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Kiwifruit seed oil prevents obesity by regulating inflammation, thermogenesis, and gut microbiota in high-fat diet-induced obese C57BL/6 mice

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ABSTRACT

Obesity is considered as a chronic disease which seriously affecting people's health and daily life. Kiwifruit (*Actinidia chinensis* Planch) seed oil (KSO), as a by-product of kiwifruit processing, is rich in fatty acids. Conventional wisdom holds that KSO has many health benefits, but there is no scientific basis. Here, the relieving effects of KSO on obesity and its potential mechanism were investigated in high-fat diet (HFD)-induced C57BL/6 mice. Mice were divided into four groups: ND (normal diet); HFD; L-KSO and H-KSO (HFD supplemented with 1.0 and 3.0 mL/kg·bw of KSO per day, respectively). Results showed that continuous supplementation KSO for 12 weeks significantly decreased bodyweight, inguinal fat tissue weight, blood glucose, and HOMA-IR index and ameliorated serum lipids accumulation (TC, TG, HDL-C, and LDL-C). Relative mRNA expression of inflammatory cytokines (TNF-α, IL-6, IL-1β, COX-2, and iNOS) was down-regulated and expression of thermogenesis-related genes (PPAR-γ, UCP1, PGC1-α, and PRDM16) was up-regulated in the inguinal fat tissue of KSO treated mice. Principal component analysis showed that the microbial community compositions of four groups were different. KSO supplementation dramatically decreased the *Firmicutes*-to-*Bacteroidetes* ratio. Together, our findings demonstrated that long-term supplementation KSO ameliorates obesity by reducing inflammation, adipose thermogenesis and gut microbiota dysbiosis.

1. Introduction

Obesity, which is defined as a certain degree of apparent overweight and fatty layer over thickness, is the result of many risk factors, such as increasing food consumption and reducing physical exercise (Rodríguezhernández et al., 2013). Previous researches have shown that obesity can increase the risk of various disorders, such as diabetes mellitus, dyslipidemia, cardiovascular disease, and non-alcoholic fatty liver disease (Li and Ji, 2018; Null and Consultation, 2000). Increase in fat intake is positively related to body weight, which can give rise to the development of obesity or other related metabolic diseases. Therefore, the prevention of obesity is one of the major challenges facing society today.

It has been confirmed that obesity is linked to inflammation, which is predominantly regulated the pro-inflammatory cytokines (Rodríguezhernández et al., 2013). Inflammation-mediated processes are thought to be important in regulating metabolism process (Stienstra et al., 2012). In obese individuals, pro-inflammatory cytokines are mainly derived from adipose tissue. According to the structure and function of fat cells, fat tissue is divided into two categories: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is mainly to regulate systemic homeostasis and store energy in the form of

Abbreviations: ALA, α-linolenic acid; BAT, brown adipose tissue; COX-2, cyclooxygenase-2; FAS, fatty acid synthase; iNOS, inducible nitric oxide synthase; IL-1β, interleukin-1β; IL-6, interleukin-6; KSO, kiwifruit seed oil; PGC1-α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PRDM16, positive regulatory domain containing 16; PPAR-α, proliferator-activated receptor alpha; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor-α; UCP1, uncoupling protein 1

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triglycerides (TG), while BAT mainly uses lipids for thermogenesis. BAT activity in humans is inversely correlated with adiposity, blood glucose concentrations, and insulin resistance, while the browning of WAT is an alternative pathway to increase energy expenditure (Liu et al., 2017; Guan et al., 2018). Uncoupling protein 1 (UCP1) is a morphological and functional marker for the production of thermogenic adipocytes, including brown and beige adipocytes. Activation of UCP1 can induce the thermogenesis pathway and then uses energy to generate heat to improve metabolic homeostasis (Pfeifer and Hoffmann, 2015).

Gut microbiota has a series of changes in developing obesity and other associated metabolic disease, and is a new direction of research for the treatment of such conditions (Liu et al., 2017). Increased ratio of *Firmicutes*-to-*Bacteroidetes* in major phyla and changes of several bacterial species showed a risk of obesity increases in the genetic and dietary models of mice and humans (Chang et al., 2015a,b). Complex intestinal flora plays an important role in intestinal health by fermenting dietary fiber (Scott et al., 2010). Thus, gut microbiota are a potential new target for preventing and treating obesity.

Vegetable oils have many health functions, such as nutritional supplements (Siano et al., 2016), hepatoprotection (Teng et al., 2017), anti-diabetic (Osman and Hussein, 2014) as well as anti-obesity properties (Miranda et al., 2013; Fotschki et al., 2015; Gonzálezmañán et al., 2017). Dietary flax seed oil in diabetic patients with coronary heart disease found significant improvement in peroxisome proliferator-activated receptor γ (PPARγ), lipoprotein, IL-1β, and TNF-α gene expression levels after 12 weeks of diet (Hashemzadeh et al., 2017). *Camellia* oil can ameliorate ethanol-induced acute gastric mucosal injury by inhibiting inflammation and oxidative stress (Tu et al., 2017). It has also reported that garden cress and its mixed oils rich in α-linolenic acid (ALA) significantly reduce the accumulation of fatty acids and lipids in *Wistar* rats (Umesha and Naidu, 2012). Rice bran oil and pumpkin seed oil afford hepatic protection against non-alcoholic fatty liver disease in a rat model fed a high fructose diet (Alokbi et al., 2014). Another study showed that bitter melon seed oil can effectively reduce fat accumulation in the body through lipid metabolism pathway (Chen et al., 2012).

Kiwifruit (*Actinidia chinensis* Planch), has many potential biological activities, such as antioxidant, anti-inflammation, and antibacterial (Deng et al., 2013, 2016, 2018). As a product of kiwifruit processing, kiwifruit seed oil (KSO) is rich in fatty acids, especially the essential fatty acid ALA, which has significant healthcare effects. ALA consumption has shown to improve the lipid metabolism levels in high-cholesterol diet-induced rats (Su et al., 2016). Furthermore, ALA-rich vegetable oils have potential anti-obesity effects, although the function of KSO in this regard remains to be established. Therefore, in this study, the effect of KSO on the risk of obesity, inflammation, and their possible roles in BAT activity and WAT browning were investigated in a HFD induced obese mouse model. Changes in the intestinal microbiota were determined by high-throughput sequencing. Our results, for the first time, give a rational explanation on the obesity prevention ability of KSO, which partly might attribute to the modification of the gut microbiota.

2. Materials and methods

2.1. Reagents and materials

The Hongyang kiwifruit seeds were collected from Lianxing Co. in Shannxi, China. RevertAid first strand cDNA synthesis kits were purchased from Thermo Scientific (Wilmington, DE, USA). The FastStart Essential DNA Green Master was bought from Roche (Roche, Germany). Kiwifruit seeds (100 g) were extracted by Soxhlet extraction with n-hexane (1 L) at 80 °C for 6 h, and the n-hexane was removed by a rotary evaporator and the prepared KSO were stored at 4 °C for future use. The composition of KSO described in our recent study (Deng et al., 2018; Qu et al., 2019).

2.2. Animals and diets

Male C57BL/6 mice (n = 40; body weight 20 ± 2 g) were obtained from the Experimental Animal Center of Xi'an Jiaotong University (China). Mice were housed at a controlled temperature (22 ± 2 °C) and humidity (50 ± 5%) with a 12-h light/dark cycle. The experiment was conducted in accordance with the guidelines of the Animal Ethics Committee of Northwest University (China). All experiments met the requirements of the National Laboratory Animal Act (China). After a week of adaptation, mice were fed a HFD to induce obesity and a normal diet (ND) as a control. Feed composition was listed in Supplementary Table S1. All food was stored at −20 °C and replaced every three days. After 8 weeks, mice were randomly divided into four groups (n = 10 per group): ND; HFD; HFD supplemented with 1.0 mL/kg·bw of KSO per day (L-KSO); HFD supplemented with 3.0 mL/kg·bw of KSO per day (H-KSO) for a further 12 weeks. Body weight and food intake were recorded weekly and daily. By the end of experiment, all mice were fasted for a night, and then anesthetized. Blood samples were collected for serum preparation and stored at −80 °C for future use. The WAT were obtained and stored at −80 °C.

2.3. Serum chemistry analysis

The serum can be obtained by centrifugation from blood for 15 min at 2500 rpm and at 4 °C. The serum concentration of glucose, insulin, TG, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were measured using commercial kits (Nanjing Jiancheng Biology Engineering Institute, Nanjing, China) according to manufacturer's instructions. The HOMA-IR index was calculated as [fasting insulin concentration (mU/L) × fasting glucose concentration (mmol/L)]/22.5.

2.4. Histology of WAT

Freshly isolated WAT from the four groups of mice was rapidly fixed with 10% formalin at 20 °C for 24 h at room temperature. The tissue was then embedded in paraffin wax and sections (5 μm thickness) were prepared to be stained with hematoxylin and eosin (H&E).

2.5. RNA extraction and mRNA quantification by real-time quantitative reverse-transcription PCR (qRT-PCR)

The qRT-PCR was used to analyzed relative mRNA expression changes. Total RNA was isolated from the frozen WAT using the Trizol reagent (Ambion, MA, USA) according to manufacturer's instructions. Then, the quality of total RNA was evaluated using a Nano-Drop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). First strand of cDNA used 1 μg of total RNA as a template with the RevertAid first strand cDNA synthesis kits. qRT-PCR was performed using SYBR green mix on a CFX thermocycler system (Bio-Rad, Hercules, CA, USA). PCR amplification was performed as follows: pre-degeneration step at 95 °C for 10 min, followed by 45 cycles of amplification degeneration step at 95 °C for 10 s, annealing step at 60 °C for 10 s, and extension step at 72 °C for 15 s. Relative quantification was calculated using the $2^{-\Delta\Delta CT}$ method. The primers used are shown in Table 1.

2.6. DNA extraction and 16S rRNA gene amplicon pyrosequencing

Extraction of bacterial genome DNA was performed by E.Z.N.ATM Mag-Bind Soil DNA Kit (OMEGA, Georgia, US) according to the manufacturer's instructions. Then, the V3-V4 region of the 16S rRNA gene was amplified as the following procedure: 95 °C for 2 min, 27 cycles of amplification at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, then followed 72 °C for 10 min using a universal forward primers 341 F (5′-CCCTACACGACGCTCTTCCGATCTG-3′) and the reverse primer 805 R (5′-GACTGGAGTTCCTTGGCACCCGAGAATTCCA-3′). The quality

Table 1
The primer sequences were used for qPCR in this study.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)
GAPDH	GCTGAGTATGTCGTGGAGT	GTTCACACCCATCACAAAC
COX-2	AAGACTTGCCAGGCTGAACT	CTTCTGCAGTCCAGGTTCAA
iNOS	CCTCCTCCACCCTACCAAGT	CACCCAAAGTGCTTCAGTCA
TNF-α	GTCTACTGAACTTCGGGGTGAT	GGCTACAGGCTTGTCACTCG
IL-6	CTCTGCAAGAGACTTCCATCC	GAATTGCCATTGCACAACTC
IL-1β	CCAACAAGTGATATTCTCCATGAG	ACTCTGCAGACTCAAACTCCA
PPAR-α	GGATGTCACACAATGCAATTCGCT	CAGCGAGTAGCGCATAGTCA
FAS	GCTTGCTGGCTCACAGTTAAG	AGGTTGGTGTACCCCCATTC
PPAR-γ	AGGCCGAGAAGGAGAAGCTGTTG	TGGCCACCTCTTTGCTCTGCTC
UCP1	GTGAACCCGACAACTTCCGAA	TGCCAGGCAAGCTGAAACTC
PGC1-α	CCAGGTCAAGATCAAGGTCTCCAG	TTCGGTGCGTGCGGTGTC
PRDM16	TGCTGACGGATACAGAGGTGT	CCACGCAGAACTTCTCGCTAC

of PCR products was assessed by 1% agarose gel, the products were purified using an AxyPrep DNA Gel Extraction kit (Axygen Biosciences, Union City) according to the manufacturer's instructions and quantified using an ABI GeneAmp1 9700 system (Carlsbad, CA). Pyrosequencing was performed on Illumina MiSeq platforms following the manufacturer's manuals at Sangon Biotech Co., Ltd., Shanghai, China.

2.7. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and evaluated by analysis of variance (ANOVA), followed by Tukey's multiple comparison test. All statistical analyses were performed using Statistical Product and Service Solutions (IBM SPSS 22.0, Chicago, IL, USA). Graphs were performed by GraphPad Prism (Version 5.00, GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Body weight, food consumption, blood glucose, and insulin

As shown in Table 2, the body weight of four groups reached 27.5 ± 1.88 (ND group), 42.3 ± 2.02 (HFD group), 40.0 ± 1.89 (L-KSO group), and 35.5 ± 1.90 (H-KSO group) at the end of experiment. Dietary KSO reduced weight in a dose dependent manner ($P < 0.05$). In comparison to ND group, food intake was lower ($P < 0.05$) and calorie intake was higher ($P < 0.05$) in the HFD group; however, KSO supplementation did not reverse this situation. Next, commercial kits were used to detect the effect of dietary supplementation of KSO on fasting blood glucose, insulin, and HOMA-IR index in HFD-induced obese mice. As shown in Table 2, HFD-fed mice showed significant increase in blood glucose after 12 weeks of dietary intervention ($P < 0.05$ *vs* ND); however, supplement with KSO significantly decreased the blood glucose levels (L-KSO, 20.8% and H-KSO, 22.4%). In addition, after 20 weeks, the HFD group showed a significant increase in insulin levels in comparison with the ND group; however, the insulin levels were reduced the in L-KSO and H-KSO groups. Thus, dietary KSO supplementation improved the insulin resistance indexes HOMA-IR. These results suggested that KSO supplementation inhibits HFD-induced obesity.

3.2. Serum biochemistry

Changes in serum biochemistry are closely associated with obesity. Therefore, we analyzed the following serum parameters: TC, TG, HDL-C, and LDL-C. As shown in Fig. 1A, the HFD group had a higher serum TC concentration (5.18 ± 0.25 mmol/L) than that in the ND group (3.57 ± 0.31 mmol/L) ($P < 0.05$), while the TC levels were significantly reduced from 4.62 ± 0.27 mmol/L to 4.26 mmol/L in the L-KSO and H-KSO groups. Furthermore, the concentrations of TG (Fig. 1B) and HDL-C (Fig. 1C) in the L-KSO and H-KSO groups were reduced in a similar manner. The LDL-C concentration in the HFD group was higher 58.1% than that in the ND group ($P < 0.05$), while the LDL-C levels were decreased by 32.4% and 54.1% in the L-KSO and H-KSO groups, respectively (Fig. 1D).

3.3. Fat tissue weight and histopathology

Furthermore, as shown in Fig. 2A, the inguinal fat tissue weight in the HFD group was higher than that in the ND group ($P < 0.05$), whereas the inguinal fat weight was reduced by 42.07% and 52.18% in the L-KSO and H-KSO groups, respectively, with no significant difference between the results in the ND and H-KSO groups. Histological analysis of inguinal WAT showed that HFD promoted adipocyte hypertrophy and significantly increased fat cell area in comparison to ND group, while supplementation of KSO prevented this phenomenon in a dose-dependent manner (Fig. 2B).

3.4. Relative mRNA expression related to inflammation

In general, obese adipocytes secrete increased levels of various pro-inflammatory cytokines during developing and progressing obesity. Here, we analyzed the relative mRNA expression of some important inflammatory factors in inguinal fat tissue. The HFD group had higher expression levels of cyclooxygenase-2 (COX-2) (Fig. 3A), inducible nitric oxide synthase (iNOS) (Fig. 3B), TNF-α (Fig. 3C), IL-6 (Fig. 3D), and IL-1β (Fig. 3E) than those in the ND group ($P < 0.05$), In contrast, compared with the HFD group, KSO supplementation decreased the expression levels of these cytokines were decreased in the L-KSO and H-KSO groups in a dose-dependent manner ($P < 0.05$). These results demonstrated that KSO supplementation reduced obesity-induced inflammation.

3.5. Relative mRNA expression related to lipid metabolism and thermogenesis

Next, we analyzed the changes in relative mRNA expression of several lipid metabolism- and thermogenesis-related genes. As shown in Fig. 4, the expression levels of proliferator-activated receptor alpha and gamma (PPAR-α and PPAR-γ), which are linked to fatty acid β oxidation, was up-regulated in the L-KSO ($P < 0.05$) and H-KSO ($P < 0.05$)

Table 2
Effect of KSO supplementation on the body weight and biochemical parameters of mice[a].

	ND	HFD	L-KSO	H-KSO
Food intake (g/day)	2.4 ± 0.3 [a]	2.2 ± 0.2 [b]	2.1 ± 0.2 [b]	2.1 ± 0.3 [b]
Calorie intake (kcal/day)	9.2 ± 0.9 [b]	11.4 ± 1.1 [a]	11.3 ± 1.1 [a]	11.4 ± 1.4 [a]
Final body weight (g)	27.5 ± 1.9 [d]	42.3 ± 2.0 [a]	40.0 ± 1.9 [b]	35.5 ± 1.9 [c]
Fasting glucose (mmol/L)	6.1 ± 0.6 [c]	10.1 ± 0.6 [a]	8.0 ± 0.3 [b]	7.8 ± 0.6 [b]
Insulin (ng/mL)	8.0 ± 0.3 [d]	14.4 ± 0.4 [a]	12.5 ± 0.3 [b]	9.4 ± 0.4 [c]
HOMA-IR	2.2 ± 0.3 [d]	6.5 ± 0.3 [a]	4.5 ± 0.1 [b]	3.3 ± 0.2 [c]

[a] ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively. Data are expressed as the mean ± SEM (n = 8). The different letters indicate a significant difference ($P < 0.05$).

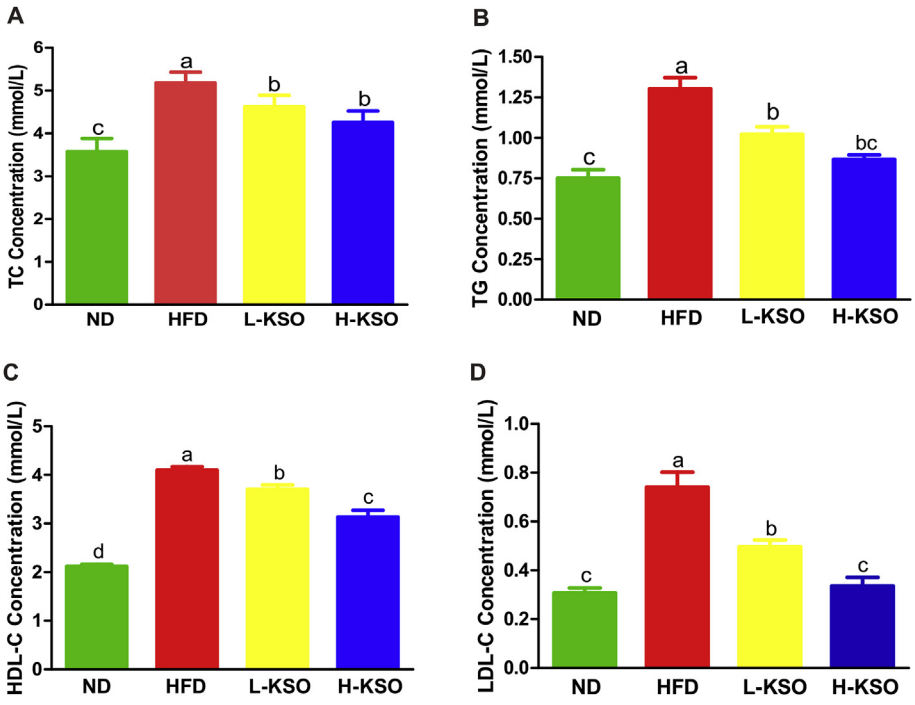

Fig. 1. KSO supplementation decreased the liver lipid levels in HFD-induced obese mice. (A) TC; (B) TG; (C) HDL-C; (D) LDL-C. ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively. Data are expressed as the mean ± SEM (n = 8). The different letters indicate a significant difference ($P < 0.05$).

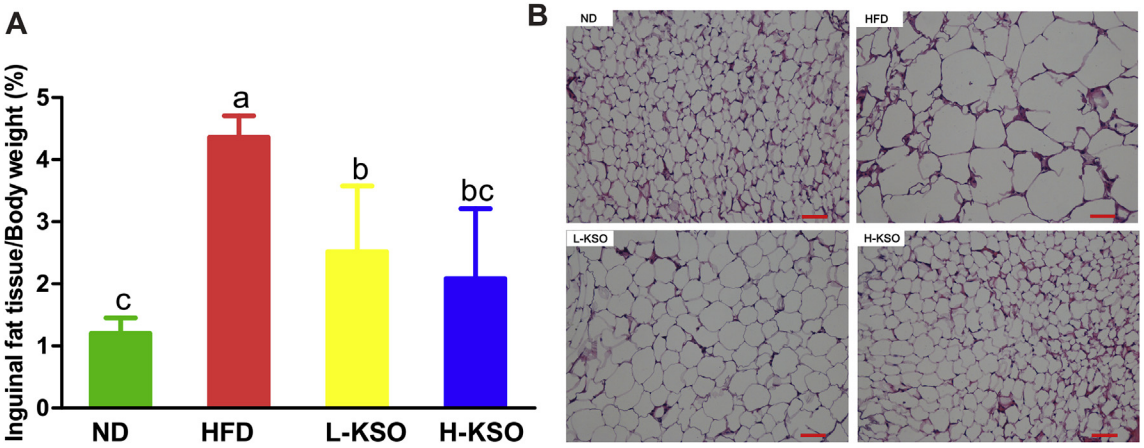

Fig. 2. KSO supplementation decreased the inguinal fat tissue weight and fat cell size. (A) Inguinal fat tissue weight; (B) Histological analysis of inguinal fat tissue. ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively. Data are expressed as the mean ± SEM (n = 8). The different letters indicate a significant difference ($P < 0.05$).

groups compared to the levels in the HFD group (Fig. 4A and B). In addition, we also investigated the ability of KSO supplementation to inhibit lipid synthesis by analyzing the expression of fatty acid synthase (FAS) (Fig. 4C). Compared with the ND group, expression levels of the FAS gene were significantly increased ($P < 0.05$), while the levels were significantly lower after 12 weeks of KSO supplementation than those in the HFD group. These observations suggested that lipid synthesis was inhibited in the inguinal fat tissue of HFD-induced obese mice by dietary KSO supplementation. Furthermore, we analyzed the mRNA expression levels of thermogenesis-related genes. Compared with the ND group, the expression levels of the UCP1 gene were significantly downregulated in the HFD group; however, the levels was dramatically recovered after 12 weeks of dietary KSO supplementation in L-KSO and H-KSO group (Fig. 4D). Similar trends were also observed in the gene's expression levels of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) (Fig. 4E) and positive regulatory domain containing 16 (PRDM16) (Fig. 4F).

3.6. Gut micrbiome dysbiosis

The intestinal flora plays an important role in the pathogenesis of obesity and related metabolic diseases. We also compared the difference in the composition of intestinal microflora in mice by 16S rRNA sequencing analysis. MiSeq sequencing analysis yielded a dataset of 742 050 high-quality 16S rDNA gene sequences from 12 samples (range, 36577-80349; average number of sequence reads per sample, 61838). The sequences were clustered with representative sequences, and the cut-off value of sequence identity was 97%. As shown in Fig. 5A, a total 15 phyla were identified, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* being the three major abundant bacterial phyla in the four groups. *Firmicutes* and *Bacteroidetes* are two typical species of bacteria in the phylum. The content of *Firmicutes* in HFD group was significantly higher than that in ND group ($P < 0.05$) (Fig. 5B). However, the content of *Firmicutes* in H-KSO group decreased compared with that in HFD group ($P < 0.05$). As shown in Fig. 5C, the abundance of *Bacteroidetes* was contrary to the content of *Firmicutes*, while the

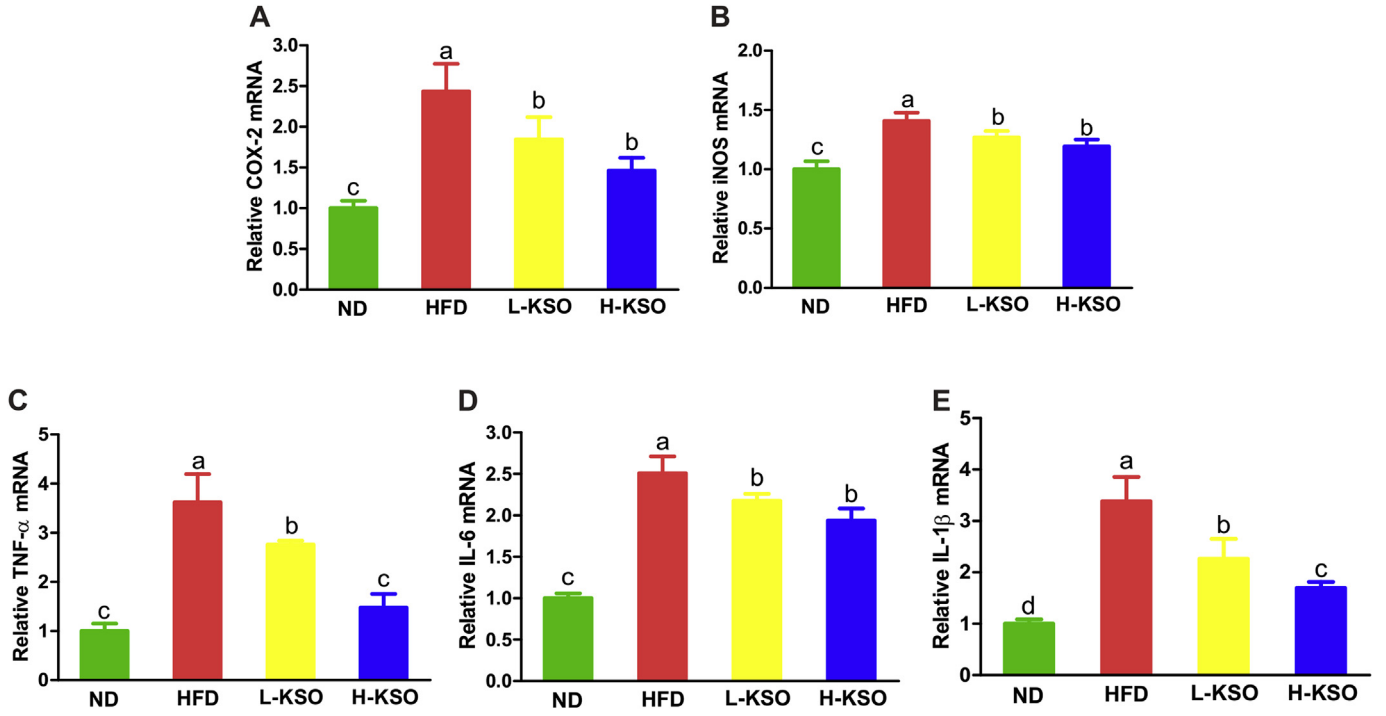

Fig. 3. KSO supplementation decreased the relative mRNA expression of inguinal fat tissue in HFD-induced obese mice. (A) COX-2; (B) iNOS; (C) TNF-α; (D) IL-6; (E) IL-1β. ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively, respectively. Data are expressed as the mean ± SEM (n = 8). The different letters indicate a significant difference ($P < 0.05$).

abundance was significantly increased in the L-KSO and K-KSO groups in comparison to that in the HFD group ($P < 0.05$). Meanwhile, the *Firmicutes*-to-*Bacteroidetes* ratio was associated with changes in host weight. Our results found that the *Firmicutes*-to-*Bacteroidetes* ratio in the HFD group dramatically increased compared with that in the ND group ($P < 0.05$); this situation was reversed in the L-KSO and H-KSO groups in a dose-independent manner ($P < 0.05$) (Fig. 5D).

3.7. Sample cluster tree map based on OUT

The principal component analysis (PCA) was shown in Fig. 6A. Different colors in the figure represent samples in different groups. The degree of similarity between samples is reflected by the level of aggregation in the graph. The results showed that HFD induced changes in intestinal microflora in mice. After 12 weeks of KSO supplementation, the structure of the intestinal flora in L-KSO and H-KSO group was

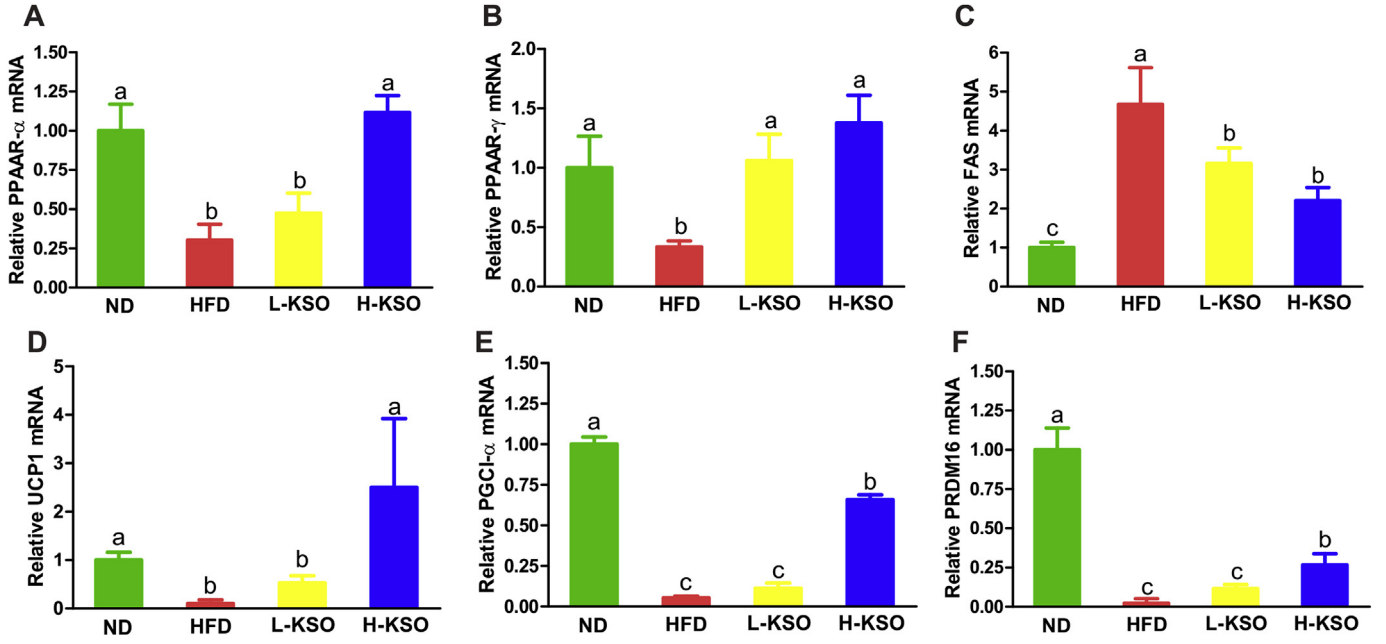

Fig. 4. Effect of KSO supplementation on the expression levels of thermogenesis-related genes in inguinal fat tissue. (A) PPAR-α; (B) PPAR-γ; (C) FAS; (D) UCP1; (E) PGC1-α; (F) PRDM16. ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively, respectively. Data are expressed as the mean ± SEM (n = 8). The different letters indicate a significant difference ($P < 0.05$).

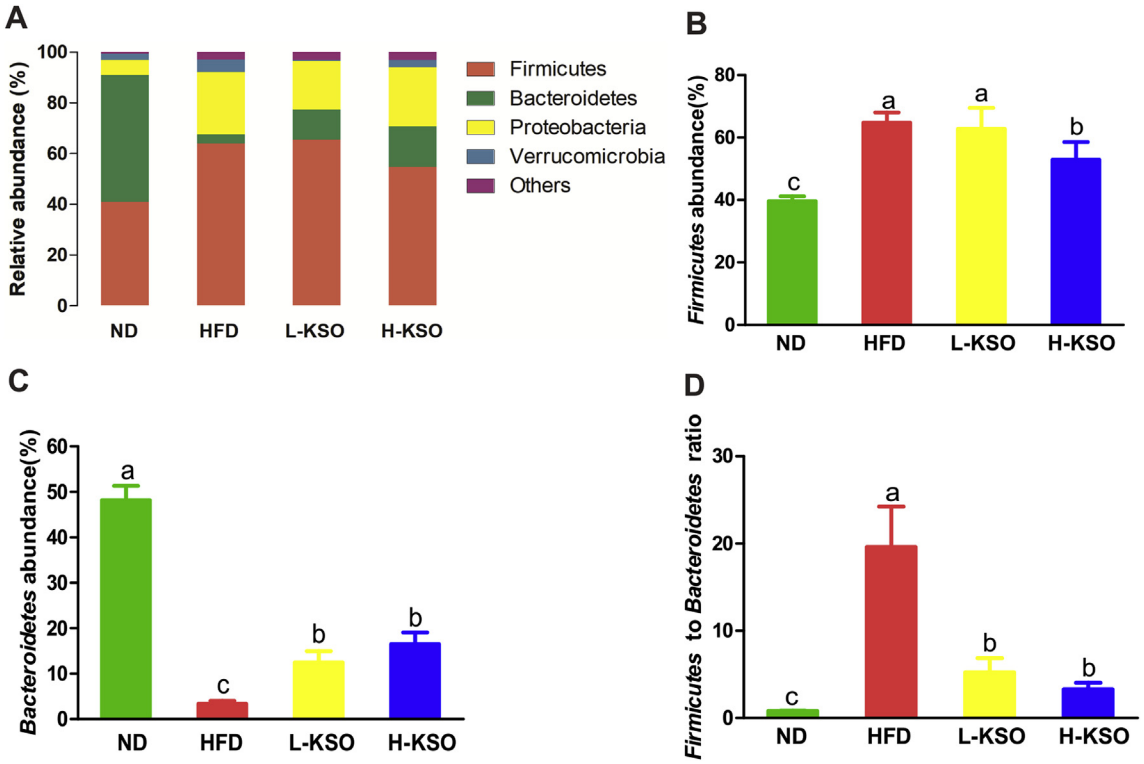

Fig. 5. (A) Fecal bacterial community at the phylum level. (B) Relative abundance of *Firmicutes*; (C) Relative abundance of *Bacteroidetes*; (D) *Firmicutes*-to-*Bacteroidetes* ratio. ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively, respectively. Data are expressed as the mean ± SEM (n = 4). The different letters indicate a significant difference ($P < 0.05$).

similar to that in the ND group. The sample clustering tree analysis is shown in Fig. 6B. The similarities and differences among several samples are intuitively reflected by the branch structure, with the length of the branch representing the distance in the structure of the intestinal flora between the samples. The results of this analysis showed that the structure of intestinal flora in ND group and HFD group was the worst, and the difference was the largest. After the administration of KSO, the structure of the intestinal flora was improved.

To further evaluate the microbiota community changes of the faecal in the obese mice, the 50 most abundant genera were presented in a heat map. The genus were represented at different levels in the four groups. Compared with the relative abundance in the ND group, *Bilophila*, *Clostridium IV*, *Acetatifactor*, *Helicobacter*, *Clostridium XIVa*, *Lachnospiracea incertae sedis*, *Akkermansia*, *Mucispirillum*, *Anaerotruncus*, *Eisenbergiella*, *Hydrogenoanaerobacteruim*, *Lactobacillus*, *Staphylococcus*, *Peptococcus*, *Marvinbryantia*, and *Acidaminobacter* were increased in the HFD group, whereas*Bacteroides*, *Barnesiella*, *Intestinimonas*, *Tannerella*, *Coprobacter*, *Ailstipes*, *Odoribacter*, *Alloprevotella*, *Parabacteroides*, *Ruminococcus2*, *Acetobacteroides*, *Macellibacteroides*, *Stomatobaculum*, and *Clostridium XVII* were decreased. These changes were reversed by KSO supplementation; thus, a heat map of the relative abundance of altered microbial species treated by KSO shows the difference between the composition of intestinal bacteria in the H-KSO and L-KSO groups and that of intestinal bacteria belonging to the HFD groups (Fig. 6C).

Furthermore, to identify the specific bacteria characteristic of four groups, linear discriminant analysis effect size (LEfSe) analysis was performed on account of the nonparametric factorial Kruskal-Wallis (KW) sum-rank test. As shown in Fig. 6D and E, the abundance of *Pseudoflavonifractor*, *Flavonifractor*, *Intestinimonas*, *Romboutsia*, and *Olsenlla* was markedly increased in the H-KSO group comparing to that in other three groups. Taken together, these data suggested that dietary KSO supplementation altered the gut microbiota.

4. Discussion

Obesity is recognized as a major worldwide health problem leading to a range of related diseases, and is also believed to increase the risk of a variety of other chronic diseases. There are many limitations in the drugs currently available for the treatment of obesity, such as greater side effects and high recurrence rates. Therefore, it is urgent to find some natural compounds to treat obesity. Kiwifruit has many biological activities, such as antioxidant, anti-inflammation, and antibacterial properties (Deng et al., 2013, 2016, 2018), and KSO, which contains ALA, is an excellent nutritional supplement. Studies have shown that *Rosa mosqueta* oil supplements prevented the obesity in HFD-fed mice by lowering the expression and secretion of inflammatory cytokines and stimulating the production of liver antioxidants and fatty acid oxidation markers (Gonzálezmañán et al., 2017). Another study showed that pomegranate seed oil improved insulin secretion without changing blood glucose in diabeticrats (Nekooeian et al., 2014). Fu et al. (2016a,b) reported that *Cinnamomum camphora* seed kernel oil ameliorated oxidative stress and inflammation in obese rats. However, the anti-obesity effect of KSO remains to be established. In this study, we evaluated the anti-obesity effects of KSO and the potential to ameliorate obesity-induced inflammation, heat production, and intestinal microflora dysbiosis in HFD-induced obese C57BL/6 obese mice.

HFD causes weight gain in the body and organs as well as elevated fasting blood glucose. In our study, KSO supplementation decreased the bodyweight gain with the increase of KSO dose compared to that HFD group ($P < 0.05$), but no effect on food and calorie intake, in consistent with our results, purslane seed oil (150 and 300 g/kg of diet) administered over 8 consecutive weeks prevented the increase in body weight in obese diabetic mice (Osman and Hussein, 2014). Black raspberry seed oil, which is also rich in ALA, lowered the bodyweight but not food intake in *db/db* mice when administered for 10 weeks (Lee et al., 2016). Conversely, pomegranate seed oil had no impact on the final bodyweight and food intake (Miranda et al., 2013). It means that

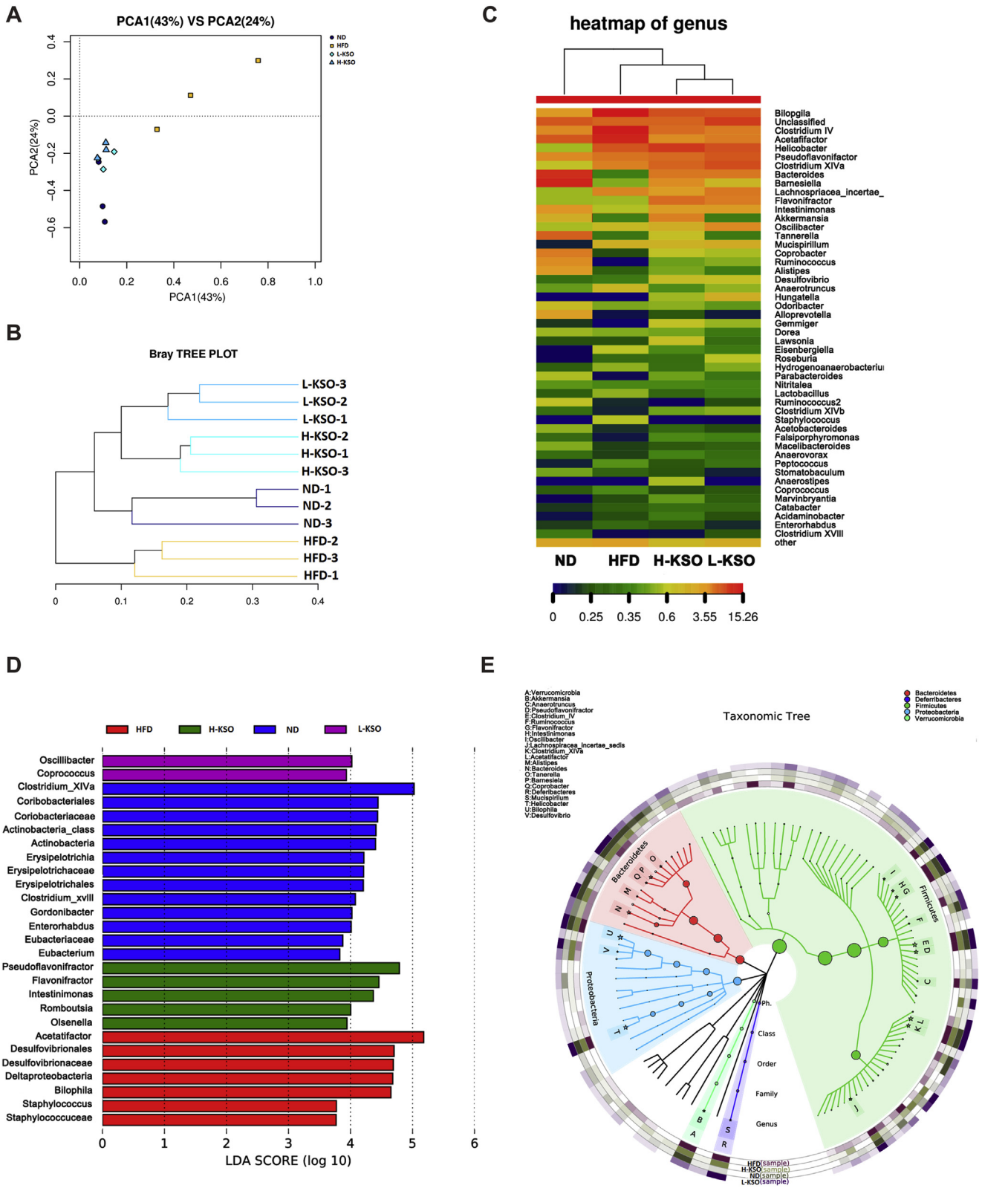

Fig. 6. LEfSe analysis of key genera of mice gut microbiota. (A) Principal component analysis (PCA) of microbiota communities; (B) Multivariate analysis of variance from PCA matrix scores; (C) Heat map of the intestinal microbiota in rats at the genus level. (D) Histogram shows the LDA scores computed for features (OTU level) differentially abundant between different treatments. The higher the score is, the more important the role is; (E) Cladogram shows that brown dots are unimportant bacteria in any groups; other colored dots are important bacteria in the group labeled with the same color. ND, mice fed a normal diet; HFD, mice fed a high-fat diet; L-KSO and H-KSO, mice fed a high-fat diet supplemented with KSO at 1.0 and 3.0 mL/kg·bw per day, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the effect of KSO on body weight is not due to reduced food consumption or calorie intake. Obesity, caused by excessive fat accumulation, is linked to numerous health risks, including a reduction in the number of insulin receptors and receptor function defects, leading to insulin resistance. In our study, long-term consumption of a HFD resulted in fasting blood glucose levels that were obviously much than ND group ($P < 0.05$), while KSO supplementation reduced the fasting blood glucose concentrations in a dose-dependent manner. The HOMA-IR and HOMA-IS indexes are common indicators used to assess insulin sensitivity and pancreatic β-cell function in diabetic patients. In the L-KSO and H-KSO groups, the HOMA-IR index was significantly reduced comparing to HFD group ($P < 0.05$), while the HOMA-IS index was increased ($P < 0.05$). Thus, our results show that KSO supplementation alleviates HFD-induced hyperglycemia and improves insulin resistance.

Previous studies have shown that HFD affects the serum levels of TC, TG, HDL-C, and LDL-C, leading to abnormal lipid metabolism and ultimately to dyslipidemia or non-alcoholic fatty liver (Liu et al., 2017). In our study, compared to the HFD group, KSO supplementation decreased the TC and TG levels in the L-KSO and H-KSO groups in a dose-dependent manner, while the levels of HDL-C and LDL-C recovered in the L-KSO and H-KSO groups. These findings are consistent with previous studies and taken together (Fu et al., 2015, 2016; Fu et al., 2016a,b), our observations indicate that KSO improves lipid metabolism effectively in C57BL/6 J mice.

Excessive accumulation of fat in the body (mainly duing to that energy intake is greater than consumption) is the core of the obesity disease, and adipose tissue plays a key role in developing obesity related complications (Mraz and Haluzik, 2014). Our results showed that a long-term HFD feeding resulted in a significant increase in the amount of inguinal fat tissue as a percentage of body weight in HFD group comparing to ND group ($P < 0.05$). In contrast, KSO supplementation significantly decreased the amount of inguinal fat tissue as a percentage of body weight ($P < 0.05$) compared to that in the HFD group; this observation confirmed by H&E staining of inguinal fat tissue. In accordance with our results, *Rosa mosqueta* oil decreased the percentage of fat tissue percentage and fat cell size in mice feeding HFD (Gonzálezmañán et al., 2017). These findings suggested that KSO reduced fat accumulation.

In recent years, it has become clear that obesity can also cause increased inflammation. Obesity in adipose tissue accompanied by the expression, production, and release of inflammatory-related adipokines, such as TNF-α, IL-6, and IL-1β (Trayhurn and Wood, 2004). Inflammation may be closely related to obesity, type 2 diabetes, cardiovascular disease, and other metabolic diseases (Tam et al., 2010). The level of TNF-α secreted by adipose tissue correlates with the degree of obesity and associated insulin resistance (Tzanavari et al., 2010). IL-6 has intrinsic pro-inflammatory activity, which can increase the TNF-α level (Park et al., 2005). The pro-inflammatory mediators IL-1β and TNF-α activate aberrant expression of inflammation-related genes (Sellam and Berenbaum, 2010). COX-2 is an important inflammatory marker that can be activated by IL-1β and TNF-α (Ahmad et al., 2002; Largo et al., 2003). iNOS is also considered to be another important inflammatory factor in inflammation (Ahmad et al., 2002). In our study, KSO supplementation decreased the mRNA expression of inflammatory cytokines (TNF-α, IL-6, IL-1β, COX-2, and iNOS) in a dose-dependent manner in the L-KSO and H-KSO groups comparing to HFD group. Previous studies showed that a water extract of *Ganoderma lucidum* mycelium decreased pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) expression in the liver and adipose tissues (Chang et al., 2015a,b). Eo et al. (2015) reported that *Ecklonia cava* polyphenol extract reduced the levels of proteins related to inflammatory responses, such as TNF-α, IL-1β, IL-6, COX-2, and NF-κB in HFD-induced obese mice. In this study, we found that a HFD resulted in up-regulated expression of inflammatory factors, while KSO decreased the expression of these factors.

WAT has become the subject of considerable focus of activity in recent years and is now a 'hot spot' in biomedical research (Trayhurn and Wood, 2005). WAT represents a major secretory organ responsible for the release of fatty acids. PPAR-α, which is highly expressed in metabolically active tissues, is activated *via* a variety of natural agonists to induce fatty acid oxidation (Su et al., 2016). FAS is a central adipogenesis enzyme involved in lipid synthesis (Noratto et al., 2016). Our results showed that KSO supplementation up-regulated the expression of PPAR-α and down-regulated the expression of FAS at the mRNA levels, which is consistent with a previous report on *Rosa mosqueta* oil (Gonzálezmañán et al., 2017). Thermogenesis by BAT and beige adipose tissue, which arises from the browning of WAT, has emerged as a promising strategy to combat obesity (Lu et al., 2016). UCP1 is a morphological and functional marker for the production of thermogenic adipocytes, including brown and beige adipocytes (Zhang et al., 2016). PPAR-γ is a key regulator of both brown and white adipocyte differentiation (Tontonoz et al., 1994), which can be activated by synthetic full agonists drives browning of WAT (Petrovic and Walden, 2010). PGC1-α as a transcriptional co-activator can mediate many energy metabolism related biological programs. PRDM16 is associated with energy expenditure in adipocyte tissue (Liu et al., 2017). Our results showed that KSO supplementation up-regulated the relative mRNA expression levels of PPAR-γ, UCP1, PGC1-α, and PRDM16. Similar study showed that supplementation with bitter melon seed oil can increase WAT browning as a potential natural compound to treat obesity (Hsieh et al., 2013). Another study showed that gypenosides could increase the progression of BAT heat production and induce the Browning of fat cells in WAT (Liu et al., 2017). These data indicate that KSO induces adipocyte browning in inguinal fat tissue.

The gut microbiota is an environmental factor that affects predisposition toward adiposity (Bäckhed et al., 2004). There is increasing evidence that the disturbance of the intestinal flora caused by long-term dietary HFD, which is a key factor in the development of indicators of obesity, insulin resistance and other metabolic syndrome (Parks et al., 2013). Changes in intestinal flora have been linked to obesity and inflammation in humans or serrated animals (Zhang et al., 2010). Mice fed HFD had more *Firmicutes* and fewer *Bacteroidetes* than mice fed low-fat diets (Qin et al., 2012). Increased proportions of major phylum *Firmicutes/Bacteroidetes* could increase developing obesity in mice (Chalkiadaki and Guarente, 2012). In our study, we observed that KSO administration decreased the abundance of *Firmicutes* compared to that in the HFD group, while the abundance of *Bacteroidetes* was increased, thus restoring the ratio of *Firmicutes/Bacteroidetes* to close to the ND group. A similar study shows that *Ganoderma lucidum* can reverse the HFD induced intestinal maladjustment, such as reducing the ratio of *Firmicutes*-to-*Bacteroidetes* and the recovery of intestinal mucosal integrity (Chang et al., 2015a,b). These results suggest that KSO may be resistant to obesity by changing the *Firmicutes-to-Bacteroidetes* ratio. The intestinal microflora species *Ruminococcaceae*, belonging to the *Firmicutes* phylum, contains several butyric acid-producing bacteria that have anti-inflammatory activity and may exert potential physiological functions beneficial to host health (Louis and Flint, 2009). In our study, in comparison to the ND group, the HFD group had a significantly reduced abundance of *Ruminococcaceae*, whereas KSO supplementation increased the level of this bacterium. Research has shown that an increase in the abundance of the *Lachnospiraceae* family of bacteria is accompanied by the increasing body weight contribute to the development of diabetes in *ob/ob* obese mice (Kameyama and Itoh, 2014). Our study also shows that the abundance of *Lachnospiraceae* is proportional to body weight. *Akkermansia*, belonging to *Verrucomicrobia*, works in treating obesity, inflammation, diabetes, and metabolic syndrome (Shin et al., 2014). Our data showed that the abundance of genus *Akkermansia* significantly increased in the H-KSO group compared with that in the ND and HFD groups. Taken together, the data suggest that alterations in the gut microbiota play an important role in the overall beneficial effect of KSO in reducing the development of obesity and

inflammation.

5. Conclusions

In summary, the present study demonstrates that dietary of KSO supplementation reduced the body weight gain, inguinal fat tissue weight, blood glucose, and HOMA-IR index in HFD-induced C57BL/6 mice. Serum analysis results showed that KSO supplementation increased HDL-C levels and decreased the levels of TC, TG, and LDL-C. Histopathological studies of inguinal fat revealed that KSO reduced the volume of fat cells. Furthermore, analysis of the relative mRNA expression of inflammation- and thermogenesis-related genes showed that KSO supplementation decreased inflammation (TNF-α, IL-6, IL-1β, COX-2, and iNOS) and improved thermogenesis (PPAR-γ, UCP1, PGC1-α, and PRDM16). Further investigation of the effect of KSO supplementation on the gut microbiota in C57BL/6 obese mice revealed that KSO supplementation decreased the *Firmicutes*-to-*Bacteroidetes* ratio and the abundance of *Lachnospiraceae* and increased the abundance of *Ruminococcaceae* related to inflammation and *Akkermansia* related to obesity. Overall, our results indicate that long-term dietary of KSO supplementation can ameliorate HFD-induced obesity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.046.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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