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Multi-omics reveals that consumption of black tea affects the host satiety, gut flora and metabolic profile to aid weight loss

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ABSTRACT

The health benefits of teas and tea preparations in assisting with weight loss have been well documented in numerous studies. Nevertheless, there is a paucity of population-based research on the effects of consumption of tea and tea preparations on human dietary intake, gut microbial and metabolic profiles, and thus on weight loss. This study therefore focused on changes in dietary intake, gut microbiota and metabolism in healthy adults who consumed black tea for 3 consecutive weeks. Our findings showed that the consumption of black tea was associated with a notable increase in satiety prior to meals, which was observed to be more pronounced in female participants than in male participants. The consumption of black tea has been shown to increase the species richness of gut microorganisms, as evidenced by a higher number of observed operational taxonomic units (OTUs) compared the test group with the control group (203 ± 35 *vs*. 229 ± 18, respectively). Moreover, it improves the microbiological structure of the gut, enhances the abundance of beneficial bacteria such as *Faecalibacterium* and *Parabacteroides*, and reduces the abundance of *Prevotella*. Metabolomic analysis revealed 13 significantly different metabolites and 9 enriched metabolic pathways in individuals who consumed black tea. Correlation analyses of body mass index (BMI), satiety, gut flora and metabolic profiles indicate that the modulatory effects of black tea on gut flora and metabolic profiles are more effective in high BMI participant. This study may provide new insights into the relationship between black tea interventions and host satiety, gut microbiota and metabolic profiles. Furthermore, it may offer new directions and options for clinical strategies and personalized nutrition for tea and tea preparations to assist in weight loss and lipid reduction.

1. Introduction

It is estimated that the global obese population may reach 1 billion by 2022[1-2]. In addition to being defined as a complex chronic disease in its own right, obesity is also an important risk factor for a number of chronic diseases such as stroke, cancer and blindness[3]. Obesity is typically characterized by metabolic disorders, energy imbalance and chronic inflammation. A number of unhealthy factors, including disrupted gut flora, imbalanced energy intake and expenditure, and metabolic disorders, may contribute to the development of obesity[4-5]. Current research indicates that many of the causes of obesity can be prevented and reversed, with dietary interventions being one of the more promising approaches.

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The gut microbiota is a complex ecosystem harbored in the human gut and contains a large number of microorganisms[6-7]. The gut microbiota plays a significant role in the host's physiology and is involved in regulating a multitude of physiological functions and activities. A dysbiosis gut microbiota can have a significant negative impact on the host, leading to a variety of diseases including obesity[8]. The gut microbiome exerts its influence on obesity primarily through its regulatory effects on host energy metabolism[9]. It is now well established that the gut microbiome can regulate host energy intake through short-chain fatty acid (SCFA)-mediated hormone secretion and direct microbial synthesis of neurotransmitters and hormone mimics that interact with the gut and the central nervous system to regulate hunger and satiety. Consequently, a number of SCFA-producing bacteria, including *Faecalibacterium*, *Bacteroides* and *Ruminococcus*, have been identified as potential contributors to obesity[10]. The gut microbiome, comprising bacteria such as *Bifidobacterium*, *Lactobacillus* and *Bacteroidetes*, can influence energy utilization through the regulation of bile acid metabolism, including fatty acid uptake, lipolysis, and thermogenesis. Modification of the gut microbiota may therefore prove an effective means of preventing and reversing obesity[11-13].

Black tea, a traditional Chinese beverage, is one of the most popular and widely consumed natural plant-based beverages worldwide. Black tea contains a variety of beneficial chemical compounds, including theanine, theobromine and tea polysaccharides[14-15]. It has been demonstrated that Black tea and high purity black tea extracts have the ability to assist in weight loss and prevent obesity[16]. In addition, the functional components of black tea have shown various beneficial effects in anti-inflammation, prevention of cardiovascular diseases, prevention of metabolic diseases (diabetes, lipid metabolism disorders, etc.)[17-18]. Current research indicates that black tea exerts its effects on fat storage and weight loss in 3 principal ways: 1) Regulate intestinal flora (gut microbiota, GM), increase the diversity of intestinal flora and increase the abundance of weight loss-friendly bacteria such as *Bacteroides*, *Faecalibacterium*. This approach may prove effective in preventing and assisting in the treatment of obesity[19]. 2) Provides a subjective sense of satiety, assists in controlling the amount of food eaten and suppresses the intake of excess energy[20]. 3) Assists in regulating the formation of mature fat cells and fat accumulation in the body and promote body fat decomposition and metabolism[21-22].

The above theoretical reasoning based on existing research and convincing evidence from animal studies suggests that tea, tea extracts, and tea-based preparations have positive outcomes as functional foods to assist in the treatment of obesity[23-25]. However, there is a paucity of data from human studies on the efficacy of tea, tea extracts, and tea-based preparations in the treatment of obesity in humans, taking into account factors such as differences in food intake, background diet quality, etc. Therefore, it is of interest to study the bioactive components of tea, tea extracts and tea preparations. Focusing on black tea, this study attempted to analyze the effects of an intervention with black tea on satiety, gut microbiology, and metabolic profiles in randomized healthy adults.

In this study, tea polyphenols (total polyphenols) and tea polysaccharides (total polysaccharides) in black tea were determined using spectrophotometric methods with reference to national standards, and theaflavin content, using body measurements, certified self-assessment questionnaires, 16S rRNA gene sequencing, and untargeted metabolomics to explore the interventional effects of daily intake of black tea on body composition, gut microbiota, metabolic profiles, and satiety in healthy participants. The findings of this study may have implications for the broader population, as they could provide nutritional recommendations that could be applied to individuals who are seeking to prevent gut flora disorders and treat obesity. Furthermore, this study may contribute to a deeper comprehension of the interrelationships between dietary habits, satiety, gut microbiota composition, and metabolic profiles.

2. Methods

The study was approved by the Ethics Committee of Nankai University (NKUIRB2024078) and adhered to the principles of the Declaration of Helsinki. The trial was conducted in Tianjin, China from December 2023 to January 2024. All participants were made aware of the details of the study and submitted written consent before the beginning of the study.

2.1 Black tea preparation

The black tea used in this study was a commercially available black tea based infused tea beverage. The infused tea beverage was prepared according to International Organization for Standardization (ISO) guidelines (ISO 3103; guidelines titled *Tea-Preparation of Liquor for Use in Sensory Test*). The preparation process can be simply described as: double boiling distilled water of volume 400 mL was added to 12.50 g of tea leaves. After 20 min, the infusion was filtered and the cooled filtrate was placed in a volumetric flask and water was used to reach a final volume of 500 mL.

2.2 Participant recruitment

Participants included 40 healthy adults. A total of 4 participants withdrew from the study after the intervention began (the sample size estimation method is described in the Additional information on sample size estimation section of the Supporting Information). The study participants were aged between 20 and 40 years and were not provided with any weight loss or fat loss program during the intervention period. The number of participants in this study was determined based on the results of previous trials investigating the gut microbiota[26]. Participants with a history of allergic reactions to caffeine or tea polyphenols, as well as those with disrupted gut flora, were excluded prior to the commencement of the study. The criteria for exclusion were as follows: 1) breastfeeding or pregnant women; 2) individuals with serious diseases of the kidneys, respiratory, endocrine, hematopoietic systems; 3) individuals who have used medications in the last 3 months that can lead to obesity as a side effect; 4) individuals who have undergone weight loss plans during the intervention cycle; 5) individuals with strict dieting and irregular eating habits; 6) individuals with allergies to test food ingredients such as tea polyphenols, theanine, and caffeine. Recruitment and screening took place in November 2023 to December 2023.

2.3 Research design

A total of 40 participants who met the eligibility criteria were randomly recruited for this study. Following the signing of the informed consent form, the participants were randomly assigned to 1 of 2 groups: the T intervention group (n = 20) and the C control group (n = 20). However, 3 participants in Group T and 4 participants in Group C elected to withdraw from the experiment prior to the commencement of the intervention. Consequently, the final division comprised Group T (n = 17) and Group C (n = 16). Participants in the study were asked to avoid tea, coffee, fermented foods and other probiotic-containing drinks, such as yoghurt, for 2 weeks before the start of the intervention. Subsequently, participants in Group T consumed 500 mL of bottled black tea every day from 1 pm to 4 pm, and participants in Group C consumed 500 mL of bottled purified water every day from 1 pm to 4 pm. The intervention lasted 3 weeks. Participants were asked to maintain their dietary and lifestyle habits during the intervention and to complete the Food Frequency Questionnaire (FFQ) dietary questionnaire midweek to demonstrate that there were no major fluctuations in participants' dietary intake during the experiment (Table S1). A total of 5 visits were conducted for the study, including a baseline assessment, 3 dietary visits (1 per week for a total of 3 visits), outcome measures; participants' satiety before dinner was tracked and assessed using a 100-mm visual analog scale (VAS) each afternoon[27] (completed 28 times). Stool samples were collected before and at the end of the intervention for gut microbiological and metabolic analysis. Additionally, throughout the course of the study, participants were instructed to document any unfavorable occurrences, including adverse effects and the use of concomitant medications.

2.4 Human body data collection

Human data was collected by a bioelectrical impedance analyzer (InBody 260, Biospace, California, USA). Including standing height, weight and body mass index (BMI). Prior to the commencement of the intervention study, participants were requested to complete anthropometric data measurements and to submit questionnaires within a week's time.

2.5 Fecal sample collection

All participants provided stool samples the day before the start of the intervention (as a baseline measurement) and the day after the end of the intervention (as an outcome measurement). Fecal samples (> 2 g) were collected at home using sterile stool collection tubes, immediately refrigerated (4 ℃), and then frozen at −80 ℃ within a day of delivery to the laboratory to avoid loss of sample quality and for subsequent analysis of gut microbiota and metabolism.

2.6 Gut flora analysis

A comprehensive examination of the gut microbiota in the above-described stool samples was conducted using appropriate analytical techniques. Paired genome libraries were constructed using total DNA from fecal samples and sequenced using the Illumina NovaSeq platform (Illumina, San Diego, CA, USA).

2.7 Determination and analysis of metabolites

Non-targeted metabolomics analysis of black tea samples and collected fecal samples were performed using a Vanquish UHPLC system (Thermo Fisher, Munich, Germany) coupled with an Orbitrap Q Exactive™ HF mass spectrometer or Orbitrap Q Exactive™HF-X mass spectrometer (Thermo Fisher, Munich, Germany). Raw data files generated by the UPLC-MS/MS were processed by Compound Discoverer 3.3 (CD3.3, Thermo Fisher) for peak alignment, peak selection and quantification of each metabolite.

2.8 Statistical analysis

SPSS version 28.0 (SPSS, IBM, USA) was used for Student's t-test. The richness and homogeneity of gut microbial communities were quantified using the α-diversity index (observed species), while biomarkers were analyzed using the β-diversity index (linear discriminant analysis effect size, LEfSe). Furthermore, clusters of samples were visualized using principal component analysis (PCA). Relative abundance of bacterial taxa was calculated at the phylum and genus level. Spearman correlation analyses were also conducted to examine the relationship between the relative abundance of dominant bacterial taxa and host parameters in the black tea treatment and control groups at baseline and at the conclusion of the intervention trial.

3. Results

3.1 Content of black tea active ingredients

Tea polyphenols (total polyphenols) and tea polysaccharides (total polysaccharides) in black tea samples were detected using spectrophotometric methods (refer to GB/T 8313-2018 for the spectrophotometric method of tea polyphenols; refer to SN/T 4260-2015 for the spectrophotometric method of tea polysaccharides). The results showed that the content of tea polyphenols (total polyphenols) was (32.087 ± 1.03) mg/100 mL, and the content of tea polysaccharides (total polysaccharides) was (307.81 ± 2.53) mg/100 mL.

3.2 Characteristics of pre-intervention participants

The eligible participants were randomly grouped into T (Test) and C (Control) groups. The baseline characteristics of the participants at the pre-and post-intervention stages are presented in Tables 1 and 2. There were no significant differences between Group T and Group C on baseline characteristics of primary interest such as age, weight, and BMI (Figs. S1-S2). During the 3 weeks intervention cycle, the physical activity levels, dietary intake of carbohydrates, proteins, and fats, and total energy intake of the participants remained consistent with those observed prior to the intervention. A total of 36 participants completed the 3 week intervention (Fig. 1). There are 85% of people in Group T and 95% of people in Group C followed the program. The data from the 36 participants who participated in the intervention were used for further statistical analyses in this study.

Table 1
Baseline characteristics (height, weight, BMI) of the participants in T (test) group before and after the intervention.

Before intervention					After intervention				
Number	Age	Height (m)	Weight (kg)	BMI	Number	Age	Height (m)	Weight (kg)	BMI
T01	27	1.77	90	28.73	T01	27	1.77	89	28.41
T02	21	1.68	55	19.48	T02	21	1.68	55	19.49
T03	21	1.77	63	20.11	T03	21	1.77	63	20.11
T04	20	1.73	87	29.07	T04	20	1.73	86	28.73
T05	21	1.75	56	18.28	T05	21	1.75	56	18.29
T06	22	1.76	60	19.37	T06	22	1.76	60	19.37
T07	28	1.80	78	24.07	T07	28	1.8	76	23.46
T08	32	1.76	64	20.66	T08	32	1.76	64	20.66
T09	23	1.65	70	25.71	T09	23	1.65	70	25.71
T10	24	1.70	65	22.49	T10	24	1.7	65	22.49
T11	20	1.75	60	19.59	T11	20	1.75	60	19.59
T12	19	1.69	53	18.56	T12	19	1.69	53	18.56
T13	27	1.68	61	21.61	T13	27	1.68	61	21.61
T14	32	1.72	56	18.93	T14	32	1.72	57	19.27
T15	23	1.73	60	20.05	T15	23	1.73	60	20.05
T16	34	1.81	70	21.36	T16	34	1.81	68	20.76

Table 2
Baseline characteristics (height, weight, BMI) of the participants in C (control) group before and after the intervention.

Before intervention					After intervention				
Number	Age	Height (m)	Weight (kg)	BMI	Number	Age	Height (m)	Weight (kg)	BMI
C01	25	1.66	55	19.96	C01	25	1.66	55	19.96
C02	34	1.66	54	19.59	C02	34	1.66	54	19.60
C03	30	1.65	58	21.30	C03	30	1.65	58	21.30
C04	24	1.65	65	23.87	C04	24	1.65	65	23.88
C05	22	1.73	51	17.04	C05	22	1.73	50	16.71
C06	23	1.73	60	20.05	C06	23	1.73	60	20.05
C07	24	1.65	75	27.55	C07	24	1.65	76	27.92
C08	37	1.82	92	27.77	C08	37	1.82	92	27.77
C09	37	1.72	75	25.35	C09	37	1.72	75	25.35
C10	34	1.71	69	23.59	C10	34	1.71	69	23.60
C11	35	1.62	45	17.15	C11	35	1.62	46	17.53
C12	24	1.57	47	19.07	C12	24	1.57	48	19.47
C13	26	1.81	79	24.11	C13	26	1.81	79	24.11
C14	33	1.68	80	28.34	C14	33	1.68	80	28.34
C15	25	1.60	50	19.53	C15	25	1.6	50	19.53
C16	26	1.65	67	24.61	C16	26	1.65	67	24.61

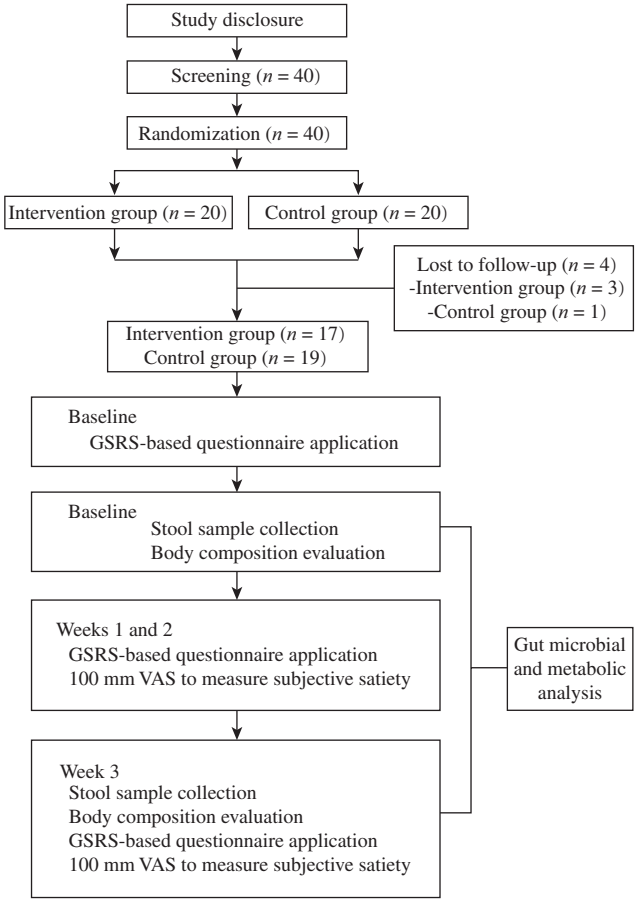

Fig. 1 Randomized clinical trial design flow chart. Healthy participants were recruited, screened and allocated. Basic physical parameters (height, weight, age) were collected and fecal samples were collected on day 0 and day 21 for analysis of gut microbiota. The effect of black tea on gastrointestinal function was assessed weekly using a gastrointestinal symptoms rating scales (GSRS)-based questionnaire. Satiety was assessed daily using the visualization VAS scale.

3.3 Effects of black tea intake on body composition, physical activity and dietary intake

The study would involve the recording of body weight parameters, dietary intake before and after the 3-week intervention period. Weight and BMI remained unchanged at the end of the intervention. It is worth noting the effect of black tea intake on dietary intake (this study was concerned with the effect of black tea intake on satiety). Data collection, data counting and scatterplot fitting resulted in a fitted graph (Fig. 2A). It can be seen that during the intervention cycle, the satiety level of Group T ranged from 65 to 50, while that of Group C ranged from 40 to 30, which in combination with the violin plot (Fig. 2B) showed that the satiety level of Group T consuming black tea tended to be higher than that of Group C (55.61 ± 6.65 *vs*. 37.23 ± 4.98, $P = 0.091$). In addition, subgroup analyses showed that black tea consumption was more effective in increasing satiety in females. During the intervention period, females in Group T who consumed black tea had significantly higher satiety scores than females in Group C who did not consume black tea (66.09 ± 5.62 *vs*. 41.7 ± 8.75, $P < 0.05$). Males in Group T who consumed black tea had slightly higher satiety levels than males in Group C who consumed black tea (50.00 ± 12.66 *vs*. 41.18 ± 4.50, $P > 0.05$). This suggests that intake of black tea increases satiety and reduces dietary intake, and that this effect is more pronounced in women than in men.

3.4 Effects of black tea consumption on participants' baseline body parameters

The effect of black tea consumption on the basic body parameters of the participants, including height, weight and BMI, was assessed by counting the data from the questionnaires returned every week during the 3-week intervention period, and the results showed that there were no significant changes in the 3 basic body parameters of height, weight and BMI after the 3-week intervention.

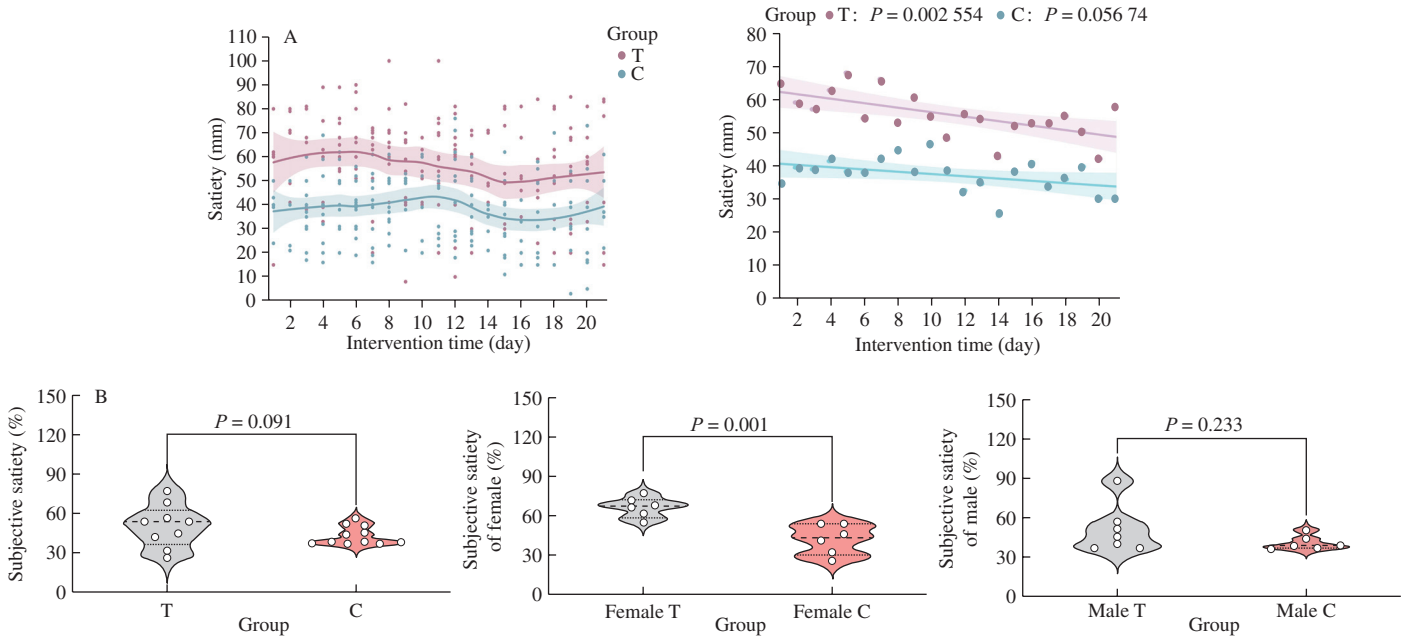

Fig. 2 Effect of black tea intervention on satiety. (A) Scatter fit plot of 21-day satiety data for intervention and control samples and scatter fit plot of mean 21-day satiety data for intervention and control samples. (B) Comparison of relative levels of satiety between men and women.

3.5 Effect of black tea consumption on the diversity of the gut flora

To investigate the effect of black tea intake on host microbial profiles, fecal bacteria were collected for 16S rRNA sequencing. We performed 16S rRNA sequencing of fecal bacteria to compare groups T and C characterized by the number of species observed to investigate the effects of black tea treatment on host gut microbes. Comparison of baseline and outcome values of α-diversity index, LEfSe index characterized by number of species observed in C and T groups. Fig. 3A shows that there was a significant increase in observed species levels in the outcome measures in Group T compared to pre-intervention (observed species mean, Group T baseline and outcome measures 203 ± 35 and 229 ± 18, respectively, $P = 0.050$).

This result suggests that 3 consecutive weeks of black tea intake increased species abundance in the participants' gut microbial communities.

3.6 Effect of drinking black tea on the gut microbiome

PCA explaining the percentage of variance with axis 1 (32.21%) and axis 2 (23.90%) showed a noteworthy difference in β-diversity between the intervention baseline group and the intervention outcome group (Fig. 3C); PCA explaining the percentage of variance with axis 1 (29.30%) and axis 2 (20.24%) do not show a marked difference in β-diversity between the control baseline group and the control outcome group (Fig. 3D).

The graphs of the density distribution demonstrate that the structure of gut bacteria in the T baseline and T outcome groups showed a tendency to be clustered into 2 groups, with differences compared to the control group. This evidence supports the conclusion that the intervention had an effect on the composition of the intestinal flora.

The impact of the intervention on the bacterial composition was more clearly illustrated in a Venn diagram (Fig. 3B), which demonstrated that post and pre-intervention participants shared 418 operational taxonomic units (OTUs), exhibited 178 and 105 unique OTUs.

Subsequently, LEfSe species difference models and species contribution models were constructed in terms of β-diversity (Figs. 3E and F). The LEfSe species difference model showed that the intervention group had differences at baseline (QT) and at outcome (HT) at the level of 5 genera including *Dietzia*, *Parabacteroides*, and unidentified_Ruminococcaceae ($P < 0.01$) and at the level of 1 phylum including Desulfobacterota ($P < 0.01$); in contrast, species contribution models serve to illustrate the principal sources of variation and to quantify the extent to which each factor contributes to the overall variance. The species visualization model facilitated the identification of the most abundant taxa, which in turn led to changes in the bacterial composition of the participants in the intervention T and control C groups. The results of the species visualization model indicated that, based on species contribution, the 4 genera providing the top 4 contributions in the intervention group were *Bacteroides*, *Escherichia-Shigella*, *Faecalibacterium*, and *Prevotella*. In contrast, the 4 genera providing the top 4 contributions in the control group were *Faecalibacterium*, *Escherichia-Shigella*, *Prevotella*, and *Bacteroides*. Subsequently, the impact of black tea intake on alterations in bacterial taxa was evaluated at the phylum and genus levels (Figs. 3G and H). At the phylum level, Bacteroidota and Firmicutes dominated. Fig. 4A shows that the black tea intervention inhibited Firmicutes ($P < 0.07$) and slightly promoted Bacteroidota (no statistically significant difference between groups). As shown in Fig. 4B, at the genus level, the black tea intervention significantly inhibited *Prevotalla* ($P < 0.05$) and promoted *Faecalibacterium* ($P < 0.05$) and *Parabacteroides* ($P < 0.01$) compared to baseline.

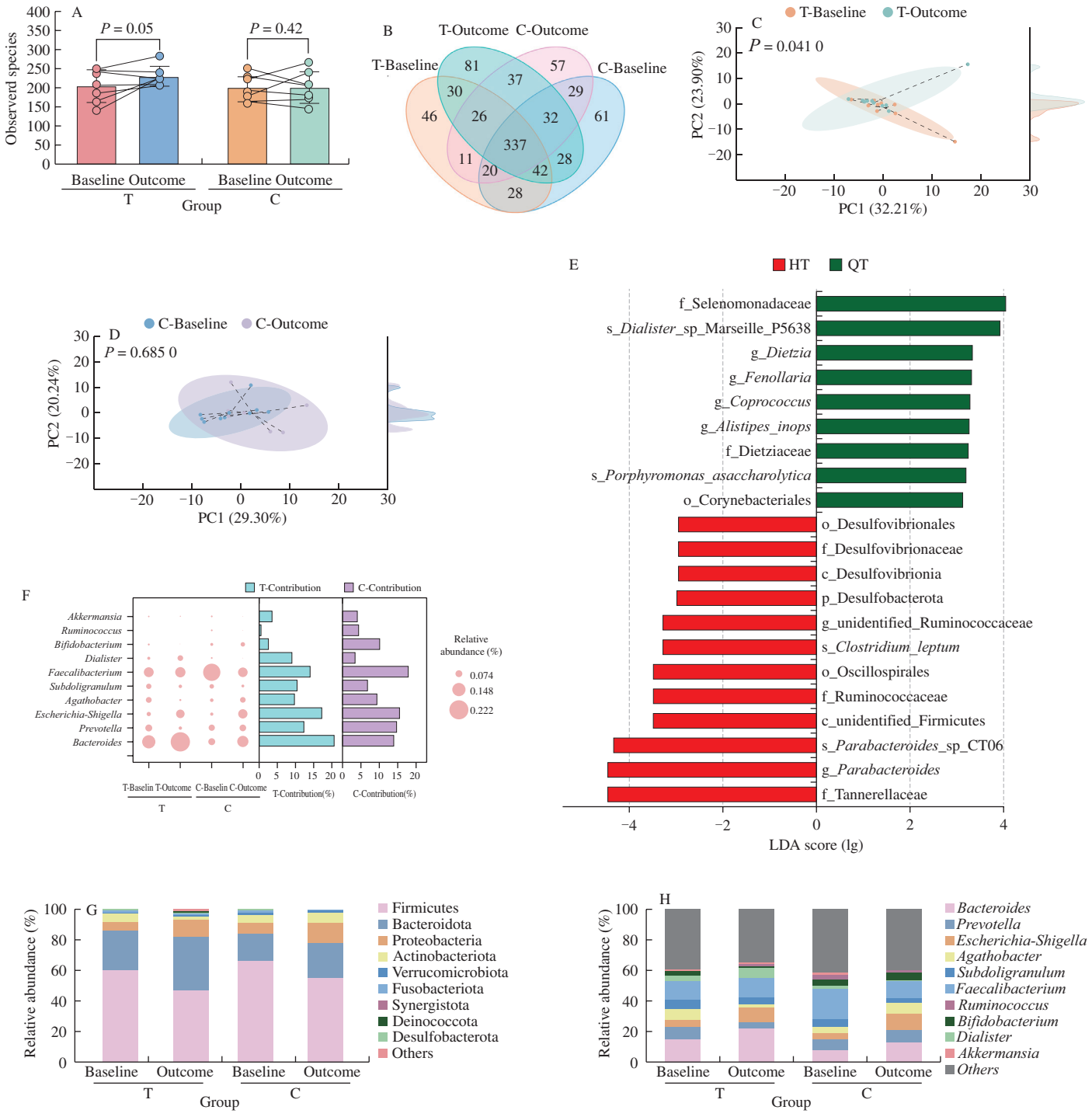

Fig. 3 Effect of black tea intervention on gut microbes. (A) Observed species. (B) Venn diagram of OTUs of gut flora in each group. (C) PCoA of Group T baseline and outcome. (D) PCoA of Group C baseline and outcome. (E) LEfSe analysis before and after intervention in Group T. (F) Combined bubble and species contribution plots. (G) Gut flora composition at the phylum level. (H) Intestinal flora composition at the genus level.

3.7 Effect of consumption of black tea on metabolic profiles

It is not sufficiently convincing to argue that black tea affects the gut flora and thus the host by altering the gut microbiota alone, and since a large part of the communication between the gut microbiota and the host takes place through metabolites with multiple functions, untargeted metabolomics analyses were used to identify the different constituent metabolites that were produced as a result of the intervention of the black tea (information related to metabolic profiles can be found in Tables S2 and S3). The samples from the intervention group at baseline and the resulting PCA plots at time showed 2 clusters with differences (Fig. 5A). As illustrated in Fig. 5B, a total of 13 differentially expressed metabolites were identified before and after the black tea intervention. (8 up-regulated and 5 down-regulated; variable important in projection (VIP) score > 1.0, $P < 0.01$ and fold change (FC) > 1.0 were used as screening criteria for differential

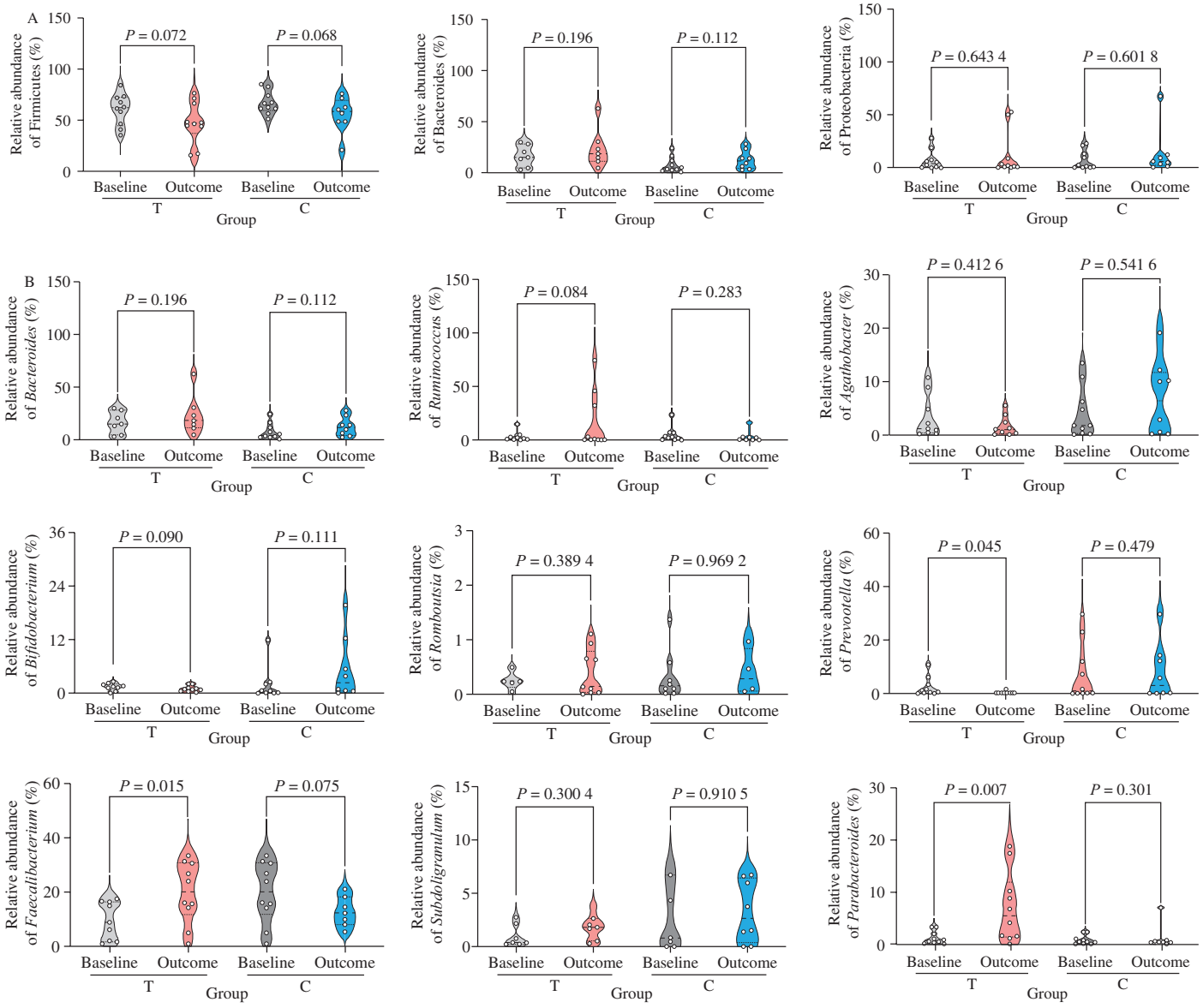

Fig. 4 Effects of a black tea intervention on gut microbial phylum and genus levels. (A) Relative abundance of 3 representative microorganisms at the phylum level. (B) Relative abundance of 9 representative microorganisms at the genus level.

metabolites). The 13 differentially expressed metabolites were classified (Fig. 5C), of which 5 differentially expressed metabolites belonged to fatty acyls, 3 differentially expressed metabolites belonged to glycerophospholipids, 2 differentially expressed metabolites belonged to organooxygen compounds, 2 differentially expressed metabolites belonged to prenol lipids, and 1 differential metabolite belonged to carboxylic acids and derivatives.

As shown in Fig. 5D, the abundance of lysophosphatidylinositol (LPI) 15:0, lysophosphatidylcholine (LPC) 15:1-SN, and lysophosphatidic acid (LPA) 18:2 was significantly up-regulated after the black tea intervention, whereas the abundance of 1-[4-(1-adamantyl)phenoxy]-3-piperidinopropan-2-ol hydrochloride and *L*-palmitoylcarnitine was significantly decreased. Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed that 9 pathways associated with differential metabolites, involving regulation of adipocyte lipolysis, amino acid biosynthesis, metabolism of glycine, serine, and threonine, biosynthesis of aminoacyl-tRNAs, and metabolism of cysteine and methionine (Fig. 5E). Fig. 5F shows the association analysis of metabolic profiles with gut microbes. Among the genera that showed significant differences in gut microbial abundance before and after the intervention, changes in the abundance of *Parabacteroides* and *Dietzia* affected the metabolic profiles, with *Parabacteroides* leading to significant up-regulation of LPC 15:1-SN1, LPI 15:0 and LPA 18:2 *Dietzia* led to significant up-regulation of 1-[4-(1-adamantyl)phenoxy]-3-piperidinopropan-2-ol hydrochloride and *L*-palmitoylcarnitine and significant down-regulation of LPC 15:1-SN1, 15-epi prostaglandin A1.

3.8 Association analysis of BMI, satiety, gut microbes and metabolic profiles

The preceding sections of this discussion indicate that the consumption of black tea may influence the sensation of satiety,

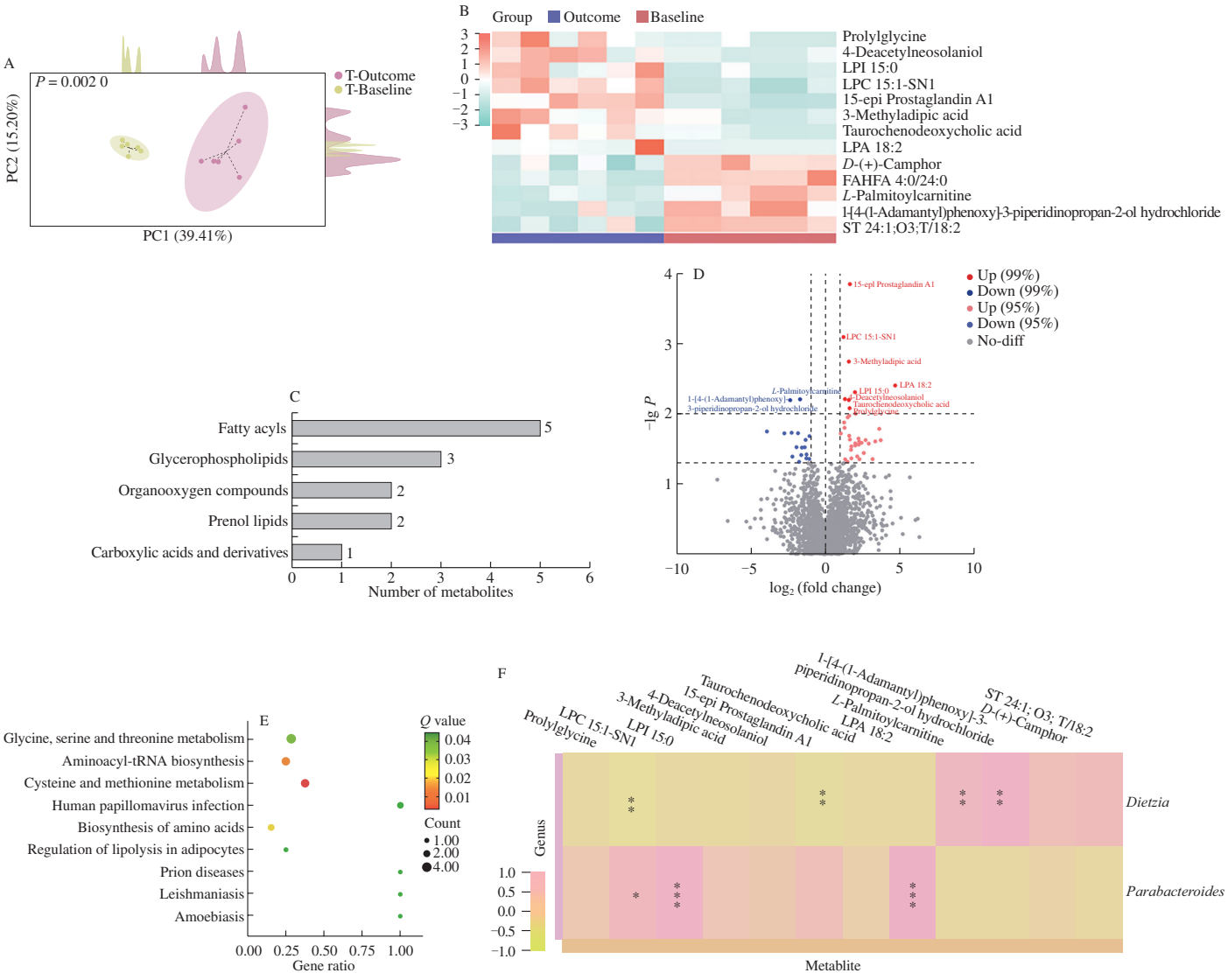

Fig. 5 Assays analysis was performed to obtain metabolite profiles for the T baseline group ($n = 6$) and the T outcome group ($n = 6$). (A) PLS-DA plot. (B) Heat map of relative abundance of different metabolites. (C) Categorization map of differential metabolites. (D) Metabolic volcano map. (E) Enrichment analysis of KEGG pathways. (F) Spearman correlation analysis of intestinal flora with differential metabolites after black tea intake. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

the composition of the gut microbiome, and metabolic profiles. Building on the previous discussion, we conducted within-group correlation analyses to explore the relationships between BMI, satiety, the top-ranked genera in terms of abundance among gut microbes, and metabolites in the metabolic profiles that showed significant differences before and after the black tea intervention. The association analysis of BMI, satiety, and gut microorganisms showed that BMI was positively correlated with satiety and negatively correlated with gut abundance of *Faecalibacterium* in this population-based experiment; whereas satiety was positively correlated with *Bacteroides* and *Parabacteroides* (Fig. 6A); After receiving an black tea intervention, correlation analyses of BMI, satiety, and metabolic profiles showed that BMI was positively correlated with *L*-palmitoylcarnitine and negatively correlated with LPC 15:1-SN1 and 15-epi prostaglandin A1. (Fig. 6B). Afterwards, we divided the high BMI population (BMI > 24) and the low BMI population (BMI < 24) in the intervention group into 2 groups for analyses. Analyses of gut microorganisms showed (Fig. 6C) that the relative abundance of *Bacteroides* was significantly lower in the high BMI population than in the low BMI population before the intervention ($P < 0.05$), and that after the black tea intervention, the relative abundance of *Bacteroides* was significantly higher in the high BMI population ($P < 0.05$), and there was no significant change in the low BMI population. Notably, the low-BMI population exhibited a higher baseline abundance of *Prevotella* than the high-BMI counter-parts ($P < 0.05$). However, the black tea intervention significantly reduced the relative abundance of *Prevotella* within the high-BMI group from baseline to outcome ($P < 0.05$); The relative abundance of *Parabacteroides* was lower in the high BMI population than in the low BMI population before the intervention, and after the black tea intervention, and after the black tea intervention, the relative abundance of Parabacteroides in the high-BMI population was significantly increased compared to the baseline ($P < 0.05$); Analysis of metabolic profiles showed (Fig. 6D) that 15-epi prostaglandin A1 expression was significantly lower in the high BMI population than in the low BMI population before intervention ($P < 0.05$), and that 15-epi prostaglandin A1

expression was significantly higher in the high BMI population after the black tea intervention ($P < 0.01$), and that although 15-epi prostaglandin A1 expression was similarly elevated in the population of the low BMI group, but the 15-epi prostaglandin A1 expression in the high BMI population was significantly higher than that in the low BMI population after the intervention ($P < 0.05$); whereas the metabolite daidzein, which did not differ significantly among the different BMI populations before the intervention, changed after the intervention, black tea intervention significantly elevated daidzein expression in the high-BMI population compared to baseline ($P < 0.05$), and the expression of daidzein in the high BMI population was significantly higher than that in the low BMI population after the intervention ($P < 0.05$). For *L*-palmitoylcarnitine, there was no significant difference in expression between high BMI and low BMI populations before intervention, but it still showed a trend of higher expression of *L*-palmitoylcarnitine in high BMI populations than in low BMI populations, and after black tea intervention, the *L*-palmitoylcarnitine expression decreased in both populations.

The association analyses of BMI, satiety, gut microbes and metabolic profiles further demonstrated that the black tea intervention not only affects the host through multiple single factors, but there is also a compounded effect on the host through the associative interplay of multiple factors.

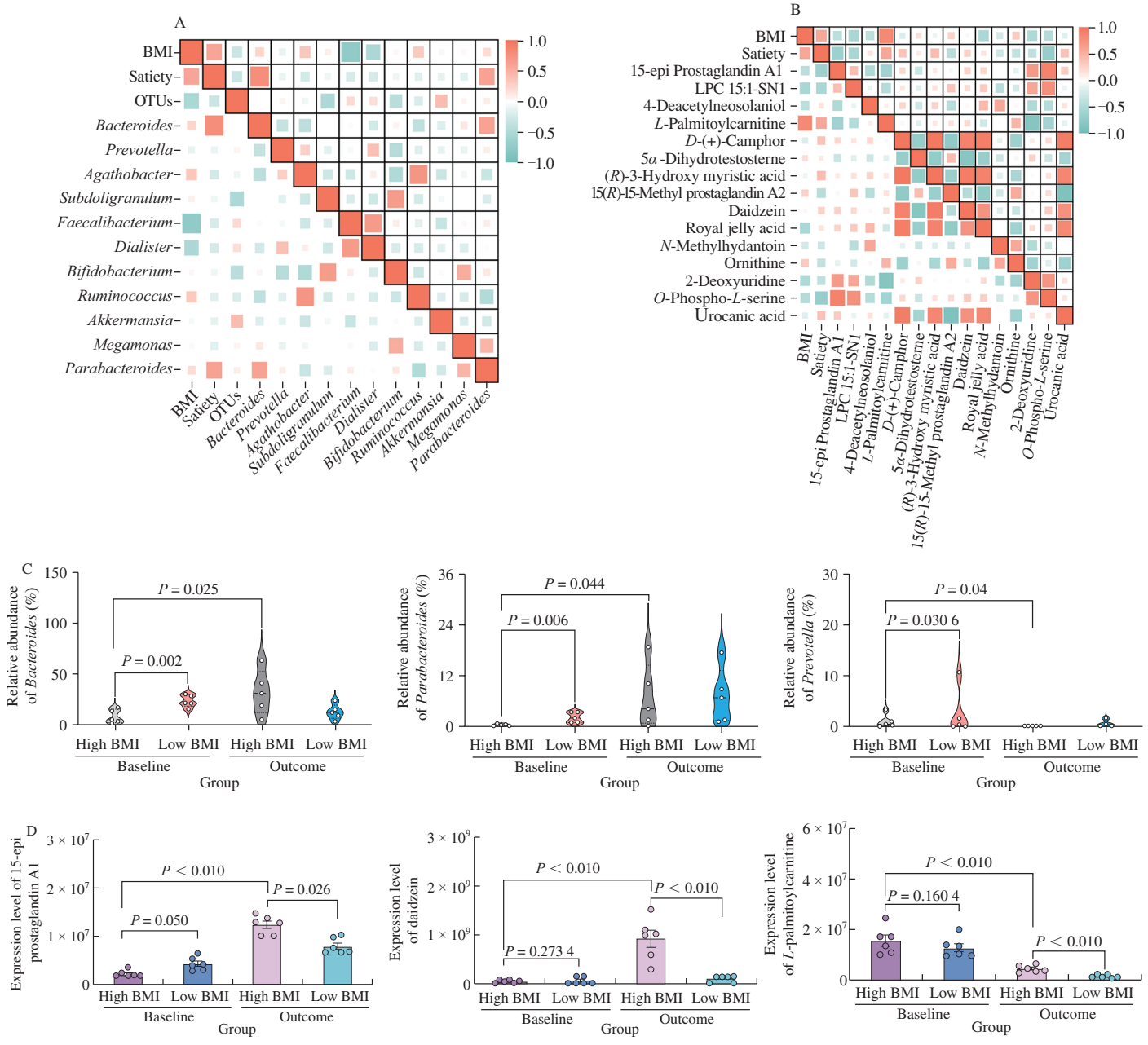

Fig. 6 Association analysis profiles of BMI, satiety, gut microbiology and metabolic profiles. (A) Within-group correlation analysis between BMI, satiety, OTUs, and genera with higher abundance in the test group. (B) Within-group correlation analysis between BMI, satiety, and pre- and post-intervention differential metabolites in the test group. (C) Differential bacteria genera appearing in high *vs*. low BMI pre- and post-intervention comparisons. (D) Differential metabolites appear in pre- and post-intervention comparisons of high *vs*. low BMI.

4. Discussion

By determining the active compounds in the black tea samples, it can be tentatively concluded that the black tea samples of this intervention are beneficial to host health based on the established studies on the probiotic properties of tea polyphenols and tea polysaccharides.

Satiety affects energy intake and has been shown to be an important factor in weight loss, and it has been shown in the literature that some tea beverages with probiotic properties, such as Kampuchea, are able to influence the satiety of drinkers, with different effects on drinkers of different BMI and different sexes[28]. In the present study, despite the fact that black tea does not provide energy, the majority of participants in the intervention group reported an increase in satiety 2–3 h after consuming black tea. Furthermore, the increase in satiety was significantly higher in female participants than in male participants. Given that black tea does not provide energy on its own, it is reasonable to assume that the increased satiety induced by black tea may have caused participants to reduce their food intake, which is recognized as the simplest and most direct way to lose weight[29]. Although we did not observe a reduction in participant BMI in this experiment, the ability of black tea consumption to enhance satiety without providing energy can reduce food intake in the context of a normal diet, which is thought to have the potential to assist in weight loss. It seems reasonable to posit that long-term consumption of black tea may facilitate weight loss through the stimulation of long-term satiety, which in turn reduces food intake. In addition, this supportive role may be more pronounced for women. The consumption of black tea was observed to increase feelings of satiety without providing energy, with this effect being more pronounced in female participants. This increase has the potential to assist in short-term weight loss, although further experiments are required to verify whether it is effective in assisting weight loss in the long term.

In established studies using obese mouse models, tea beverages have been shown to contribute to weight loss by modulating the gut microbiota with beneficial effects on host metabolism[30]. Moreover, related studies have demonstrated a correlation between the gut microbiota and the regulation of satiety and energy intake. The administration of prebiotics and probiotics has been shown to modulate the gut microbiota, which in turn modulates food intake and ameliorates obesity and related metabolic disorders in humans[31].

The current study found that the black tea intervention significantly increased the observed levels of gut microbial species, suggesting a beneficial effect on gut microbial diversity. Consistent with the accumulating evidence that low levels of gut microbial diversity may be a marker of disease states or a potential causative factor, the black tea intervention affected the composition of the gut microbial community, suggesting that it may have a deeper effect on the structure of the gut flora by inhibiting harmful bacteria and enriching beneficial bacteria[32]. Metabolic diseases such as obesity and bulimia have been shown to be associated with some specific bacterial taxa[33]. The contribution of the characteristic bacterial taxa could be accurately identified during the treatment of the T intervention group using a taxonomy-based contribution model, with an increase in the abundance of *Bacteroides* and a decrease in the abundance of *Prevotella* contributing to the changes in the bacterial population of the intervention group. In the calibration of obesity-dominated metabolic diseases, these 2 bacteria are important references, *Bacteroides* has a wide range of metabolic potential in the gut microbiota to rapidly adjust its carbohydrate metabolism to available nutrients and produce SCFAs, and the increased abundance of *Bacteroides* facilitates the energy metabolism of the host[34]; it is generally believed that low levels of *Prevotella* is generally believed to alleviate insulin resistance in the host[35]. *Agethobacter*, *Dialister*, *Akkermansia*, *Bifidobacterium*, and *Ruminococcus* are considered to be the major contributors to differences in the composition of gut microbes, and are in the top 10 of the list of contributors.

A more convincing treatment would be to compare the species that showed significant differences between the T and C groups before and after the trial by analyzing the pre- and post-intervention flora of the T-intervention and C-control groups (*P*-values were calculated for the top 50 species in the OUTs group rankings, with $P < 0.05$ considered significant). The results demonstrated that at the genus level, there were no statistically significant changes in species before and after the intervention in the C control group. In contrast, the T intervention group exhibited 3 significant changes in species before and after the intervention, characterized by a reduction in the relative abundance of *Prevotella* and an increase in the relative abundance of *Faecalibacterium* and *Parabacteroides*. It has been demonstrated that healthy overweight adults who consume an *ad libitum* diet rich in whole grains and fiber for a period of 6 weeks experience a greater reduction in body fat in those with a high abundance of *Prevotella* than in those with a low abundance of *Prevotella*[36]. However, high *Prevotella* abundance has been identified as a potential biomarker for obesity, BMI in non-diabetic patients, insulin resistance, hypertension and non-alcoholic fatty liver disease (NAFLD). *Faecalibacterium* is a well-studied, common, beneficial gut microorganism that has been associated with the production of a variety of SCFAs. It has been demonstrated that obesity, NAFLD, and type 2 diabetes are associated with low *Faecalibacterium* abundance[37-38]. Furthermore, *Faecalibacterium* has been demonstrated to promote bile secretion, indicating that an elevated abundance of *Faecalibacterium* is associated with a reduced prevalence of obesity and other metabolic disorders. This is supported by evidence that suggests high *Faecalibacterium* abundance is beneficial for weight loss and lipid lowering[39]. Similarly, *Parabacteroides* are also beneficial microorganisms that are commonly found in the human gut. The use of *Parabacteroides distasonis* and *Parabacteroides goldstein* in the treatment of obesity has been demonstrated to be effective[40]. This is due to the fact that *Parabacteroides* promotes the production of intestinal succinic acid and secondary bile acids through the administration of gavage. This, in turn, facilitates intestinal gluconeogenesis and protects intestinal permeability. With regard to the specific bacterial taxa at the phylum and genus level associated with the black tea intervention, although there was only a trend for changes in phylum levels that were not statistically significant, an increase in the abundance of *Bacteroides* and *Parabacteroides* at the phylum level and a down-regulation of Firmicutes at the phylum level were noted in the participants after ingestion of black tea. Notably, at the generic level, intake of black tea increased the associated abundance of *Escherichia-Shigella*. Although *Escherichia-Shigella* is thought to be associated with a variety of diseases, within normal levels, the relative abundance of *Escherichia-Shigella* is positively

correlated with that of *Parabacteroides* and negatively correlated with that of *Prevotella*[41], which is in line with the trend of relative abundance of *Parabacteroides* and *Prevotella* in the present study. These results indicate that black tea can inhibit the bacteria that are positively correlated with obesity abundance and enrich the bacteria that are negatively correlated with obesity abundance, and that black tea not only increases the abundance of host gut microbial species, but also modifies and alters the structure of the host gut microbiota, and increases the abundance of weight loss-friendly genera such as *Faecalibacterium* and *Parabacteroides*. This will ultimately make the host gut microbiota develop in the direction of weight loss.

Whether alterations in the gut microbiota affect host metabolism requires further analyses using untargeted metabolomics to clarify the effect of the black tea intervention on host metabolism. In PLS-DA analyses, clustering of T baseline and T results that were clearly separated indicated that consumption of black tea had an effect on host metabolism. The abundance of LPI 15:0, LPC 15:1-SN1, and LPA 18:2 was significantly up-regulated, and the abundance of 1-[4-(1-adamantyl) phenoxy]-3-piperidinopropan-2-ol hydrochloride and *L*-palmitoylcarnitine was significantly decreased, with the differences all being statistically significant ($P < 0.01$). According to the available studies, lysophosphatidylinositol 15:0 (LPI), lysophosphatidylcholine 15:1 (LPC 15:1-SN1), and lysophospha tidylphosphatidylinositol 18:2 (LPA 18:2) have been associated with metabolic disorders such as obesity and diabetes mellitus; LPI and GPR55 comprise the LPI-GPR55 axis, whereas the LPC-like metabolite and the LPA-like metabolite both belong to lysophospholipid metabolites[42]. Both phospholipid metabolites and their abundance are proportional to insulin expression. Early studies have shown that LPI and LPC have pro-inflammatory effects and some negative effects, while more recent studies have shown that low levels of LPI, LPC and LPA are associated with metabolic disorders such as insulin resistance[43]. *L*-Palmitoyl carnitine, a metabolite of carnitine, and its metabolites have been reported to be involved in fatty acid metabolism, and elevated circulating concentrations of carnitine metabolites have been observed in obese and insulin-resistant patients[44]; while the exact effect of 1-[4-(1-adamantyl)-phenoxy]-3-piperidinopropan-2-ol hydrochloride on host metabolism has not been clarified. In light of the aforementioned metabolite modulation, it can be postulated that black tea intervention may exert an influence on lipid metabolism in the host. Furthermore, KEGG enrichment analysis indicates that the consumption of unsweetened black tea may impact the regulation of lipolysis in adipocytes. amino acid biosynthesis, glycine, serine and threonine metabolism, aminoacyl-tRNA biosynthesis, and cysteine and methionine metabolism. The regulation of lipolysis in adipocytes is closely related to obesity[45]. It was also found that the biosynthesis and metabolism of amino acids (including glycine, serine, threonine and tyrosine) were also affected by the black tea intervention, which is closely related to diet-induced obesity[46-47]. Aminoacyl-tRNA biosynthesis involves aminoacyl-tRNA synthetase (FARS), and overexpression of FARS leads to insulin resistance and glucose intolerance, suggesting that black tea interventions may have an effect on the host's insulin-resistant and glucose-intolerant state[48]; whereas cysteine and methionine metabolism are mainly associated with high-fat diets, methionine- and choline-deficient diets (MCDs), and NAFLD[49]. Correlation analyses of metabolic profiles and gut flora serve as more direct evidence that changes in gut microbial structure leads to changes in metabolic profiles. From the above discussion, we can conclude that the black tea intervention affected the metabolic profile of the host, especially lipid metabolism and amino acid metabolism, and that this alteration to the metabolic profile was associated with changes in the gut microbial structure.

And further correlation analyses of BMI and satiety with gut microbes and metabolic profiles provide even more compelling evidence that black tea intake assists in weight loss. The correlation analysis of BMI, satiety and intestinal microorganisms showed that after the intake of black tea, the participants' BMI was positively correlated with satiety, which, combined with the previous conclusion that black tea has the ability to enhance satiety, points to the fact that the enhancement of satiety by black tea is correlated with participants' BMI, and that the enhancement of satiety is higher for participants with a higher BMI. This is conducive to dietary control during weight loss for people with a high BMI, assisting in weight loss; Meanwhile, BMI was more significantly negatively correlated with gut abundance in *Faecalibacterium*, and satiety was more significantly positively correlated with *Bacteroides* and *Parabacteroides*. The former fits with the existing research that the gut abundance of *Faecalibacterium* is negatively correlated with obesity; the latter points to the fact that the satiety provided by black tea may not only provide virtual satiety in the brain through the lubricating effect of the oral cavity[50], but also affect the brain through the gut-brain axis by altering the intestinal flora, which in turn affects the feeling of satiety[51]. Correlation analysis of BMI, satiety, and metabolic profiles showed that BMI was positively correlated with *L*-palmitoylcarnitine was negatively correlated with LPC, 15-epi prostaglandin A1, which is consistent with our previous discussion. Interestingly, when we regrouped the population by BMI height (BMI > 24 was considered high BMI and BMI < 24 was considered low BMI) and then examined the gut microbial and metabolic profiles in this experiment, we found that in terms of gut microbes, the relative abundance of weight loss-favoring *Bacteroides* and *Parabacteroides* in the high BMI population was both low, but the relative abundance of both *Bacteroides* and *Parabacteroides* increased significantly and dramatically after the black tea intervention, whereas the relative abundance of *Bacteroides* did not change much before and after the black tea intervention in the low BMI population, and the relative abundance of *Parabacteroides* was slightly, but not significantly, elevated, and positively correlated with obesity. Interestingly, despite the statistical variance in *Prevotella* abundance between the 2 BMI categories at baseline, the primary impact of the black tea intervention was distinctively manifested in the high-BMI population, where a significant reduction was observed. Previous analyses have concluded that black tea can assist weight loss by modulating the gut microbial structure, and it can be further argued that this modulation of the gut microbial structure to assist weight loss is even more effective and significant in obese populations with a high BMI. This situation also appeared in the metabolic profiling analysis, the expression of 15-epi prostaglandin A1 in the high BMI population was significantly higher than that in the low BMI population after the black tea intervention; the expression of the metabolite daidzein, while showing no significant difference between the BMI groups at baseline, was significantly elevated in the high-BMI population compared to the low-BMI population after the black tea intervention;

the expression of the metabolite *L*-palmitoylcarnitine before the intervention was not significantly different between the high BMI population and the low BMI population, but it still showed a trend of higher expression of *L*-palmitoylcarnitine in the high BMI population than in the low BMI population after the black tea intervention, the expression of *L*-palmitoylcarnitine declined in both populations. 15-epi Prostaglandin A1 and daidzein are endogenous metabolites that are beneficial for weight loss and fat metabolism, and *L*-palmitoylcarnitine expression was positively correlated with obesity. The above analyses allow us to extend our previous conclusions that black tea interventions modulate the metabolic profiles of the host in order to aid weight loss, and that this modulation is more potent and pronounced in the high BMI population. In conclusion, through further correlation analyses, we were able to extend our previous conclusion that black tea interventions can assist in weight loss through modulation of dietary intake, gut flora and metabolic profiles, to conclude that this modulation of black tea is more effective and significant for people with a high BMI, which provides more targeted nutritional recommendations for the use of black tea to assist in weight loss.

In this 3-week population-based experiment, we also monitored the BMI and percentage body fat (PBF) of the participants in both groups before and after the intervention. By comparing baseline and outcome (Fig. S3), we did not find significant changes in BMI and PBF. However, it is worth discussing that the mean values of BMI and PBF for baseline and outcome in both groups were calculated by looking at the median line of the violin plots as well as through the tabular data (BMI: Group T 21.75 ± 3.32 *vs*. 21.66 ± 3.19; Group C 22.43 ± 3.59 *vs*. 22.48 ± 3.60; PBF: Group T 19.51 ± 6.00 *vs*. 19.46 ± 5.82; Group C 20.60 ± 6.54 *vs*. 20.61 ± 6.53), we still found a slight downward trend in BMI and PBF in Group T who ingested black tea for 3 weeks (Table S4). Combined with the discussion of satiety, gut microbes, and metabolic profiles above, it is reasonable to believe that black tea ingestion has a potential probiotic function to assist in weight loss.

Practice has proved that tea and tea products in tea polyphenols, tea polysaccharides and other active ingredients have the health effect of assisting in weight loss, while tea and tea products provide almost no calories, as a kind of assisted weight loss of health food tea and tea products have the potential for development and research significance[52]. Current research on tea and tea products involves various varieties of tea such as green tea, Pu-erh tea, etc. A substantial body of evidence from animal and population studies indicates that long-term consumption of catechin-rich green tea can regulate host metabolism and facilitate weight loss by enhancing energy metabolism and the rate of fat oxidation[53]; compared with post-fermented tea (Pu-erh tea) or fermented tea (black tea), the higher content of tea polyphenols in green tea leads to an increase in the abundance of specific genera of intestinal flora capable of metabolizing tea polyphenols, such as *Flavonifractor plautii*, and further research is needed to find out whether these tea polyphenol-preferring genera are beneficial for weight loss[54]. Despite the loss of more easily oxidizable active substances, such as tea polyphenols, during deep fermentation, Pu-erh tea's rich theaflavins have been demonstrated to regulate intestinal flora and bile acid metabolism. Consequently, they are postulated to have the potential to assist in lipid-lowering and weight loss[55]. Our study undertook a comprehensive examination of the effects of black tea, which is rich in tea polysaccharides, on satiety, the composition of the host gut microbiota and metabolic profiles. In a 3-week intervention trial, black tea was shown to provide satiety and reduce dietary intake in a healthy and sensible manner, while having the ability to improve host gut microbiota, further influencing host metabolic profiles (the mentioned probiotic properties are more pronounced in the high BMI population). As a functional food, especially for weight loss and obesity treatment populations, this supports personalized treatment recommendations and nutritional counselling. However, there are some limitations of this study. Firstly, this study was conducted in a small Chinese population, and although randomized recruitment was used to recruit participants, individual differences due to other factors such as age, gender, and personal nutritional habits are unavoidable, which may have an impact on the accuracy of the trial and may be difficult to detect and eliminate; Subgroup analyses require large sample sizes, and there were too few sample sizes for subgroup analyses in this experiment. Given those 2 points, for subsequent experiments, can attempts to increase the number of recruits and the sample size of the test population, and further sample the participants to extract more information in depth in an attempt to minimize the bias of the research effect. Secondly, this experiment emphasized to participants on a daily basis the need to ensure a normal dietary background for individuals during participation, but did not keep strict track of each participant's daily diet (e.g., by recording each participant's daily food intake), and thus there may have been some factors that slightly affected the accuracy of the experiment. Consequently, future studies in larger multi-ethnic populations are necessary to validate the generalizability of this study's findings that black tea intake is associated with satiety, gut microbiology and metabolic profiles. Future studies may be possible to record and standardize the daily diet of each participant, if necessary, in order to further improve the accuracy of the experiment.

5. Conclusion

Black tea, as a traditional beverage, has also received increasing attention and development due to its theobromine, theanine and theaflavin content, which are considered to be biologically active substances. This 3-week preliminary study explored the relationship between black tea intake, satiety, gut microbiota, metabolic profiling. The results suggest the consumption of black tea provides the host with a sense of satiety in order to reduce the host's dietary intake in a healthy and rational manner, and that this sense of satiety was increased in female participants more than in male participants. Black tea intake enriched the intestinal microbial diversity, improved the composition of the intestinal flora, and helps enrich some beneficial flora, such as *Parabacteroides*, *Faecalibacterium*. Black tea intervention also improves host metabolic health. Our findings may not only provide a public nutritional strategy for black tea intake to assist in weight loss and lipid reduction, but also personalized nutritional advice for the general population.

Conflicts of interest

Shuo Wang is an editorial board member for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. The authors declare no conflict of interest.

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Institutional review board statement

The Cohort Pilot Study protocol was approved by the Ethics Committee of Nankai University (program code NKUIRB2024078).

Data availability statement

The data that supports the findings of this study are accessible in the Supplementary Materials of this article. Further information might be available from the corresponding author upon reasonable request.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2025.9250505.

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