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Matcha alleviates obesity by modulating gut microbiota and its metabolites

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ABSTRACT

Matcha shows promise for diabetes, obesity, and gut microbiota disorders. Studies suggest a significant link between gut microbiota, metabolites, and obesity. Thus, matcha may have a positive impact on obesity by modulating gut microbiota and metabolites. This study used 16S rDNA sequencing and untargeted metabolomics to examine the cecal contents in mice. By correlation analysis, we explored the potential mechanisms responsible for the positive effects of matcha on obesity. The results indicated that matcha had a mitigating effect on the detrimental impacts of a high-fat diet (HFD) on multiple physiological indicators in mice, including body weight, adipose tissue weight, serum total cholesterol (TC), and low-density lipoprotein (LDL) levels, as well as glucose tolerance. Moreover, it was observed that matcha had an impact on the structural composition of gut microbiota and gut metabolites. Specifically, matcha was able to reverse the alterations in the abundance of certain obesity-improving bacteria, such as *Alloprevotella*, *Ileibacterium*, and *Rikenella*, as well as the abundance of obesity-promoting bacteria *Romboutsia*, induced by a HFD. Furthermore, matcha can influence the levels of metabolites, including formononetin, glutamic acid, pyroglutamic acid, and taurochenodeoxycholate, within the gastrointestinal tract. Additionally, matcha enhances caffeine metabolism and the HIF-1 signaling pathway in the KEGG pathway. The results of the correlation analysis suggest that formononetin, theobromine, 1,3,7-trimethyluric acid, and Vitamin C displayed negative correlation with both the obesity phenotype and microbiota known to exacerbate obesity, while demonstrating positive correlations with microbiota that alleviated obesity. However, glutamic acid, pyroglutamic acid, and taurochenodeoxycholate had the opposite effect. In conclusion, the impact of matcha on gut metabolites may be attributed to its modulation of the abundance of *Alloprevotella*, *Ileibacterium*, *Rikenella*, and *Romboutsia* within the gastrointestinal tract, thereby potentially contributing to the amelioration of obesity.

1. Introduction

Obesity is one of the major health problems endangering human health in the world. Obesity currently affects more than 2 billion people worldwide, and the prospects for controlling the obesity epidemic are not good (Caballero, 2019). In addition to many metabolic diseases such as diabetes (Ng et al., 2021), cardiovascular and cerebrovascular diseases (Kim et al., 2021), fatty liver (Hashem et al., 2021), and chronic kidney disease (Vasylyeva and Singh, 2016), obesity can also lead to reproductive disorders (Crujeiras and Casanueva, 2015) and mental diseases such as depression (Milaneschi et al., 2019). Thus, there is an urgent need to address the health crisis of obesity.

Matcha, a powder derived from green tea leaves, is commonly utilized in the food realm. In addition to its unique flavor profile, matcha

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has shown promise in possessing anti-inflammatory, anti-diabetic, and anti-obesity characteristics (Ye et al., 2023). Studies have shown that matcha has the potential to mitigate weight gain, elevated blood lipids, and liver damage caused by a high-fat diet (HFD) in mice (J. Zhou et al., 2021). In addition, matcha can also inhibit JAK2/STAT3 signaling pathway to prevent obesity-induced hypothalamic inflammation (J. Zhou et al., 2020). A recent prospective study has suggested that incorporating matcha into one's diet may result in notable decreases in body weight, body mass index, waist circumference, and fasting blood glucose levels in individuals who are obese (El-Elimat et al., 2022). Nevertheless, the specific mechanism by which matcha alleviates obesity remains to be further explored.

The gut microbiota, also known as the "second genome", is distinguished by its dynamic and variable composition and function, which can interact with dietary components to extensive impact various physiological processes in the host (Schoeler and Caesar, 2019; B. Zhu et al., 2010). Among them, the host energy metabolism and balance is considered to be important targets for the role of gut microbiota (Cani and Van Hul, 2024). Gut microbiota and their metabolites play an important role in the pathogenesis of obesity and related diseases (M. Zhou et al., 2023a). Specifically, the gut microbiota has been shown to regulate metabolic pathways such as branched-chain amino acid metabolism and bile acid metabolism in the gut, thus playing a significant role in the onset and progression of obesity (Allegretti et al., 2020; Miyamoto et al., 2019; Zeng et al., 2020). Recent studies have shown that green tea can change the gut microbiota to promote thermogenesis, as well as prevent or improve obesity (D. Li et al., 2023a; Tian et al., 2024). Matcha served as a powder of green tea processing, and it has also been suggested that the supplementation of matcha may help alleviate alterations in stool bile acid composition and gut microbiota caused by a HFD (Y. Wang et al., 2022b). Consequently, the gut microbiota and gut metabolites are believed to be significant factors in the potential efficacy of matcha in addressing obesity.

This research utilized a HFD to induce obesity in a mouse model and administered matcha via gavage to assess its effects on obesity. Then, 16S rDNA sequencing and untargeted metabolomics analysis were employed to investigate the impact of matcha on gut microbiota and metabolites. Subsequently, correlation analysis was conducted to explore the metabolic interactions between gut microbiota and metabolites, with the goal of elucidating the potential mechanism by which matcha may mitigate obesity.

2. Materials and methods

2.1. Animal feeding and sample collection

The C57BL/6J male mice (6 weeks old) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. All the animal experiments were performed in accordance with the requirements of the Laboratory Animal-Guideline for ethical review of animal welfare. And the animal experiments were approved by the Animal Ethics Committee of Southwest Medical University (No. swmu20220181). The mice were housed in the specific pathogen-free facility (SPF) barrier system at the Experimental Animal Center of Southwest Medical University, with an ambient temperature of 22 ± 2 °C, humidity of 50%–60%, and a 12-h dark/light cycle. The experimental procedures complied with the ethical guidelines for laboratory animal welfare. After one week of adaptation, 30 mice were randomly divided into a control group (CK group, n = 10) and a high-fat diet group (HFD group, n = 20). The CK group was fed a normal diet, while the HFD group was fed a high-fat diet (HFD). The normal diet contained carbohydrates 65.08 kcal%, protein 23.07 kcal%, and fat 11.85 kcal%; the high-fat diet contained carbohydrates 20 kcal%, protein 20 kcal%, and fat 60 kcal%. Detailed compositions of the normal and HFD diets are provided in Supplement Table 1. During the experiment, all experimental animals ate and drank freely. In the 12th week, mice in the HFD group were randomly divided into a model control group (MK group, n = 10) and a matcha group (M group, n = 10). Over the following 5 weeks, mice in the M group were orally administered matcha physiological saline solution (1 g/kg body weight, provided by Sichuan University of Science and Engineering), while mice in the CK and MK groups were orally administered an equivalent amount of physiological saline. Detailed compositions of the matcha is provided in Supplement Table 1. In the 16th week, 4 mice from each group were randomly selected for glucose tolerance tests and insulin tolerance tests. The remaining mice continued to be orally administered their respective solutions until the 17th week. This was to avoid the potential impact of intraperitoneal glucose and insulin injection on 16S rDNA sequencing and untargeted metabolomics results. At the end of week 17, these 6 mice were fasted overnight, euthanized by cervical dislocation under 1% pentobarbital sodium anesthesia (50 mg/kg body weight), and immediately collected and weighed the peritesticular adipose tissue. The contents of the cecum below the cecum valve were collected, frozen at −80 °C, and used for 16S rDNA sequencing and untargeted metabolomics analysis.

2.2. Glucose tolerance test (GTT) and insulin tolerance test (ITT)

In the 16th week, four randomly selected mice from each group were fasted for 12 h. Blood samples were collected via tail vein puncture, and blood glucose concentrations were measured using a blood glucose meter (Roche, ACCU-CHEK). Subsequently, 10% glucose solution (2 g/kg body weight) was injected intraperitoneally. Blood glucose concentrations were then measured at 30, 60, 90, and 120 min after administration. After the experiment, mice resumed their original diet and oral administration. In the 17th week, the aforementioned randomly selected mice were fasted for 4 h, and blood glucose concentrations were measured using the same method. Subsequently, insulin solution at a dose of 0.75 IU/kg was injected intraperitoneally at a concentration of 0.0075 IU/ml. Blood glucose concentrations were measured at 30, 60, 90, and 120 min after administration.

2.3. Biochemical analysis of serum

Blood samples were collected in the morning and centrifuged at 4 °C at 3500 r/min for 10 min. Serum was collected, and the concentrations of testing triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured using a fully automatic veterinary biochemical analyzer (Jiangxi Tekang Technology Co., Ltd., TC220).

2.4. Fat histopathology

The peritesticular adipose tissue was immersed in 4% paraformaldehyde for fixation, followed by washing, dehydration, paraffin embedding, sectioning (4 μm), and staining with hematoxylin-eosin. The tissue samples were evaluated for histopathological characteristics under a microscope, with three photographs taken for each sample. The area of adipocytes in the sample images was measured and analyzed using Image Pro Plus 6.0.

2.5. 16S rDNA sequencing

The gut microbial genomic DNA from the cecal contents was extracted using the HiPure Stool DNA Kit (D3141, Guangzhou Meiji Biological Co., LTD., China). The V3-V4 region of the 16S rDNA (341F: CCTACGGGRBGCASCAG; 806R: GGACTACNNGGGTATCTAAT) was amplified using specific primers with barcode. The amplified products were recovered, quantified, purified, and used for library construction and subsequent sequencing. After obtaining raw reads, low-quality reads were filtered using FASTP (V0.18.0, https://github.com/OpenGene/fastp), followed by joining reads into tags using FLASH (V1.2.11, http://www.cbcb.umd.edu/software/flash), filtering and removing

chimeras from tags to obtain effective tags. OTU abundance was calculated based on effective tags. The sequence information of OTUs was compared with the SILVA database to obtain species annotation information for each OTU. Alpha diversity analysis was conducted using QIIME (V1.9.1, http://qiime.sourceforge.net/), while beta diversity analysis was performed using R studio. The LEfSe software was employed for Linear discriminant analysis effect size (LEfSe) analysis (V1.0, http://huttenhower.sph.harvard.edu/lefse/), with species having an LDA value > 4 considered as biomarkers.

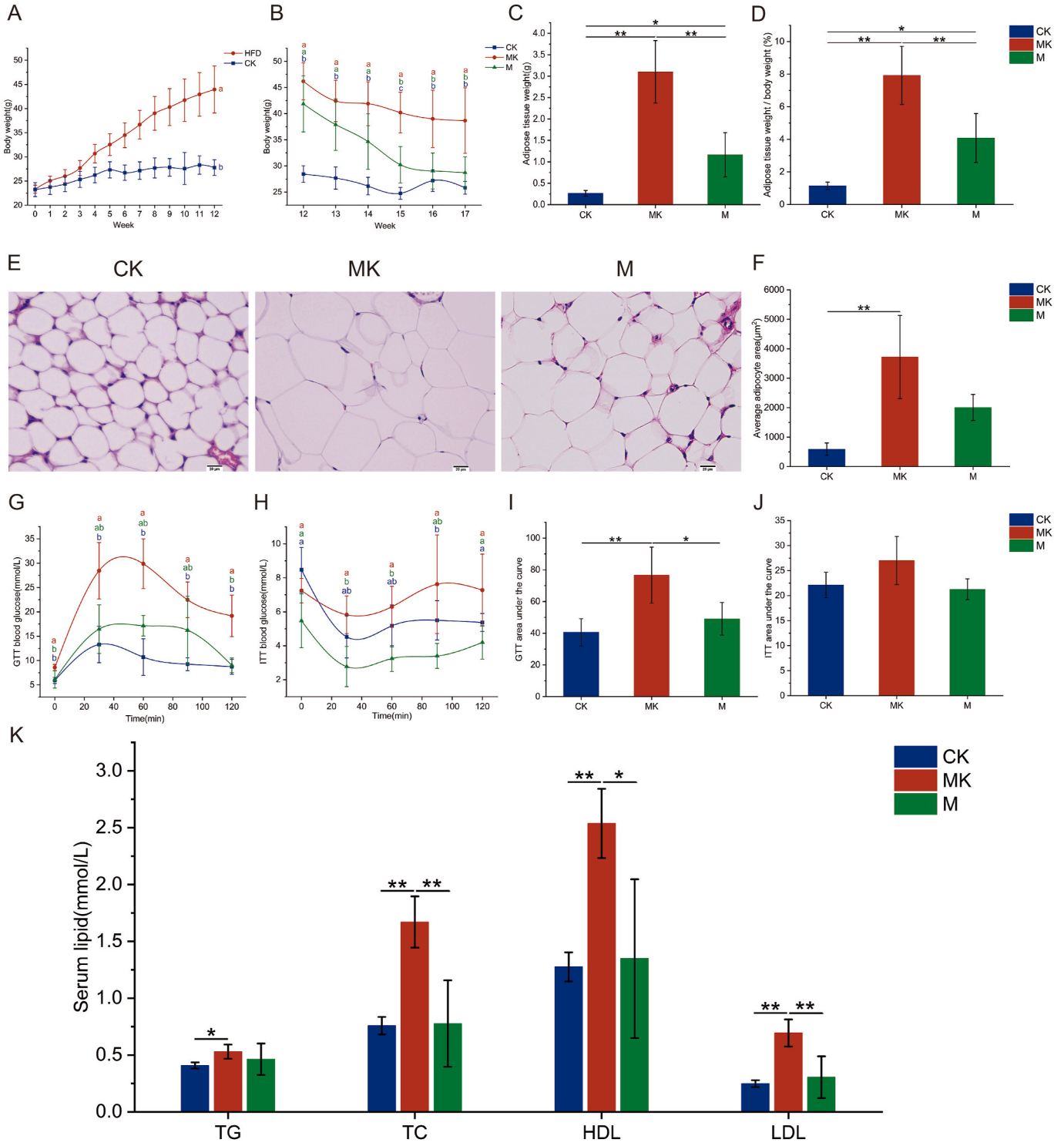

Fig. 1. Effect of matcha on obesity-related phenotypes in high-fat diet (HFD)-induced obese mice. **(A)** Body weight of mice during the obesity model induced by high fat diet. **(B)** Effect of matcha on body weight, **(C)** adipose tissue weight, **(D)** adipose tissue weight as a percentage of body weight, **(E)** size of adipocytes under HE staining, **(F)** average adipocyte area, **(G, I)** glucose tolerance test (GTT) and **(H, J)** insulin tolerance test (ITT), and **(K)** serum lipid profile including triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Different lowercase letters indicate significant differences between groups ($P < 0.05$), and data are expressed as mean ± SD (n = 6), *$P < 0.05$ and **$P < 0.01$.

2.6. Untargeted metabolomics

The cecal contents were slowly thawed at 4 °C, and an appropriate amount of sample was added to a solution of methanol/acetonitrile/water (2:2:1, v/v), mixed, and left at −20 °C for 10 min. Subsequently, the mixture was centrifuged at 14000g, 4 °C for 20 min, and the supernatant was taken for analysis by LC-MS. An equal volume of each sample was mixed to create a pooled sample used as a quality control (QC). Samples were analyzed using Ultra-High Performance Liquid Chromatography (UHPLC, 1290 Infinity LC, Agilent Technologies) with a HILIC column for separation (Column temperature: 25 °C, flow rate: 0.5 mL/min, sample volume:2 μL). The mobile phase contained A: 25 mM ammonium acetate and 25 mM ammonium hydroxide in water and B: acetonitrile. The gradient elution program was as follows: 0–0.5 min, 95% B; 0.5–7 min, linear decrease of B from 95% to 65%; 7–8 min, linear decrease of B from 65% to 40%; 8–9 min, B held at 40%; 9–9.1 min, linear increase of B from 40% to 95%; 9.1–12 min, B held at 95%. Throughout the analysis, samples were kept in an autosampler at 4 °C. Samples were analyzed consecutively in random order, with QC samples inserted into the sample queue. AB Triple TOF 6600 mass spectrometer was used to collect the primary and secondary spectra of the samples. The ESI source conditions after HILIC chromatographic separation were as follows: Ion Source Gas1 (Gas1): 60, Ion Source Gas2 (Gas2): 60, Curtain gas (CUR): 30, source temperature: 600 °C, IonSpray Voltage Floating (ISVF) ±5500 V; TOF MS scan m/z range: 60–1000 Da, product ion scan m/z range: 25–1000 Da, TOF MS scan accumulation time 0.20 s/spectra, product ion scan accumulation time 0.05 s/spectra; second-level mass spectrometry was conducted using information dependent acquisition (IDA) and high sensitivity mode, Declustering potential (DP): ±60 V, Collision Energy: 35 ± 15 eV, IDA settings as follows: Exclude isotopes within 4 Da, Candidate ions to monitor per cycle: 10. Peak identification, filtering, and alignment were performed on the mass spectra to obtain data results including mass-to-charge ratio, retention time, and peak area. Metabolites were annotated using databases such as Mass Bank, Metlin, and MoNA.

2.7. Statistical analysis

In this study, we performed statistical analysis using SPSS 25.0 software. The *Shapiro-Wilk test* was employed to assess the normality of the data. For normally distributed data, *ANOVA test* was conducted, while the *Kruskal-Wallis rank sum test* was used for non-normally distributed data. Spearman correlation analysis was utilized to assess the correlation between different types of data samples. GraphPad Prism 9.0.0 was used to calculate the area under the curve. $P < 0.05$ indicated a statistically significant difference between the experimental results.

3. Results

3.1. Matcha can alleviate obesity-related phenotypes in mice

Following a 12-week period of consuming a high-fat diet (HFD), compared to CK mice, the HFD mice exhibited a significant increase in body weight (Fig. 1A, $P < 0.05$). Upon commencement of matcha gavage treatment, the body weight of M mice continued to decrease (Fig. 1B). The body weight of M mice was significantly lower than MK mice from the 15th week (Fig. 1B, $P < 0.05$). And during the period of matcha gavage treatment, there was no significant difference in food intake and energy intake between M and MK mice (Supplement Figs. 1a–b). After a high-fat diet, the adipose tissue weights of MK mice were significantly higher than that of CK mice (Fig. 1C, $P < 0.05$). However, after matcha supplementation, the adipose tissue weight of M mice was significantly lower than that of MK mice (Fig. 1C, $P < 0.05$). Adipose tissue weight as a percentage of body weight showed the same results (Fig. 1D, $P < 0.05$). These results suggest that the supplementation of matcha may have the potential to counteract weight gain and adipose tissue accumulation induced by a HFD. Additionally, compared to CK mice, the mean adipocyte area of MK mice showed a significant increase ($P < 0.05$), whereas the mean adipocyte area of M mice did not differ significantly from that of MK and CK mice (Fig. 1E and F).

Furthermore, obesity induced by a HFD has been shown to negatively impact glucose and insulin tolerance in mice. To assess this, glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were conducted at weeks 4 and 5 of supplementation. In the GTT, compared to CK mice, the blood glucose levels of MK mice were increased significantly. But the blood glucose levels of M mice were significantly lower than that of MK mice (Fig. 1G, $P < 0.05$). However, no significant differences were observed among the three groups in the ITT (Fig. 1H). Additionally, the area under the curve in the GTT showed that MK mice had significantly larger values than CK and M mice (Fig. 1I, $P < 0.05$). However, the analysis of the area under the curve in ITT revealed no statistically significant difference between the groups of mice (Fig. 1J). This suggests that while matcha may have a significant impact on alleviating the impaired glucose tolerance of mice, its effect on enhancing insulin sensitivity is not statistically significant. In comparison to CK mice, MK mice exhibited significantly elevated serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) ($P < 0.05$). Conversely, following supplementation of matcha, compared to MK mice, M mice demonstrated significantly reduced serum levels of TC, HDL, and LDL (Fig. 1K, $P < 0.05$). These results indicate that matcha supplementation can effectively mitigate HFD induced increases in body weight, adipose tissue weight, and lipid levels, as well as enhance glucose tolerance.

3.2. Effect of matcha on gut microbiota composition in mice

3.2.1. Alpha and beta diversity

In the Alpha diversity analysis, the ACE, Chao1, and Shannon indices of MK mice were found to be significantly lower than those of CK mice (Fig. 2A, $P < 0.05$). However, compared to MK mice, the ACE, Chao1, and Shannon indices of M mice did not exhibit significant differences (Fig. 2A). Additionally, there was no statistically significant variance in the Simpson index among the three groups (Fig. 2A). These results indicated that a HFD led to a significant reduction in microbial richness and evenness, while the supplementation of matcha did not result in a significant improvement in microbial richness and evenness. In the analysis of Beta diversity, Principal Co-ordinate Analysis (PCoA) based on linear microbial community structure and Non-metric analysis of microbial structure Multidimensional Scales (NMDS) based on nonlinear microbial structure revealed a distinct dispersion trend across the three groups of mice, suggesting significant differences in gut microbiota structure among the groups (Fig. 2B). The NMDS stress value of 0.08, below the threshold of 0.1, could reflect the differences between samples. These results suggest that matcha supplementation can induce significant alterations in the gut microbiota structure of mice fed a HFD.

3.2.2. Species composition analysis of gut microbiota

The Venn diagram revealed that there were 280 identical OTUs present in all three groups of mice, with 490 OTUs unique to CK mice, 129 OTUs unique to MK mice, and 246 OTUs unique to M mice (Fig. 2C). The composition of gut microbiota at the phylum level in the three groups of mice primarily consisted of Firmicutes, Bacteroida, Desulfobacterota, Verrucomicrobiota, Proteobacteria, and Actinobacteriota (Fig. 2D). Among genera with a relative abundance exceeding 0.1, seven genera exhibited significant alterations compared to MK mice following matcha supplementation. The relative abundance of *Alloprevotella*, *Ileibacterium*, *Rikenella*, and Chlamydia in MK mice exhibited significant down-regulation compared to CK mice (Fig. 2E, $P < 0.05$), while *Romboutsia* showed a significant up-regulation (Fig. 2E, $P < 0.05$). However, supplementation with matcha significantly reversed this situation ($P < 0.05$). Furthermore, matcha supplementation led to a significant increase in the relative abundance of *Eubacterium_fissicatena_group* and a

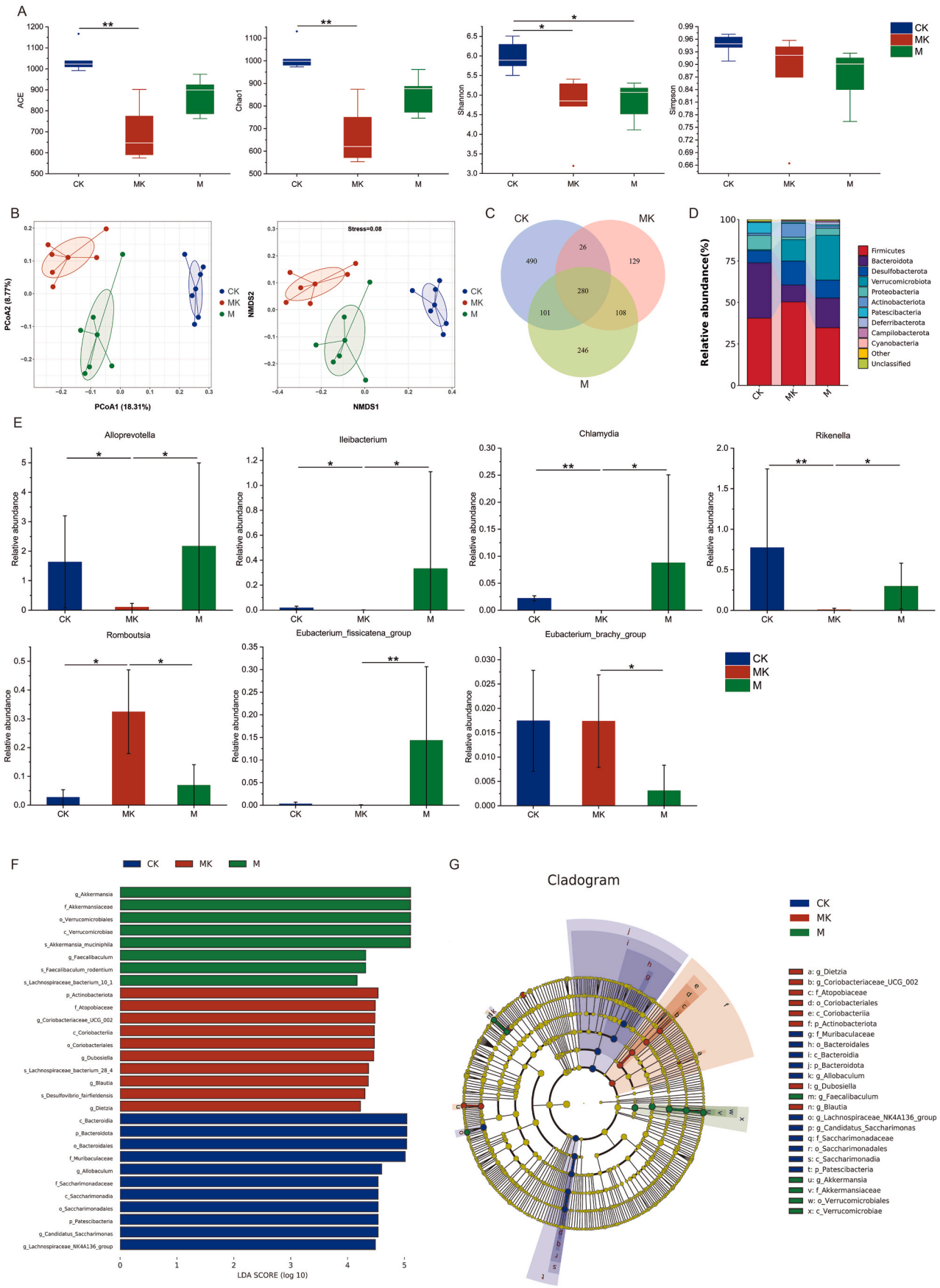

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Fig. 2. Matcha altered the gut microbiota structure of high-fat diet (HFD)-induced mice. **(A)** Alpha diversity analysis consisting ACE, Chao1, Shannon, and Simpson indices of gut microbiota among groups. **(B)** Principal Co-ordinate Analysis **(**PCoA) and Non-metric analysis of microbial structure Multidimensional Scales (NMDS) in Beta diversity analysis. **(C)**The Venn diagram shows the number of unique and shared OTUs and **(D)** the stack plot shows species composition and relative abundance at phylum level. **(E)** The bar chart shows the relative abundance of different genera. **(F)** Histogram and **(G)** cadogram of LDA values of biomarkers in the linear discriminant analysis effect size (LEfSe) analysis. In the histogram and cadogram, p, phylum; c, class; o, order; f, family; g, genus. In the cadogram, the circles radiating from inside to outside represent the taxonomic level from kingdom to species, with each circle at different taxonomic levels representing a species at that taxonomic level, and the size of the circle was proportional to the relative abundance. Data are expressed as mean ± SD (n = 6), $*P < 0.05$ and $**P < 0.01$.

significant decrease in the relative abundance of *Eubacterium_brachy_group* (Fig. 2E, $P < 0.05$).

3.2.3. LEfSe analyze

The LEfSe analysis was employed to identify biomarkers with LDA scores exceeding 4 within each group. At the genus level, the biomarkers identified in CK mice included *Allobaculum*, *Candidatus_Saccharimonas*, and *Lachnospiraceae_NK4A136_group*. Conversely, the biomarkers in MK mice were *Coriobacteriaceae_UCG_002*, *Dubosiella*, *Blautia*, and *Dietzia*, while M mice exhibited *Akkermansia* and *Faecalibaculum* as biomarkers (Fig. 2F and G).

3.3. Effects of matcha on gut metabolites of mice

In both positive and negative ion modes, the results of principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) demonstrate a notable segregation trend among the three groups of mice. Additionally, all comparison models had a Q2Y value surpassing 0.5, indicating the reliability of the model predictions (Supplement Figs. 2a–c). Metabolites with VIP >1 and $P < 0.05$ were identified as differential metabolites, resulting in the screening of a total of 2797 distinct metabolites. In comparison to CK mice, a total of 970 metabolites were found to be up-regulated and 982 down-regulated in MK mice, while 569 metabolites were up-regulated and 1262 down-regulated in M mice (Supplement Fig. 2d, $P < 0.05$). Furthermore, when compared to the MK mice, a total of 217 metabolites were up-regulated and 693 were down-regulated in M mice (Supplement Fig. 2d, $P < 0.05$).

The distribution of differential metabolites is demonstrated in the cluster heat map (Fig. 3A). An investigation into the significance of these metabolites was carried out through KEGG pathway enrichment analysis. The results indicate that, compared to CK mice, there were significant alterations in nine and seven KEGG pathways in MK and M mice, respectively (Fig. 3B, $P < 0.05$). Among these pathways, steroid hormone biosynthesis, eicosanoids, ABC transporters, ovarian steroidogenesis, and steroid biosynthesis were found to be significantly modified in both MK and M mice when compared to CK mice (Fig. 3B, $P < 0.05$). In comparison to MK mice, M mice exhibited significant alterations in five KEGG pathways, namely Vitamin B6 metabolism, caffeine metabolism, HIF-1 signaling pathway, cyanoamino acid metabolism, and glycine, serine, and threonine metabolism (Fig. 3B, $P < 0.05$). Specifically, caffeine metabolism and HIF-1 signaling pathway are hypothesized to play a significant role in the development of obesity.

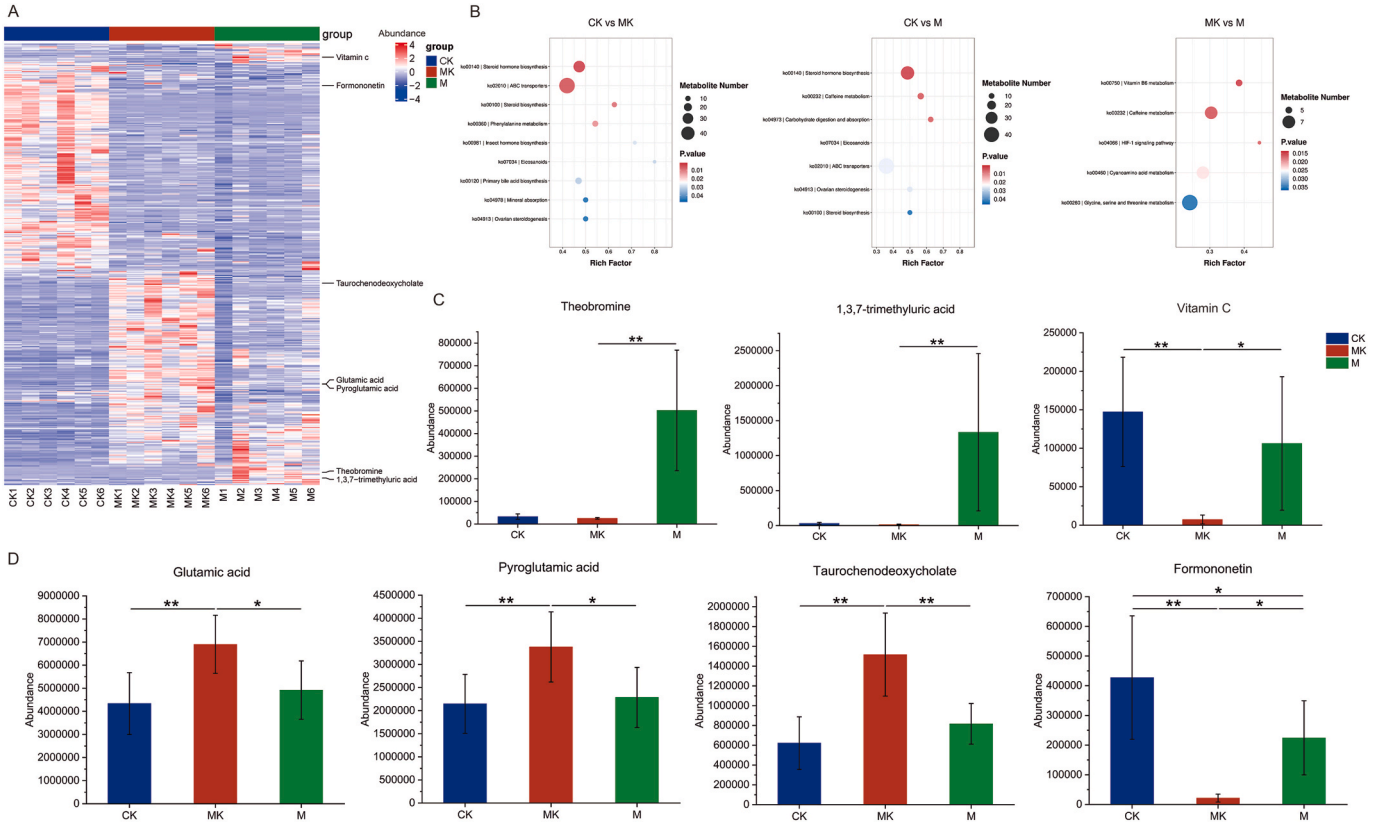

Fig. 3. There was a significant difference in gut metabolites of mice after the treatment with matcha and high-fat diet (HFD). **(A)** Cluster analysis heat map. **(B)** Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis: CK vs MK, CK vs M, and MK vs M. **(C)** Abundance of metabolites theobromine, 1,3,7-trimethyluric acid, Vitamin C, **(D)** glutamic acid, pyroglutamic acid, taurochenodeoxycholate, and formononetin. Data are expressed as mean ± SD (n = 6), $*P < 0.05$ and $**P < 0.01$.

Meanwhile, compared to MK mice, caffeine metabolism and HIF-1 signaling pathway were significantly up-regulated in M mice (Supplement Fig. 3, $P < 0.05$). Additionally, compared to CK mice, the abundance of Vitamin C in the HIF-1 signaling pathway was significantly decreased in MK mice (Fig. 3C, $P < 0.05$). In comparison to MK mice, the abundance of Vitamin C in the HIF-1 signaling pathway, as well as theobromine and 1,3,7-trimethyluric acid in caffeine metabolism, were found to be significantly elevated in M mice (Fig. 3C, $P < 0.05$). Furthermore, upon analyzing the physiological impacts of metabolites, it was observed that glutamic acid, pyroglutamic acid, and taurochenodeoxycholate, which are known to promote obesity (Bagheri et al., 2019; Cai et al., 2021). were significantly up-regulated in MK mice when compared to CK mice (Fig. 3D, $P < 0$). Conversely, formononetin, a metabolite associated with the improvement of obesity, exhibited a significant downregulation (Gautam et al., 2017). The supplementation of matcha significantly reversed the above change (Fig. 3D, $P < 0.05$).

3.4. Correlation analysis of gut microbiota, metabolites, and phenotypes

To explore the relationship between gut metabolites and gut microbiota, the differential metabolites in the five KEGG pathways with significant differences between MK and M mice were correlated with genera having a relative abundance greater than 0.1 and significant differences between MK and M mice. The results indicate significant positive correlations between theobromine and *Alloprevotella* and *Ileibacterium* (Fig. 4A, $P < 0.05$), as well as between 1,3,7-trimethyluric acid

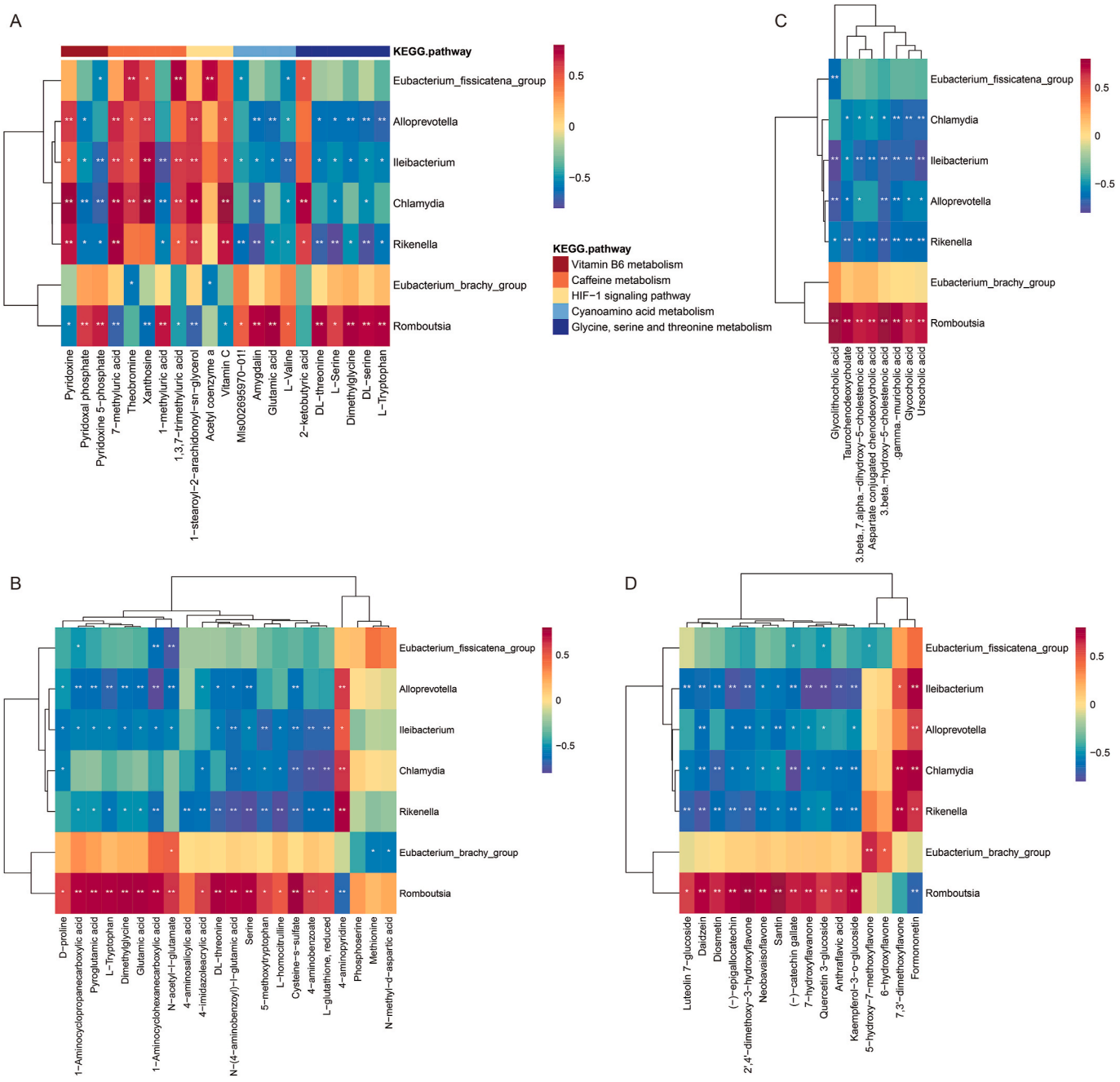

Fig. 4. Heat map for correlation analysis between gut biomarkers and differential metabolites. **(A)** Heat map for metabolites in Kyoto encyclopedia of genes and genomes (KEGG) pathway, **(B)** amino acids and their derivatives, **(C)** bile acid and their derivatives and **(D)** flavonoids. The correlation coefficient r is shown in color. $r > 0$ represents a positive correlation and is shown in red; $r < 0$ represents a negative correlation and is shown in blue. The darker the color, the stronger the correlation. $^{*}P < 0.05$, and $^{**}P < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and *Ileibacterium* and *Rikenella* (Fig. 4A, $P < 0.05$). Additionally, 1,3,7-trimethyluric acid was found to have a significant negative correlation with *Romboutsia* (Fig. 4A, $P < 0.05$). Furthermore, Vitamin C showed significant positive correlations with *Alloprevotella*, *Ileibacterium*, and *Rikenella* (Fig. 4A, $P < 0.05$), while also exhibiting a significant negative correlation with *Romboutsia* (Fig. 4A, $P < 0.05$). In addition, we performed correlations between all 2797 metabolites and microbial genera with relative abundance greater than 0.1. From this analysis, a total of 841 metabolites were identified with a correlation coefficient $r > 0.6$ or $r < -0.6$, and had a statistically significant difference between MK and M mice. To aid in the interpretation of the results, these 841 differential metabolites were categorized into groups including amino acids and their derivatives, bile acids, flavonoids (Fig. 4B–D), and other metabolites (Supplement Fig. 4). The results indicate significant negative correlations between glutamic acid, pyroglutamic acid, and taurochenodeoxycholate with *Alloprevotella*, *Ileibacterium*, and *Rikenella* (Fig. 4B–C, $P < 0.05$), as well as positive correlations with *Romboutsia* (Fig. 4B–C, $P < 0.05$). Conversely, formononetin had significant positive correlations with *Alloprevotella*, *Ileibacterium*, and *Rikenella* (Fig. 4D, $P < 0.05$), and negative correlations with *Romboutsia* (Fig. 4D, $P < 0.05$).

In this study, correlation analysis was performed for key genera, specific differential metabolites, and phenotypes. The results reveal significant positive correlations between glutamic acid, pyroglutamic acid, taurochenodeoxycholate, and *Romboutsia* with various phenotypes including body weight, adipose tissue weight, average adipocyte area, LDL, HDL, and TC (Fig. 5A, $P < 0.05$). Conversely, theobromine and 1,3,7-trimethyluric acid were found to have negative correlations with LDL, HDL, and TC (Fig. 5A, $P < 0.05$), with the latter also showing a negative correlation with body weight (Fig. 5A, $P < 0.05$). Vitamin C had a negative correlation with all phenotypes except TG (Fig. 5A, $P < 0.05$). Formononetin, *Ileibacterium*, and *Rikenella* had negative correlations with body weight, adipose tissue weight, average adipocyte area, TG, and LDL (Fig. 5A, $P < 0.05$). Additionally, *Alloprevotella* formed negative correlations with various obesity-related phenotypes, excluding LDL (Fig. 5A, $P < 0.05$).

The correlation network diagram provided a visual representation of the complex relationships between microbial genera, metabolites, and obesity-related phenotypes. Glutamic acid, pyroglutamic acid, taurochenodeoxycholate, theobromine, 1,3,7-trimethyluric acid, Vitamin C, and formononetin had significant correlations with multiple microorganisms, with glutamic acid, pyroglutamic acid, and taurochenodeoxycholate showing positive correlations with obese-related phenotypes, while theobromine, 1,3,7-trimethyluric acid, Vitamin C, and formononetin instead had inverse correlations (Fig. 5B). This indicates that matcha may be able to reduce the abundance of glutamic acid, pyroglutamic acid, and taurochenodeoxycholate, while increasing the abundance of theobromine, 1,3,7-trimethyluric acid, Vitamin C, and formononetin by the action of gut microbiota, thereby alleviating obesity.

4. Discussion

Obesity is one of the major health problems in the world today. How to effectively fight the obesity epidemic remains a problem to be solved. Disturbance of glucose and lipid metabolism and abnormal energy metabolism are the common symptoms of obesity, as well as the early symptoms of diabetes (A. A. Li et al., 2022a). Matcha, a fine powder made from green tea leaves, has potential anti-inflammatory, anti-diabetic, and anti-obesity effects (Ye et al., 2023). Studies have shown that oral green tea decoction may regulate intestinal absorption of nutrients to improve glucose tolerance in HFD-induced obese mice (Snoussi et al., 2014). This is similar to the results in our experiments where HFD mice showed significant improvement in glucose tolerance after matcha supplementation. In addition, our results show that matcha supplementation can reduce body weight, adipose tissue weight, and serum TC and LDL levels in mice, which is consistent with previous studies (Y. Wang et al., 2022b; P. Xu et al., 2016; J. Zhou et al., 2021).

Our matcha contains high levels of theanine and catechins. Research

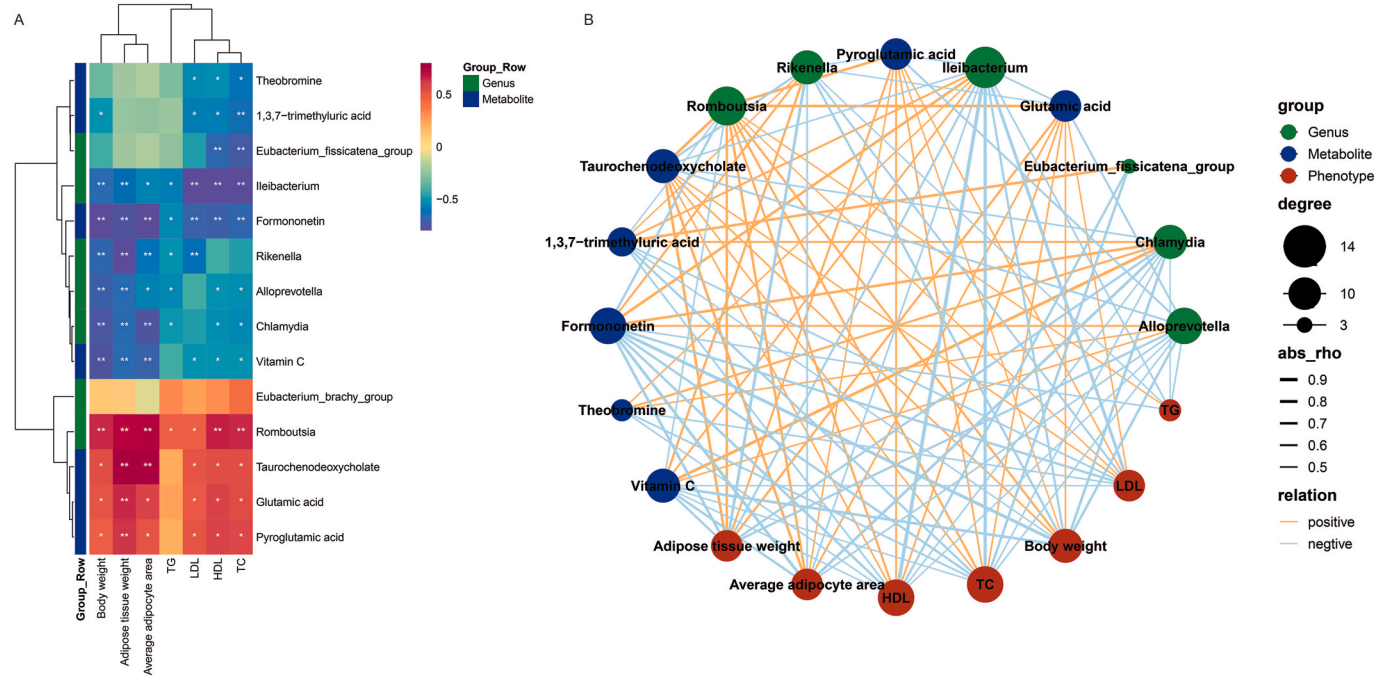

Fig. 5. (A) Heat map of correlation analysis between bacterial genera and some differential metabolites with phenotype. The correlation coefficient r is shown in color. $r > 0$ represents a positive correlation and is shown in red; $r < 0$ represents a negative correlation and is shown in blue. The darker the color, the stronger the correlation. $^{*}P < 0.05$ and $^{**}P < 0.01$. **(B)** Network diagram for correlation analysis of bacterial genera, metabolites, and obesity related phenotypes. The size of a node represents the number of connected nodes, and the thickness of a line represents the size of the correlation coefficient r. $r > 0$ represents a positive correlation and is shown in orange; $r < 0$ represents a negative correlation and is shown in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

has demonstrated that theanine has the ability to stimulate browning of white adipocytes through the AMPK/α-Ketoglutarate/Prdm16 Axis (Peng et al., 2021). Additionally, theanine is capable of modulating the composition of gut microbiota and reducing the abundance of *Enterorhabdus*, *Clostridium* and other bacteria (Peng et al., 2021; W. Xu et al., 2023). Green tea catechins have been shown to potentially upregulate GLUT4 expression, enhance glucose uptake by adipocytes and skeletal muscles, and ameliorate glucose tolerance (Snoussi et al., 2014). Furthermore, research has indicated that green tea catechins may modulate the composition of gut microbiota by reducing the Firmicutes/Bacteroidetes ratio, thereby exerting anti-obesity effects (J. Liu et al., 2023; Remely et al., 2017). The observed effects of these bioactive compounds on gut microbiota and obesity consistent with our results.

In our study, the high-fat diet resulted in a disturbance of the gut microbiota structure, leading to reduced homogeneity and richness. The high-fat diet also led to a decrease in the relative abundance of beneficial bacteria such as *Alloprevotella*, *Ileibacterium*, *Rikenella*, and *Eubacterium_fissicatena_group*, while increasing the relative abundance of harmful bacteria *Romboutsia*. Studies have shown that *Alloprevotella* is negatively correlated with body weight, fat weight, serum TG, TC and HDL-C (Hu et al., 2021; S. L. Li et al., 2021a), and *Ileibacterium* is negatively correlated with fat weight, which is consistent with our results (Meng et al., 2023). *Alloprevotella*, *Ileibacterium*, and *Eubacterium_fissicatena_group* have been shown to produce a variety of short-chain fatty acids (SCFAs) metabolites (Huang et al., 2023; L. Li et al., 2021a; Zhao et al., 2021), while SCFAs can activate GPR41 and GPR43 in adipose tissue and increase the expression of PGC-1 and UCP-1 proteins in brown adipose tissue to improve lipid metabolism, thereby reducing lipid deposition (He et al., 2020; M. Zhu et al., 2023). SCFAs can also improve obesity by activating the PPAR pathway to increase energy expenditure, reduce body weight, and reduce liver TG accumulation to regulate lipid metabolism (Ai et al., 2022). In addition, *Alloprevotella* was negatively correlated with fasting glucose, insulin resistance index (HOMA-IR), and the area under the curve in the GTT and could enhance satiety and maintain glucose homeostasis by producing enterokinin YY and glucagon-like peptide 1 (S. S. Li et al., 2021; Ni et al., 2023). Studies have shown that *Rikenella* may also play a key role in the improvement of glucose and lipid metabolism parameters (L. Zhou et al., 2019). It was negatively correlated with body weight gain, serum TG, and area under the abdominal glucose tolerance curve (S. Li et al., 2022b; L. Zhou et al., 2019; L. Zhou et al., 2018). *Romboutsia* was positively correlated with body weight gain, fasting blood glucose, HOMA-IR, LDL-C, and IL-6 and negatively correlated with UCP-1, PGC-1α, PPAR-γ, propionic acid, and butyric acid (X. Li et al., 2023b). Reducing *Romboutsia* is beneficial to reduce obesity, reduce blood lipid level, and regulate glucose homeostasis (Zhao et al., 2022). In our study, matcha treatment enriched beneficial bacteria *Alloprevotella*, *Ileibacterium*, *Rikenella* and *Eubacterium_fissicatena_group*, and reduced the abundance of harmful bacteria *Romboutsia*. Thus, matcha may alleviate obesity by affecting the gut microbiota.

Studies have shown that the gut microbiota may be involved in caffeine metabolism to alleviate HFD-induced fat deposition and metabolic disorders (Jing et al., 2020). Theobromine in the caffeine metabolism pathway has the effect of browning white fat and increases the lipolysis and heat production of brown fat cells by inhibiting the activity of PDE4 in adipose tissue and cells, playing a key role in alleviating obesity caused by HFD (Jang et al., 2020). In addition, 1,3,7-trimethyluric acid in this KEGG metabolic pathway can inhibit the accumulation of intracellular lipids after complete differentiation of adipocytes(Nakabayashi et al., 2008). The HIF-1 signaling pathway, which is associated with oxidative stress and inflammation, plays a key role in improving insulin resistance (P. Li et al., 2024). Vitamin C in the HIF-1 signaling pathway can reduce HFD-induced weight gain in mice and reduce fat mass by modulating Tet1 activity, inhibiting the hypertrophy of white fat cells (Yuan et al., 2021). In addition, Vitamin C improves glucose homeostasis in obese hyperglycemic mice (Abdel-Wahab, O'Harte, Mooney, Barnett and Flatt, 2002). In our results, the KEGG pathway caffeine metabolism and HIF-1 signaling pathway were significantly up-regulated in M mice compared to MK mice. At the same time, theobromine, 1,3,7-trimethyluric acid, and Vitamin C were also significantly enriched in M mice compared to MK mice and were significantly negatively correlated with multiple obesity phenotypes, as well as with microorganisms that influence obesity. In summary, the theanine and catechins in matcha may affect caffeine metabolism and HIF-1 signaling pathway by regulating gut microbiota, thus playing a role in alleviating obesity and metabolic disorders.

In our study, both glutamic acid and pyroglutamic acid were significantly up-regulated after HFD and significantly down-regulated after matcha supplementation. The free pyroglutamic acid can act as a repository for glutamic acid, which may be the reason why they share a similar trend. Glutamic acid levels have been shown to increase significantly in obese individuals and are significantly positively correlated with obesity-related indicators such as body mass index, waist circumference, insulin resistance, lipid metabolism (TC, TG, LDL), and blood glucose (Bagheri et al., 2019; R. Liu et al., 2017; S. Wang et al., 2022a). In addition, glutamic acid can enhance appetite by promoting glucagon secretion from islet alpha cells and participating in the formation of gamma-aminobutyric acid (S. M. Wang et al., 2018). A decrease in glutamic acid concentration can reduce food intake, which can alleviate weight gain and obesity (R. Liu et al., 2017). Glutamate metabolism is also closely related to gut microbes. In the study conducted by Ruixin Liu, *Ruminococcus* sp., *Dorea longicatena,* and *Coprococcus comes* were enriched in the obese group, possessed genes encoding the enzyme required for glutamine production, and were positively correlated with the level of circulating glutamate. The decreased *Bacteroides thetaiotaomicron* in the obese group, which can encode glutamate decarboxylase, is negatively correlated with circulating glutamate levels, suggesting that gut microbes may influence serum glutamate and affect obesity phenotype (R. Liu et al., 2017). In our study, glutamic acid and pyroglutamic acid were significantly positively correlated with several obesity-related phenotypes and *Romboutsia* and were significantly negatively correlated with *Alloprevotella*, *Ileibacterium*, and *Rikenella*. It appears that theanine and catechins in matcha may alleviate obesity by affecting gut microbes leading to a reduction in levels of glutamic acid and pyroglutamic acid.

As a primary bile acid, taurochenodeoxycholate (TCDCA) is considered an important biomarker associated with obesity (Cai et al., 2021). High levels of TCDCA promote trimethylamine-N-oxide (TMAO) production in the liver by activating the FXR/FMO3 signaling pathway in the liver, thereby increasing serum TMAO content, which is positively associated with obesity (Wei et al., 2022). In addition, low levels of circulating TCDCA also help lower blood sugar levels and improve glucose metabolism (Heianza et al., 2022). Numerous studies have shown that a high-fat diet (HFD) can significantly increase TCDCA levels in various tissues, while transplanting gut microbes from healthy mice can significantly reduce TCDCA levels, thereby reducing TCDCA stimulation of FXR in the dorsal vagal complex of the brain and gut, hence improving glucose tolerance (Waise et al., 2021; Zhang et al., 2021). Other studies have shown that green tea extract can significantly reduce TCDCA and thus improve obesity and hyperlipidemia in HFD mice (X. M. Zhou et al., 2023b). In our study, significantly down-regulated TCDCA in M mice was significantly negatively associated with multiple obesity-related phenotypes. TCDCA was also negatively correlated with *Alloprevotella*, *Ileibacterium*, and *Rikenella*, and positively correlated with *Romboutsia*. This suggests that reducing TCDCA levels by regulating gut microbiota may be the key to the theanine and catechins in matcha in the treatment of obesity and improvement of glucose tolerance.

As an o-methylated isoflavone, formononetin has been shown to reduce weight gain and visceral fat accumulation caused by HFD (Gautam et al., 2017). The study showed that formononetin can reduce weight gain induced by HFD in mice by binding and activating the PPARγ and AMPK/β-catenin pathways, thereby increasing Ucp1

expression in adipocytes in a dose dependent manner, reducing adipogenesis and promoting adipocyte thermogenesis (Gautam et al., 2017; Nie et al., 2018). Other research has shown that formononetin can specifically reduce adipose tissue weight (Nie et al., 2018). Additionally, formononetin improves insulin sensitivity and glucose metabolism in mice by down-regulating the mRNA expression levels of pro-inflammatory cytokines IL-6, IL-22, and TNF-α in the liver (Naudhani et al., 2021). Furthermore, it effectively regulates the gut microbiota, leading to an increase in *Clostridium aldenense*, *unclassified Clostridiaceae*, and *Eubacterium plexicaum* (Naudhani et al., 2021). In addition, formononetin and catechin belong to the flavonoid metabolites together, and both of them are in the KEGG metabolic pathway Biosynthesis of phenylpropanoids. In correlation analysis, formononetin was significantly negatively associated with multiple obesity-related phenotypes. In addition, formononetin was positively correlated with *Alloprevotella*, *Ileibacterium*, and *Rikenella* and negatively correlated with *Romboutsia*. This suggests that the theanine and catechins in matcha may play an important role in the treatment of obesity by regulating gut microbiota through formononetin.

The findings of this study offer novel evidence that matcha can mitigate obesity by modulating gut microbiota and metabolites, identifying several noteworthy bacteria and metabolites. However, the association between the aforementioned gut microbiota and its metabolites with obesity requires further validation, representing a limitation of this study. Furthermore, our study did not include an experimental group that received a normal diet supplemented with matcha. The inclusion of such a group would have rendered this study more comprehensive in investigating the impact of matcha on regulating gut microbiota and its metabolites to alleviate obesity.

5. Conclusion

In conclusion, matcha demonstrated efficacy in mitigating the deleterious effects of a high-fat diet on body weight, adipose tissue weight, and lipid levels in mice, while also enhancing glucose tolerance in obese mice. Matcha could also regulate the composition and structure of gut microbiota, up-regulate the relative abundance of *Alloprevotella*, *Ileibacterium* and *Rikenella*, and down-regulate the relative abundance of *Romboutsia*. The caffeine metabolism and HIF-1 signaling pathways in KEGG metabolic pathways were significantly up-regulated after matcha supplementation. Furthermore, the modulation of gut bacteria by matcha may contribute to the mitigation of obesity by up-regulating formononetin and Vitamin C, while down-regulating glutamic acid, pyroglutamic acid, and taurochenodeoxycholate in gut metabolites. In summary, the modulatory effects of matcha on gut microbiota and its metabolites may play an important role in the anti-obesity mechanism of matcha.

Data availability statement

Data on 16S rDNA sequencing in this study are publicly available at https://www.ncbi.nlm.nih.gov/(BioProject ID: PRJNA1084780), and data on the untargeted metabolomics are publicly available at https://www.ebi.ac.uk/metabolights/MTBLS9862/(ID: MTBLS9862).

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

I have shared the link to my data in the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100823.

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