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Apple polyphenol product, phlorizin or procyanidin B_2, alleviates transplanted obese patient fecal microbiota-induced obesity in mice by modulating the gut microbiota and related phages

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ABSTRACT

Apples are popular fruits worldwide and rich in phenolic compounds that can alleviate obesity and related metabolic diseases. However, the mechanisms underlying the anti-obesity actions of apple polyphenols (AP) like phlorizin (PZ) and procyanidin B_2 (PB2) on transplanted obese patient fecal microbiota (TOPFM)-induced obesity and related syndromes have not yet been fully examined *in vivo*. Herein, a commercial AP product, PZ compound or PB2 compound was used to ameliorate TOPFM-induced obesity in mice. The results indicated that the AP, PZ or PB2 supplementation markedly alleviate TOPFM-induced obesity in mice through effectively suppressing body weight gain and fat accumulation, alleviating insulin resistance and liver inflammation, regulating gut microecology and lipid synthesis/metabolism, and improving gut barrier function and antioxidant capacity. The gut barrier function and integrity were improved through regulating the expression of intestinal pro-inflammatory cytokines, tumor necrosis factor-alpha (*TNF-α*), interleukin-1beta (*IL-1β*) and interleukin-6 (*IL-6*), and gut barrier function-related genes, zonula occludens-1 (*ZO-1*) and *Occludin*, and raising the glucagon-like peptide 2 (GLP-2) level via increasing the contents of short-chain fatty acids (SCFAs). Interestingly, the AP, PZ or PB2 supplementation could significantly improve the production of SCFAs and restore the microbial community structure and diversity in mice with TOPFM-induced obesity, in particular, increased the abundance of Lachnospiraceae and Bifidobacteriaceae possibly by inhibiting *Blautia* and *Bifidobacterium* phages. The influences of AP, PZ or PB2 on gut microorganisms and phases of the mice upon TOPFM were species-specific. This study was the first report on the ability of an AP, PZ or PB2 supplementation to promote the production of SCFAs by modulating gut microbiota possibly via regulating gut phages.

1. Introduction

Obesity, a major public health issue worldwide, is caused by the abnormal or excessive accumulation of ectopic fat[1]. Obesity makes direct contributions to metabolic disorders, including type 2 diabetes and increase the risk of cardiovascular diseases[2]. Currently there exist some anti-obesity drugs including those with Food and Drug Administration (FDA) approval (e.g. liraglutide) that can alleviate obesity probably via mechanisms such as fat absorption reduction, increase of energy expenditure and thermogenesis, suppression of digestive enzymes and inhibition of appetite[3-4]. However, these drugs have limitations and can cause adverse reactions and side effects,

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including addictive potential, reduction of food intake, emergence of tachyphylaxis, damage to immune system, diarrhea, and liver/kidney failure, etc.[4-6]. Therefore, to avoid the adverse health risks associated with these drugs, especially for long-term treatments, natural anti-obesity agents/products as alternatives to the conventional drugs against obesity are being highly sought.

The gut microbiota as a complex microbial community living in the gastrointestinal system is known for its close symbiotic relationship with the host. A growing body of research shows an intimate functional relationship between the gut microbiota and obesity[7]. The onset and progression of obesity were found in close association with gut microbiota, particularly the microbial diversity and the Firmicutes/Bacteroidetes ratio[8]. Tailoring the structure and composition of gut microbiota is an effective and novel approach for alleviating obesity and related metabolic diseases, through methods such as intake of probiotics and/or prebiotics, or fecal microbiota transplantation (FMT), to suppress the potentially harmful microbial species (e.g., Desulfovibrionaceae), promote the favorable species (e.g., *Akkermansia*), and alter the level of lipopolysaccharide (LPS) and the profile and amounts of short-chain fatty acids (SCFAs) in the gut[9-10]. Phytochemicals that naturally occur in edible plants, such as fruits and vegetables, exhibit anti-inflammatory, antioxidant and anti-obesity effects[11-12]. Recent studies showed that the function and structure of gut microbiota can be modulated by some natural phytochemicals in obese mice, including polysaccharides, pigments and polyphenols[13-15]. The microbial diversity and community structure could be restored by salidroside, which could alleviate high-fat diet (HFD)-induced obesity in mice[1]. Dihydromyricetin was found able to ameliorate obesity in obese and diabetic mice through reducing the relative abundance of *Lactobacillus*, thereby altering the activity of bile salt hydrolase[16]. Administration of epigallocatechin-3-gallate (EGCG) could greatly reduce the ratio of Firmicutes/Bacteroidetes in HFD mice[17].

Apple polyphenols (AP) refer to the polyphenolic substances extracted from apple, including compounds like quercetin, phloretin (PT), phlorizin (PZ), and procyanidin B_2 (PB2)[18]. It was found that AP could exert an anti-obesity effect by regulating the structure and composition of gut microbiota[10-19]. Extracts of AP could prevent high-carbohydrate diet (HCD)-induced obesity by increasing considerably the relative abundance of *Akkermansia*, *Frisingicoccus* and *Parasutterella*, and Verrucomicrobia[20]. An apple polyphenol extract was also reported to regulate the diversity and composition of gut microbiota by significantly increasing the level of *Akkermansia* while reducing the relative abundance of *Lactobacillus* in mice with HFD-induced hepatic steatosis[21]. Rutin administration could increase the growth of Amoebacteria, Firmicutes and *Trichophyton* while counteracting HFD-induced ecological dysregulation in male C57BL/6J mice[22]. PZ was found to ameliorate HFD-induced obesity by increasing the levels of Bacterioidia, Bacteroidales, Muribaculaceae while reducing the levels of Clostridia, Desulfovibrionales and *Desulfovibrion* in mice[10]. PB2 was reported to alleviate high-fat-cholesterol diet-induced obesity while ameliorating insulin resistance through increasing the levels of *Akkermansia* and Bacteroidetes in rabbits[23]. Taken together, AP preparations/products and the individual phenolic compounds such as PZ and PB2 have demonstrated their abilities to ameliorate obesity by modulating the structure and composition of gut microbiota. However, whether these AP products and individual phenolic compound can ameliorate transplanted obese patient fecal microbiota (TOPFM)-induced obesity in mice, along with the underlying mechanism(s), remains unknown.

Phages deserves attention when modulators of the gut ecosystem and the treatment efficacy of FMT are discussed[24-25]. Phages, also known as bacteriophages, are viruses occurring abundantly within the human biome, with a size from approximately 3.5 kb to 540 kb and a density range of 10^8–8×10^{10} phage virions per gram of feces[26]. Phages are pivotal components of the gastrointestinal ecosystem, as they are key players in shaping the gut microbiota, restoring the dynamic balance of gut microbiota, maintaining gastrointestinal tract's homeostasis, and controlling the progression of gastrointestinal disorders and related diseases, through modulating specifically gut bacterial community via predation of bacteria or promotion of bacterial survival by the transfer of fitness-enhancing genes, as well as interacting directly with the immune cells thereby regulating the host immune system and immune response[25,27-31]. It was found that HFD-induced obesity could significantly change the diversity and relative abundances of viruses and gut microbiota in mice, indicating the close relationship between HFD-induced intestinal dysbacteriosis and the changes of viruses[32]. Green tea polyphenols were found to alter the diversity and relative abundances of phages by reducing the intestine's temperate phages and increasing lytic phages in mice[33]. Previous studies also indicated the need to consider the influence of phages on long-term microbial dynamics for the therapeutic approaches involving FMT, as the abundance of and actions of phages affect the outcome of FMT[25,34]. So far, no studies have been reported on whether an AP product, PZ or PB2 can prevent or alleviate obesity by modulating gut microbiota via regulating phages.

This study was set up to help fill the above-mentioned knowledge gaps. The effects of a commercially available AP product, PZ compound and PB2 compound on TOPFM-induced obesity, along with the potential underlying mechanisms, in particular, the roles of gut microbiota and intestinal bacteriophages in controlling obesity, were examined in mice. Through investigating the changes in phage, gut microbiota, gut barrier and body weight gain affected by the AP product, PZ compound or PB2 compound, a novel and effective therapeutic approach for treating obesity without adverse effects is presented.

2. Materials and methods

2.1 Reagents and materials

An AP product (CAS: 85251-63-4, HPLC ≥ 98%) was purchased from Tianjin Jianfeng Natural Product R&D Co., Ltd. (Tianjin, China). PZ (CAS: 60-81-1, HPLC ≥ 98%) and PB2 (CAS: 29106-49-8, HPLC ≥ 98%) were purchased from YuanYe Biotechnology Co., Ltd. (Shanghai, China). Ampicillin (CAS: 69-53-4), metronidazole (CAS: 443-48-1) and vancomycin (CAS: 1404-90-6) were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Neomycin and gentamicin were purchased from Shanghai Yingxin Laboratory Equipment Co., Ltd. (Shanghai, China).

2.2 Animal experiments

Fifty male C57BL/6J mice (aged 6 weeks) were purchased from Pengyue Experimental Animal Breeding Co., Ltd. (SCXK(Lu) 20230002, Jinan, China), and housed for 1 week in an environmentally controlled room (temperature: (22 ± 1) °C; relative humidity: 60%; 12 h

dark/12 h light cycle), with *ad libitum* access to food (standard chow) and water throughout the acclimatization.

Antibiotic administration after the acclimatization: in order to better implant the donor microbiota, administration of a combination of several antibiotics was employed to reduce the gut microbial load of the recipient mice[35-36]. The antibiotic administration experiments were performed followed the procedure described by Kelly et al.[37]. In brief, the combination of ampicillin + neomycin + metronidazole + vancomycin + gentamicin (1.0 g/L + 1.0 g/L + 1.0 g/L + 0.5 g/L + 1.0 g/L) was diluted with 0.9% sterile saline. Then, each mouse (200 μL/day) was given the diluted combination via gavage three times a week (for 1 week). The feces of the mice before and after the 1-week antibiotic treatment were collected for real-time reverse transcription-polymerase chain reaction (RT-qPCR) analysis. The primers used to detect all bacteria were based on 16S rRNA gene sequences: forward 5′-ACTCCTACGGGAGGCAG CAGT-3′ and reverse 5′-ATTACCGCGGCTGCTGGC-3′. During the antibiotic treatment, the mice had free access to food (standard chow) and water.

FMT: fecal samples were collected at (22 ± 1) ℃ from 6 healthy volunteers (3 male and 3 female; aged 25−30 years, 19 kg/m^2 > body mass index (BMI) > 23 kg/m^2) and 6 obese volunteers (3 male and 3 female; aged 25−30 years, BMI > 30 kg/m^2) (the volunteers had no known health problems/conditions and did not take any medications in the past 6 months). The freshly collected fecal samples were immediately suspended in 0.9% sterile saline (100 mg/mL), homogenized, and centrifuged at 600 × *g* and 22 ℃ for 5 min (Eppendorf 5804R, Hamburg, Germany), to obtain the supernatants (suspensions of microorganisms, termed "fecal microbial suspensions"). After the 1-week antibiotic treatment, fifty mice were randomly divided into 5 groups (n = 10 mice per group): the normal control group (NC), obese group (OC), obesity + apple polyphenol group (OC + AP), obesity + phloridzin group (OC + PZ), and obesity + PB2 group (OC + PB2). Among them, the NC group was transplanted with the fecal microbiota from the healthy volunteers, and the OC, OC + AP, OC + PZ and OC + PB2 groups were transplanted with obese volunteers' fecal microbiota. To complete intestinal microbiome colonization, each recipient mouse was intragastrically given the corresponding fecal microbial suspension (10 μL/(g·day), 3 times a week, for 2 weeks). Mice had free access to food (standard chow) and water during FMT.

The commercial AP product, PZ compound and PB2 compound were constituted separately in sterile water. After 2 weeks of FMT, the mice were fed daily as follows: the NC group (a standard diet + 0.2 mL sterile water), the OC group (a standard diet + 0.2 mL sterile water), the OC + AP group (a standard diet + 0.2 mL of the aqueous AP solution (dose: 200 mg AP/kg body weight)), the OC + PZ group (a standard diet + 0.2 mL of the aqueous PZ solution (dose: 60 mg PZ/kg body weight)), the OC + PB2 group (a standard diet + 0.2 mL of the aqueous PB2 solution (dose: 30 mg PB2/kg body weight)). The sterile water, the aqueous AP solution, the aqueous PZ solution or the aqueous PB2 solution was given intragastrically to an experimental mouse on a daily basis (for 8 weeks).

The entire mouse trial lasted 12 weeks (Fig. 1). All the animal experiments were carried out in strict compliance with Chinese legislation guidelines on the use and care of laboratory animals, and approved by the Animal Care and Use Committee of Shandong Agricultural University (SDAU-ACU: 2007005).

2.3 Serum and sample collection

During the animal trial, the body weight and food intake of mice were recorded weekly. At the 12th week, the feces of the mice were collected and stored at −80 ℃. At the end of the animal trial, mice were deeply anesthetized with sodium pentobarbital, and blood samples were collected from their angular vein and placed into 1.5 mL centrifuge tubes. Centrifugation of the collected blood was performed at 3 000 × *g* and 4 ℃ for 15 min to obtain blood serum, and the serum samples were stored at −80 ℃.

All the mice were then sacrificed by cervical dislocation, and their organs and tissues, such as the liver, colon, and subcutaneous and epididymal fat, were quickly collected and weighed. About one

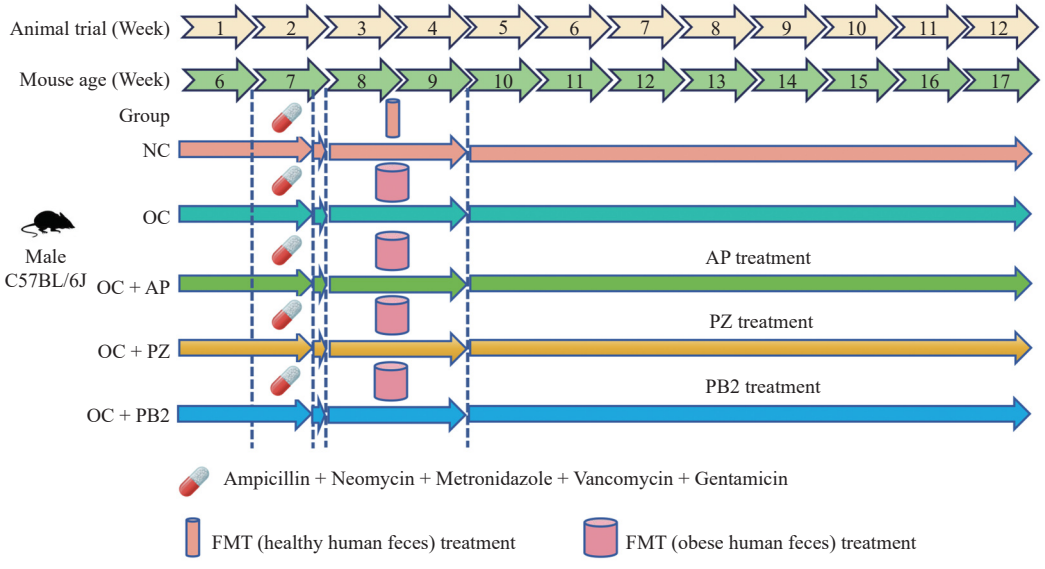

Fig. 1 Experimental design. NC: mice transplanted with healthy human fecal microbiota; OC: mice transplanted with obese human fecal microbiota; OC + AP: mice transplanted with obese human fecal microbiota + 200 mg/(kg·day) commercial AP product; OC + PZ: mice transplanted with obese human fecal microbiota + 60 mg/(kg·day) PZ compound; OC + PB2: mice transplanted with obese vhuman fecal microbiota + 30 mg/(kg·day) PB2 compound.

third of the epididymal adipose tissue, liver and colon tissue were fixed in 10% formalin buffer solution for histopathological analysis. A part of the liver tissue was placed in phosphate buffered saline (0.2 mol/L, pH 7.4) at a ratio of 1:9 (g/mL), and the mixture was subjected to homogenization then centrifugation at 3 000 × *g* and 4 °C for 15 min. The resulting supernatant was collected for analysis. The remaining epididymal adipose tissue, colon tissue and liver tissue were wrapped in foil, flash-frozen in liquid nitrogen, and stored at −80 °C for further use.

2.4 Biochemical analysis

2.4.1 Determination of serum biochemical indices

The levels of serum total cholesterol (TC), total triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), LPS, leptin, and adiponectin (ADPN) were measured by corresponding serum assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by corresponding commercial assay kits (Beyotime Biotechnology, Guangzhou, China) following the instructions of the manufacturer.

2.4.2 Determination of hepatic biochemical indices

The levels of hepatic TC and TG were measured by corresponding commercial assay kits, and the levels of inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β)) were measured by corresponding commercial assay kits (Shanghai Lengton Bioscience Co., Ltd., Shanghai, China). The activities/levels of superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA), ALT and AST were determined by corresponding commercial assay kits following the manufacturer's instructions.

2.5 Analysis of SCFAs by gas chromatography (GC)

The contents of SCFAs were measured following the procedure of Zhang et al.[10]. In brief, certain amounts (10−30 mg) of fecal samples were suspended in 25% aqueous methanol (fecal sample: aqueous methanol, 1:2.5 (*m*:*V*), before the homogenization at 65 Hz and 4 °C for 120 s then centrifugation at 12 000 × *g* and 4 °C for 15 min. The resulting supernatants were filtered respectively through membranes with a 0.22-μm pore size. An aliquot (100 μL) of each filtrate was used for analysis by a PerkinElmer gas chromatography-mass spectrometer (30.0 m × 0.25 mm, 0.25 μm, a polar DB-FFAP capillary column). In terms of chromatographic separation, high-purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min, and high-purity hydrogen (99.999%) was used as the auxiliary gas. The injection volume was 1 μL, and the split ratio was set to 10:1. Both the flame ionization detection temperature and the injection temperature were 250 °C. The temperature gradient was as follows: the initial temperature was 100 °C; increased at 5 °C/min to 180 °C; held at 180 °C for 2 min; further increased at 20 °C/min to 250 °C; held at 250 °C for more than 5 min. The amounts of SCFAs were determined using a mixture of individual commercial SCFAs as the external standard.

2.6 Histopathological analysis

The epididymal fat, liver and colon tissues were examined by hematoxylin and eosin (H&E) staining, after being fixed in 10% formalin, dehydrated with alcohol and embedded in paraffin. The embedded tissues were sectioned into 4-μm sample slices, dewaxed, stained and sealed. Examinations on these treated tissues were carefully performed under an optical microscope (Olympus-ix53, Tokyo, Japan). Histopathological analysis was conducted based on the degrees of inflammation, epithelial damage and steatosis.

2.7 Quantitative RT-qPCR analysis

Total RNA of the liver or colon tissue sample was extracted using the TRIzol reagent (Thermo Scientific, USA) following the manufacturer's instructions. The cDNA was synthesized using the PrimeScript RT Master Mix reagent kit (TaKaRa, Japan). The RT-qPCR analysis was performed following the procedure of Li et al.[38]. The relative levels of mRNA expression were determined by the $2^{-\Delta\Delta Ct}$ method with *GAPDH* being used as an internal reference gene. All the sequence-specific primers used for RT-qPCR are listed in Table S1.

2.8 Microbial community analysis by 16S rRNA sequencing

All the fecal samples from the mice were sent to MeiGe Biological Technology Co., Ltd. (Guangzhou, China) for 16S rRNA sequencing. Briefly, total DNA of each of the samples was isolated, and the microbial 16S rRNA gene sequence libraries were created using the two primers, 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′), on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The amplicon was prepared, pooled and quantified by the Illumina MiSeq platform. In order to ensure the accuracy of the analysis results, raw sequencing data were strictly filtered and spliced. Sequences with a similarity threshold of 97% were clustered and assigned to the same operational taxonomic units (OTUs). All offline data were collected and analyzed by MeiGe Biological Technology Co., Ltd. (Guangzhou, China).

2.9 Sequencing and analysis of gut viruses/phages

All the fecal samples from the mice were sent to MeiGe Biological Technology Co., Ltd. (Guangzhou, China) for virus sequencing. The fecal samples were pre-treated following the procedure of Dong et al.[33]. The total viral DNA was extracted and amplified using the Magen kits (R6662-02 MagPure Viral DNA/RNA Mini LQ Kit) and Qiagen kit (150054 REPLI-g Cell WGA & WTA Kit), respectively, following the procedure of Li et al.[39]. Sequencing libraries were constructed using an NEB Next® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA). The library quality was assessed using a Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY, USA) and an Agilent 4200 (Agilent, Santa Clara, CA, USA) system. Finally, the sequencing library was established using an Illumina NovaSeq 6000 platform, and 150 bp paired-end reads were generated[40-41]. The virome data analysis and phage host prediction were performed following the procedure of Dong et al.[33].

2.10 Statistical analysis

All obtained data were presented as mean ± standard deviation of three biological replicates and analyzed via One-way ANOVA using the SPSS 16.0 software. SigmaPlot 12.0 software was used to draw the graphs. A P value of 0.05 or 0.001 was regarded as statistically significant.

3. Results

3.1 AP, PZ or PB2 ameliorated TOPFM-induced obesity

The effects of the main components of the AP product (including PZ, PB2, chlorogenic acid (CGA), PT and epicatechin (EC)) on obesity in mice were examined during the preliminary experiments prior to the research reported here. The results of the body weight of mice showed that PZ and PB2 exhibited the greatest anti-obesity effects among the PZ, PB2, CGA, PT and EC (Fig. S1). Therefore, PZ and PB2 were chosen for further investigations reported in this paper. As shown in Fig. S2A, over 99% of the gut microbiota of the mice was removed after the antibiotic treatment. Moreover, the abundance of mouse's fecal microbiome after the FMT and the abundance of human fecal microbiome in the NC group or OC group differed insignificantly (Fig. S2B). The microbial community composition of mouse's fecal microbiome after the FMT (based on OTU) was similar to that of the human fecal microbiome in the NC group or OC group, respectively (Fig. S2C). The average body weights of the mice in the OC group were higher than those of the NC group after the first week of TOPFM (5 weeks of feeding), indicating that the model mice with TOPFM-induced obesity were successfully created. Moreover, the AP, PZ or PB2 supplementation significantly suppressed TOPFM-induced body weight gain, as these supplementations made the body weight markedly lower than those of the mice with TOPFM-induced obesity from the second week of the AP, PZ or PB2 supplementation (after 6 weeks of feeding) (Figs. 2A and B). The food intake of the OC group was much higher than the NC group, and the TOPFM-induced increase of food intake was significantly reduced by the AP, PZ or PB2 supplementation (Fig. 2C). As shown in Figs. 2D and E, the weights of subcutaneous fat and epididymal fat were remarkably higher in the OC group compared with the NC group, and the AP, PZ or PB2 supplementation considerably suppressed the TOPFM-induced accumulation of subcutaneous fat and epididymal fat. Images with H&E staining revealed that the TOPFM-induced cell expansion of epididymal fat was markedly suppressed by the AP, PZ or PB2 treatment, with the PB2 treatment being the most effective (Fig. 2F).

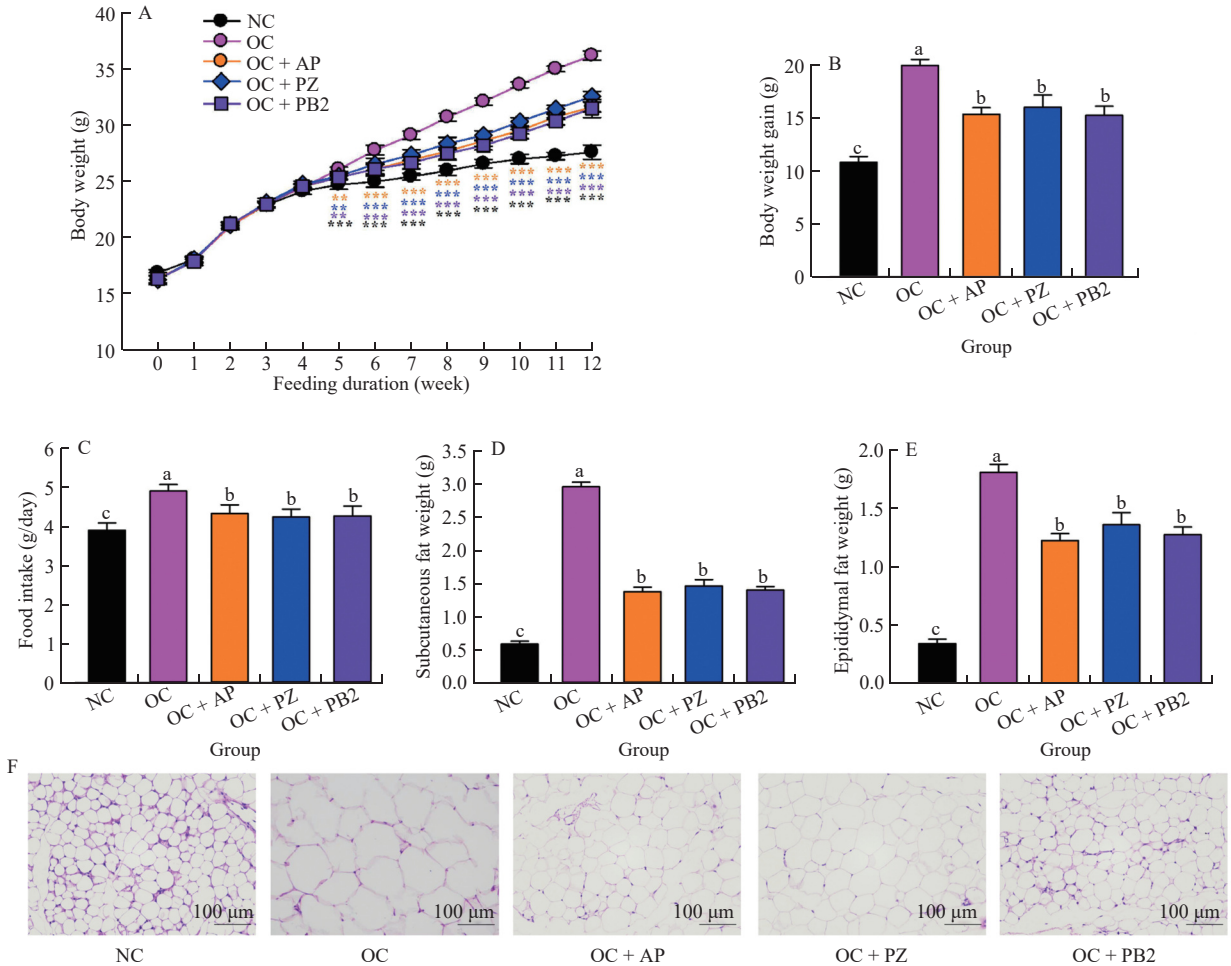

Fig. 2 AP, PZ or PB2 supplementation ameliorated TOPFM-induced obesity in mice. Effect of AP, PZ or PB2 on the body weight (A), body weight gain (B), food intake (C), subcutaneous fat weight (D), epididymal fat weight (E), and epididymal adipocyte stained with H&E (F). Different letters on the bars in the figure indicate $P < 0.05$.

3.2 AP, PZ or PB2 counteracted the abnormalities in the serum biochemical indices of the mice with TOPFM-induced obesity

Glucose metabolism and lipid metabolism are known in close association with obesity[42]. As shown in Figs. 3A-D, the OC group had significantly higher levels of fasting blood glucose, fasting blood insulin and homeostatic model assessed insulin resistance (HOMA-IR) but a lower level of homeostatic model assessed insulin sensitivity (HOMA-IS), compared with the NC group. However, such differences were significantly reduced by the AP, PZ or PB2 supplementation. Meanwhile, the increases in the serum TC, TG, LDL-C levels and decrease of the HLD-C level in the mice with TOPFM-induced obesity were significantly inhibited by the AP, PZ or PB2 supplementation. As for TC, TG, LDL-C and HDL-C, the counteracting effects provided by the PB2 treatment seemed greater than those exerted by the AP or PZ treatment (Figs. 3E-H).

Obesity is known in close association with hormonal derangements, including the changes in the levels of leptin and ADPN[43]. Herein, the level of leptin was higher in the OC group than in the NC group, and the AP, PZ or PB2 supplementation markedly suppressed the level of leptin (Fig. 3I). As for ADPN, the OC group had a considerably lower level than the NC group, but the presence of an AP, PZ or PB2 supplementation counteracted the decrease of ADPN level (Fig. 3J). LPS is one of the triggering factors that can promote the onset and progression of systemic inflammation[44] and the LPS level can be raised by HFD[45]. In this study, the LPS level in the OC group was higher than that in the NC group, but the AP, PZ or PB2 supplementation suppressed the increase of LPS level (Fig. 3K). As one of the key gut hormones, GLP-1 ameliorates obesity-induced metabolic syndromes by stimulating glucose-dependent insulin secretion, whilst GLP-2 is able to repair gut barrier and support barrier integrity[46]. In this study, the levels of serum GLP-1 and GLP-2 were lower in the OC group compared with the NC group. However, the presence of an AP, PZ or PB2 supplementation counteracted the deceases in these two hormones (Figs. 3L and M). As for ALT and AST, the OC group had considerably increased levels compared with the NC group, and such increases were suppressed effectively by the AP, PZ or PB2 supplementation (Figs. 3N and O).

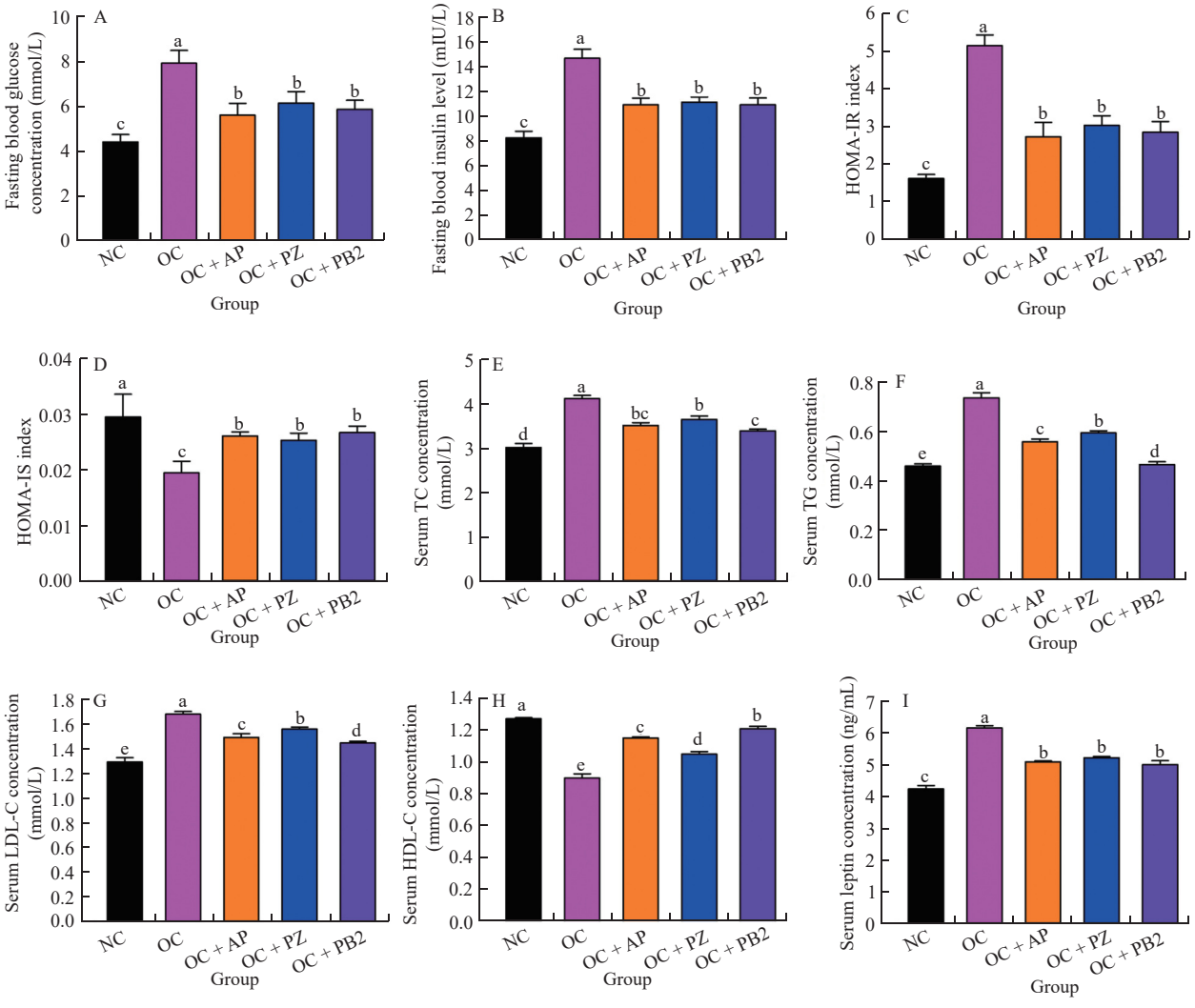

Fig. 3 AP, PZ or PB2 supplementation ameliorated TOPFM-induced abnormalities in serum biochemical indices of mice. (A) Fasting blood glucose, (B) fasting blood insulin, (C) HOMA-IR index, (D) HOMA-IS index. The levels of TC (E), TG (F), LDL-C (G), HDL-C (H), leptin (I), ADPN (J), LPS (K), GLP-1 (L), GLP-2 (M) in serum. The activities of ALT (N), and AST (O) in serum. Different letters on the bars indicate $P < 0.05$.

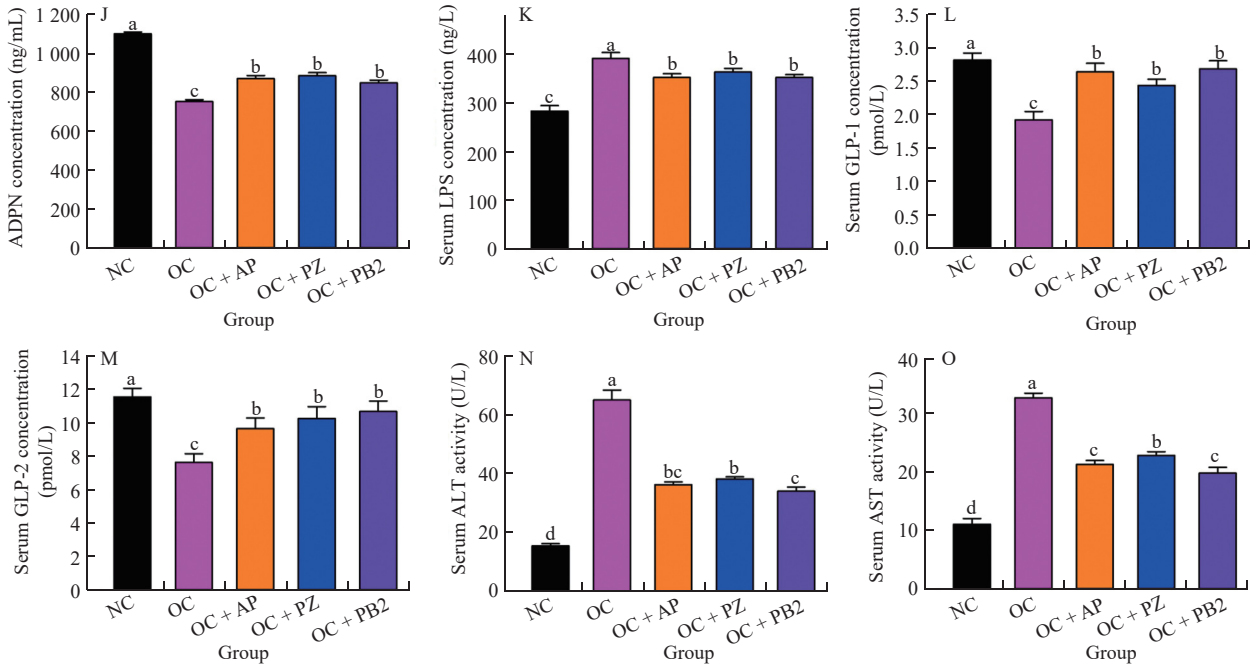

Fig. 3 (*Continued*)

3.3 AP, PZ or PB2 ameliorated hepatic inflammation of TOPFM-induced obesity in mice

As shown in Fig. 4A, the liver weight was significantly higher for the OC group than for the NC group, and such an obesity-induced increase in the liver weight was markedly suppressed by the AP, PZ or PB2 supplementation. Compared with the NC group, the fat droplets in the liver of the OC group were larger, indicating the occurrence of fatty liver. The presence of the AP, PZ or PB2 supplementation induced a counteracting effect against the fat droplet enlargement (Fig. 4B). In terms of TNF-α, IL-1β, TC, TG, AST and ALT, the OC group had higher levels than the NC group, but the AP, PZ or PB2 supplementation significantly inhibited the obesity-induced increases of these indices (Figs. 4C-H). As for the lipid synthesis-related proteins, including fatty acid synthase (*FAS*), acetyl-coenzyme A carboxylase 1 (*ACC-1*), sterol regulatory element

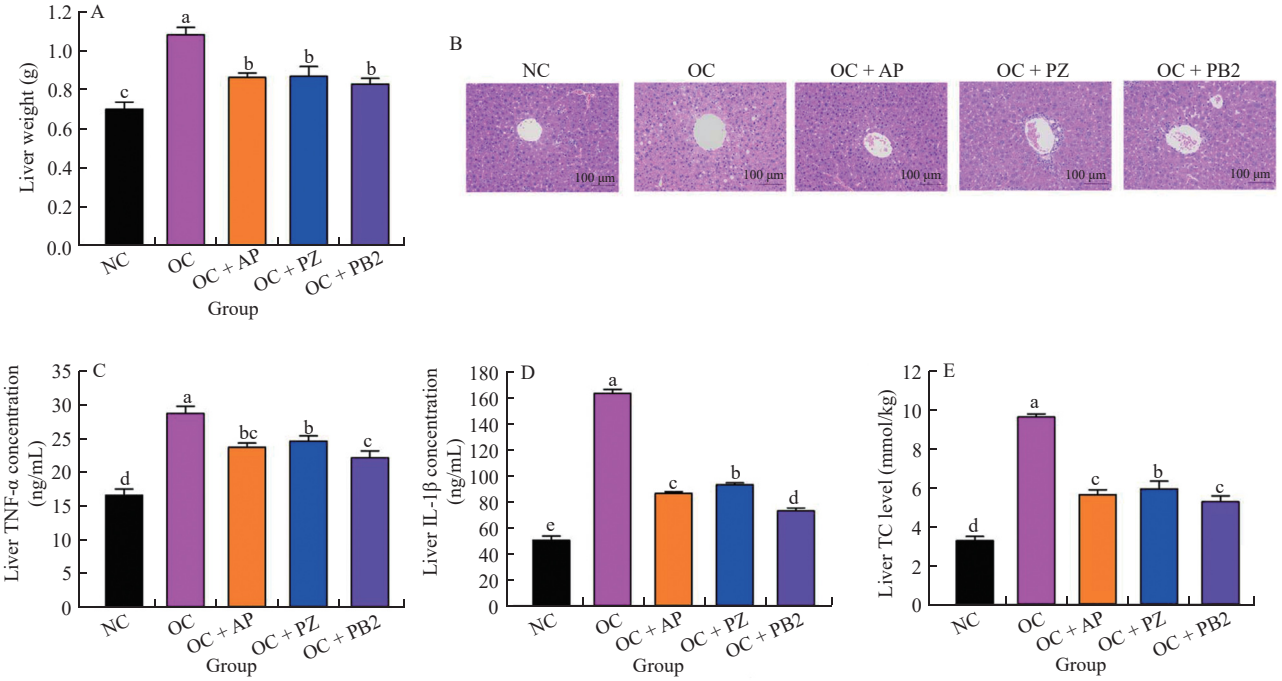

Fig. 4 AP, PZ or PB2 supplementation ameliorated TOPFM-induced liver injury in mice. (A) Liver weight. (B) Liver tissue stained with H&E. The levels of TNF-α (C), IL-1β (D), TC (E) and TG (F). The activities of AST (G) and ALT (H). The expression levels of *FAS* (I), *ACC-1* (J), *SREBP-1c* (K), *PPAR-γ* (L), *CYP7A1* (M), *LDLR* (N), *ASBT* (O) and *HMGR* (P). Different letters on the bars indicate $P < 0.05$.

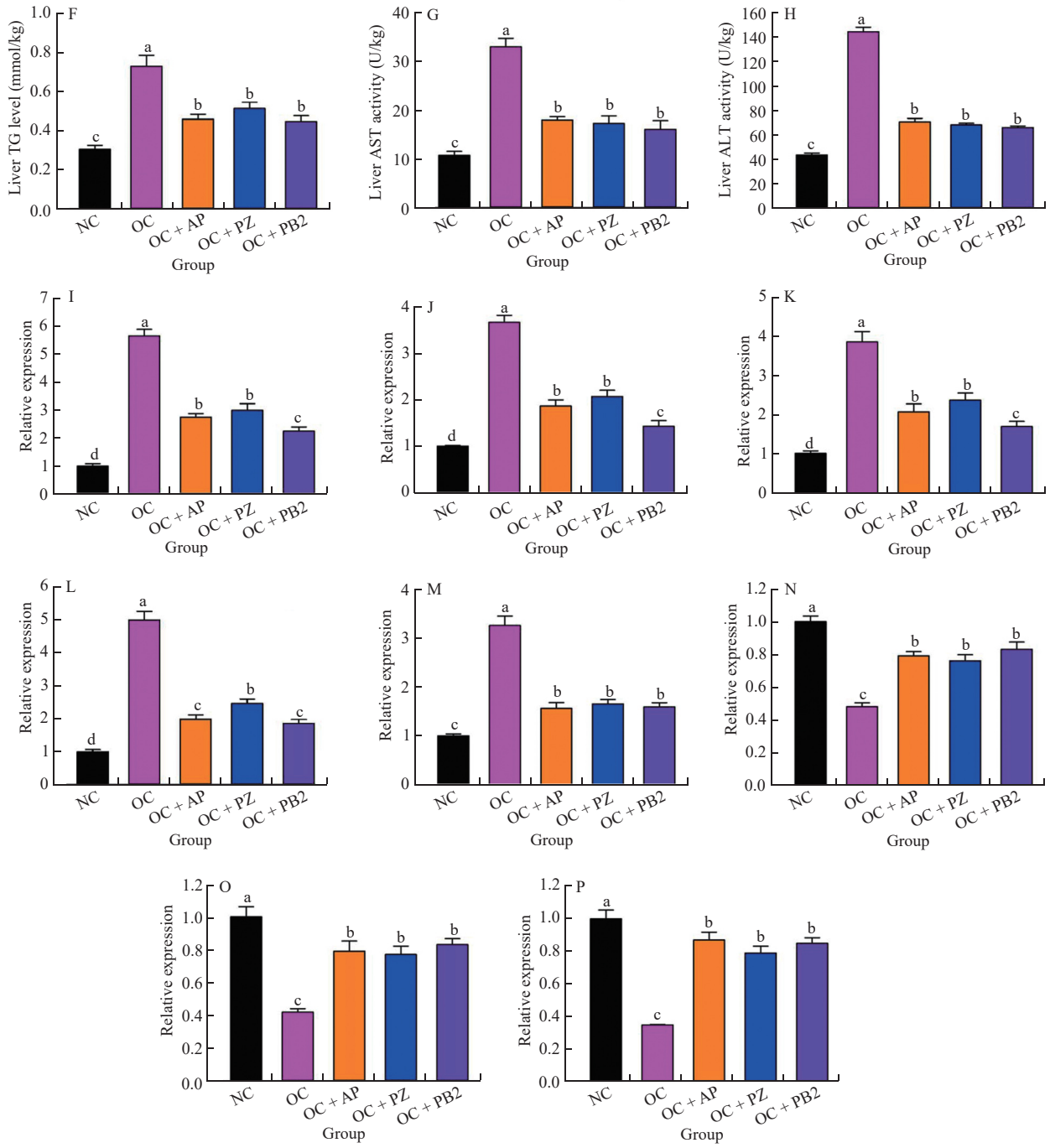

Fig. 4 (*Continued*)

binding protein-1c (*SREBP-1c*), peroxisome proliferators-activated receptor γ (*PPAR-γ*) and cholesterol 7*α*-hydroxylase (*CYP7A1*), remarkable increases in their gene expressions were detected in the OC group compared with the NC group, but such increases were greatly suppressed by the AP, PZ or PB2 supplementation (Figs. 4I-M). However, the gene expressions of lipid metabolism-related proteins, including low-density lipoprotein receptor (*LDLR*), apical sodium-dependent bile acid transporter (*ASBT*) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGR*), were considerably lower in the OC group compared with the NC group, but such obesity-induced lowering effects were ameliorated by the AP, PZ or PB2 supplementation (Figs. 4N-P).

3.4 AP, PZ and PB2 improved hepatic antioxidant capacity of the mice with TOPFM-induced obesity

The antioxidant capacity of the liver is one of the main factors counteracting hepatic inflammation[47]. Herein, considerable decreases in the activities of SOD, GSH, GSH-Px and CAT were detected in the OC mice compared with the NC group, but the AP, PZ or PB2 supplementation effectively counteracted such decreases (Figs. S3A-D). Moreover, the MDA content in the OC group was much higher than that of the NC group, and the AP, PZ or PB2 supplementation was able to suppress greatly such an obesity-induced increase of MDA level (Fig. S3E)

3.5 AP, PZ or PB2 ameliorated colon inflammation of the mice with TOPFM-induced obesity

As shown in Fig. 5A, the colon length was much shorter for the OC group than for the NC group, and the AP, PZ or PB2 supplementation markedly reduced such a shortening effect on the colon in TOPFM-treated mice. The examinations on the colons of mice revealed that the AP, PZ or PB2 supplementation significantly increased the length of intestinal villi and alleviated the separation of mucosal epithelium from lamina propria (Fig. 5B). In terms of the intestinal barrier function-related genes, considerable decreases in the expression levels of *ZO-1* and *Occludin* were detected in the OC group compared with the NC group, but such decreases in gene expression were counteracted by the AP, PZ or PB2 supplementation (Figs. 5C and D). As for the pro-inflammatory factor-related genes, such as those for *IL-6*, *TNF-α* and *IL-1β*, remarkable upregulation was found in the OC group compared with the NC group, but such upregulation related to the three pro-inflammatory cytokines was greatly suppressed by the AP, PZ or PB2 supplementation (Figs. 5E-G).

3.6 AP, PZ or PB2 attenuated TOPFM-induced gut microbial dysbiosis and metabolic changes

Gut microbiota plays a vital role in the onset and development of obesity, its comorbidities and other metabolic syndromes, thereby acting as an important regulator of obesity[48]. The composition of fecal microbiota in this study was analyzed by high-throughput sequencing. The principal coordinates analysis (PCoA) revealed the differences in the fecal microbiota structure among all the groups (Fig. 6A; PCo1 and PCo2 were 71.5% and 17.5%, respectively). The score plot of the OC group differed significantly from that of the NC group, and the AP, PZ or PB2 supplementation effectively counteracted the TOPFM-induced drastic shift in gut microbiota. These results clearly indicate that the AP, PZ or PB2 supplementation could influence significantly the gut microbiota. However, there were no statistically significant differences in the Chao1 index (one of the *α*-diversity indices) among all the groups, suggesting that the taxa richness was not changed significantly by the AP, PZ or PB2 supplementation (Fig. 6B).

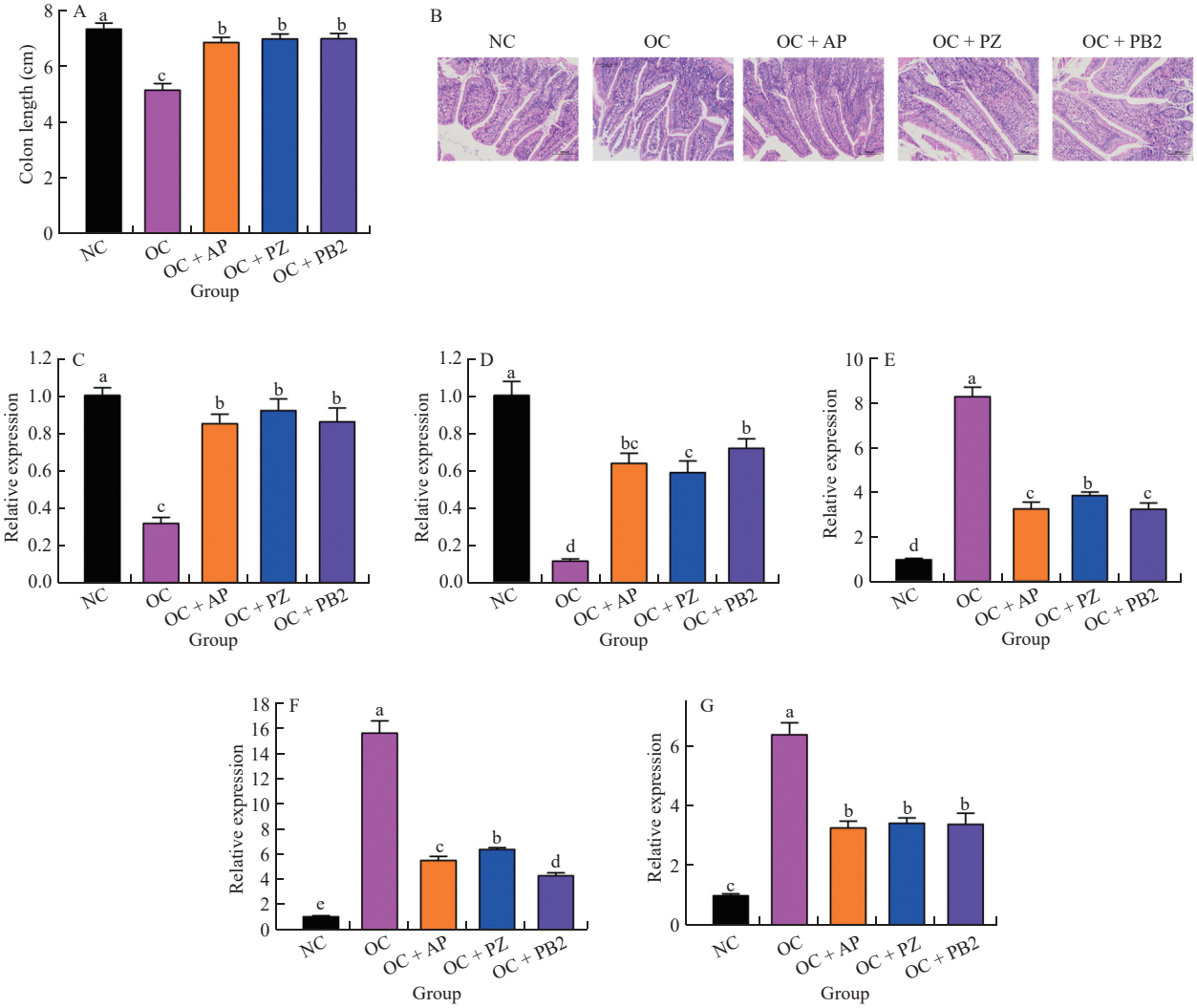

Fig. 5 AP, PZ or PB2 supplementation ameliorated TOPFM-induced colon inflammation in mice. (A) The length of colon. (B) The colon tissue stained with H&E. The expression levels of *ZO-1* (C), *Occludin* (D), *IL-6* (E), *TNF-α* (F), and *IL-1β* (G). Different letters on the bars indicate $P < 0.05$.

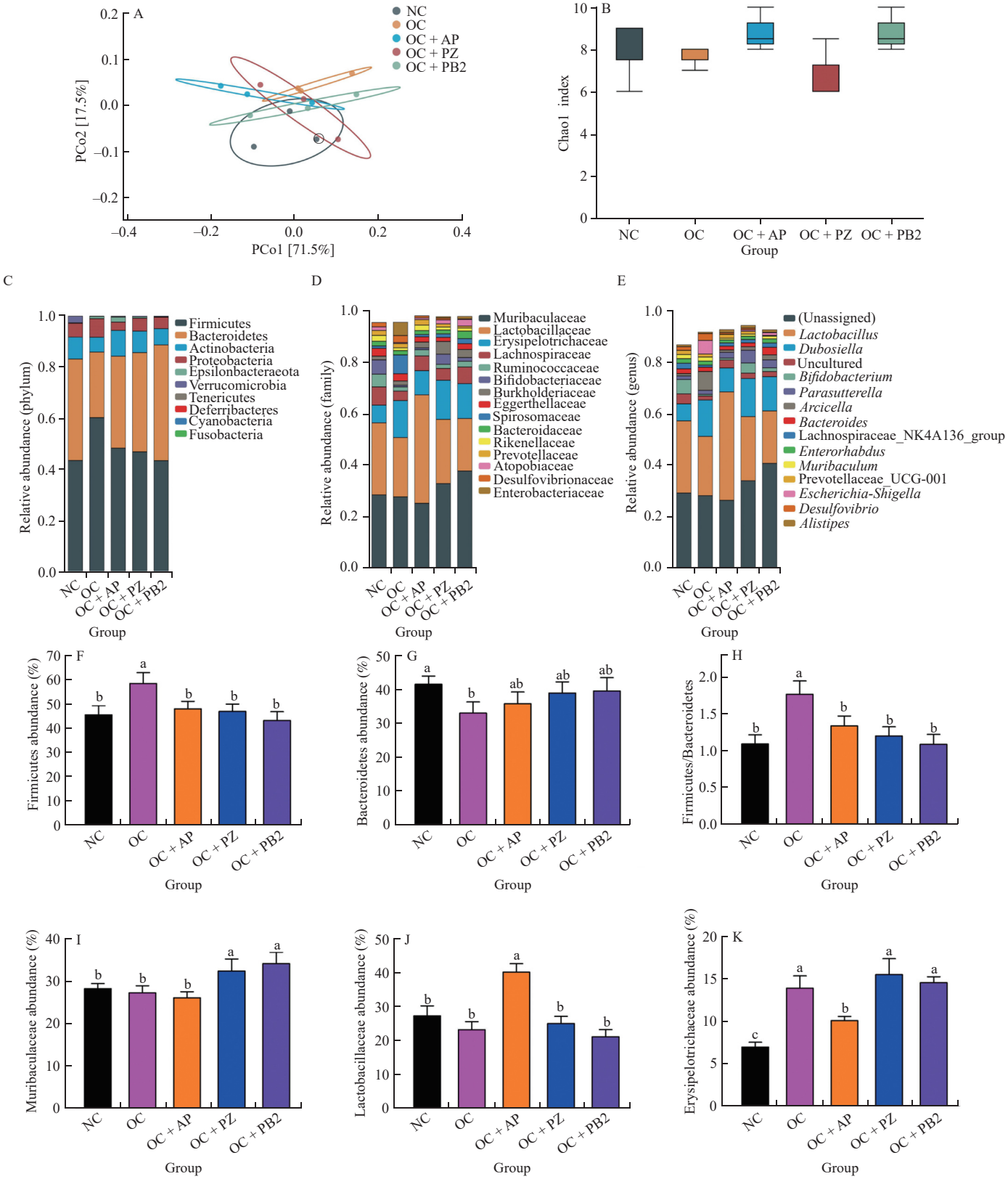

Fig. 6 AP, PZ or PB2 supplementation ameliorated TOPFM-induced gut microbiota dysbiosis in mice. (A) PCoA of fecal microbiota. (B) The Chao1 indice of the fecal microbiota. (C) Top 10 most abundant microbiota taxa at the phylum level. (D, E) Top 15 most abundant microbiota taxa at the family and genus levels. (F, G) The abundance of Firmicutes and Bacteroidetes in fecal microbiota at the phylum level. (H) The ratio of Firmicutes/Bacteroidetes in fecal microbiota. (I-N) The abundance of Muribaculaceae (I), Lactobacillaceae (J), Erysipelotrichaceae (K), Lachnospiraceae (L), Ruminococcaceae (M) and Bifidobacteriaceae (N) at the family level. Different letters on the bars indicate $P < 0.05$.

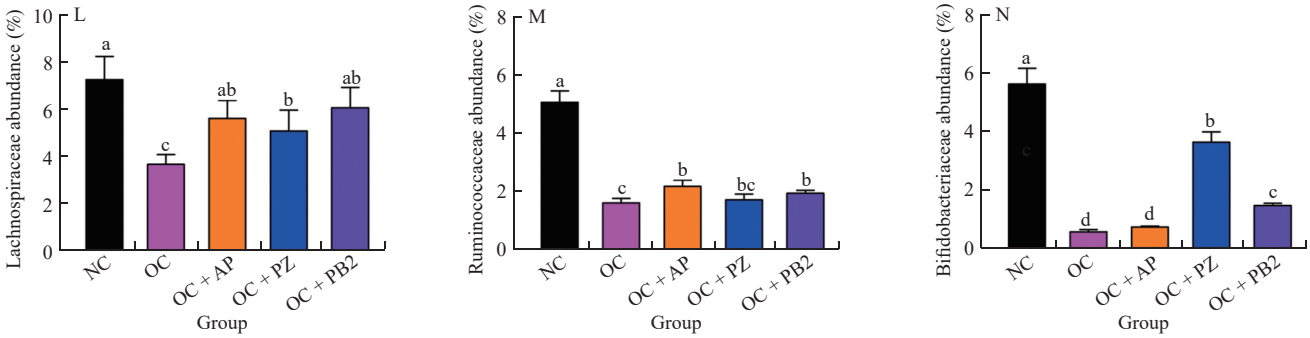

Fig. 6 (*Continued*)

To gain insights into the specific changes in the microbial communities, comparisons were made on the relative abundance at the phylum, family and genus levels among all the groups (Figs. 6C-E). At the phylum level, Bacteroidetes and Firmicutes were the main phyla for all the groups, accounting for over 80% of the total sequence. At the genus level, the OC group had lower relative abundance of *Lactobacillus* and *Bifidobacterium* compared with the NC group. The presence of an AP supplementation could counteract such a TOPFM-induced decrease in the relative abundance of *Lactobacillus*, whilst any of these 3 phenolic treatments could suppress the TOPFM-induced decrease in the relative abundance of *Bifidobacterium*. The relative abundance of *Arcicella* was higher in the OC group than in the NC group, and the AP, PZ or PB2 supplementation could impart a significant suppressive effect (Fig. 6E). Moreover, TOPFM raised the relative abundance of Firmicutes but decreased the relative abundance of Bacteroidetes, though such changes were counteracted by the AP, PZ or PB2 supplementation (Figs. 6F and G). The ratio of Firmicutes/Bacteroidetes (an important indicator of obesity) was significantly higher for the OC group than for the NC group, but the AP, PZ or PB2 supplementation could effectively make the ratio down towards that of the NC group (Fig. 6H). At the family level, there were no statistically significant differences in the relative abundance of Muribaculaceae and Lactobacillaceae between the OC and NC groups, but the PZ or PB2 supplementation could raise the relative abundance of Muribaculaceae whilst the AP supplementation was able to increase the relative abundance of Lactobacillaceae (Figs. 6I and J). The relative abundance of Erysipelotrichaceae was higher in the OC group compared with the NC group, and the AP supplementation could effectively suppressed such a TOPFM-induced increase of abundance, though either the PZ or PB2 supplementation exerted an insignificant effect (Fig. 6K). Moreover, the OC group had a much lower relative abundance of Lachnospiraceae, Ruminococcaceae or Bifidobacteriaceae compared with the NC group (Figs. 6L-N). While all the three phenolic treatments could effectively counteract the TOPFM-induced decrease in the relative abundance of Lachnospiraceae, the relative abundance of Ruminococcaceae could only be improved (significantly but slightly) by the AP or PB2 supplementation, and the relative abundance of Bifidobacteriaceae could only be improved significantly by the PZ or PB2 supplementation (Figs. 6L-N). Taken together, the influences of the AP, PZ or PB2 supplementation on TOPFM-induced changes in microorganisms were species-specific.

SCFAs were one major class of microbial metabolites produced by the gut microbiome. In this study, the changes in SCFAs were monitored over the 8 weeks of AP, PZ or PB2 supplementation (from the 12th week of feeding). As shown in Figs. 7A-E, the contents of total SCFAs, acetic acid, propionic acid, *n*-butyric acid and *i*-butyric acid were all lower in the OC group compared with the NC group, but the presence of an AP, PZ or PB2 supplementation counteracted effectively such TOPFM-induced decreases in these acid contents in mice. As for the contents of *n*-valeric acid and *i*-valeric acid, all the groups of mice essentially had the same amounts (Figs. 7F and G).

3.7 AP, PZ or PB2 attenuated TOPFM-induced changes of intestinal bacteriophages in mice

As shown in Fig. 8A, the Shannon index for the OC group was essentially the same as those for the NC, AP and PZ groups, but significantly higher than that of the PB2 group. The Shannon index of

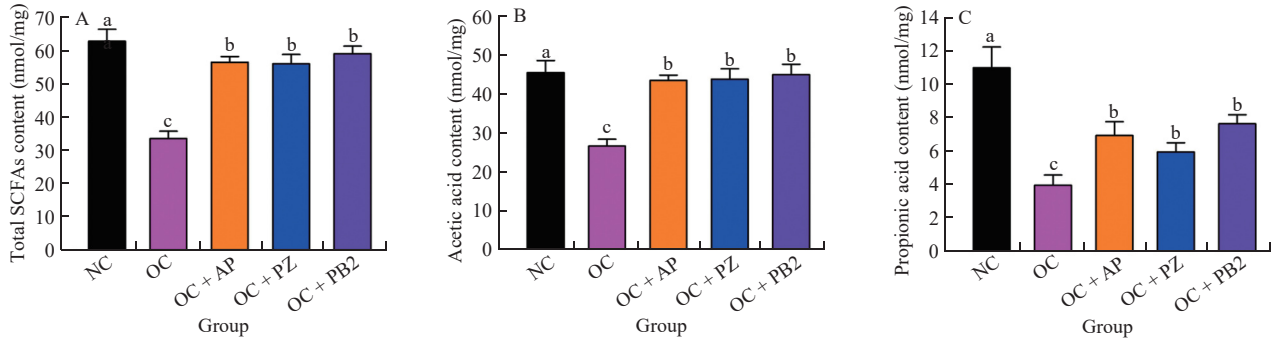

Fig. 7 AP, PZ or PB2 supplementation increased the contents of SCFAs. (A-G) The contents of SCFAs, (A) Total SCFAs, (B) acetic acid, (C) propionic acid, (D) *n*-butyric acid, (E) *i*-butyric acid, (F) *n*-valeric acid, and (G) *i*-valeric acid. Different letters on the bars indicate $P < 0.05$.

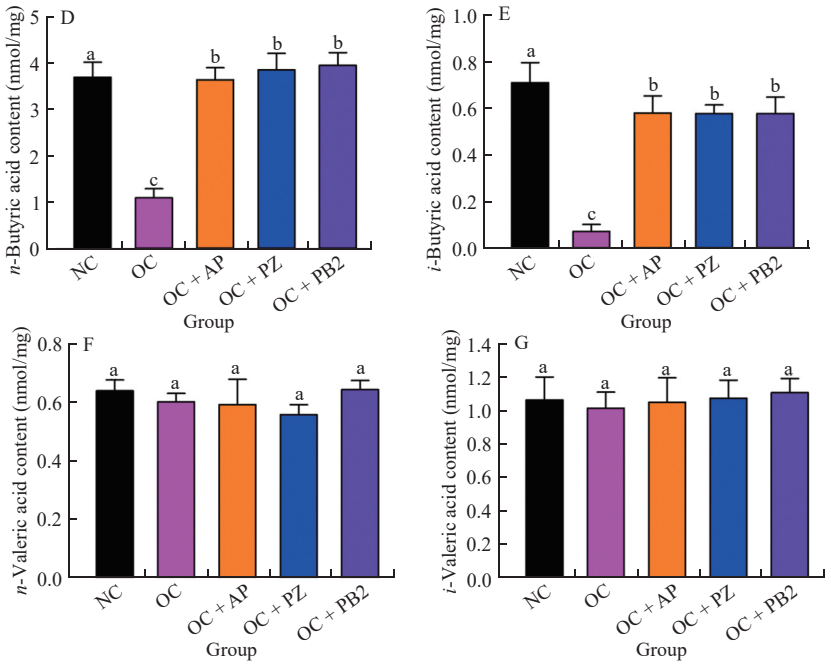

Fig. 7 (*Continued*)

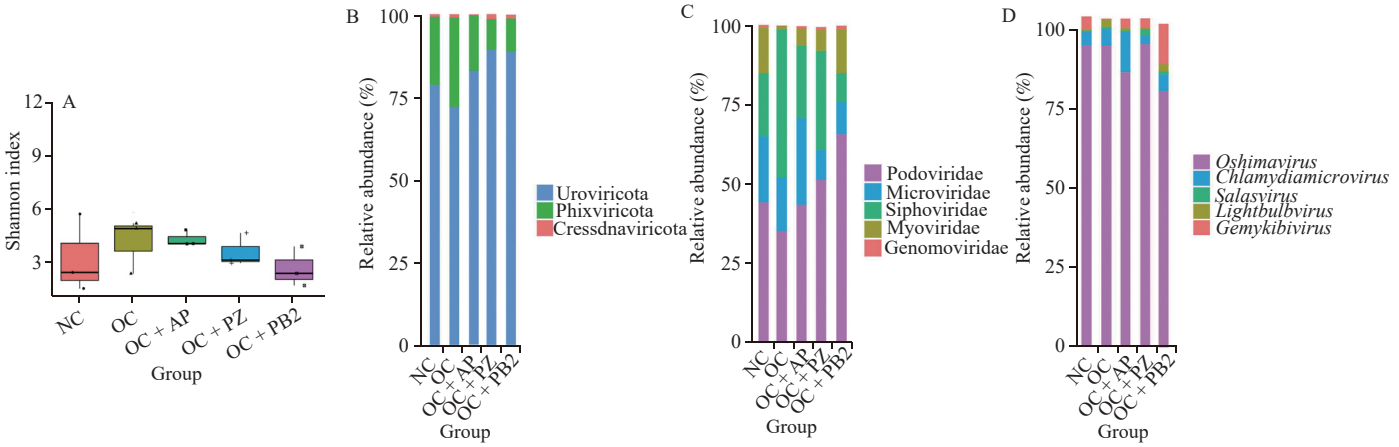

Fig. 8 AP, PZ or PB2 supplementation regulated the composition intestinal bacteriophages in mice. (A) The Shannon index of intestinal bacteriophages. (B-D) The intestinal bacteriophages at the phylum (B), family (C) and genus (D) levels.

the NC group differed insignificantly from the other 4 groups (Fig. 8A). At the phylum level (Fig. 8B), Uroviricota was the main phylum for all the groups of mice, accounting for over 70% of the total reads. The relative abundance of Uroviricota was lower in the OC group compared with NC group, but was increased by the AP, PZ or PB2 supplementation to that above the level of the NC group. The changes of the phylum, Phixviricota, exhibited an opposite trend in response to TOPFM and AP/PZ/PB2 supplementation. The relative abundance of Cressdnaviricota was very low, TOPFM tended to increase its relative abundance, and the AP supplementation seemed able to counteract such a TOPFM-induced increase (Fig. 8B). At the family level (Fig. 8C), decreases in the relative abundance of Podoviridae, Myoviridae and Genomoviridae were detected in the OC group compared with the NC group, but such decreases were counteracted by the AP, PZ or PB2 supplementation, with the AP supplementation increasing the relative abundance of Microbiridae, while the PZ or PB2 supplementation increasing the relative abundance of Podoviridae, to the levels above those of the NC group. As for the family Siphoviridae, opposite changing trends were detected in response to TOPFM and AP/PZ/PB2 supplementation. At the genus level (Fig. 8D), *Oshimavirus* was the most abundance phage. There were no significant differences in the relative abundance of *Oshimavirus* among the NC, OC and PZ groups, but the relative abundance of *Oshimavirus* was significant reduced in the presence of the AP or PB2 supplementation. *Chlamydiamicrovirus* had the second highest relative abundance, and TOPFM made its relative abundance increase slightly but insignificantly. For such a TOPFM-induced increase of abundance, the AP supplementation led to a large enhancement, the PZ treatment exerted a significant suppression, whilst the PB2 treatment exhibited no effect. The relative abundance of *Lightbulbvirus* was significantly higher in the OC group compared with the NC group, and such a TOPFM-induced increase of abundance was suppressed by the AP or PZ supplementation (but not by the PB2 supplementation). The relative abundance of

Gemykibivirus of the OC group was significantly lower than that of the NC group, and all the three phenolic treatments were able to counteract such a TOPFM-induced decrease, with the PB2 supplementation could even increase the abundance well above that of the NC group. *Salasvirus* had very little abundance, thus, the change in its abundance in response to TOPFM or AP/PZ/PB2 supplementation could hardly be distinguished.

The host range prediction revealed that at the family level, the top 10 most abundant species were Campylobacteraceae, Enterobacteriaceae, Moraxellaceae, Pseudomonadaceae, Xanthomonadaceae, Oscillospiraceae, Streptococcaceae, Lactobacillaceae, Bifidobacteriaceae and Lachnospiraceae (Fig. 9A). At the family level, there were significant differences in the relative abundance of Erysipelotrichaceae, Ruminococcaceae, Lachnospiraceae and Bifidobacteriaceae between the NC group and OC group (Figs. 6K-N). Accordingly, the phages related to the gut microbiota, Erysipelotrichaceae, Ruminococcaceae, Lachnospiraceae (11 species) and Bifidobacteriaceae (14 species), were selected (Figs. 9B and C). Most of the Lachnospiraceae and Bifidobacteriaceae phages had higher abundance in the OC group than in the NC group, especially the F_Lachnospiraceae. G_*Blautia*-phage1 and F_Bifidobacteriaceae. G_*Bifidobacterium*-phage8, and such TOPFM-induced increases in abundance were significantly suppressed by the AP, PZ or PB2 supplementation (Figs. 9B and C). Interestingly, the AP or PZ supplementation could increase greatly the abundance of F_Bifidobacteriaceae-phage-1, F_Bifidobacteriaceae-phage-2 and F_Bifidobacteriaceae-phage-3 (Fig. 9C). At the genus level, the OC group had remarkably lower abundance of *Blautia* and *Bifidobacterium* than the NC group, and such TOPFM-induced decreases could be alleviated by the AP, PZ or PB2 supplementation (Figs. 9D and E)

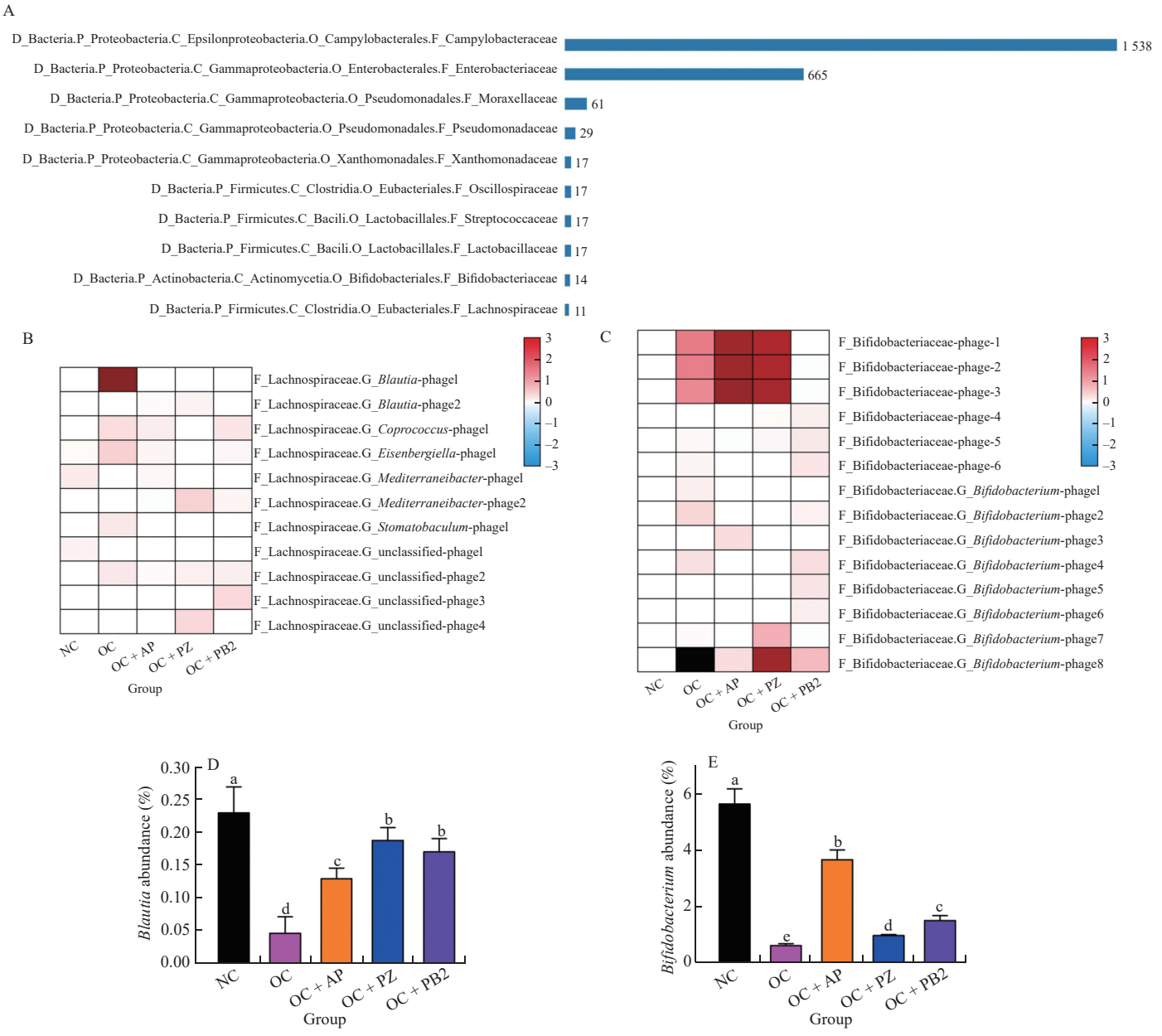

Fig. 9 AP, PZ or PB2 supplementation influenced the abundance of intestinal bacteriophages in mice. (A) Prediction of bacteriophages at the family level. The abundance of phages of Lachnospiraceae (B) and Bifidobacteriaceae (C). The abundance of *Blautia* (D) and *Bifidobacterium* (E).

4. Discussion

Previous research has shown that TOPFM could induce obesity in mice[49]. As indicated in the Introduction section, this research aimed to examine the effects of the commercial AP product, PZ compound or PB2 compound on TOPFM-induced obesity, while investigating the potential mechanisms, especially those involving gut barrier, intestinal phages and gut microbiota. Herein, the mice with TOPFM-induced obesity mice had significantly higher body weight, insulin resistance and accumulated fat content compared with the NC group, and the AP, PZ or PB2 supplementation could significantly counteract such TOPFM-induced increases (Figs. 2 and 3). The results were in agreement with the previously published findings that an AP or PZ supplementation could ameliorate HFD-induced obesity by reducing body weight and fat accumulation in mice[10,50], and that a PB2 supplementation could reduce high-fat-high-cholesterol diet-induced obesity in rabbits[23].

A growing body of evidence has shown that obesity could cause liver inflammation and abnormal lipid metabolism, and plant-derived phenolic compounds could exert anti-obesity effects through counteracting liver inflammation and lipid metabolism abnormalities[51-52]. Cranberry polyphenols could ameliorate liver inflammation and lipid metabolism abnormalities, while improving antioxidant capacity in the mice with high-fat/high-sucrose diet-induced[51]. Tea polyphenol, EGCG, could ameliorate liver inflammation and lipid metabolism disorders by regulating the expression of genes related to lipid synthesis/metabolism in the mice with HFD-obesity[53]. Complex plant extract rich in polyphenols could tackle HFD-initiated liver inflammation and abnormal lipid metabolism via inhibiting the activity of FAS in C57BL/6 mice[46]. In this study, the AP, PZ or PB2 supplementation markedly ameliorated TOPFM-induced liver inflammation and abnormal lipid metabolism via inhibiting the expression of genes related to lipid synthesis such as *FAS*, *ACC-1*, *SREBP-1c*, *PPAR-γ* and *CYP7A1*, promoting the expression of genes related to lipid metabolism such as *LDLR*, *ASBT* and *HMGR*, increasing the activities of SOD, GSH, GSH-Px and CAT, and reducing the content of MDA (Figs. 4 and S3). Therefore, the AP, PZ or PB2 supplementation can improve liver function by regulating the expression of genes related to lipid synthesis/metabolism while enhancing oxidation resistance.

Obesity is closely associated with the changes in the function and structure of gut microorganisms and their metabolites[7-8]. LPS contributes to the occurrence and development of systemic inflammation through going across the damaged intestinal barrier then circulating along with the blood to various organs[54]. Long-term systemic inflammation can lead to insulin resistance and glucose tolerance, which contribute greatly to the occurrence and development of obesity[10]. In this study, TOPFM caused the increase of LPS level, and such an increase was markedly suppressed by the AP, PZ or PB2 supplementation (Fig. 3). Moreover, microbial metabolites such as SCFAs can exert anti-obesity effects by protecting gut barrier integrity and improving physiological function[55]. HFD was reported to cause significant decreases in the levels of SCFAs, and various natural plant-based extracts could increase the contents of SCFAs, especially acetic acid, propionic acid and *i*-butyric acid[56-57]. Sugarcane polyphenols could increase the contents of SCFAs in a pig fecal fermentation model[56]. Bean polyphenols have been found to increase significantly the contents of SCFAs including butyric acid, propionic acid and acetic acid in mice[57]. In this study, TOPFM led to decreases in the contents of total SCFAs, acetic acid, propionic acid, *n*-butyric acid and *i*-butyric acid, but such decreases were counteracted by the AP, PZ or PB2 supplementation (Fig. 7).

Some hormones including GLP-1 and GLP-2 are modulated by SCFAs[58]. GLP-1 can stimulate glucose-dependent insulin secretion, and GLP-2 is known as a peptide hormone secreted by L cells for repairing disrupted intestinal barrier and inhibiting LPS leakage into the blood[59]. Previous studies showed that HFD caused significant suppression of GLP-1 and GLP-2 levels in mice, and the supplementation of GLP-1 mimetics (supaglutide) could markedly reduce HFD-induced body weight gain[10,46,59]. In this study, the levels of GLP-1 and GLP-2 were also significantly suppressed after TOPFM, and such suppression was alleviated by the AP, PZ or PB2 supplementation, which was consistent with the changes in the contents of SCFAs (Figs. 3 and 7). Previous research also showed that PZ could improve the GLP-1 and GLP-2 levels by increasing the contents of SCFAs in HFD-fed mice[10]. Furthermore, this study revealed that TOPFM inhibited significantly the expression of intestinal barrier function-related genes, *ZO-1* and *Occludin*, and the AP, PZ or PB2 supplementation could raise the transcript levels of *ZO-1* and *Occludin* (Figs. 5C and D). Similar results were obtained in previous studies. Black chokeberry polyphenols ameliorated HFD-induced intestinal barrier dysfunction via promoting *ZO-1* and *Occludin* expression[60]. In terms of the pro-inflammatory cytokines, *TNF-α*, *IL-1β* and *IL-6*, high levels of these cytokines were found in obese animals, which may be responsible for the development of insulin resistance and chronic inflammation[61]. Tea polyphenols were able to attenuate obesity by inhibiting the expression of *TNF-α*, *IL-1β* and *IL-6* in dogs[62]. In this study, TOPFM increased the transcript levels of *TNF-α*, *IL-1β* and *IL-6* in mice, and such increases were suppressed by the AP, PZ or PB2 supplementation (Figs. 5E-G). Therefore, the AP, PZ or PB2 supplementation can alleviate TOPFM-induced intestinal barrier dysfunction via suppressing the LPS level and the expression of *TNF-α*, *IL-1β* and *IL-6*, and increasing the levels of GLP-1, GLP-2, SCFAs, *ZO-1* and *Occludin*.

Previous studies have shown the intimate relationship between the gut microbiota and obesity[7-8]. SCFAs are the metabolites of gut microorganisms, therefore, the profile of SCFAs depends highly on gut microbiota[10]. Moreover, Lachnospiraceae, Ruminococcaceae, Eubacteriaceae, Bifidobacteriaceae, Enterobacteriaceae were found as the SCFA-producing bacteria[55,57]. In this study, after TOPFM, the relative abundance of Lachnospiraceae was significantly increased by the AP, PZ or PB2 supplementation, the relative abundance of Ruminococcaceae was markedly raised by the AP or PB2 supplementation, and the relative abundance of Bifidobacteriaceae was increased by the PZ or PB2 supplementation (Fig. 6). These results indicated that the AP, PZ or PB2 supplementation raised the contents of SCFAs by increasing the relative abundance of *Bifdobacterium*, Ruminococcaceae and Lachnospiraceae. The influences of the AP, PZ or PB2 supplementation on the gut microorganisms of the mice subjected to TOPFM were microbial species-specific.

As stated in the Introduction section, phages should be taken into account when the gut ecosystem is under investigation, especially when FMT is also involved[24-25]. The diversity and relative abundance of intestinal phages differ significantly between the obese patients and normal individuals[49]. Some phenolic compounds were able to inhibit phages[63]. Green tea polyphenols could alter the diversity and

the relative abundance of intestinal phages in mice[33]. This study was the first study to show that the intestinal phages could be regulated by the AP, PZ or PB2. At the family level, TOPFM caused the decreases in the relative abundance of Podoviridae, Myoviridae and Genomoviridae, and such decreases were counteracted by the AP, PZ or PB2 supplementation, with the family Siphoviridae showing an opposite trend (Fig. 8). Accordingly, the AP, PZ or PB2 supplementation could change the profile of intestinal bacteriophages. Moreover, the phages related to SCFA-producing bacteria (*Bifdobacterium*, Ruminococcaceae and Lachnospiraceae) were selected, including 11 Lachnospiraceae and 14 Bifidobacteriaceae phages. Compared with the NC group, the OC group had significantly higher relative abundance of F_Lachnospiraceae. G_*Blautia*-phage1 and F_Bifidobacteriaceae. G_*Bifidobacterium*-phage8, and such TOPFM-induced increases of abundance were suppressed effectively by the AP, PZ or PB2 supplementation (Figs. 9B-C). At the genus level, TOPFM caused decreases in the relative abundance of *Blautia* and *Bifidobacterium*, and the AP, PZ or PB2 supplementation alleviated such decreases (Figs. 9D and E). Therefore, the AP, PZ or PB2 supplementation raised the levels of SCFAs by increasing the relative abundance of *Blautia* and *Bifidobacterium* possibly via inhibiting the relative abundance of *Blautia*- and *Bifidobacterium*-related phages. Fig. 10 shows the possible mechanisms underlying the anti-obesity effects of the AP, PZ and or PB2 supplementation.

The "phage, gut microbiota and gut barrier" route (including the intestinal bacteriophages and gut microbiota inhabiting there) and hepatic inflammation (along with the lipid metabolism and oxidation resistance events) might be the alternative targets for the beneficial action of the AP product, PZ compound or PB2 compound. The involved intestinal barrier function-related genes included *ZO-1* and *Occludin*. The involved pro-inflammatory cytokines included *IL-6*, *TNF-α* and *IL-1β*. The changes in SOD, GSH, GSH-Px, CAT and MDA were involved in the counteracting effect against the rising oxidative stress. Lipid synthesis-related genes (*FAS*, *ACC-1*, *SREBP-1c*, *PPAR-γ* and *CYP7A1*) and metabolism-related genes (*LDLR*, *ASBT* and *HMGR*) might also participate in the actions of the AP product, PZ compound or PB2 compound.

5. Conclusions

In this study, the supplementation of a commercial AP product, PZ compound or PB2 can could ameliorate TOPFM-induced obesity in mice by alleviating insulin resistance and liver inflammation, regulating gut microecology, and improving gut barrier function. The alleviation of insulin resistance was achieved by increasing the GLP-1 level and decreasing the LPS level. Amelioration of liver inflammation was realized by regulating the expression of the genes related to lipid synthesis/metabolism while increasing oxidation resistance. The gut barrier function was improved through regulating the expression of the intestinal pro-inflammatory cytokines, *TNF-α*, *IL-1β* and *IL-6*, and the gut barrier function-related genes, *ZO-1* and *Occludin*, and raising the GLP-2 level via increasing the contents of SCFAs. Furthermore, the AP, PZ or PB2 supplementation could increase the levels of SCFAs in the mice with TOPFM-induced obesity through restoring the microbial community structure and diversity, in particular, increasing significantly the abundance of Lachnospiraceae and Bifidobacteriaceaca possibly by inhibiting the relative abundance of *Blautia* and *Bifidobacterium* phages. The influences of the AP, PZ or PB2 supplementation on the gut microorganisms and associated phases of the mice subjected to TOPFM were species-specific. Taken together, there might be close relationships among the gut microbiota, gut phages and the production of SCFAs. The AP, PZ and PB2 supplementation used in this study could alleviate TOPFM-induced obesity by regulating the gut barrier, gut microbiota and gut phages. This study was the first

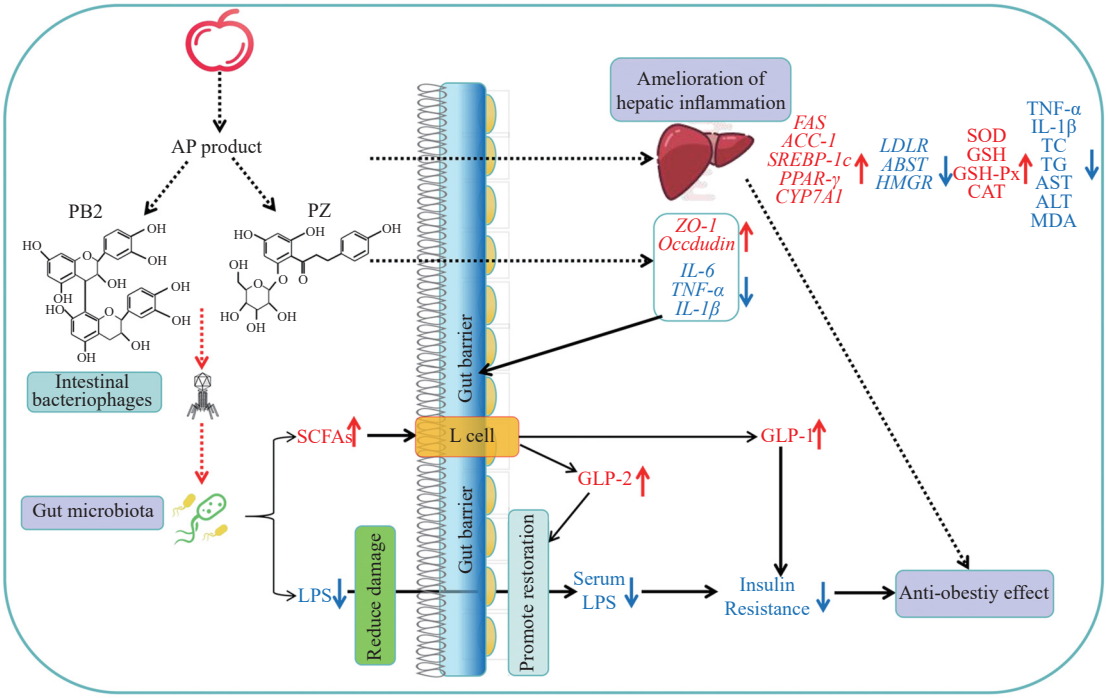

Fig. 10 Schematic diagram showing the possible mechanisms underlying the anti-obesity effects of the supplementation of the AP product, PZ compound or PB2 compound.

report on the ability of an AP, PZ or PB2 supplementation to change the profiles of gut phages and gut microbiota, and the possibility of manipulating gut phages to modulate gut microbiota in obese individuals through an AP, PZ or PB2 supplementation.

Conflict of interest

Dongxiao Sun-Waterhouse is a senior editor for *Food Science and Human Wellness* and was not involved in the editorial review on the decision to publish this article. The authors declare that they have no conflict of interest.

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Data Availability Statement

Data will be made available on request.

Ethics statement

All animal experiments were carried out in strict accordance with Chinese legislation guidelines on the use and care of laboratory animals, and approved by the Animal Care and Use Committee of Shandong Agricultural University (SDAU-ACU: 2007005).

Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250266.

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