignment included a 5 ppm mass tolerance, a maximum shift of 0.2 min, isotope search with 30% tolerance, a signal-to-noise ratio of 3, a minimum peak intensity of 1,000,000, and at least seven scans per peak. The mass error was calculated using the following formula: $Mass\ error\ (ppm) = \frac{Measured\ Value - Theoretical\ Value}{Theoretical\ Value} \times 10^6$. Metabolites were annotated through spectral similarity in databases, with a mass error below 5 ppm. The quality control (QC) threshold was set at a coefficient of variation (CV) of less than 30%, with testing every 30 samples plus a QC sample. Metabolites were considered valid if detected in at least 25% of samples within each group, and the background ratio was limited to 3. A total of 116 samples from 58 participants, collected before and after the synbiotic supplementation, were analyzed across

two batches: 102 in Batch 1 and 14 in Batch 2. Batch effect correction was performed using MetaboAnalyst 6.0, which selected ComBat based on principal component analysis (PCA) evaluation to reduce batch variation. PCA plots before and after correction showed improved clustering of QC and biological samples, confirming effective adjustment.

2.8. Metabolite annotation and confidence level classification

The raw data output from Compound Discoverer 3.3.0 was first normalized to internal standards to minimize matrix effects and ensure comparability across samples. The normalized intensity was calculated as: $Normalized\ Area = \frac{Metabolite\ Peak\ Area}{Internal\ Standard\ Peak\ Area}$. The normalized dataset was then processed using in-house software (Leeuwenhoek Laboratories Co. Ltd., Taipei, Taiwan), which generated a metabolite annotation table. Metabolites were assigned confidence levels from 1 to 4, with 1 indicating the highest confidence (Benjamini & Hochberg, 1995). Each level is defined as follows: Level 1 indicates a match with the LWHK Database, where accurate mass, fragmentation pattern, and retention time match standards; Level 2 signifies a match with the mzCloud online database, with accurate mass and fragmentation patterns aligned; Level 3 includes putative metabolites identified solely by accurate mass without fragmentation data from online databases; Level 4 corresponds to unknown peaks.

2.9. Measurement of plasma GLP-1

GLP-1 concentrations were measured using a commercial GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (EIA-GLP1, RayBiotech, GA, USA), following the manufacturer's instructions. Absorbance was read at 450 nm with a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA).

2.10. Animal experiments

The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University (approval number: NTU112-EL-00094) and the IACUC of Leeuwenhoek Laboratories Co. Ltd. (approval number: LL-2023001). Fourteen seven-week-old male C57BL/6 mice were purchased from the National Center for Biomodels in Taipei, Taiwan. The mice were housed four per cage under Specific pathogen-free (SPF) conditions and maintained at 20–22 °C with a 12-h light/dark cycle at the Animal Resource Center of National Taiwan University, Taipei, Taiwan. After a one-week adaptation, the mice were used for experiments. The mice were randomly divided into two groups: a control group ($n = 7$) and a synbiotic group ($n = 7$). Both groups were fed ground chow diets (5010, LabDiet, USA). Mice in the synbiotic group received ground chow containing 1.5% FLORA LE LIGHT™ powder, while those in the control group received ground chow with 1.5% maltodextrin (Grain Processing Corporation, USA). The mice had free access to food and water, except during plasma GLP-1 measurements. In the sixth week of the experiment, fecal and blood samples were collected to measure the relative fecal abundance of *A. muciniphila* and plasma GLP-1 concentrations. To measure plasma GLP-1, the mice were gavaged with glucose dissolved in ddwater at 2 g/kg BW after fasting for five hours (Yoon et al., 2021). Blood was collected 10 min later via retro-orbital sinus puncture into tubes containing EDTA (600,215, Cayman Chemical, Ann Arbor, MI, USA) and a DPP-4 inhibitor (DPP4-M, Sigma-Aldrich, USA). Plasma samples were obtained after centrifugation at 1800 xg for 15 min at 4 °C. The plasma was then collected and stored at −80 °C for further analysis. At the end of the experiments, the mice were sacrificed using CO2.

2.11. Real-time PCR analysis

Fresh mouse stools were collected in sterilized 1.5 mL tubes and immediately stored at −80 °C. DNA from the stool samples was extracted using the QIAamp PowerFecal Pro DNA Kit (51,804, QIAGEN, Germantown, MD, USA). DNA concentrations were assessed using a spectrophotometer (NanoDrop™ One, Thermo Fisher Scientific, MA, USA) at 260/280 nm. Real-time PCR analysis was performed using a SensiFast™ SYBR Hi-ROX Kit (Bioline, London, UK) on a QuantStudio 6 Pro Real-Time PCR System (A43180, ThermoFisher Scientific, Waltham, USA). The PCR program consists of an initial polymerase activation at 95 °C for two minutes, followed by 40 cycles of denaturation at 95 °C for five seconds, annealing at 60 °C for 10 s, and extension at 72 °C for 20 s. Primers for *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *A. muciniphila* and universal 16 s rRNA for normalization are listed in Table 1 (Dao et al., 2016; Ennis, Shmorak, Jantscher-Krenn, & Yassour, 2024; Roopchand et al., 2015; Tso, Bonham, Fishbein, Rowland, & Klepac-Ceraj, 2021; Wang, Qin, et al., 2022). Each sample was run in triplicate. Threshold cycle (Ct) values were determined using Design & Analysis 2 Software version 2.7.0 (ThermoFisher Scientific, Waltham, USA). A Ct value >40 was defined as undetectable. The relative abundance of target bacterial species was calculated using the $2^{-\triangle\triangle CT}$ method, and then normalized to the 16 s rRNA gene within the same sample, using the equation as follows: $\Delta Ct =$ Ct of target bacterial species − Ct of 16s (Livak & Schmittgen, 2001). Subsequently, fold changes of relative abundance of the target bacterial species were calculated using the equation as follows: fold change $= 2^{-(\Delta Ct\ of\ experimental\ group - \Delta Ct\ of\ control\ group)}$.

2.12. Statistics

Paired *t*-tests were employed to compare values before and after the intervention in human trials. Wilcoxon matched-pairs tests were utilized to analyze visceral fat levels. In animal studies, unpaired t-tests were applied to assess differences between treatment groups. A *p*-value of <0.05 was considered statistically significant.

To reduce false-positive results in untargeted metabolomic analysis, we applied the false discovery rate (FDR) method to all *p*-values from the two-tailed Student's t-test, with a cutoff set at FDR < 0.05 (Benjamini & Hochberg, 1995). Fold change and statistical analyses were conducted using Python (3.11). PCA, partial least squares discriminant analysis (PLS-DA), heatmap, and pathway analysis were performed using the web-based metabolomics data processing platform MetaboAnalyst 6.0 (Canada), accessible at http://www.metaboanalyst.ca.

3. Results

3.1. Supplementation with a multi-component synbiotic reduced body weight and fat mass

To assess the effects of the synbiotic supplement on body weight and fat mass, a 5-week intervention trial was conducted with 58 participants aged 18 to 68 years (average age: 41.83 ± 11.22), of whom 70.69% were female (Table 2). After daily intake of the synbiotic supplement,

Table 1
Primer sets for real-time PCR.

Target	Primer sequence
Bifidobacterium longum subsp. *infantis*	Forward: GCGCCATCCTGGTGATGTTATT Reverse: CTACGTGATCTGGGAGAGTTTC
Bifidobacterium longum subsp. *longum*	Forward: GTGTGGATTACCTGCCTACC Reverse: GTCGCCAACCTTGACCACTT
Akkermansia muciniphila	Forward: CAGCACGTGAAGGTGGGGAC Reverse: CCTTGCGGTTGGCTTCAGAT
Universal bacterial 16 s rRNA	Forward: ACTCCTACGGGAGGCAGCAG Reverse: ATTACCGCGGCTGCTGG

Table 2
Effects of the synbiotic supplementation on body composition in participants.

Characteristics			
Age (years)	Range	Mean	
	18–68	41.83 ± 11.22	
Gender	Male	Female	Female sex (%)
	17	41	70.69
Body composition	Week 0	Week 5	*p* value
Body weight (kg)	69.86 ± 16.05	68.59 ± 15.92	<0.0001
Fat mass (kg)	23.76 ± 8.497	22.69 ± 8.446	<0.0001
Body fat (%)	33.6 ± 6.296	32.64 ± 6.544	<0.0001
Visceral fat level	9.5 (7.0–13.0)	9.0 (7.0–12.0)	<0.0001
Skeletal muscle mass (kg)	25.4 ± 6.146	25.24 ± 6.178	0.0646

Body weight, fat mass, body fat percentage, visceral fat level, and skeletal muscle mass were measured at baseline (week 0) and after 5 weeks of synbiotic supplementation (week 5). The data are presented as mean ± SD, except for visceral fat levels, which are shown as median and interquartile range (IQR), $n = 58$. Statistical significance was assessed using paired t-tests.

participants showed a significant decrease in body weight from 69.86 ± 16.05 kg at baseline to 68.59 ± 15.92 kg after 5 weeks ($p < 0.01$). Similarly, fat mass significantly declined from 23.76 ± 8.497 kg to 22.69 ± 8.446 kg ($p < 0.01$), and body fat percentage dropped from 33.6 ± 6.296% to 32.64 ± 6.544% ($p < 0.01$). Additionally, visceral fat levels decreased from 9.5 (7.0–13.0) to 9.0 (7.0–12.0) following supplementation ($p < 0.01$). In contrast, skeletal muscle mass remained unchanged, indicating that the weight loss was mainly due to fat loss rather than muscle loss. The results demonstrate that the synbiotic supplement effectively decreased both total and visceral fat mass in overweight individuals.

3.2. The synbiotic supplementation improved blood metabolic parameters

To evaluate the metabolic effects of the synbiotic supplement, fasting blood parameters were analyzed before and after 5 weeks of supplementation (Table 3). The results showed that, although fasting glucose levels remained stable, HbA1c levels decreased from 5.548 ± 0.3455% to 5.484 ± 0.3422% ($p < 0.01$), and fasting insulin levels declined from 10.69 ± 7.464 μU/mL to 9.21 ± 7.496 μU/mL ($p < 0.01$). The HOMA-IR index, which indicates insulin resistance, also significantly dropped from 2.384 ± 1.981 to 1.964 ± 1.752 ($p < 0.01$). These results suggest that the synbiotic supplement improved blood glucose regulation and insulin sensitivity.

Blood lipid analyses (Table 3) showed that total cholesterol levels decreased from 197.1 ± 38.24 mg/dL to 184.8 ± 39.38 mg/dL ($p < 0.01$), triglycerides from 94.1 ± 43.56 mg/dL to 79.45 ± 43.82 mg/dL ($p < 0.01$). A reduction in LDL-C levels was also observed, dropping from 123.3 ± 36.13 mg/dL to 114.9 ± 35.75 mg/dL ($p < 0.01$), while HDL-C levels declined from 62.9 ± 17.72 mg/dL to 59.62 ± 17.76 mg/dL ($p < 0.01$). The ratio of apolipoprotein B to apolipoprotein A1 (APO-B/APO-A1) remained unchanged. These findings suggest that the synbiotic supplementation caused beneficial changes in blood lipid profiles, although a decrease in HDL-C was also observed.

Two cardiovascular risk markers, homocysteine and high-sensitivity C-reactive protein (hsCRP) (Ganguly & Alam, 2015; Oemrawsingh et al., 2016), were also assessed (Table 3). No significant changes were observed in either marker, suggesting limited effects on systemic inflammation or cardiovascular risk factors within the 5-week period.

3.3. The synbiotic supplementation selectively changed microbial-derived metabolites

To further explore microbiota-related changes, we measured circulating levels of SCFAs and tryptophan-derived metabolites, two important classes of microbial metabolites involved in host metabolic regulation (Table 4). After five weeks of supplementation, significant increases were observed in plasma concentrations of acetic acid (AA, from 21.48 ± 19.04 μM to 30.69 ± 14.29 μM, $p < 0.01$), isobutyric acid (iBA, from 0.4283 ± 0.1714 μM to 0.4814 ± 0.141 μM, $p < 0.05$), and isovaleric acid (iVA, from 0.4188 ± 0.3649 μM to 0.5897 ± 0.5475 μM, $p < 0.01$). Other SCFAs detected, including propionic acid, butyric acid, and valeric acid, did not show significant changes.

Regarding tryptophan metabolism, plasma tryptophan levels remained stable. However, notable increases were observed in two downstream microbial metabolites, serotonin (from 0.6978 ± 0.3011 μM to 0.7784 ± 0.3201 μM, $p < 0.05$) and indolelactic acid (ILA) (from 0.6514 ± 0.2182 μM to 0.7228 ± 0.2521 μM, $p < 0.05$). No significant changes occurred in indoleacetic acid (IAA) and indolepropionic acid (IPA). These findings suggest that the synbiotic supplementation selectively influenced certain microbial metabolic pathways, boosting the production of specific SCFAs and tryptophan-related compounds.

3.4. Global metabolomic profiling reveals subtle but specific changes following synbiotic supplementation

To investigate broader changes in metabolites induced by the synbiotic supplementation, untargeted metabolomics analysis was performed on plasma samples collected before and after supplementation. A total of 2029 compounds were identified across all samples. Of these, 61 metabolites were annotated with Level 1 (the highest confidence), 107 as Level 2, 667 as Level 3, and 1194 as Level 4. PLS-DA analysis was used to visualize overall metabolite changes before and after supplementation (Fig. 1a). Only partial separation between before- and after-supplementation samples was observed, indicating modest changes in the metabolomics profile. Similarly, hierarchical clustering analysis also did not show clear group separation (Fig. 1b). However, volcano plot analysis identified 35 significantly altered metabolites (6 upregulated,

Table 3
Effects of the synbiotic supplementation on blood parameters in participants.

Parameter	Week 0	Week 5	*p* value
Glucose-AC (mg/dL)	82.62 ± 10.53	82.83 ± 8.738	0.8046
HbA1c (%)	5.548 ± 0.3455	5.484 ± 0.3422	0.0003
Insulin (μU/mL)	10.69 ± 7.464	9.21 ± 7.496	0.0024
HOMA-IR index	2.384 ± 1.981	1.964 ± 1.752	0.0157
T-Cholesterol (mg/dL)	197.1 ± 38.24	184.8 ± 39.38	< 0.0001
Triglyceride (mg/dL)	94.1 ± 43.56	79.45 ± 43.82	0.0004
LDL-C (mg/dL)	123.3 ± 36.13	114.9 ± 35.75	0.0004
HDL-C (mg/dL)	62.9 ± 17.72	59.62 ± 17.76	<0.0001
APO-B/APO-A1	0.5953 ± 0.1963	0.5912 ± 0.1801	0.6626
Homocysteine (μmol/L)	10.96 ± 3.019	10.95 ± 3.289	0.9665
hsCRP (mg/dL)	0.1334 ± 0.1465	0.1466 ± 0.223	0.5926

Blood parameters related to glucose homeostasis, lipid metabolism, and cardiovascular disease risk were measured at weeks 0 and 5 of the synbiotic supplementation. Data are presented as mean ± SD, n = 58. Statistical significance was assessed using paired t-tests.

Table 4
Effects of the synbiotic supplementation on levels of gut microbes-derived metabolites in participants.

Metabolite (μM)	Week 0	Week 5	*p* value
Acetic acid	21.48 ± 19.04	30.69 ± 14.29	0.0022
Propionic acid	1.621 ± 1.224	1.824 ± 0.8019	0.1598
Butyric acid	0.4767 ± 0.617	0.5079 ± 0.2478	0.666
Isobutyric acid	0.4283 ± 0.1714	0.4814 ± 0.141	0.0266
Valeric acid	0.0769 ± 0.08682	0.09086 ± 0.05910	0.1965
Isovaleric acid	0.4188 ± 0.3649	0.5897 ± 0.5475	0.0058
Tryptophan	60.21 ± 11.2	61.5 ± 12.72	0.5623
Serotonin	0.6978 ± 0.3011	0.7784 ± 0.3201	0.0254
Indoleacetic acid	2.297 ± 1.291	2.59 ± 1.761	0.1028
Indolepropionic acid	5.442 ± 17.34	5.867 ± 16.6	0.692
Indolelactic acid	0.6514 ± 0.2182	0.7228 ± 0.2521	0.0165

SCFAs, tryptophan (TRP), and TRP-derived microbial metabolites were analyzed at week 0 and week 5 of the synbiotic supplementation. Data are presented as mean ± SD, n = 58. Statistical significance was assessed using paired t-tests.

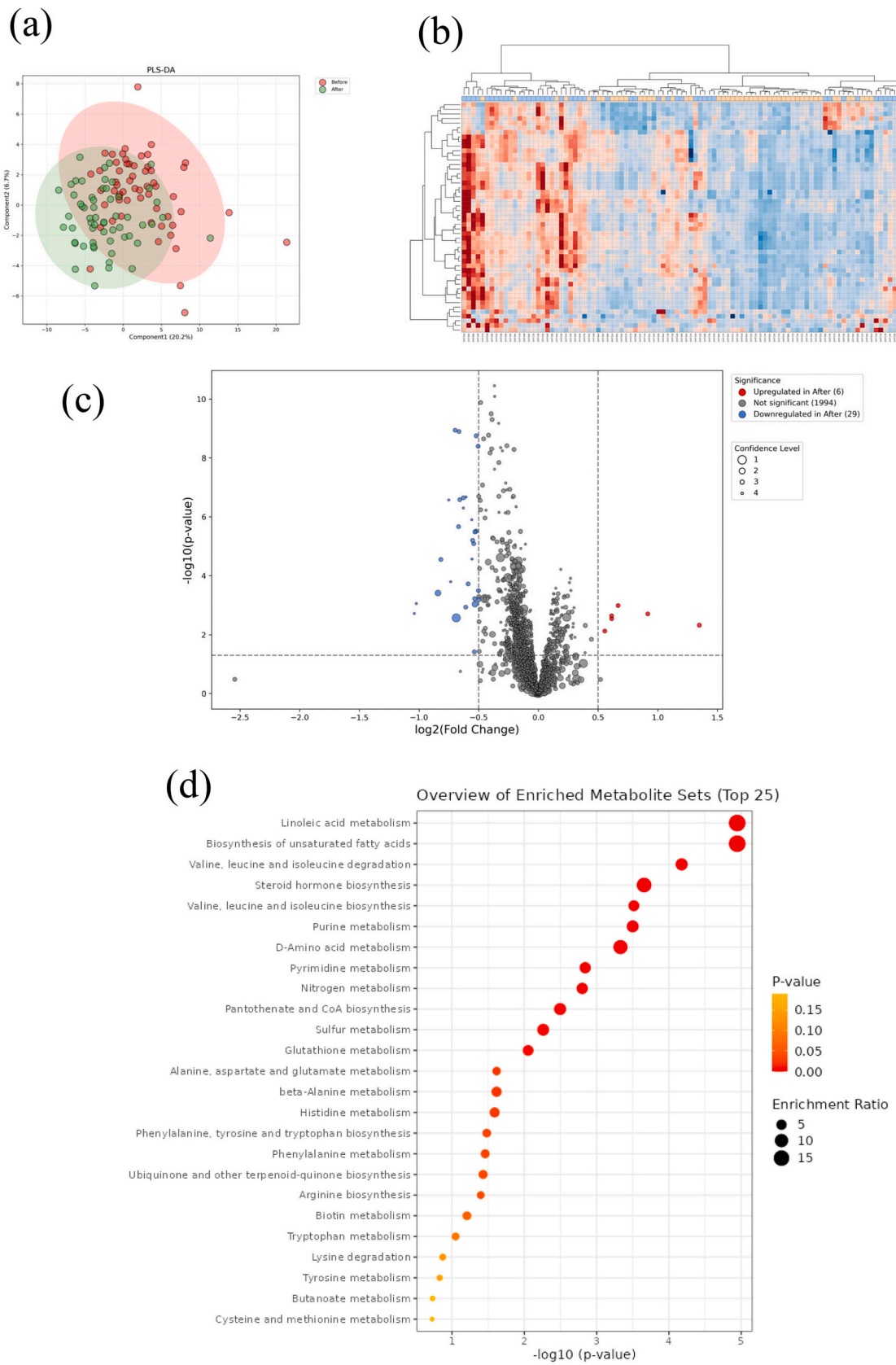


Fig. 1. Plasma metabolomic profiles before and after synbiotic supplementation.

29 downregulated) based on thresholds of $\log_2$ fold change >0.5 or < -0.5, with $p < 0.05$ (Fig. 1c). Pathway enrichment analysis revealed that linoleic acid metabolism and unsaturated fatty acid biosynthesis were notably enriched following the synbiotic supplementation (Fig. 1d).

To further characterize the high-confidence metabolic changes, we focused on Level 1 metabolites, which have the highest confidence level based on accurate mass, MS/MS fragmentation patterns, and retention time matched to authentic standards. Among these, fifteen metabolites showed significant changes after synbiotic supplementation (Fig. 2a–o). Notably, several lipid-related metabolites were significantly decreased, including sphingosine-1-phosphate, sphinganine-1-phosphate, leukotriene B4, 1-heptadecanoyl-2-hydroxy-sn-glycero-3-PC, and 1-docosahexaenoyl-2-hydroxy-sn-glycero-3-PC. In parallel, several amino acids and their related metabolites were also affected, with decreases observed in alanine, tyrosine, glutamate, L-kynurenine, and *N*-methyl-aspartic acid, while glutamine levels increased. Additionally, levels of α-ketoglutaric acid and pseudouridine were elevated, whereas acetylcholine and γ-glutamylphenylalanine levels were reduced. Although the overall metabolome remained relatively stable, these specific changes suggest that synbiotic supplementation selectively influenced pathways related to lipid and amino acid metabolism.

3.5. The synbiotic supplementation increased plasma GLP-1 levels

GLP-1 is a crucial incretin involved in regulating glucose, suppressing appetite, and stimulating insulin secretion. It is triggered by nutrients and specific microbial metabolites like SCFAs, as well as certain gut microbes, including *A. muciniphila* (Larraufie et al., 2018; Yoon et al., 2021; Zeng, Wu, Zhang, & Xiao, 2024). Given the observed rise in SCFAs following the synbiotic supplement, both fasting and postprandial plasma were measured to evaluate changes in GLP-1 levels (Fig. 3). Results showed that postprandial GLP-1 levels were significantly higher than fasting levels both before and after the intervention ($p < 0.01$), consistent with previous research (Brynes et al., 1998; Jakubowicz et al., 2014; Lee et al., 2015; Vilsbøll et al., 2003; Watkins, Koumanov, & Gonzalez, 2021). Notably, both fasting and postprandial GLP-1 levels increased at week 5 compared to baseline ($p < 0.01$), suggesting that the synbiotic supplementation boosted GLP-1 secretion in both states. These findings suggest that synbiotic supplementation enhances GLP-1 secretion in humans, likely through increased SCFA production, changes in gut microbiota, or both.

3.6. The synbiotic supplementation enhanced GLP-1 secretion and increased gut levels of a. muciniphia and B. longum in mice

Although the clinical trial data showed that the synbiotic supplementation significantly increased both fasting and postprandial GLP-1 levels in participants, it should be noted that GLP-1 measurements were not taken in all participants. To further validate the effects, we conducted a follow-up experiment in mice. In this experiment, mice were divided into a control group fed a ground chow diet containing 1.5% maltodextrin and an experimental group fed a chow diet supplemented with 1.5% of the synbiotic supplement. After six weeks of treatment, plasma GLP-1 levels were significantly higher in the synbiotic group compared to the control group ($p < 0.01$) (Fig. 4a), confirming that the synbiotic intervention stimulated GLP-1 secretion in mice. Given the established link between *A. muciniphila* and GLP-1 secretion (Yoon et al., 2021), we further investigated whether the synbiotic supplementation could affect the abundance of this next-generation probiotic. Quantitative PCR analysis of fecal samples showed that mice receiving the synbiotic had significantly higher levels of *A. muciniphila* compared to the control group ($p < 0.05$) (Fig. 4b). These results suggest that the synbiotic supplement not only enhances GLP-1 secretion but also increases the abundance of *A. muciniphila*, which may contribute to the elevated GLP-1.

In addition to evaluating *A. muciniphila*, we further assessed the fecal abundance of two subspecies of *B. longum*. Notably, *B. longum* subsp. *infantis*, the strain included in the synbiotic supplement, was undetectable in all control group samples but was consistently present in the synbiotic group (Ct value: 37.29 ± 1.53), indicating that it was introduced through supplementation rather than being a constituent of the native gut microbiota (Fig. 4c). On the other hand, *B. longum* subsp. *longum*, which was detectable in both groups, exhibited a significantly higher abundance in the synbiotic group compared to the control group ($p < 0.05$) (Fig. 4d). This finding suggests that, beyond introducing *B. longum* subsp. *infantis*, the synbiotic may also promote the growth or colonization of endogenous *B. longum* subsp. *longum*. These results align with previous studies reporting that lychee-derived polyphenols can stimulate the proliferation of *B. longum* strains in the gut (Lyu et al., 2024), further supporting the potential of the synbiotic to modulate the gut microbial ecosystem in favor of beneficial taxa.

4. Discussion

In this pilot clinical trial, five weeks of supplementation with the new synbiotic formulation resulted in significant improvements in obesity-related parameters. Our results showed notable reductions in body weight, total fat mass, and visceral fat, while skeletal muscle mass remained stable in the participants. These findings suggest that the synbiotic specifically decreased adiposity rather than causing overall weight loss, although further confirmation is necessary. Improvements in glycemic control, indicated by decreases in HbA1c, fasting insulin levels, and HOMA-IR, support its metabolic benefits. Additionally, positive changes in lipid profiles were observed, including lower levels of total cholesterol, triglycerides, and LDL-C, although HDL-C levels also declined. These results align with previous clinical studies demonstrating that probiotics, especially *Bifidobacterium* species, and polyphenols can positively influence metabolic health in overweight or obese individuals (Ben Othman et al., 2023; Chaiyasut et al., 2021; Kilic Yildirim et al., 2022; Sergeev et al., 2020).

Microbial-derived metabolites play a vital role in regulating host metabolism. In this study, targeted metabolite analysis revealed that supplementation with the synbiotic supplement significantly increased plasma levels of several SCFAs, including acetic acid, isobutyric acid, and isovaleric acid, as well as tryptophan-derived metabolites such as serotonin and indolelactic acid. Although some clinical studies have shown a positive association between higher SCFA levels and obesity (Gilbert et al., 2023; K. N. Kim, Yao, & Ju, 2019; Lu et al., 2016), increasing evidence also highlights the beneficial metabolic effects of specific SCFAs. For instance, acetic acid has been demonstrated to reduce body weight and improve glucose and lipid metabolism, possibly through mechanisms involving the modulation of bile acid metabolism and increased energy expenditure (Valdes, So, Gill, & Kellow, 2021; Wang, Fan, et al., 2022; Yamashita, 2016). Likewise, branched-chain SCFAs like isobutyric acid and isovaleric acid have been associated with better glucose regulation and insulin sensitivity in both animal and human studies (Aslamy et al., 2024; Heimann, Nyman, Pålbrink, Lindkvist-Petersson, & Degerman, 2016).

In addition to their metabolic effects, SCFAs are also known to stimulate the secretion of GLP-1, a gut hormone that is crucial for glucose regulation and suppressing appetite. Acetic acid, in particular, may produce this effect through activation of free fatty acid receptor 2 (FFAR2) or via cAMP-dependent pathways (Christiansen et al., 2018; Tolhurst et al., 2012). We also observed an increase in serotonin levels after synbiotic supplementation. Previous studies have shown that activation of the serotonin receptor 4 (5-HT_4 receptor) can promote GLP-1 secretion (Vanslette, Toft, Lund, Moritz, & Arora, 2023), suggesting that the increased serotonin levels in this study may also help raise GLP-1 concentrations. Overall, these findings indicate multiple potential mechanistic links between microbial metabolites and the improved metabolic outcomes seen in our research.

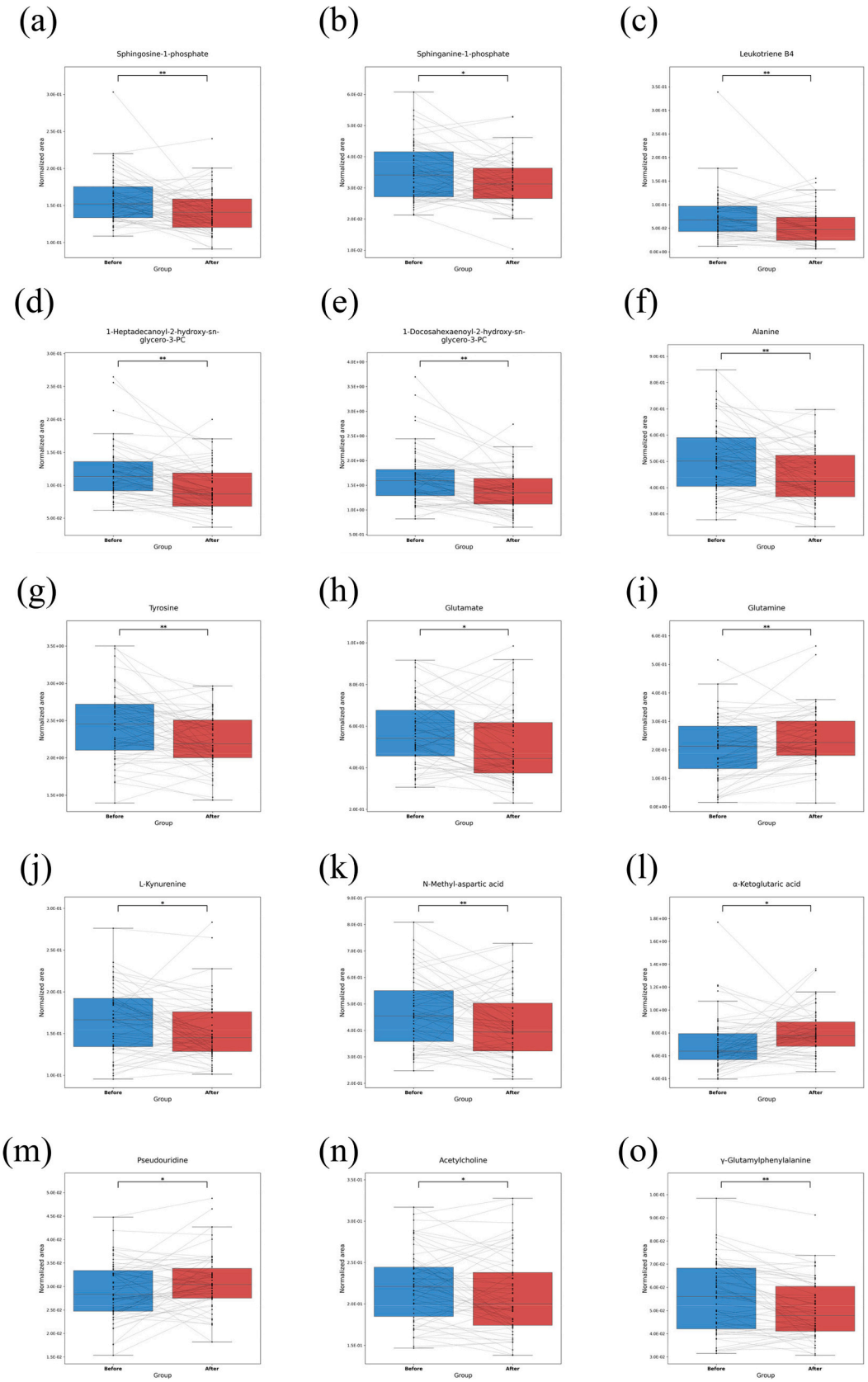


Fig. 2. Significant Changes in Well-Annotated Metabolites Before and After Synbiotic Supplementation.

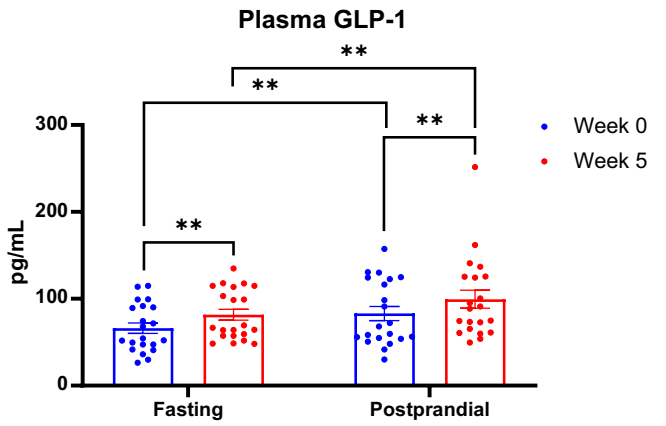


Fig. 3. The synbiotic supplementation increased plasma GLP-1 levels in participants.

One of the most notable findings is the consistent increase in both fasting and postprandial plasma GLP-1 concentrations after supplementation in human participants. GLP-1 plays a central role in glycemic control, appetite suppression, and insulin secretion (Drucker, 2018). Because of these functions, GLP-1 receptor agonists (GLP-1 RAs) have been widely used in clinical medicine to treat type 2 diabetes and obesity, showing significant benefits in lowering blood glucose and promoting weight loss (Ard, Fitch, Fruh, & Herman, 2021; Zheng et al., 2024). The rise in endogenous GLP-1 observed in this study suggests that the synbiotic supplement produces similar metabolic benefits by naturally stimulating GLP-1 secretion. This increase is likely linked to higher SCFA levels and may also involve changes in the microbiota, including increased levels of *A. muciniphila,* a next-generation probiotic known to promote GLP-1 secretion (Yoon et al., 2021). Supporting evidence from the mouse study further confirmed the synbiotic's ability to raise both GLP-1 and *A. muciniphila* levels, suggesting that these microbial and hormonal changes may work together to improve host metabolism. However, due to the absence of human microbiota profiling, this possible link remains speculative.

Furthermore, we observed a significant increase in *B. longum* subsp. *infantis* in the synbiotic group, while this subspecies was undetectable in the control group. This indicates that *B. longum* subsp. *infantis* was introduced through the synbiotic supplement. Additionally, *B. longum* subsp. *longum* was present in both groups, but its levels were significantly higher in the synbiotic group, indicating that the synbiotic also helped promote the growth of native *B. longum* strains. These results suggest that the synbiotic may influence gut microbial composition by introducing or enriching specific probiotic strains. It's worth noting that lychee-derived polyphenols, one of the synbiotic components, have been reported to promote the growth of *B. longum* in the gut microbiota of mice (Lyu et al., 2024). These earlier findings align with our observation of increased *B. longum* abundance in the synbiotic group. However, while our results show a shift in certain bacterial taxa, the current study does not provide a thorough analysis of the entire gut microbiota. Therefore, although these targeted qPCR results suggest a potential microbiota-modulating effect of the synbiotic, we recognize that further studies using high-throughput sequencing are needed to fully characterize these changes and determine whether they are long-lasting or lead to broader ecological shifts. The possible link between these microbial changes and the observed metabolic improvements also remains unclear.

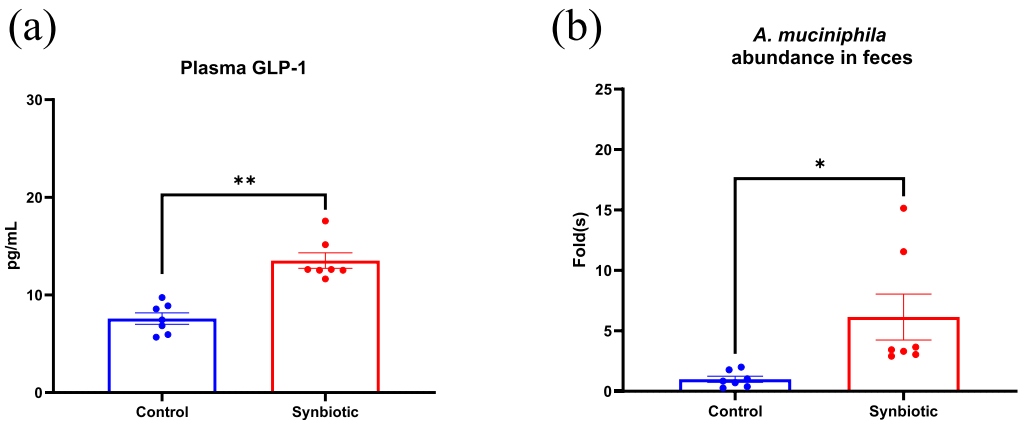
To expand the analysis beyond targeted metabolites, untargeted metabolomics profiling of plasma samples was performed, revealing subtle but specific metabolic changes. Although overall metabolome alterations were limited, thirty-five metabolites showed significant differences, as shown by volcano plot analysis. Pathway enrichment analysis indicated changes in lipid-related pathways, especially linoleic acid metabolism and the biosynthesis of unsaturated fatty acids. Among the high-confidence (Level 1) annotated metabolites, several lipid-related molecules were significantly decreased after synbiotic supplementation. Similarly, multiple amino acid-related metabolites were affected, with decreases in alanine, tyrosine, glutamate, L-kynurenine, and *N*-methyl-aspartic acid, and increases in glutamine and α-ketoglutaric acid. These findings suggest that the synbiotic may have systemic metabolic effects by influencing both lipid and amino acid pathways. Prior research has shown that elevated sphingosine-1-phosphate levels are positively linked to obesity and metabolic issues (Fan et al., 2005; Kowalski, Carey, Selathurai, Kingwell, & Bruce, 2013), while leukotriene B4 acts as a proinflammatory lipid mediator capable of impairing insulin signaling and promoting insulin resistance (Filgueiras, Serezani, & Jancar, 2015; Li et al., 2015; Ying et al., 2017). Likewise, L-kynurenine, a metabolite derived from tryptophan, has been linked to increased adiposity and glucose intolerance (Cussotto et al., 2020; Huang et al., 2022), whereas α-ketoglutaric acid is known to enhance glucose metabolism and has been associated with lifespan extension (Naeini, Mavaddatiyan, Kalkhoran, Taherkhani, & Talkhabi, 2023; Yuan et al., 2022). Although these specific changes provide insight into how the synbiotic might influence host metabolism, the causal relationship between these metabolite shifts and physiological improvements, such as reduced adiposity and improved insulin sensitivity, remains unclear. Further mechanistic and long-term studies are necessary to determine whether these metabolite changes serve as active mediators or are simply downstream markers of the synbiotic ‘s metabolic benefits.

These findings suggest that the synbiotic combining *B. longum*, lychee polyphenols, and methionine may be an effective strategy for targeting gut microbiota and improving obesity-related outcomes. While each component has been independently linked to metabolic benefits in previous studies, our results show that this combined synbiotic formulation can reliably improve body composition, metabolic markers, and GLP-1 secretion. The effects are likely due to microbiota modulation and increased production of beneficial metabolites. However, since the study did not test each component individually, it cannot confirm whether the effects are additive or synergistic. Further research with comparative groups is needed to clarify the specific roles and interactions of these components.

Despite promising results, this study has some key limitations that should be acknowledged. As a pilot clinical trial, the small sample size, short duration, and lack of a placebo-controlled design may impact the generalizability and reliability of the findings. Additionally, while improvements in host metabolism and microbe-derived metabolites were observed, the absence of detailed gut microbiota profiling limits our understanding of which specific microbial taxa are involved. Furthermore, although methionine and *B. longum* were selected based on prior evidence linking their interaction to the production of MTA, the role of MTA in driving these metabolic benefits has not yet been directly confirmed in humans. Future studies with larger participant groups, longer intervention periods, placebo controls, and comprehensive microbiome and metabolite analysis will be necessary to validate these results and to better understand the interactions among microbial communities, host responses, and bioactive metabolites. These efforts will help clarify the mechanisms behind this synbiotic approach and its potential application in managing obesity.

5. Conclusion

This study demonstrates that a novel synbiotic supplement, including lychee polyphenols, *B. longum*, *B. animalis*, methionine, prebiotics, and postbiotics, has positive effects on obesity-related indicators in humans. Reductions in body fat, visceral fat, HbA1c, insulin resistance, and blood lipid levels were observed in the 5-week pilot clinical trial. Metabolite analyses, using both targeted and untargeted approaches, revealed increases in short-chain fatty acids and tryptophan-related metabolites, along with shifts in amino acid and lipid



(c)

Group	Ct value	
	Control	Synbiotic
B. longum supsp. *infantis*	All ND	37.29 ± 1.53
Universal bacterial 16s rRNA	16.18 ± 0.14	16.49 ± 0.18

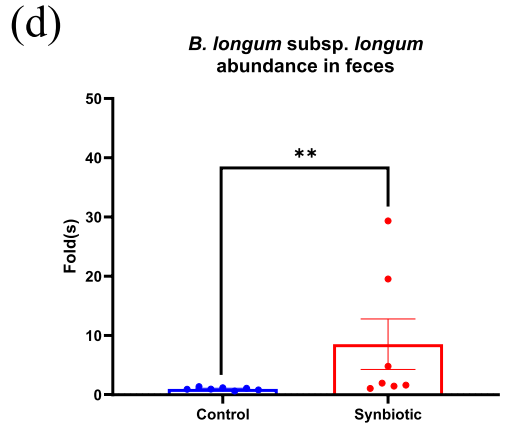


Fig. 4. The synbiotic supplementation increased plasma GLP-1 levels and the abundance of fecal *B. longum* subspecies and *A. muciniphila* in mice.

metabolism. Both human and animal studies also showed higher plasma GLP-1 levels. Additionally, mice given the synbiotic showed increased levels of gut *A. muciniphila*. These results suggest that this synbiotic could be a novel approach to enhance metabolic health and alleviate obesity by modulating gut microbiota-derived metabolites and incretin hormones. More long-term and detailed studies are necessary to better understand the mechanisms and improve clinical applications.

CRediT authorship contribution statement

Chiao-Wei Lin: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hsin-Bai Zou:** Methodology, Data curation. **Rou-An Chen:** Methodology, Conceptualization. **Chi-Shan Li:** Supervision, Project administration. **Chia-Ying Lin:** Formal analysis. **Cheng-Chih Hsu:** Supervision, Funding acquisition, Conceptualization.

Ethics statement

The clinical study protocol was reviewed and approved by the Institutional Review Board of the Development Center for Biotechnology, Taiwan (Approval No. IRB-AP-11305). All participants provided written informed consent before enrollment in accordance with the Declaration of Helsinki. Animal experiments were conducted following

the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University (Approval No. NTU112-EL-00094) and the IACUC of Leeuwenhoek Laboratories Co., Ltd. (Approval No. LL-2023001).

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Declaration of competing interest

This study was funded by Leeuwenhoek Laboratories Co., Ltd., and all authors are employees of the company. The product evaluated in this study was developed by the company.

Data availability

Data will be made available on request.

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