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Distinct effects of three tea varieties made green teas on improving diabetic dyslipidemia induced by high-fat diet in C57BL/6J mice

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ABSTRACT

Currently, cultivated tea plants are classified into three varieties, namely, *Camellia sinensis* var. *sinensis*, *C. sinensis* var. *assamica*, and *C. sinensis* var. *kucha.* Three tea varieties made green teas (TVGTs) are widely consumed worldwide. However, a comparative investigation of the beneficial effects, mechanism, and characteristic compounds of TVGTs has not been reported. Here, three representative tea plant varieties, namely, *C. sinensis* var. *sinensis* cv. Fuding (FD), *C. sinensis* var. *assamica* cv. Yunkang 10 (YK), and *C. sinensis* var. *kucha* (KC), planted in the same garden, were used to make green teas utilizing the same standard procedure. Our findings show that the TVGTs effectively improved hyperglycemia, obesity, dyslipidemia, fatty liver, and mesenteric artery (MA) hypercontractility in mice with diabetes and obesity induced by a high-fat diet (HFD) after 11- and 22-week interventions, with YK being the most effective at 22 weeks. Liver lipidomics indicated that the TVGTs restored the glycerophospholipid and sphingolipid balance and reduced triglycerides, with YK demonstrating superior effects. The TVGTs, particularly YK, suppressed triglyceride synthesis through the ARV1-FXR-SHP-SREBP-1c pathway. A chemical profile analysis revealed that YK green tea had enriched active compounds, including caffeine, quinic acid, *L*-theanine, gallic acid, and catechins, compared with FD and KC green teas, which may contribute to ameliorating diabetic dyslipidemia via synergistic action. As a representative tea variety of *C. sinensis* var. *assamica*, YK green tea was found to be superior to the other two green teas in preventing and treating diabetic dyslipidemia, especially with long-term consumption. These data provide important information for tea plant breeders and tea consumers.

1. Introduction

During recent decades, the prevalence of diabetes has undergone a substantial surge, primarily fueled by the persistent upswing in the occurrence of type 2 diabete[1]. It is widely recognized that hyperlipidemia is one of the major characteristics of diabetes mellitus and obesity[2-3]. Hyperlipidemia is strongly associated with excessive fatty acid flux to the liver, which results in excess production of triglyceride (TG)-based lipids[4-5]. In diabetic and obese patients, dyslipidemia is closely related to liver steatosis and vascular complications[6-9]. Therefore, prevention of diabetic dyslipidemia by consuming natural foods and beverages would be a preferable public health strategy.

Green tea, which is widely consumed in Asian countries, is made from the leaves and buds of the *Camellia sinensis* plant. China is the world's largest green tea producer[10-11]. At present, a widely recognized classification of cultivated tea plants includes three varieties, namely, *C. sinensis* var. *sinensis* (Sinensis variety), *C. sinensis*

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var. *assamica* (Assamica variety), and *C. sinensis* var. *kucha* (Kucha variety)[12]. The Fuding tea plant (*C. sinensis* var. *sinensis* cv. Fuding) has been recognized as one national superior breeder for improving Chinese variety breeding, as well as a standard control species by the National Tea Tree Variety Research Association[13-14]. Yunkang 10 (*C. sinesis* var. *assamica* cv. Yunkang 10) is a dominant variety of Assam variety, and is widely cultivated in Southwestern China[15]. Compared to other cultivars, Yunkang 10 has a wide range of adaptability, low cultivation cost, and fast growing. Kucha (*C. sinensis* var. *kucha*) is a unique variety[12] of tea plants cultivated in Yunnan, Guangdong, Guangxi, Hunan, and Jiangxi Provinces[16-17]. These three tea varieties are cultivated in most regions of China. Therefore, investigating their health benefits can provide theoretical guidance for tea consumption in China.

Studies have demonstrated that green tea polyphenols, especially epigallocatechin gallate (EGCG), significantly improve metabolic syndrome (MetS) and prevent obesity in animals[15,18-19]. *L*-Theanine, a functional compound in green tea, has been demonstrated to regulate glucose and lipid metabolism[20-21]. Moreover, green tea extract, as well as its major functional components, have been demonstrated to have lipid-lowering and weight-loss effects in recent studies[19,22-23]. Different planting locations, picking times, processing methods, etc., can lead to different phytochemical components, which can alter the health benefits. The effectiveness of different types of tea in reducing lipid levels and body weight has recently been comparatively investigated. Studies have shown that six types of tea extracts ameliorate high-fat diet (HFD) induced MetS by modulating gut microbiota with equal effectiveness, and white tea performed better efficiency in body weight control[24]. It has been found that polyphenol extracts from green, black, and oolong tea are effective at reducing body weight, and promoting angiogenesis, even though their amounts of polyphenols are significantly different[25].

Most previous studies were based on teas from different origins, using different processing and intervention methods. Therefore, it is challenging to compare the effects of different tea varieties on lipid and glucose modulation under identical conditions, such as cultivation in the same garden and manufacture using the same standard process. Consequently, it is important to systematically evaluate the effects of three tea varieties made green teas (TVGTs) on lipid and glucose metabolism. However, a comparative investigation of the effectiveness and mechanism of action of TVGTs in modulating lipid and glucose metabolism has not been reported. The aim of this study was to comparatively explore the hypoglycemic and dyslipidemic effects and mechanism of TVGTs from tea plants cultivated in the same garden and produced using the same standard protocol. Furthermore, ultra-high performance liquid chromatography-Orbitrap-tandem mass spectrometry (UPLC-Orbitrap-MS/MS) based un-targeted metabolomics combined with chemometrics analysis were employed to identify distinct functional components among TVGTs. These evaluation results will provide important information for tea breeders in selecting the right variety and for tea consumers.

2. Materials and methods

2.1 Sample preparation

Three tea varieties, including Fuding (*C. sinensis* var. *sinensis* cv. Fuding), Yunkang 10 (*C. sinensis* var. *assamica* cv. Yunkang 10), and Kucha (*C. sinensis* var. *kucha*), were cultivated at the National Germplasm Resources Center for Assam Tea Plant in Menghai County, Yunnan Province, China. One bud and two leaves were selected from plants of the three tea varieties and made green tea using a standard steaming protocol (fresh leaves were immersed in 100 °C steam for 30 s and then cooled).

2.2 Animal experiments

Male C57BL/6J mice (5-week old) were purchased from GemPharmatech Co., Ltd. (SCXK(Su)2024-0016, Nanjing, China) and acclimated to a specific pathogen-free (SPF) environment for 1 week at the Anhui Agricultural University's Animal Facility Center. Then, the mice were divided into 5 groups: a low-fat diet (LFD) group (LF, n = 12), a HFD group (HF, n = 12), a HFD plus 5% Fuding green tea powder group (FD, n = 12), a HFD plus 5% Yunkang green tea powder group (YK, n = 12), and a HFD plus 5% Kucha green tea powder group (KC, n = 12). FD, YK, and KC green teas were ground into powder, then 5% (m/m) of each tea powder was added to a HFD to make the specific intervention diets. All diets were specifically ordered from Trophic Animal Feed High-Tech Co., Ltd. (Nantong, China). Based on manufacture information, the LFD contained 11% energy from fat (3.5 kcal/g), whereas the HFD contained 60% energy from fat source (5.0 kcal/g) (Trophic Animal Feed High-Tech Co., Ltd., Nantong, China). The mice were monitored every day for food intake and water consumption, body weight on a weekly basis. For the investigation of the effects and mechanism during different intervention periods, 6 mice from each group were sacrificed at the 11th (middle-term intevention) and 22nd (long-term intervention) week of the intervention period. The Institutional Animal Care and Use Committee (IACUC) of Anhui Agricultural University approved all animal experiments (ethical approval code: AHAU2018020).

2.3 Sample collection

After fasting overnight at the 11th or 22nd week of treatment, the mice were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg) and sacrificed after peripheral blood samples were collected. The serum was centrifuged at 3 000 r/min for 10 min at 4 °C, and immediately stored at −80 °C until use. The liver weight was measured, and a small piece of liver tissue from the same area across various groups of mice was preserved in RNA stabilization solution (Thermo Fisher Scientific, Waltham, MA, USA) for gene expression analysis and in formaldehyde fixation solution (ZHANYUN, Wuxi, China) for histological examination.

2.4 Glucose tolerance and insulin tolerance tests

Fasting blood glucose measurements were carried out at 11 and 22 weeks using a Nova StatStrip XpresstM Glucose CR Meter (Nova Biomedical, Waltham, UK). The glucose tolerance test (GTT) and insulin tolerance test (ITT) were conducted after 10 and 20 weeks of treatment, respectively. The specific experimental methods were described in our previous publication[26].

2.5 Serum biochemical analysis

The concentrations of TG, total cholesterol (T-CHO), low density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum were measured using microtesting kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and OD values were measured using a SpectraMax microplate reader (Molecular Devices, LLC, USA).

2.6 Hematoxylin-eosin (H&E) staining

H&E staining was performed following our previous protocol[26] with slight modifications. Briefly, liver tissues were dehydrated and embedded in paraffin (Paraplast Tissue Embedding Medium, LEICA, USA). Then, 4 μm sections were cut with a fully automated rotary microtome (LEICA RM2255, USA). Staining was performed using an H&E staining kit (Beijing Solarbio Science & Technology Co., Ltd., China), and images were captured using a microscope (LEICA DM500, Wetzlar, Germany) with a supporting camera (LEICA ICC50W, Wetzlar, Germany). Infiltration cells of adipocytes in the liver were counted manually using ImageJ software.

2.7 Measurement of mesenteric artery (MA) contraction

MA contraction was measured following a previous method[27]. Briefly, the mice were sacrificed, MAs were isolated from the mesentery beds, and the contraction of the second branch of the MA was measured using a myograph system (DMT 620 M; Aarhus N, Denmark).

2.8 Hepatic lipidomics analysis

For lipidomics analysis, a 0.01 g frozen liver sample from each group of mice was thawed on ice, 200 μL of phosphate buffered saline (PBS) was added, and it was homogenized in a sample homogenizer. A mixture of 1.2 mL methanol-methyl tert-butyl ether (MTBE)-water (4:5:5, *V/V*) was added to the homogenate, and this was incubated for 1 h on ice. Then, the mixture was centrifuged at 2 000 r/min for 5 min and dried on a rotary evaporator. After that, the mixtures were suspended in 200 μL isopropanol and centrifuged for 5 min at 17 000 r/min at 4 °C. Finally, 2 μL of supernatant was injected for UPLC analysis, and 3 μL of each sample was mixed together as a quality control (QC) sample.

The samples' lipids were separated by UPLC (Thermo Fisher Scientific, USA) using a Q Exactive Focus Orbitrap MS/MS system with heated electrospray ionization and an XBridge BEH HILIC column (2.1 mm × 100 mm, 2.5 μm) (Waters, USA). Mobile phase A included 5 mmol/L ammonium formate-acetonitrile solution (acetonitrile:water = 6:4, *V/V*), and mobile phase B had 5 mmol/L ammonium formate-acetonitrile isopropanol solution (acetonitrile:isopropanol = 1:9, *V/V*). Positive and negative ion modes were both operated with an electrospray voltage of 3.5 kV, ion transfer capillary temperature of 300 °C, nitrogen as sheath gas, and auxiliary gas at 45 and 10 psi. Collision energy was divided into 25, 35, and 40 stages to facilitate the collection of as many ion fragments as possible. Data processing was carried out on Xcalibur version 3.1 software (Thermo Fisher Scientific, USA).

2.9 Quantitative real-time polymerase chain reaction (PCR)

Total RNA was extracted from liver tissue using TRIzol reagent (Vazyme Biotech Co., Ltd., Nanjing, China). A Hiscript II 1st strand cDNA Synthesis Kit was used for the reverse transcription of 2 μg of total RNA into cDNA (Vazyme Biotech Co., Ltd., Nanjing, China). As previously described[28], real-time PCR was performed with SYBR Green Master Mix (CFX96 Touch, Bio-Rad, USA), with 18S rRNA was used as a housekeeping gene to normalize the expression level of the target gene. The primer sequences used are listed in Table 1.

Table 1
Primer sequence used for real-time PCR.

Gene name	Primers	Sequences
18S rRNA	Forward (5′-3′)	GGGTCGGGAGTGGTAATTT
	Reverse (5′-3′)	AGAACGGCCACATCCAA
ARV1	Forward (5′-3′)	GATGACGTGATCCGGTACGC
	Reverse (5′-3′)	ACAGGAAAGCAAATATGCCAGT
FXR	Forward (5′-3′)	GGCAGAATCTGGATTTGGAATCG
	Reverse (5′-3′)	GCCCAGGTTGGAATAGTAAGACG
SHP	Forward (5′-3′)	GGCAGAATCTGGATTTGGAATCG
	Reverse (5′-3′)	GCCCAGGTTGGAATAGTAAGACG
Srebf1	Forward (5′-3′)	GATCCAGCCTTTGAGGATAGCC
	Reverse (5′-3′)	CCGTAGCATCAGAGGGAGTGAG
Fasn	Forward (5′-3′)	CGTGTGACCGCCATCTATATCG
	Reverse (5′-3′)	TGAGGTTGCTGTCGTCTGCAGTCT T
Acaca	Forward (5′-3′)	AGGAGGGAAAGGGATCAGAAAAG
	Reverse (5′-3′)	CAGAGCAGTCACGACCAAACAAA
Ppar-γ	Forward (5′-3′)	GAA AGA CAA CGG ACA AAT CAC CAT
	Reverse (5′-3′)	CGG CTT CTA CGG ATC GAA ACT G

Note: *ARV1*, ARV1 homolog, fatty acid homeostasis modulator; *Srebp-1c*, sterol regulatory element binding protein-1c; *FXR*, farnesoid X receptor; *SHP*, nuclear receptor subfamily 0 group B member 2; *Fasn*, fatty acid synthase; *Acaca*, acetyl-CoA carboxylase alpha; *Ppar-γ*, peroxisome proliferator-activated receptor γ.

2.10 Western blotting

Western blots were performed according to our previous description[28]. Briefly, liver tissue was frozen at –80 °C in 10% trichloroacetic acid solution for 24 h. Trichloroacetic acid was removed from the liver sample by washing with 100% acetone. After that, the liver tissue was then homogenized using 2× sodium dodecyl sulfate (SDS) buffer, and the concentration of protein was determined with a BCA protein assay kit (Vazyme Biotech Co., Ltd., Nanjing, China). Total proteins were electrophoresed on SDS polyacrylamide gels and transferred to membranes. After blocking with nonfat milk buffer, the membranes were incubated with appropriate primary antibodies overnight at 4 °C. The primary antibodies included ARV1 (N-term) rabbit polyclonal antibody (Origene, USA), nuclear receptor subfamily 1, group H, member 4 (FXR) (Proteintech, USA), NROB2 rabbit polyclonal antibody (SHP) (ZEN-BIOSCIENCE, Chengdu, China), SREBP-1c, and *β*-actin (Santa Cruz Biotechnology, CA, USA). A secondary antibody conjugated with HRP (Santa Cruz Biotechnology, CA, USA) was incubated with the membranes for 1 h at room temperature. ECL developing solution was used to visualize protein bands. *β*-Actin was used as an internal control. ImageJ software was used to analyze the intensity of protein expression.

2.11 Determination of the un-target metabolite profiling of tea samples

In accordance with our previously described method, UPLC-Orbitrap-MS/MS was utilized to analyze the metabolite profile of tea samples[29].

2.12 Analysis of major chemical components of tea samples

The quantities of caffeine (CAF), EGCG, epicatechin gallate (ECG), catechin, theacrine, and theanine were measured via HPLC (Waters, Milford, MA, USA) using individual standards, as described previously[26]. Chromatographic separation was conducted using a Phenomenex Gemini C_{18} column (250 mm × 4.0 mm, 3 μm), with the column temperature maintained at 25 °C. The sample was injected at

a volume of 5 μL, eluted at a rate of 1 mL/min, and detected at a wavelength of 280 nm (Waters 2 489 UV/visible detector). The mobile phase consisted of two components: mobile phase A (deionized water with 0.17% acetic acid) and mobile phase B (100% acetonitrile). The linear gradient proceeded as follows: mobile phase B transitioned from 8% to 28.4% for 30 min, then from 28.4% to 100% for 8 min, and finally from 100% to 8% for 10 min.

2.13 Statistical analysis

The results are presented as mean ± standard error of the mean (SEM). GraphPad Prism 8 software, MetaboAnalyst 5.0 online tool, and RStudio were used for statistical analysis. Multiple groups were compared using One-way or Two-way ANOVA with Tukey's test when necessary. The differences were considered statistically significant at $P < 0.05$ and $P < 0.01$.

3. Results

3.1 Distinct effect of TVGTs on ameliorating obesity and dyslipidemia in diabetic mice

To explore the potential effects of short- and long-term interventions with TVGTs on obesity and dyslipidemia, HFD-induced obese mice were treated with TVGTs for 11 and 22 weeks. The results showed that HFD-induced obese mice exhibited significant increases in body weight (Fig. 1A) and fat tissue mass (Figs. 1B-D), and a decrease in lean body mass (Fig. 1E). However, treatment of the obese mice with TVGTs at 11 and 22 weeks significantly improved those obese phenotypes. The data showed that there was no significant difference in decreases of body weight or fat mass between YK and FD groups at 11 weeks of intervention (Figs. 1A-B). Interestingly, YK green tea exhibited more profound effect on reducing obesity complication than that of FD or KC green teas at 22-week of intervention.

We also measured serum TG, T-CHO, and LDL-C levels in mice, since hyperlipidemia is characterized as substantial lipid disorder in type 2 diabetes patients. The results showed that serum T-CHO, TG, and LDL-C levels of the HF group mice were significantly higher than those in the LF group at both 11 and 22 weeks (Figs. 1F-H). This indicates that hyperlipidemia developed in the HF group. However, FD, YK, or KC green tea-treated groups all showed significant improvements in serum TG and LDL-C levels at both 11- and 22-week intervention (Figs. 1G-H), and T-CHO was significantly improved at 22 weeks (Fig. 1F). Interestingly, we found that YK green tea showed best effect on improving serum levels of TG and LDL-C and FD had a better effect than KC green tea (Figs. 1G-H). In addition, long-term 22-week intervention was found to be more effective at reducing obese phenotypes and hyperlipidemia than short-term 11-week intervention with TVGTs.

Food intake is an important contributor to obesity complications. Therefore, the food consumption in each group of mice was monitored. The data indicate no statistical difference in food intake among each group of mice (Fig. S1A). Furthermore, the energy expenditure of mice at 17 weeks of intervention, measured by using Comprehensive Lab Animal Monitoring System (CLAMS), showed that the respiratory exchange ratio (RER) of mice in the LF group was between 0.85 and 1.00, indicating that the main energy-supplying substance was carbohydrates, and the RER of mice in the HFD and HFD plus tea groups was between 0.7 and 0.8, indicating that they were primarily utilizing fat as an energy source. The primary energy source between TVGTs treated groups did not differ significantly

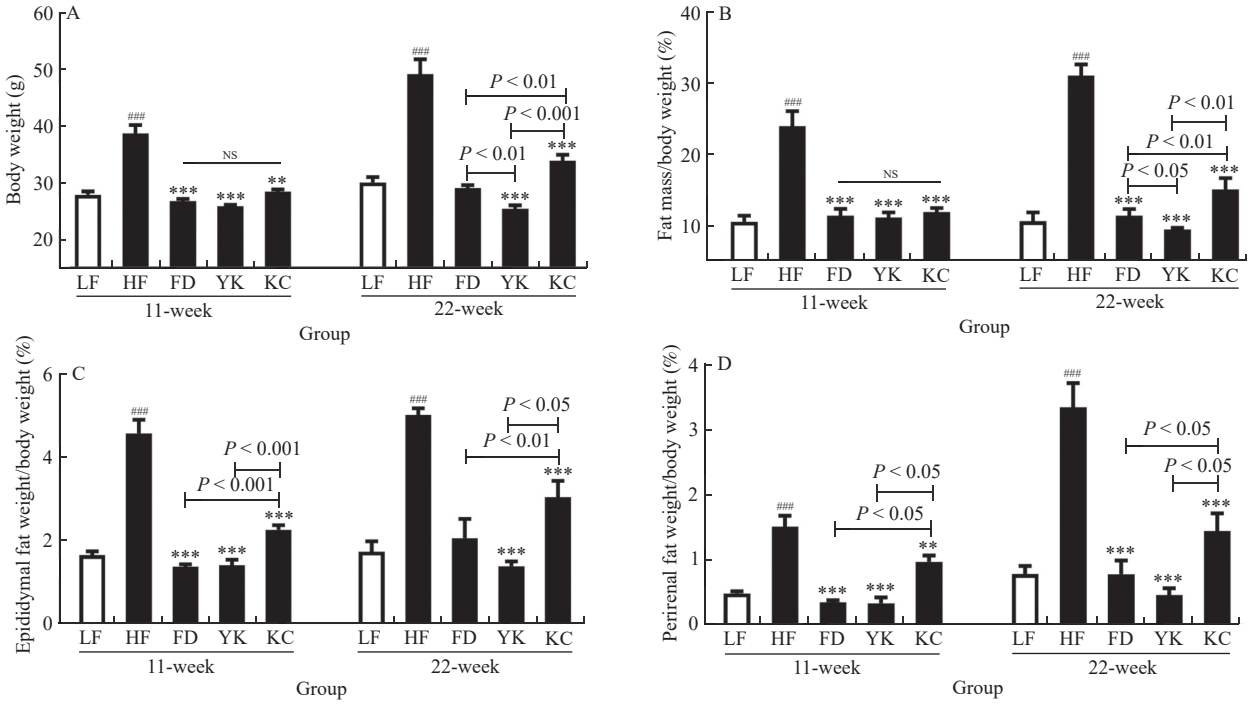

Fig. 1 Distinct effect of TVGTs ameliorated obese phenotypes and dyslipidemia of HFD-induced diabetic mice for 11- and 22-week treatment. A total of 6 mice from each group were sacrificed at 11-week and 22-week intervention, respectively. Body weight (A), fat mass rate (B), epididymal fat weight rate (C), perirenal fat weight rate (D), lean body mass rate (E), T-CHO (F), TG (G), LDL-C (H). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared to LF group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to HF group; NS, not significant; The same below. Values are means ± SEM, $n = 6$.

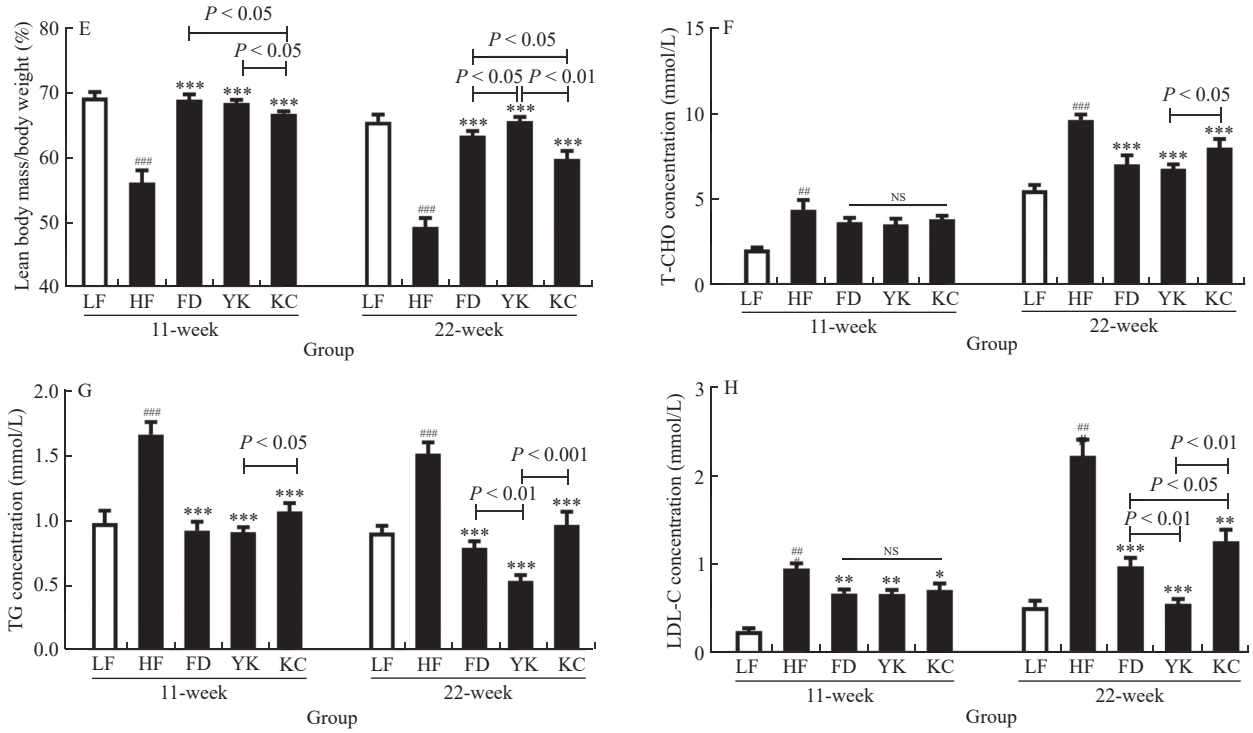

Fig. 1 (*Continued*)

(Fig. S1B). Generally, a heat map can also indicate energy consumption. The results indicate that heat consumption was significantly lower in the HF group than the LF group, and YK and FD green tea groups significantly increased the heat consumption of mice compared with HF group (Fig. S1C), which suggests that TVGTs intervention is pivotal in accelerating energy expenditure.

3.2 Distinct effect of TVGTs on improving dysglycemia in HFD-induced diabetic mice

As shown in Fig. 2A, blood glucose levels in the HF mice were significantly elevated compared LF mice group, indicating that the mice developed dysglycemia. Treatment with TVGTs for 11 and 22 weeks significantly decreased blood glucose levels compared to HF mice (Fig. 2A). At 22-week of intervention, YK green tea reduced blood glucose levels more effectively than FD and KC green teas (Fig. 2A). Both GTT and ITT area under curve (AUC) levels were significantly reduced after 10 and 20 weeks of intervention with TVGTs compared to HF mice (Figs. 2B-G). YK and FD green tea were more efficient at decreasing GTT levels than that of KC green tea at 10 weeks (Figs. 2B, F). YK green tea was more efficient at reducing GTT and ITT levels than that of FD and KC green tea during 20 weeks of intervention (Figs. 2C, E-G). These results suggest that long-term intervention with TVGTs improved dysglycemia more effectively, especially treatment with YK green tea.

3.3 Distinct effect of TVGTs on ameliorating vascular hypercontractility in diabetic mice

Diabetes patients are at increased risk of developing microvascular complications, such as diabetic retinopathy, diabetic nephropathy, and neuropathy, which can lead to blindness, renal failure, and decreased quality of life. We investigated the efficacy of TVGTs in improving vascular complications by analyzing MA hypercontractility. The

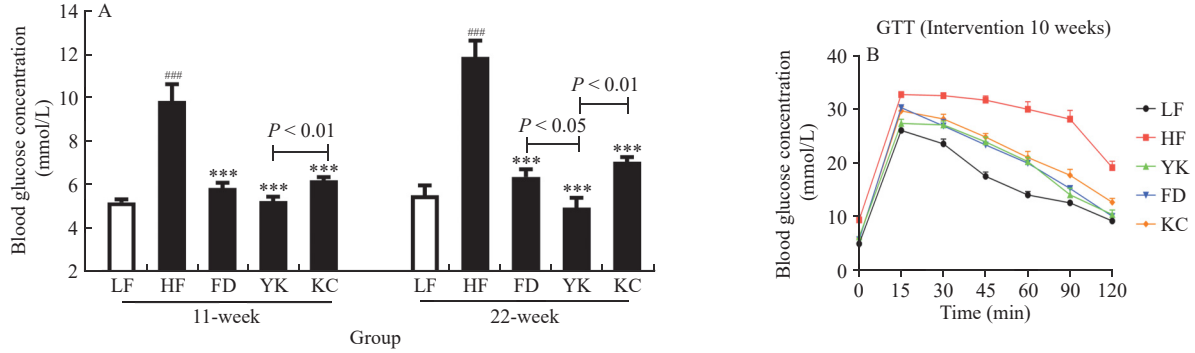

Fig. 2 Distinct effect of TVGTs on improving dysglycemia in HFD-induced diabetic mice. Blood glucose levels (A), 10-week (B) and 20-week (C) intervention GTT curve, 10-week (D) and 20-week (E) intervention ITT curve, AUC of GTT (F) and ITT (G). NS indicates no significant difference between FD, YK, or KC. Values are means ± SEM, $n = 6$.

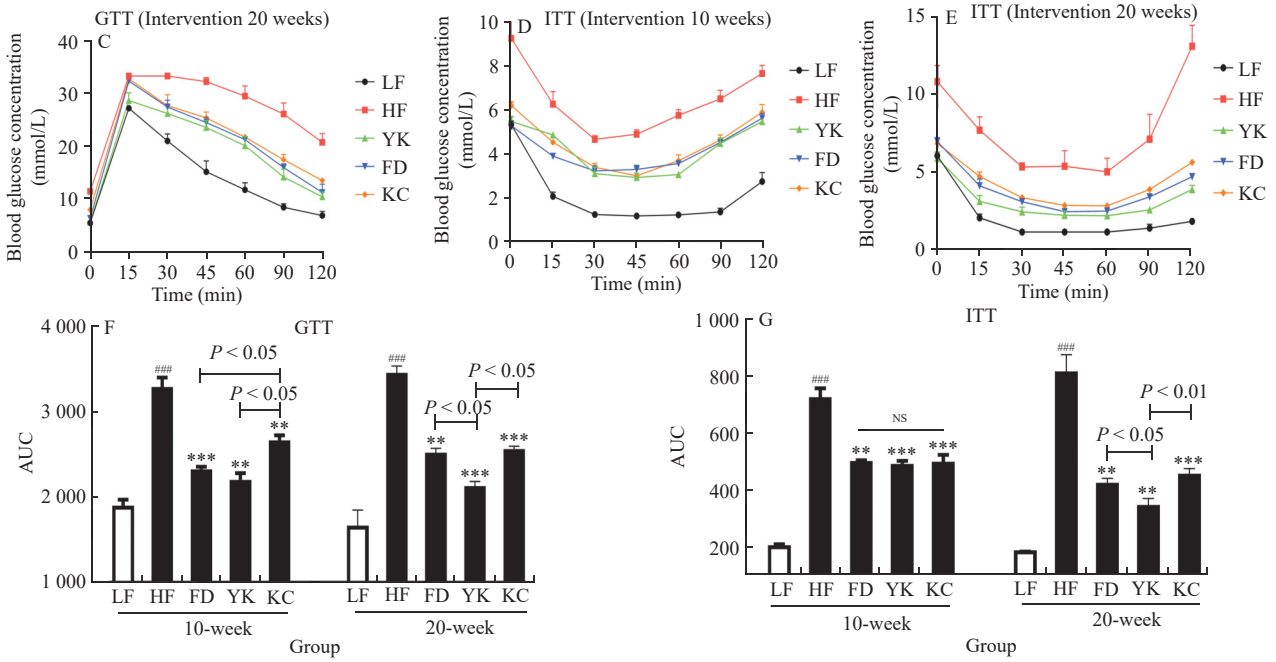

Fig. 2 (*Continued*)

results show that MA contraction was significantly higher in response to angiotensin II (100 nmol/L) and KCl (143 mmol/L) stimulation in the HF group than the LF group of mice (Figs. 3A-B). However, the MAs isolated from TVGT-treated mice had significantly decreased angiotensin II- and KCl-induced contractions compared to HF group. The contractions of MAs isolated from FD and YK groups demonstrated a better decreasing effect compared to KC group (Figs. 3A-B). In addition, stimulation of MA isolated from HF group mice by 5-hydroxytryptamine (5-HT) (10^{-7}–10^{-5} mol/L) caused stronger contraction than that of LF group of mice, and the contraction of MA isolated from FD and YK groups were showed more profound suppressed effects than that of KC group mice (Fig. 3C). Furthermore, U46619 is an agonist of thromboxane A2, and norepinephrine (NE) is an α-receptor agonist, both of them induce vascular contraction. The results show that contraction of MA from all three TVGTs treated mice response to NE and U46619 stimulation was significantly inhibited compared to HF group of mice, and the effects were more suppressed for FD- and YK-treated mice than KC mice (Figs. 3D-E). These results indicate that TVGTs could potentially contribute to improving vascular contractility in diabetic mice, and FD and YK green teas showed more efficiency than KC green tea.

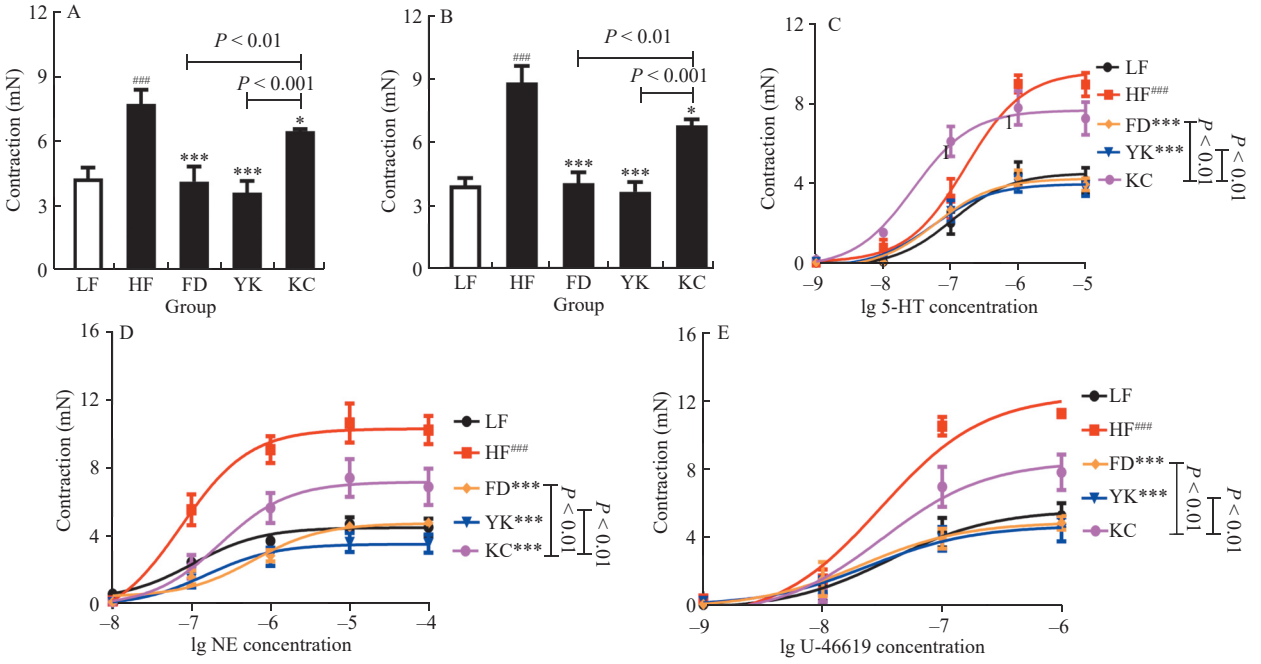

Fig. 3 Distinct effects of TVGTs on ameliorating MA vascular hypercontractility in diabetic mice. MA was isolated from each group of mice, and was stimulated by 100 nmol/L angiotensin II (A) and 143 mmol/L KCl (B), 5-HT (C), NE (D) and U46619 (E), respectively. Values are means ± SEM, n = 4–6.

3.4 YK green tea performs better protective effect on fatty liver formation in diabetic mice

Liver is the main organ responsible for maintaining glucose and lipid metabolism within the body. Fatty liver is primarily characterized by increased liver volume, fat deposition, and impaired liver function. The data show that liver weight was significantly higher in the HF group compared to the LF group. TVGTs intervention significantly decreased liver weight compared to the HF mice, and YK green tea showed better intervention effect on fatty liver than that of FD and KC green tea (Fig. 4A). In comparison to the LF group, there were significantly higher serum ALT and AST levels in the HF group (Figs. 4B-C). However, supplementation with all TVGTs obviously reduced serum ALT and AST levels, and YK green tea was particularly effective at reducing AST activity (Fig. 4C). Hepatic sections revealed normal polygonal cell structure with clear round nuclei in the liver tissues of LF group mice (Fig. 4D). The HF group mice exhibited severe hepatic steatosis, with diffuse fatty infiltration of intrahepatic vesicles, resulting in structural disruption of the hepatic lobules (Fig. 4E). However, TVGTs treatment significantly inhibited fatty liver formation (Figs. 4F-H), and YK was the most effective at preventing fatty liver (Fig. 4I).

3.5 Distinct effects of TVGTs on improving hepatic lipid profiles and lipid classes in diabetic mice

Our data showed that TVGTs intervention significantly improved the overall lipid panel. Therefore, a lipidomics approach was used to further analyze the effects of TVGTs on hepatic lipid profiles and specific lipid classes. Principal component analysis (PCA) was performed to investigate the differences between groups. Lipid profiling results showed significant differences between the HF group and the LF group. Interestingly, the hepatic lipid profiles of TVGTs treatment groups were close to that of the LF group (Fig. 5A), suggesting that TVGTs intervention improves hepatic lipid homeostasis in diabetic mice. In addition, orthogonal partial least squares-discrimination analysis (OPLS-DA) showed clear cluster separation between HF *vs.* FD (Fig. S2A), HF *vs.* YK (Fig. S2B), and HF *vs.* KC (Fig. S2C). A total of 1 002 lipid metabolites were identified in hepatic samples between the LF, HF, YK, FD, and KC groups of mice, including 256 TGs, 151 phosphatidylcholines (PCs), 111 phosphatidylethanolamines (PEs), 67 cardiolipins (CLs), 55 sphingomyelins (SMs), 53 phosphatidylserines (PSs), 51 diglycerides (DGs), and others (Fig. 5B). Specifically, the total contents of simple

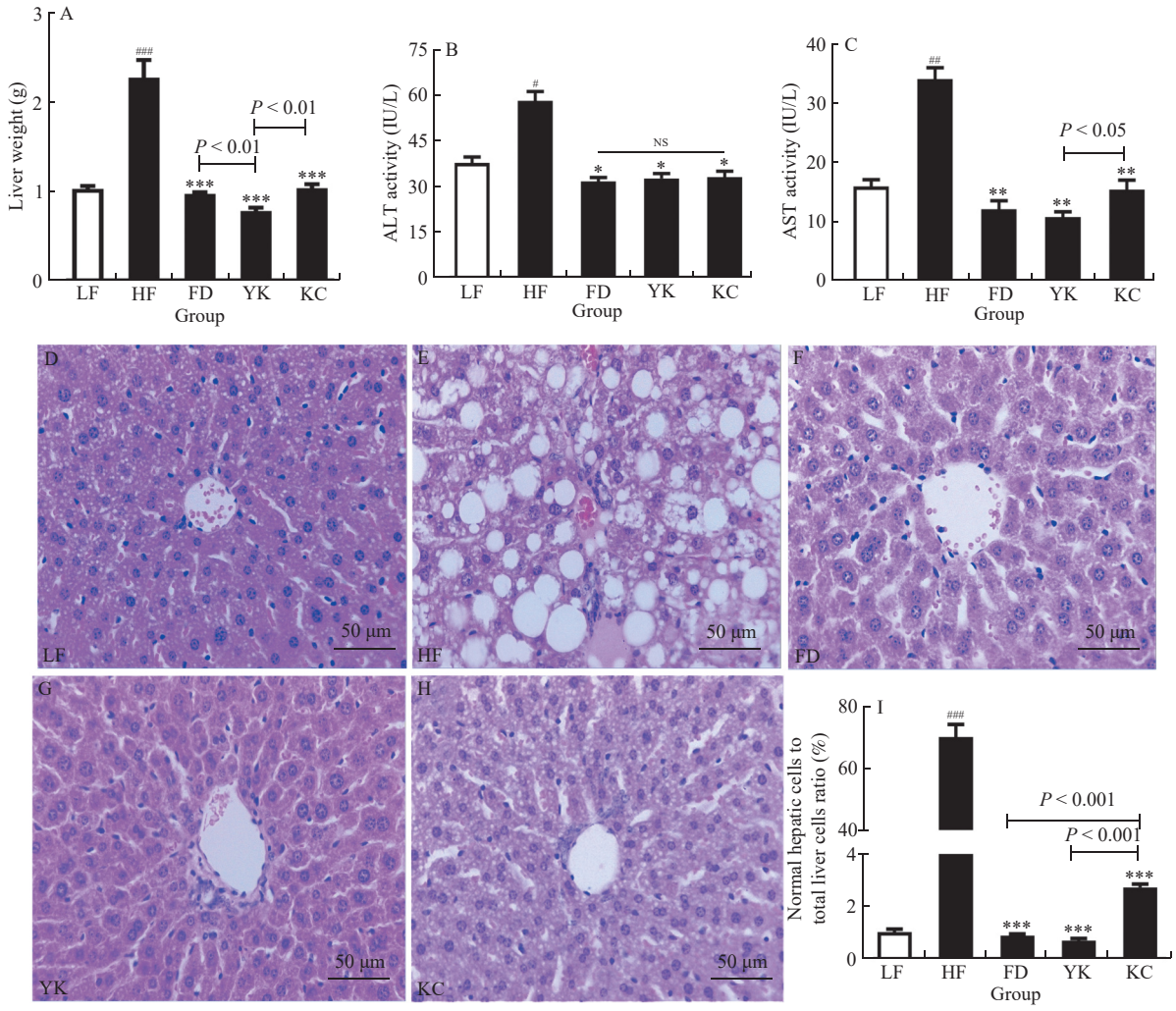

Fig. 4 YK green tea performs better protective effect on ameliorating fatty liver formation in diabetic mice after 22 weeks of treatment. Liver weight (A), serum ALT activity (B), serum AST activity (C), H&E staining image of the liver (D-H), normal hepatic cells to total liver cells ratio (I) (5 fields were randomly selected from each tissue section for statisticanalysis). Values are means ± SEM, n = 4–6.

Glc series (CerG2s), SMs, phosphatidylglycerols (PGs), PCs, and PEs were significantly decreased, while the total contents of phosphatidic acids (PAs), phosphatidylmethanols (PMes), CLs, lysophosphatidylinositols (LPIs), phosphatidylinositols (PIs), PSs, DGs, and TGs were significantly enhanced in the HF group compared to the LF group, indicating severe liver lipid disorder in diabetic mice (Fig. 5C). Interestingly, supplementing the mice with TVGTs significantly improved liver lipid homeostasis by restoring the balance of glycerophospholipids (GPs) and sphingolipids and decreasing glycerolipid (GL) levels (Fig. 5C).

The criteria variable importance in projection (VIP) > 1, fold change (FC) > 2, and $P < 0.05$ were used to screen out candidates for differential hepatic lipid biomarkers between each group of mice. A total of 272 significantly differential lipid species (DLSs) were identified between HF and LF groups, of which 172 lipid species were downregulated and 100 lipid species were upregulated (Fig. S3A). However, compared with HF group, DLSs in the TVGTs-treated groups exhibited significant changes (FD *vs*. HF: 257 down, 64 up; YK *vs*. HF: 303 down, 48 up; KC *vs*. HF: 252 down, 28 up) (Figs. S3B-D). Thus, analyzing co-regulated DLSs between groups could provide useful information related to diabetic dyslipidemia.

A total of 100 DLSs were significantly upregulated in the HF group compared to the LF group, whereas 65 DLSs were obviously co-downregulated by all TVGTs treatments compared to the HF group (Fig. S3E). Similarly, 172 DLSs were downregulated in HF group compared to LF group, while 12 DLSs were co-upregulated by all TVGTs treatments (Fig. S3F). Further analysis revealed that the 65 DLSs co-downregulated by TVGTs intervention included 23 GPs (7 CLs, 5 PIs, 3 PSs, 2 LPIs, 5 PEts and phosphatidylcarbinols (PMes), and 1 PA and PE) (Fig. 6A) and 42 GLs (12 DGs and 30 TGs) (Figs. 6C, D), and 12 co-upregulated DLSs by TVGTs intervention

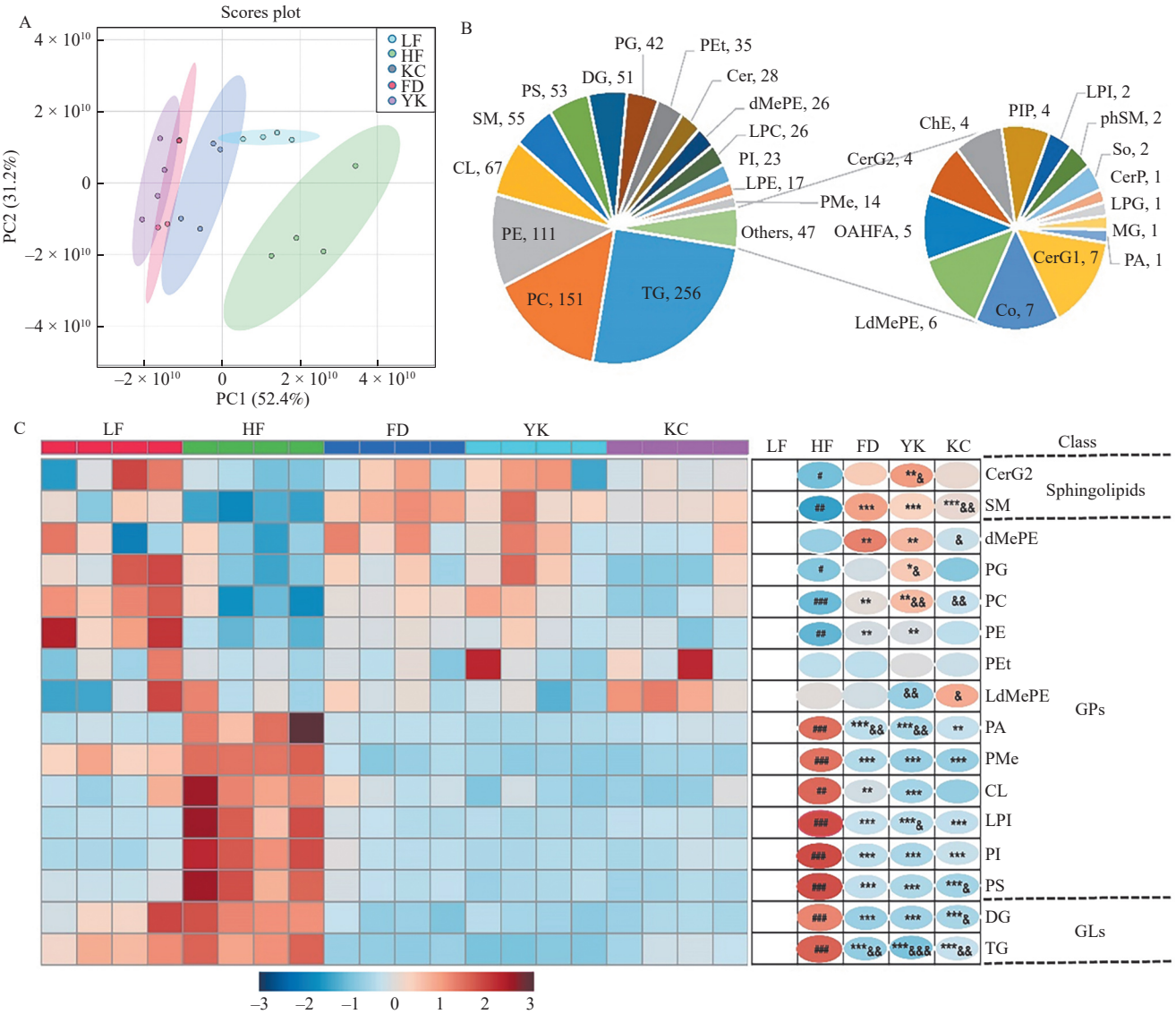

Fig. 5 Distinct effects of TVGTs on improving hepatic lipid profiles in diabetic mice after 22 weeks of intervention. Cer: ceramides; dMePE: dimethylphosphatidylethanolamine; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; ChE: cholesterol ester; PIP: phosphatidylinositol; phSM: sphingomyelin (phytosphingosine); So: sphingosine; CerP: ceramides phosphate; LPG: lysophosphatidylglycerol; MG: monoglyceride; PA: phosphatidic acid; CerG1: simple Glc series; Co: coenzyme; LdMePE: lysodimethylphosphatidylethanolamine; OAHFA: (*O*-acyl)-1-hydroxy fatty acid. PCA score plots (A), distribution of lipid classes (B), the heatmap of various lipid levels (C). The red and blue boxes represent values that were above and below the mean value, respectively. $^{\#}P < 0.05$ when compared to LF group. The significance of FD *vs*. YK, FD *vs*. KC, YK *vs*. KC is indicated on FD, KC and YK as $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$, respectively. The same below. Values are means ± SEM, $n = 4$.

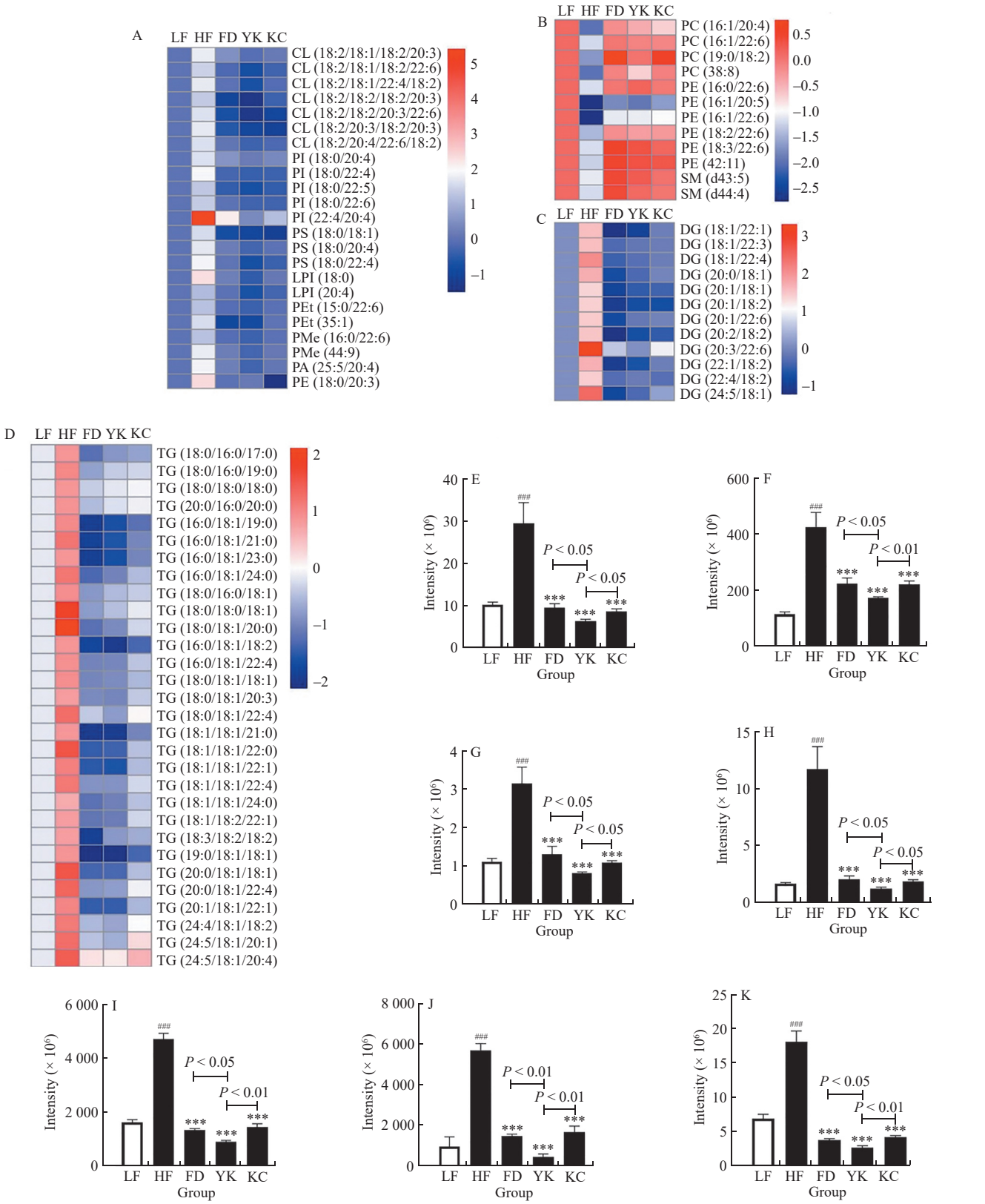

Fig. 6 Distinct effect of TVGTs restored liver GPs and sphingolipids homeostasis and decreased the hepatic specific GLs. TVGTs decreased 23 GP species (A), increased 12 GP and sphingolipid species (B), and decreased the specific 12 DGs (C) and 30 TGs (D) species. Seven specific lipid species in the liver: CL (18:2/18:1/18:2/22:6) (E), PI (18:0/20:4) (F), LPI (20:4) (G), LPI (18:0) (H), TG (18:0/16:0/18:1) (I), TG (18:3/18:2/18:2) (J), and TG (18:0/16:0/17:0) (K). Values are means ± SEM, n = 5−6.

included 10 GPs (4 PC and 6 PE lipids) and 2 SM lipids (Fig. 6B). The heatmap results indicate that the TVGTs were able to maintain hepatic lipid homeostasis by re-establishing the balance of GPs and SPs. DGs and TGs are the primary components of fat deposition and risk factors for hepatic steatosis. Our data show that TVGTs treatment significantly reduced glyceride deposition (Figs. 6C-D). Further analysis revealed that TVGTs significantly reduced the levels of 4 TGs: TG (16:0/18:1/19:0), TG (16:0/18:1/21:0), TG (16:0/18:1/23:0), and TG (16:0/18:1/24:0) (Fig. 6D). Interestingly, YK green tea showed a better decreasing effect than FD and KC green teas on seven lipid species: CL (18:2/18:1/18:2/22:6), PI (18:0/20:4), LPI (20:4), LPI (18:0), TG (18:0/16:0/18:1), TG (18:3/18:2/18:2), and TG (18:0/16:0/17:0) (Figs. 6E-K).

3.6 Distinct effects of TVGTs on suppressing hepatic lipid synthesis efficiency in diabetic mice

Next, in order to determine the underlying mechanism of TVGTs for improving diabetic dyslipidemia, the key genes for hepatic lipid synthesis in each group of mice were determined by using real-time PCR. The data show that the expression levels of key hepatic adipogenic genes (*Arv1*, *Srebp-1c*, *Acaca*, *Fasn*, and *Ppar-γ*) were significantly decreased by TVGTs intervention compared to the HF group, and YK group had better improvement than FD and KC groups (Figs. 7A-B, E-G). The expression levels of key hepatic lipolysis genes (*Fxr* and *Shp*) were significantly upregulated by YK and FD compared to the HF group. Similarly, YK group showed better effects than FD and KC groups (Fig. 7C). ARV1 homolog, fatty acid homeostasis modulator (ARV1) regulates lipid transport in the endoplasmic reticulum and plays a critical role in maintaining lipid homeostasis. SREBP-1c is a transcription factor that regulates the synthesis of TGs and fatty acids by regulating the expression of downstream genes *Acaca* and *Fasn*. Based on Western blotting results, TVGTs, especially YK green tea, dramatically inhibited ARV1 and SREBP-1c protein expression in the liver compared to the HF group (Figs. 7H-K). The expression of FXR and SHP protein is closely associated with the inhibition of de novo lipogenesis. The result show that TVGTs significantly increased the expression of FXR and SHP protein compared to the HF group (Figs. 7L-O). These sets of data suggest that TVGTs improve dyslipidemia by regulating the synthesis and lipolysis of hepatic lipids.

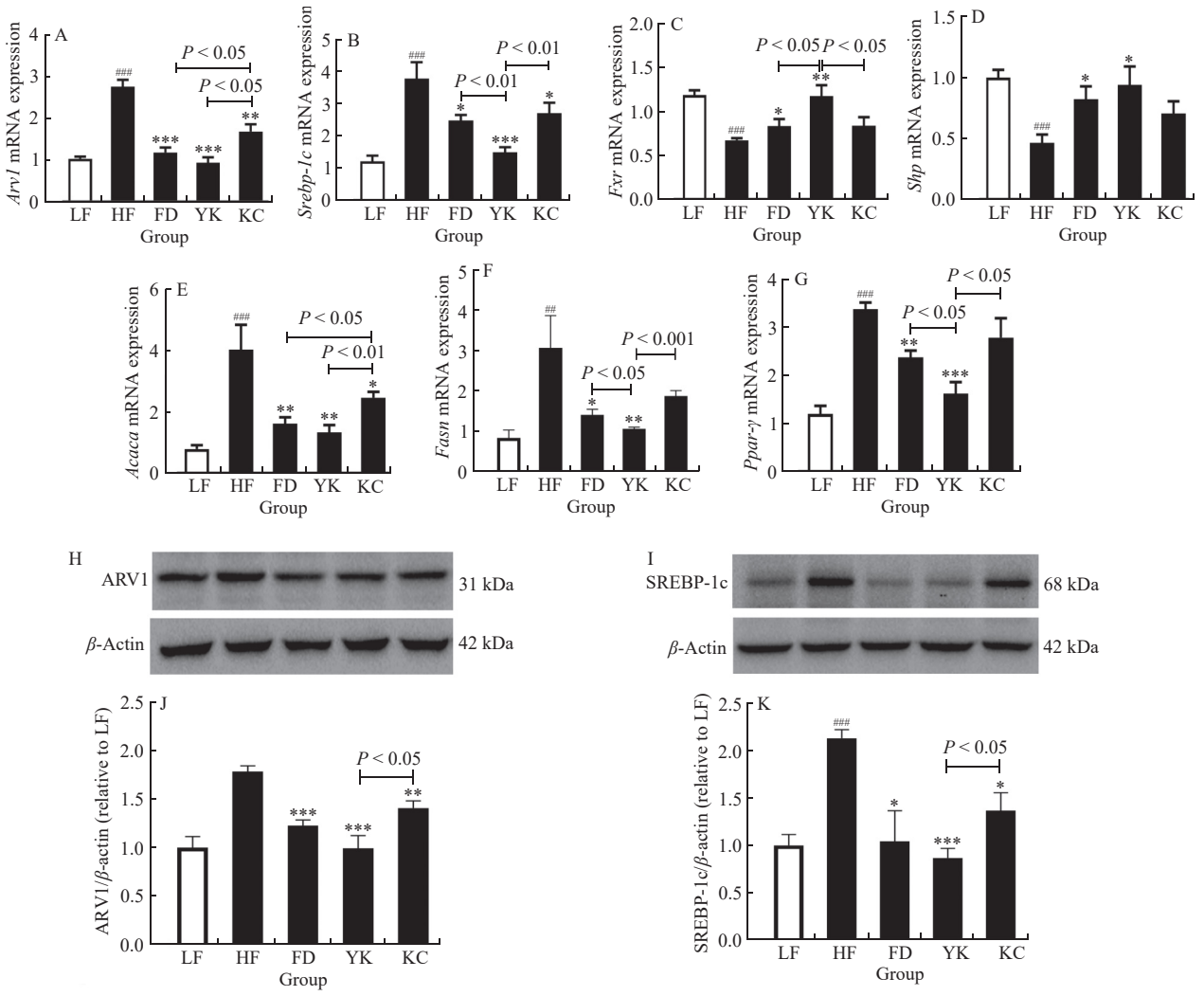

Fig. 7 Distinct effects of TVGTs on suppressing hepatic lipid synthesis efficiency in diabetic mice. The mRNA expression of *Arv1* (A), *Srebp-1c* (B), *Fxr* (C), *Shp* (D), *Acaca* (E), *Fasn* (F) and *Ppar-γ* (G) were presented. Values are means ± SEM. $n = 5-6$. Representative image and summary data for ARV1 (H, J), SREBP-1c (I, K), FXR (L, N), and SHP (M, O) protein expression. Values are means ± SEM, $n = 3-4$.

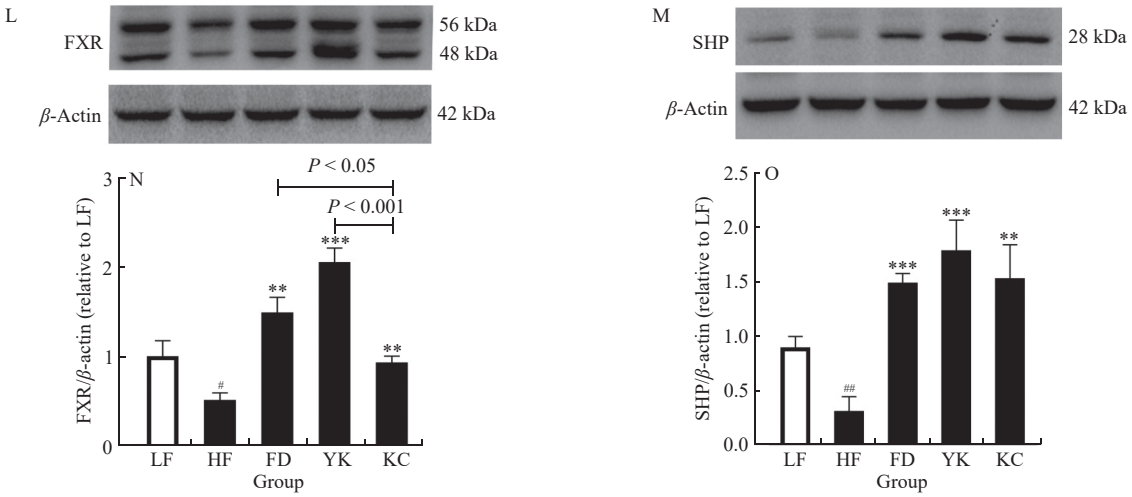

Fig. 7 (*Continued*)

The lipidomics pathway analysis revealed that DG may play a crucial intermediate role in regulating hepatic lipid metabolism in diabetic mice (Fig. S4). Supplementation with TVGTs reduces liver GL synthesis by modulating the hepatic ARV1-FXR/SHP-SREBP1c-Fasn/Acaca pathway, enhances the metabolic efficiency of DG, and consequently restores the balance of GPs and sphingolipids in HFD-induced diabetic mice.

3.7 Distinct metabolic profiles and differential metabolites of TVGTs

In order to identify the potential chemical compounds in TVGTs that respond to modulate lipid homeostasis in mice with HFD-induced dyslipidemia, TVGT extracts were analyzed using UPLC-Orbitrap-MS/MS based metabolomics, and data were acquired in both positive and negative ion mode. The typical total ion chromatography of TVGTs are shown in Figs. S5A, B. After peak extraction and matching, a data matrix containing 3 089 characteristic ions (1 982 positive, 1 107 negative) was obtained. Unsupervised PCA showed a clear separation between the FD, YK, and KC samples, indicating that their chemical profiles differed significantly (Fig. S5C). In addition, the QC samples clustered together, indicating that the metabolomics analysis was stable and reliable (Fig. S5C). Furthermore, the supervised PLS-DA results show that each TVGT sample grouped into a separate cluster; YK samples were grouped in quadrants I and II, while FD and KC samples were grouped in quadrants IV and III, respectively (Fig. S5D). Additionally, R^2_X, R^2_Y, and Q^2 were calculated to be 0,978, 0.987, and 0.98, indicating that the models were successfully established (Fig. S5D). Permutation tests showed that the PLS-DA validation model ($R^2 = 0.145 < 1$, $Q^2 = -0.457 < 0$) did not overfit, indicating that these data had high reliability (Fig. S5E).

The criteria VIP > 1, $P < 0.05$, false discovery rate (FDR) < 0.05, and FC > 1.5 were set to select potential differential metabolites. Based on the comparison of retention time (RT), *m/z*, secondary mass spectrometry fragments, and various metabolome databases, and references, a total of 65 differential metabolites were identified and divided into 6 categories: 12 catechins, 20 flavonoids and flavonoid glycosides, 4 alkaloids, 14 phenolic acids, 8 amino acids, and other substances (Fig. 8). The heatmap shows that YK green tea possesses the higher content of catechins and phenolic acids than that of FD and KC green tea, and FD green tea has higher content of flavonoids, flavone glycosides, and amino acids than that of YK and KC green tea (Fig. 8). In order to quantify the accurate concentrations of characteristic compounds, HPLC was used to determine the polyphenol, CAF, theacrine, and theanine content of the teas. The results show that YK clearly has the highest concentrations and FD has high concentrations of EGCG, ECG, and CAF among TVGTs (Table 2). The theanine content was significantly higher in FD and YK samples than that in the KC samples (Table 2). However, theacrine, a characteristic component of KC, was only detected in KC samples. Overall, the TVGTs exhibited distinct chemical profiles and characteristic compounds.

3.8 Correlation analysis between the active components of TVGTs and dyslipidemia-related indicators

In order to determine the relationship between the main active components and of TVGTs and improved dyslipidemia, a Pearson correlation analysis was conducted. The data revealed that CAF, quinic acid, *L*-theanine, gallic acid, EGCG, (−)-epigallocatechin (EGC), theogallin, and (−)-epiafzelechin-3-gallate were significantly negatively correlated with hyperlipidemia (Fig. 9). CAF, *L*-theanine, and EGCG were all negatively correlated with body weight, fat mass, liver weight, serum TG, TC and LDL-C, and liver TG, suggesting that these chemical components may play significant roles in improving dyslipidemia (Fig. 9). In addition, our results indicate that CAF, quinic acid, *L*-theanine, EGCG, EGC, and (−)-epiafzelechin-3-gallate were significantly negatively correlated with blood glucose, GTT AUC, and ITT AUC, suggesting that these chemicals play significant roles in regulating dysglycemia (Fig. 9). Furthermore, the data indicate that CAF, *L*-theanine, gallic acid, EGCG, and pyruvic acid significantly ameliorated MA hypercontractility in response to 100 nmol/L angiotensin II and 143 mmol/L KCl (Fig. 9). Correlation analysis showed that CAF, quinic acid, *L*-theanine, gallic acid, EGCG, and ECG were all negatively correlated with ARV1 protein expression in the liver, and positively correlated with FXR protein expression, suggesting that these compounds may contribute to

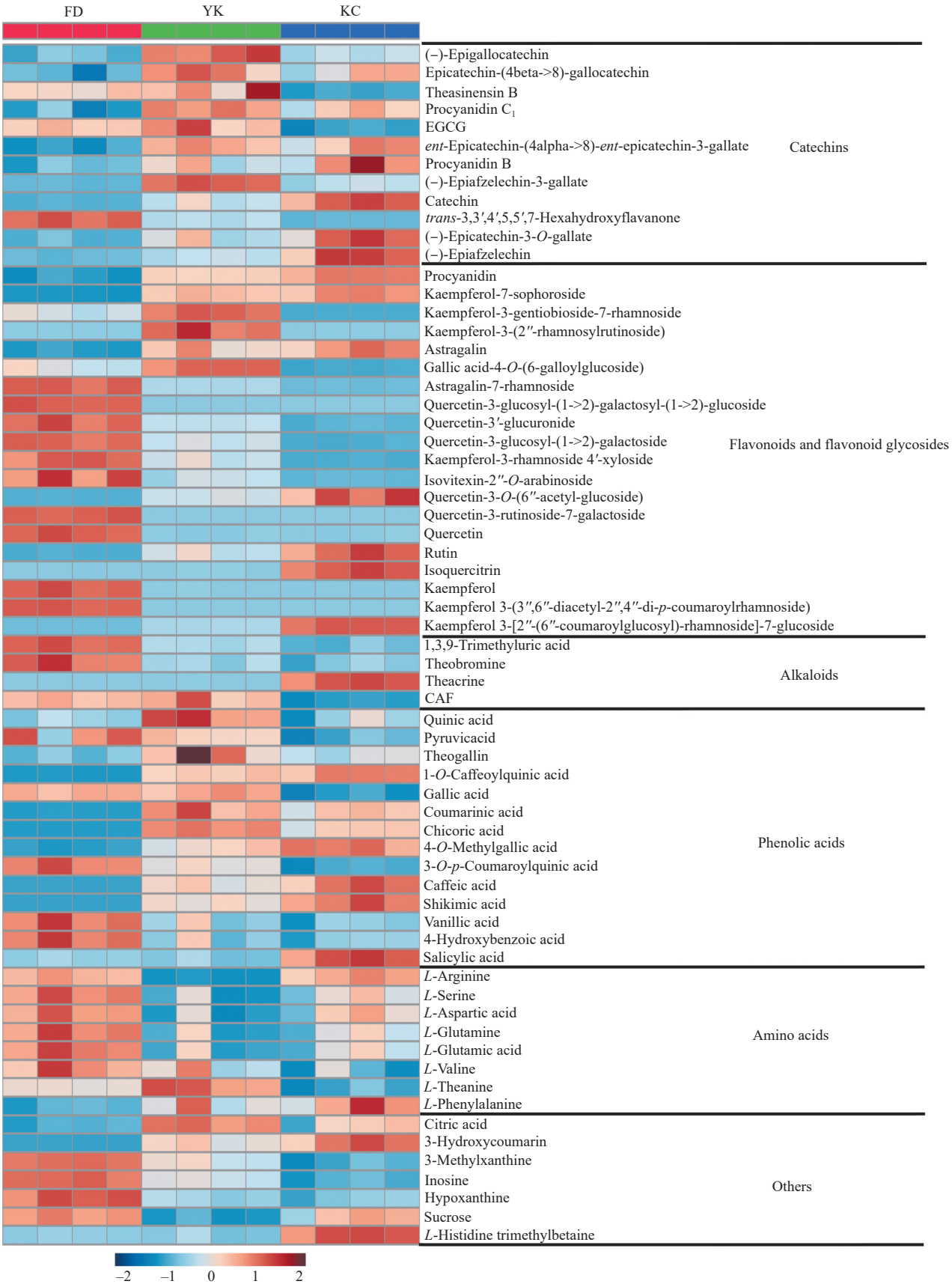

Fig. 8 Heatmap of differentiated metabolites were screened from TVGTs based on UPLC-Orbitrap-MS/MS data. The red and blue boxes represent values that were above and below the mean value, respectively.

Table 2
Analysis of the main characteristic components in YK, FD and KC (%, dry weight).

Components	FD	YK	KC
EGCG	10.02 ± 0.46*	12.43 ± 0.28**$^{\#}$	4.83 ± 0.22
ECG	1.82 ± 0.15	3.11 ± 0.18*$^{\#}$	1.76 ± 0.17
Catechin	0.08 ± 0.01***	0.23 ± 0.05***$^{\#\#\#}$	0.53 ± 0.04
CAF	3.23 ± 0.18*	3.59 ± 0.03**	1.13 ± 0.06
Theacrine	-	-	1.46 ± 0.28
Theanine	1.086 8 ± 0.052 7***	1.057 9 ± 0.025 1***	0.822 9 ± 0.024 7

Note: "-" indicates not detectable. Compared to KC group, *P < 0.05, **P < 0.01, ***P < 0.001; compared to FD group, $^{\#}P$ < 0.05, $^{\#\#\#}P$ < 0.001. Values are means ± SEM, n = 6.

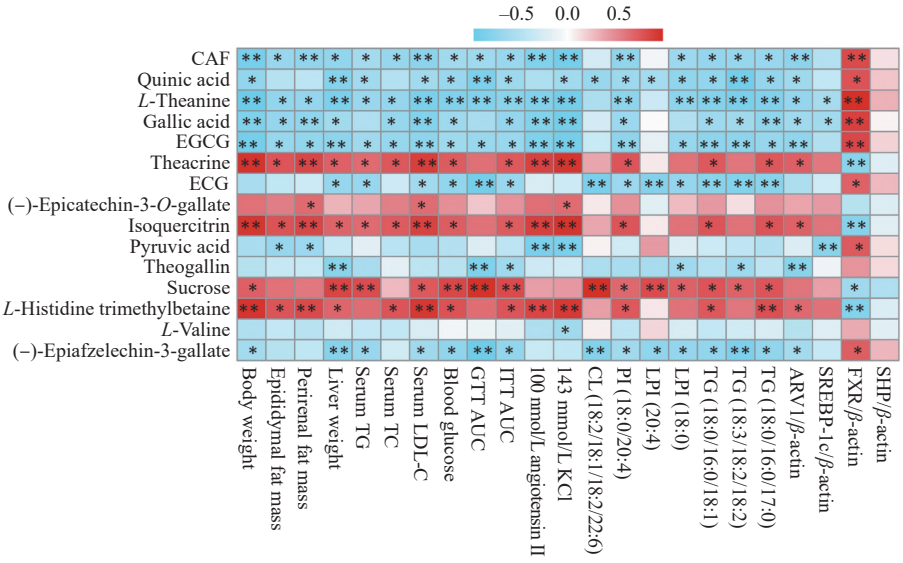

Fig. 9 Correlation analysis between the active components of TVGTs and dyslipidemia-related indicators. The significant correlations are marked as *P < 0.05, **P < 0.01. Values are the mean ± SEM, n = 4.

reduce liver lipid transport and synthesis (Fig. 9). Therefore, CAF, quinic acid, *L*-theanine, gallic acid, and polyphenols may be the primary active ingredients responsible for improving dyslipidemia in diabetic mice.

4. Discussion

Dyslipidemia is strongly correlated with obesity-related type 2 diabetes[5,8,30]. In obese and diabetic individuals, dyslipidemia is associated with an increased risk of hepatic steatosis and vascular complications[5-6,8-9]. Recent papers reported that tea supplementation improves insulin sensitivity and controls hyperglycemia and hyperlipidemia[15,26-27,31]. Previously, most studies on the health benefits of tea focused on the different types and grades of tea[25,32-33]. However, there is no information available on the comparative effectiveness of three tea varieties grown in the same place and made by the same manufacturing process on improving diabetic dyslipidemia. The main finding of this study is that all TVGTs improved lipid metabolism disorders in HFD-induced diabetic mice, and YK green tea was the most effective in long-term treatment. Long-term intervention with TVGTs showed a better protective effect than short-term intervention.

Obesity-induced type 2 diabetes is characterized by hyperlipidemia and insulin resistance. In previous studies, different types of tea were comparatively investigated for their health effects on diabetic dyslipidemia[25,32]. Liu et al.[32] compared the effects of 6 types of tea, including green, yellow, white, black, raw Pu-erh, and oolong, processed from the same tea cultivar (*C. sinensis* var. *assamica* cv. Yinghong 9) on reducing fat accumulation in mice with diabetes induced by HFD. The results showed that white tea, green tea, and raw Pu-erh tea prevented obesity by inhibiting the synthesis of fats, and white, oolong, and yellow tea exerted their effects by increasing energy expenditure and fatty acid oxidation. Polyphenol extracts from green, black, and oolong tea were found to be effective in promoting weight loss and angiogenesis[25]. Our previous study found that supplementing with YK green tea was effective in reversing preexisting obesity and hyperlipidemia and preventing fatty liver formation caused by HFD[15]. Previous papers reported the chemical components of FD white tea infusions[34-35]. Currently, there is no report available on improving obesity and hyperlipidemia with FD tea. Kucha tea is reported to have a hypoglycemic effect, and its primary compound, theophylline, is hypolipidemic[36-37]. Our data clearly demonstrate that all TVGTs prevented obese complication and improved lipid disorders in HFD-induced diabetic mice. Interestingly, YK green tea was more effective than FD and KC green teas. Long term intervention with TVGTs showed a better protective effect than short term intervention.

Diabetes is strongly associated with vascular complications such as cardiovascular disease (CVD), diabetic retinopathy, diabetic nephropathy, and neuropathy, which are the main causes of morbidity and mortality in patients with diabetes[38-39]. Our group previously found that dietary supplementation with Huangshan Maofeng green tea ameliorated MA

hypercontractility in old mice induced by desoxycorticosterone acetate and salt[27]. However, there is no report on a comparative investigation of TVGTs improving the vascular complications associated with diabetes in mice. Thus, our results not only confirm that TVGTs effectively mitigate the MA contractility response to stimulation by various agonists in diabetic mice, but also identify that YK green tea demonstrated the best effect on alleviating MA over-contractility.

Lipidomics is an advanced approach that is used for comprehensive investigation of lipid profiles, lipid classes, and species changes. Previous studies reported that liver lipidomes can be associated with hyperlipidemia, hepatic steatosis, and vascular complications[40-44]. Hence, an investigation of hepatic lipidomes can provide direct evidence explaining the effect of intervention with TVGTs on ameliorating dyslipidemia in diabetic mice. Previous hepatic lipidomics analysis showed that HFD-induced obese mice presented lipid metabolic disorders involving unbalanced changes in the CL, PI, PS, LPI, PC, PE, SM, DG, and TG profiles. GPs are the main components of cellular membranes that regulate fluidity, dynamics, and homeostasis[45-46]. GP homeostasis contributes to the biogenesis of mitochondria and the metabolism of lipids and fatty acids[47-48]. Among the GPs, CLs are characteristic phospholipids of the inner mitochondrial membrane and are central to the control of mitochondrial protein homeostasis[49-51]. In this study, TVGTs treatment all restored the homeostasis of hepatic GPs (CL, PI, PC, PE, PS, LPI, PEt, and PMe). Particularly, TVGTs significantly decreased CL levels, which were significantly increased in the HF group. Interestingly, in the current study we found that YK green tea had a greater decreasing effect on CL levels, especially CL (18:2/18:1/18:2/22:6), compared to FD and KC green tea. Furthermore, YK green tea was more effective in restoring phospholipid homeostasis, especially PI (18:0/20:4), LPI (20:4), and LPI (18:0), compared to FD and KC. A greater increase in liver phospholipid content by YK green tea intervention may cause increased mitochondrial content and more energy expenditure, which are correlated with better effectiveness in attenuating obesity complications. It should be pointed out that this is the first report showing that tea treatment can regulate liver CL homeostasis disrupted by HFD feeding.

In previous studies, elevated SM lipid levels were found in adipose tissue from obese and diabetic patients, which resulted in many complications[52-54]. However, Alexaki et al.[55] found that *de novo* SM biosynthesis is required for normal metabolic function and adipocyte cell viability. In this study, SM content decreased in the HF group compared with the LF group as a result of liver lipid metabolic dysfunction. Nevertheless, TVGTs treatment increased SM levels, especially SM (d43:5) and SM (d44:4) content, which may contribute to alleviate lipid metabolic dysfunction. Generally, as hepatic TG levels increase, apolipoprotein levels rise as well, resulting in an increase in LDL production[56-57]. In the liver, fatty acids produced by *de novo* lipogenesis are one of the major sources of fasting triacylglycerols[56]. In mammals, *Arv1* encodes an apolipoprotein that is commonly found in the endoplasmic reticulum and is involved in lipid homeostasis[58-60]. Gallo-Ebert et al.[60] reported that *Arv1* knock-out mice fed HFD were resistant to obesity, had lower blood T-CHO and TG levels, and retained insulin sensitivity and glucose tolerance. Studies have shown that *Saccharomyces cerevisiae* cells lacking ARV1 have defects in phospholipid and sphingolipid synthesis, suggesting that ARV1 is essential for biosynthesis of GP and SP[61-63]. The present study shows that HFD feeding increased the hepatic mRNA and protein levels of ARV1. However, TVGTs treatment, particularly YK treatment, effectively reduced the liver mRNA and protein levels of ARV1. The results suggest that TVGTs reduce the mRNA and protein levels of ARV1 in the hepatic tissue, which may contribute to improved GP and SP homeostasis.

A significant elevation in TG species is indicative of obesity and hyperlipidemia[64-65]. Accumulating clinical evidence indicates that the serum TG level is a better predictor of CVD than LDL-C, surpassing hemoglobin A1c in terms of predictive power[56,66-68]. TGs constitute the majority of lipid species and are the main substrates for lipid oxidation[69-70], and hepatic TG species were significantly regulated by tea treatment. Tea and its compounds have been reported to alter TG species in the liver. Huang et al.[31] demonstrated that HFD reduced more unsaturated fatty acids of TG species, and EGCG partially prevented this decline. It has been observed that long-lived worms had higher mono-unsaturated fatty acid (MUFA)/poly-unsaturated fatty acid (PUFA) ratio, and their offspring had the same results, suggesting that higher PUFA levels may be detrimental[71-72]. In this study, the content of PUFAs ($C_{18:2}$, $C_{18:3}$, $C_{22:4}$) was 2.2−2.7 fold higher in the HF group than the LF group. However, each of TVGTs reduced the excessive accumulation of PUFAs in the liver. It is possible that appropriate PUFA accumulation in the liver may improve liver function, whereas excessive PUFA accumulation may result in liver damage and complications. A genetic correlation analysis of plasma lipids showed that triacylglycerol containing palmitate ($C_{16:0}$) was associated with the risk of type 2 diabetes and obesity[73]. In this study, TVGTs were found to reduce four TGs containing palmitate, suggesting that one possible mechanism for their protective effects on dyslipidemia and obesity may be through balancing triacylglycerols containing palmitate. Further TG species analysis showed that TVGTs, particular YK green tea, significantly decreased HFD-induced TG accumulation in the liver, including potential biomarkers of TG (18:0/16:0/18:1), TG (18:3/18:2/18:2), and TG (18:0/16:0/17:0).

Arv1, *Srebp-1c*, *Acaca*, and *Fasn* are the major regulators of lipogenesis genes, while *Fxr* and *Shp* are important regulators of lipolysis genes[74-75]. In the liver, *Srebp-1c* regulates the synthesis of TGs and fatty acids by mediating the expression of downstream genes *Acaca* and *Fasn*[76-77]. Studies have shown that activating *Fxr* leads to several clinically relevant changes, including increased insulin sensitivity and decreased plasma and hepatic TG levels by suppressing *de novo* liver lipogenesis through the FXR-SHP-SREBP-1c pathway[78-79]. As shown in this study, consumption of TVGTs, especially YK green tea, suppressed the protein expression levels of ARV1 and SREBP-1c, and elevated the protein expression levels of FXR and SHP, consequently inhibiting hepatic TG synthesis in mice. Furthermore, TVGTs treatment significantly decreased *Ppar-γ* mRNA expression. In adipose tissue, *Ppar-γ* stimulates adipocyte differentiation, whereas in liver tissue, it increases insulin sensitivity and TG synthesis[80-82]. Therefore, the amelioration of dyslipidemia in HFD-induced diabetic mice by TVGTs may occur through multiple pathways.

Many studies have demonstrated that green tea extracts, which contain high levels of polyphenols, showed effective for weight loss in rodent models[18,25,31-32,70]. Tea polyphenols, particularly EGCG, have been demonstrated to prevent excessive body weight gain in HFD-fed mice[15,31,83]. In addition, CAF, catechins, and gallic acid in green tea have all been shown to improve hyperlipidemia and obesity[84-88]. The research of Zhou et al.[24] indicated that CAF and EGCG have synergistic anti-obesity effects, which may be due to the

synergistic interactions among the active ingredients in tea. Quinic acid has been proven to improve obesity. *D*-(−)-quinic acid showed significant hypolipidemic activity by preventing increases in serum cholesterol, TG, LDL, and very low-density lipoprotein (VLDL) levels induced by HFD. *L*-Theanine, a unique free amino acid, which provides the unique flavor of tea, is also a bioactive compound with numerous benefits, including metabolic regulation, cardiovascular protection, and anti-obesity and hypoglycemic properties[89]. *L*-Theanine treatment enhanced AMP-activated protein kinase (AMPK) α phosphorylation in inguinal white adipose tissue and adipocytes of mice, improved insulin sensitivity, and reduced plasma TG, TC, and free fatty acids in HFD-induced obese mice[90]. Furthermore, The research results of Liang et al.[91] suggested that *L*-theanine is beneficial for improving HFD-induced non-alcoholic fatty liver in mice by modulating the hepatic lipid CaMKKβ-AMPK signaling pathway.

In conclusion, many chemical components found in TVGTs, including CAF, gallic acid, EGCG, EGC, quinic acid, and *L*-theanine, have been shown to have lipid-reducing, weight-loss, and anti-hyperglycemic effects. Our data show that YK green tea possessed the higher levels of CAF, quinic acid, gallic acid, EGCG, EGC, ECG, and theogallin than that of FD and KC green teas. Furthermore, the Pearson correlation study indicated that these components were negatively correlated with obesity, fat accumulation, and hyperlipidemia. Therefore, higher levels of those compounds may contribute to better preventive effects on obesity, hyperglycemia, and dyslipidemia in HFD-induced diabetic mice. We propose that the weight-loss, lipid homeostasis, and anti-hyperglycemic effects of TVGTs may be attributed to the synergistic effect of multiple active compounds rather than a single active component, and the multiple active ingredients in YK green tea have a better synergistic effect compared to FD and KC green teas (Fig. 10).

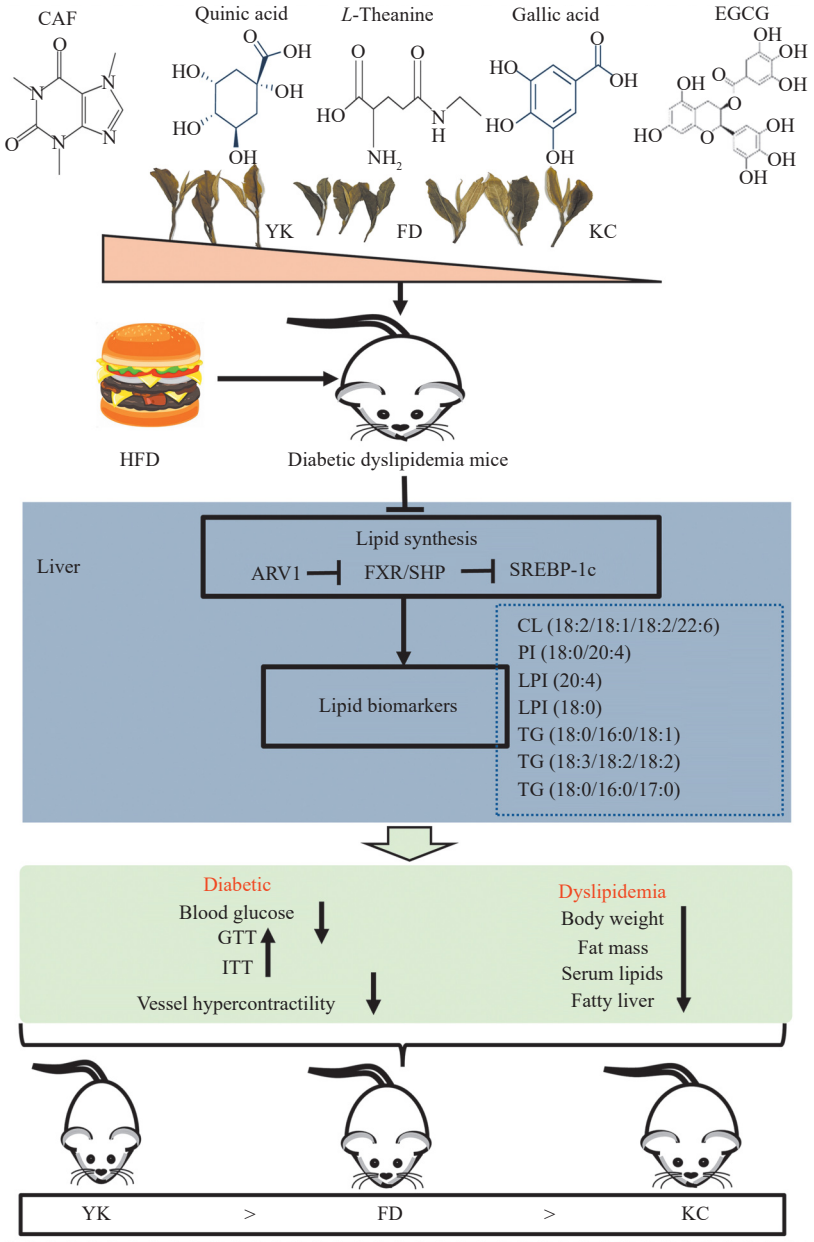

Fig. 10 Schematic diagram of TVGTs reducing liver lipid synthesis and improving diabetic hyperlipidemia related complications by regulating the ARV1-FXR-SHP-SREBP-1c pathway.

5. Conclusion

In conclusion, the dietary consumption of TVGTs significantly attenuated obesity and dyslipidemia in HFD-induced diabetic mice. Overall, YK green tea performed the best, and FD green tea had better efficiency in improving obesity and diabetic phenotypes among the TVGTs interventions. The results also suggest that long-term intervention (22 weeks) exhibited more profound effect than short-term intervention (11 weeks). In addition, lipidomics studies showed that the consumption of the TVGTs improved hepatic lipid disorders by restoring the balance of GPs and SPs, and decreased TG synthesis. A mechanistic study revealed that supplementation with the TVGTs reduced lipid synthesis by down-regulating the expression of apolipoprotein ARV1 and lipogenic protein SREBP-1c and upregulating the expression of lipolysis proteins FXR and SHP, thereby improving diabetic dyslipidemia. Furthermore, metabolomics data show that the TVGTs have distinct chemical profiles. YK green tea has high amounts of active compounds, including CAF, gallic acid, EGCG, and quinic acid, and all of them were positively correlated with ameliorating obese phenotypes and diabetic dyslipidemia induced by HFD. Therefore, among the TVGTs, YK green tea should be considered the most effective at improving obesity and diabetic dyslipidemia. Further research should be carried out to identify which chemical components in YK green tea have the most significant synergistic effect on improving diabetic dyslipidemia. Our results provide critical information for tea plant breeders and tea consumers in selecting the Assam variety as a resource plant.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250271.

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