Polygonatum sibiricum F. Delaroche polysaccharide ameliorates HFD-induced mouse obesity via regulation of lipid metabolism and inflammatory response

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Abstract. The present study sought to elucidate the role of *Polygonatum sibiricum F. Delaroche* polysaccharide (PSP) in high-fat diet (HFD)-induced mouse obesity and investigated the primary molecular mechanism underlaying these effects. An obese mouse model was established by feeding HFD and three doses of PSP were administered intragastrically. Changes in body weight, serum lipids and parameters were recorded and the mechanism was explored by reverse transcription-quantitative PCR and western blotting. Body weight, blood lipids, blood glucose, insulin, resistin, adiponectin, liver weight and abdominal fat pads weight were reduced by PSP and abnormal expression levels of inflammatory factors such as TNF-α, IL-6, IL-1β and iNOS and lipid metabolism genes such as FAS, SREBP-1, PPARα and CPT-1were also reversed by PSP. The 5' adenosine monophosphate-activated protein kinase (AMPK) signaling pathway was activated in PSP mouse liver, leading to lipid-lowering and anti-inflammatory effects. The results therefore suggested that PSP exhibited lipid-lowering and anti-inflammatory effects by activating the AMPK signaling pathway.

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Introduction

Obesity, which is defined as a body mass index >30 kg/m^2, has become one of the major public health problems worldwide and its prevalence has increased in recent years. The World Health Organization reported that the total number of obese individuals is four times the number 30 years ago (1). Obesity and overweight are known to adversely affect human health and the risks and prognosis for a number of serious medical conditions, such as type 2 diabetes and cardiovascular disease, liver diseases, cardiovascular diseases and even cancer (2,3).

Obesity involves pathological changes in a number of tissues, such as adipose tissue and liver. Expanded visceral fat deposits are also linked to dyslipidemia, which is characterized by high triacylglycerol (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and insulin resistance, which are often accompanied by impaired metabolic regulation in adipose tissue, leading to an over-production of free fatty acids (FFAs) (4). The flux of FFAs toward the liver results in increased TG deposition and secretion of TG-rich lipoproteins, which in turn affect lipolipase lipase (LPL) activity and the distribution of lipoprotein subtypes (5).

Currently, several drugs, such as sibutramine and orlistat, have been approved by the Food and Drug Administration of the United States, and have been applied for long-term use to prevent obesity (6). Sibutramine increases energy consumption and reduces fat accumulation by inhibiting the re-uptake of monoamine signaling elements (7). Orlistat is a gastrointestinal lipase inhibitor that inhibits intestinal triglyceride absorption (8). However, these drugs inevitably cause side effects such as uneven cardiac rhythm and chest pain, which restrict their clinical application (9).

Polysaccharides are widely distributed in nature: Plants, animals and fungi are valuable sources of polysaccharides. Several types of polysaccharides have been determined to exert multiple functions, such as anti-inflammatory, anti-hyperlipidemic and anti-hypertension effects (10). Among these functions, the lipid-lowering effect of polysaccharides has become a focus of research in recent years. Polysaccharides from soybean (11), flaxseed (12) and *Momordica charantia* (13)

have been demonstrated to be effective in treating or preventing obesity.

Polygonatum sibiricum F. Delaroche (P. sibiricum) is a perennial herb of the Asparagaceae family. It is mainly distributed in the Northern Hemisphere, including China, Mongolia, North Korea and the USA (14). *P. sibiricum* is a well-known traditional medicinal herb in China (15), as well as a health-improving substance (16). *P. sibiricum* has been widely used in a number of Chinese herbal medicine compounds treating different diseases as recorded in *Ben Cao Gang Mu* (*Compendium of Materia Medica*) and its effects in treating diabetes (17), cardiovascular and cerebrovascular diseasess18), somnipathy (19), dyslipidemia and pathological fatigue (20) have also been determined by modern medical methods.

A number of the functional components of *P. sibiricum* have been documented, including polysaccharides, phytosterols, triterpenoids, alkaloids, lignans, flavonoids and phytosterols (21). Among them, polysaccharides and phytosterols are the most abundant medicinal components. *P. sibiricum* polysaccharide (PSP) is mainly composed of galactose, mannose, glucose and galacturonic acid, in molar ratios of 29.63:36.10:15.09:10.20 (22). PSP has been verified to exert a variety of medicinal functions, including anti-inflammatory (23) and anti-tumor effects (24), lowering blood glucose (25), enhancing immunity and promoting the differentiation of bone cells (20).

To investigate if PSP ameliorates high-fat diet (HFD)-induced obesity mice were fed an HFD to establish a high-fat model and PSP was administered intragastrically. The molecular mechanisms were investigated by reverse transcription-quantitative PCR (RT-qPCR) and western blotting.

Materials and methods

Reagents. PSP (purity 90%) were obtained from Shaanxi Yikanglong Biotechnology Co., Ltd. Triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) kits were obtained from Nanjing Jiancheng Bioengineering Institute. The glucometer was from Sannuo Bio-Sensing Technology Co., Ltd., TRIzol® was obtained from Invitrogen; Thermo Fisher Scientific, Inc. and high-capacity cDNA reverse transcription kits were obtained from Applied Biosystems (Thermo Fisher Scientific, Inc.). Antibodies against fatty acid synthase (FAS; cat. no. 4233), phosphorylated (p-) 5' adenosine monophosphate-activated protein kinase (AMPK)α (Thr172; cat. no. 50081), AMPKα (cat. no. 5832), p-acetyl-coenzyme A carboxylase (ACC; Ser79; cat. no. 11818), TNF-α (cat. no. 11948), IL-6 (cat. no. 12912), IL-1β (cat. no. 31202), inducible nitric oxide synthase (iNOS; cat. no. 13120), β-actin (cat. no. 4970), carnitine palmitoyl transferase I (CPT1; cat. no. 12252) were purchased from Cell Signaling Technology, Inc. The peroxisome proliferator-activated receptor (PPARα; cat. no. ab245119) antibody was purchased from Abcam. The sterol regulatory-element binding protein (SREBP-1; cat. no. PA1-337) antibody and the HRP-conjugated goat anti-rabbit IgG (cat. no. 31460) antibody were obtained from Thermo Fisher Scientific, Inc. RIPA Lysis and Extraction

Buffer was obtained from Beyotime Institute of Biotechnology and protease and phosphatase inhibitors were obtained from Roche Diagnostics. Polyvinylidene fluoride (PVDF) membranes were obtained from Cytiva. An enhanced chemi-luminescence (ECL) detection kit was purchased from Pierce (Thermo Fisher Scientific, Inc.).

Experimental animals. A total of 50 eight-week-old C57BL male mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. The mice were housed in a constant temperature and humidity animal room at 23±2°C and a relative humidity of 40-45%, with a 12-h light/dark cycle, and were acclimated for 7 days. Prior to the animal experiments, the mice were fed a normal diet (Hunan SJA Laboratory Animal Co., Ltd.) and provided water *ad libitum*. The 50 mice were randomly divided into 5 groups: A normal diet (control) group; a high fat diet (HFD) group and the three PSP groups (mice separately received 200, 400 and 800 mg/kg of PSP daily by intragastric administration for 3 days before HFD feeding and for the whole experiment). The animal body weights and food intake were recorded weekly. The care and use of the animals and experimental protocols complied with the Guidelines for the Care and Use of Experimental Animals of Hunan University of Chinese Medicine. The project was approved by the Office of Animal Experiment Ethics of Hunan University of Chinese Medicine (approval no. 20190165). At the end of the experiments, the mice were fasted for 12 h and sacrificed with CO_2 for 5 min as recommended by Pritchett *et al* (26). The CO_2 flow rate was 40% chamber volume/min. Blood samples were gathered from the eye for glucose test and biochemical analysis. The liver and epididymal fat pad were immediately removed, weighed, imaged, flash frozen in liquid nitrogen and stored at -80°C for further experiments.

According to the results of body weight changes, the body weights of the 400 and 800 mg/kg PSP groups were significantly lower compared with the HFD group. However, no significant difference was observed between these two groups. Therefore, the 400 mg/kg PSP group was chosen for further research.

Biochemical analysis. Blood samples were placed in refrigerator at 4°C overnight and then centrifuged at 1,000 x g for 15 min at 4°C. The supernatants were collected as plasma samples. TG, TC, LDL-c and HDL-c levels were measured by commercial assay kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer's instructions. Serum adiponectin (cat. no. MRP300), leptin (cat. no. MOB00B) and resistin (cat. no. MRSN00) levels were measured by ELISAs (R&D Systems, Inc.), and insulin levels were measured using a mouse insulin-1 ELISA kit (Sigma-Aldrich; Merck KGaA; cat. no. RAB0327).

Histopathological examinations. Samples (epididymal fat pads and liver tissues) were fixed with 10% neutrally buffered formalin for 2 days at room temperature, dehydrated in an ascending series of alcohol, and then embedded in paraffin by the paraffin embedding machine for 16 h. Paraffin-embedded samples were then cut into 5 μm sections. The sections were then deparaffinized with xylene, rehydrated in a descending series of alcohol, rinsed with distilled water for 1 min and

Table I. Primers sequences used in reverse transcription-quantitative PCR.

Gene	Forward	Reverse
β-actin	5'-ATCATGTTTGAGACCTTCACACC-3'	5'-TAGAGCAACATAGCACAGCTTCTCTT-3'
Peroxisome proliferator-activated receptor α	5'-TAAAGTACGGTGTGTATGAAGCCAT-3'	5'-ATGTAGCCTATGTTTAGAAGGCCAG-3'
Sterol regulatory-element binding protein-1C	5'-GCCATCGACTACATCCGCTTCTT-3'	5'-TGGGCTTTGACCTGGCTATCCTC-3'
Fatty acid synthase	5'-AGCACTGCCTTCGGTTCAGTC-3'	5'-AAGAGCTGTGGAGGCCACTTG-3'
Carnitine palmitoyltransferase I	5'-CAGCTCGCACATTACAAGGA-3'	5'-TGCACAAAGTTGCAGGACTC-3'
IL-1β	5'-GCAACTGTTCCTGAACTCAACT-3'	5'-ATCTTTTGGGGTCCGTCAACT-3'
TNF-α	5'-CCCTCACACTCAGATCATCTTCT-3'	5'-GCTACGACGTGGGCTACAG-3'
IL-6	5'-TAGTCCTTCCTACCCCAATTTCC-3'	5'-TTGGTCCTTAGCCACTCCTTC-3'
Inducible nitric oxide synthase	5'-CCTTACGAGGCGAAGAAGGACAG-3'	5'-CAGTTTGAGAGAGGAGGCTCCG-3'

Data are expressed as the mean ± standard deviation (n=6). PSP, *Polygonatum sibiricum F. Delaroche* polysaccharide; HFD, high-fat diet.

stained with hematoxylin (20 min) and eosin (1 min) (H&E) at room temperature. Images were captured under a light microscope (magnification, x200) in five randomly selected fields of view.

RNA isolation and RT-qPCR. Total RNA was isolated from the liver tissues by using RNA extract reagent (TRIzol®). A total of 2 µg of RNA was reverse transcribed into cDNA with cDNA reverse transcription kits (Thermo Fisher Scientific, Inc.) at 42°C for 30 min and then 80°C for 10 min for enzymatic inactivation. qPCR was performed on a CFX96 Real Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the TransStart Green qPCR SuperMix UDG kit (Beijing Transgen Biotech Co., Ltd.; cat. no. AQ111-01). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95°C for 1 min; followed by 40 cycles of amplification (94°C for 30 sec, 58°C for 25 sec and 72°C for 1 min), followed by an extension at 72°C for 3 min. The primer sequences were described in our previous publication and are shown in Table I (27). The $2^{-\Delta\Delta Cq}$ method was used for quantification of the expression levels (28). All these methods were according to the manufacturer's protocols.

Western blot analysis. The liver tissues were homogenized in RIPA Lysis and Extraction Buffer (Beyotime Institute of Biotechnology) on ice for 30 min and then the suspension was centrifuged at 12,000 x g for 5 min at 4°C. The protein concentration was determined using a BCA Protein assay kit (Abcam). The protein-containing supernatants were equally (15 µg) subjected to 10% SDS-PAGE and then transferred onto a PVDF membrane. The membrane was first blocked with 0.1% TBS-Tween 20 (TBST) containing 5% skimmed milk at room temperature for 1 h, followed by incubations with the following primary antibodies: Anti-PPARα (1:2,000), anti-SREBP-1C (1:2,000), anti-FAS (1:2,000), anti-CPT-1 (1:2,000), anti-IL-1β (1:2,000), anti-TNF-α (1:2,000), anti-IL-6 (1:2,000), anti-iNOS (1:2,000) and β-actin (1:5,000) overnight at 4°C. Subsequently the membranes were washed in

TBST (3x15 min) and incubated with secondary antibodies (1:10,000) at room temperature for 1 h. Finally, the membrane was developed with an ECL Plus™ western blotting detection system. The images were captured using an imaging system (ChemiDoc™ XRS+, Bio-Rad Laboratories, Inc.). The densitometric analysis was performed using Adobe Photoshop 7.01 (Adobe Systems, Inc.).

Statistical analysis. SPSS v13.0 statistical software (SPSS, Inc.) was used to analyze the data which were expressed as the mean ± standard deviation. A one-way ANOVA was used for statistical analyses among three groups with the Tukey HSD post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

PSP prevents HFD-induced obesity. To evaluate whether PSP prevents HFD-induced obesity, the mice were administered PSPs as described in the methods section. The body weights of mice in the control group increased steadily up to the end of the experiment. The body weights of the HFD group mice increased quickly from 25.33±0.44 g at the beginning to 34.46±0.89 g on week 7 and then plateaued until the end of the experiment. The body weights of the different PSP dosages increased more slowly than those of the HFD group, as the 200 mg/kg PSP group increased from 25.42±0.46 to 34.97±0.88 g, which was lower compared with the HFD group (P<0.05), while the 400 mg/kg PSP group was from 25.70±0.46 to 33.19±0.73 g and the 800 mg/kg PSP group was from 25.55±0.48 to 32.81±0.71 g, all lower compared with the HFD group (P<0.01), However, no significant difference was observed between these two groups. Thus, the 400 mg/kg PSP group was chosen for further experiments and this group was defined as the PSP group hereafter (Fig. 1A).

The average food intake of control group increased steadily and slowly, while the HFD group grew quickly
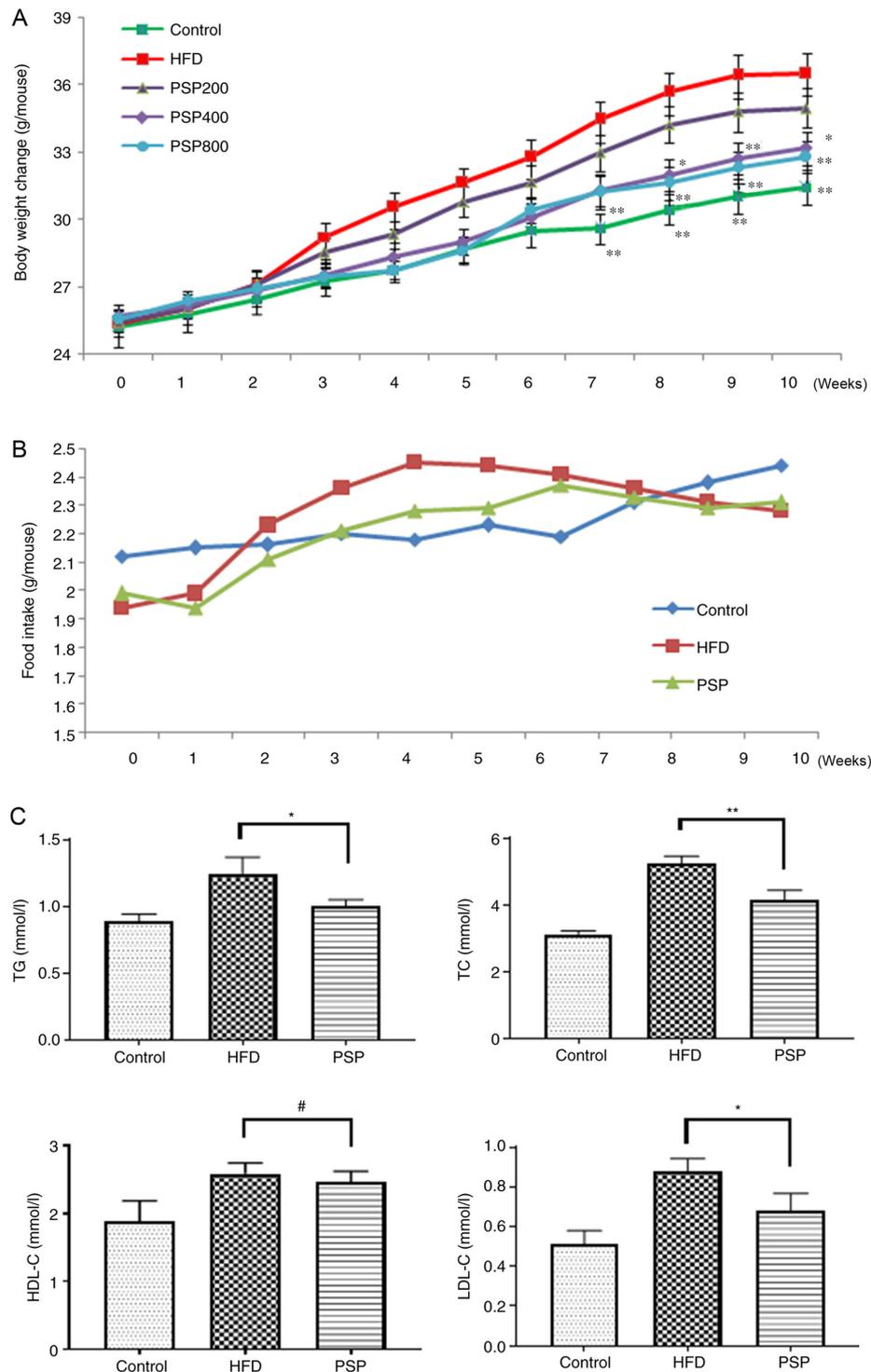
Figure 1. Inhibitory effects of PSP on HFD-induced obesity in mice. C57BL mice were divided into 5 groups. Control group mice received a normal diet, HFD group mice received HFD and three PSP groups mice received HFD and 200, 400 and 800 mg/kg of PSP by intragastric administration. Body weight and food intake were recorded weekly, Serum lipid index were measured after the mice were sacrificed. (A) Body weight changes and (B) food intake. (C) Serum TG, TC, HDL-C and LDL-C. [#]P>0.05, [*]P<0.05, [**]P<0.01. PSP, *Polygonatum sibiricum F. Delaroche* polysaccharide; HFD, high-fat diet; TG, triacylglycerol; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

in the first 4 weeks and plateaued with a slight decrease toward the end of the experiment at a level that was higher compared with the control group until week 8. The PSP group mice received less food than the HFD group during weeks 2 and 6 (Fig. 1B). Serum lipid profiles were analyzed using a detection kit. Compared with the levels in the control group, TC, TG, HDL-C and LDL-C were increased following HFD feeding. PSP significantly decreased the levels of TG, TC and LDL-C, while HDL-C was not significantly affected by PSP (Fig. 1C). The serum glucose of the HFD group was 9.62±0.98 mmol/l, much higher compared with the control group (5.63±0.53 mmol/l) while the PSP group was 7.23±0.55 mmol/l. The insulin concentration was also increased from 0.48±0.06 to 0.97±0.07 ng/ml by HFD, while

Table II. Effects of PSP on serum parameters.

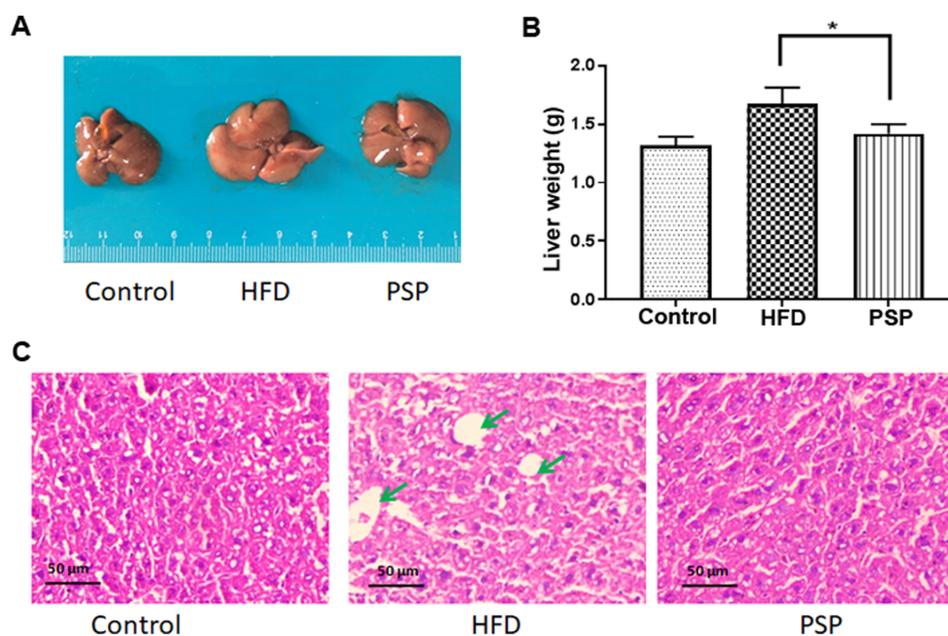| Parameters	Control	HFD	PSP	P-value; Control vs. HFD	P-value; HFD vs. PSP
Glucose (mmol/l)	5.63±0.53	9.62±0.98	7.23±0.55	<0.01	<0.01
Insulin (ng/ml)	0.48±0.06	0.97±0.07	0.68±0.09	<0.01	<0.01
Resistin (ng/ml)	0.71±0.08	2.03±0.13	1.47±0.16	<0.01	<0.01
Leptin (ng/ml)	4.07±0.58	17.05±2.03	10.34±0.64	<0.01	<0.01
Adiponectin (μg/ml)	6.39±0.72	4.50±0.39	5.83±0.80	<0.01	<0.05

Figure 2. Effect of PSP on relative liver weights and hepatic lipid accumulation in HFD-induced mice. Mice were fed a HFD for 10 weeks and then sacrificed. The livers were removed immediately, weighed, images captured and then stored at -80˚C. (A) Liver morphology. (B) Liver weight. (C) Liver sections with hematoxylin and eosin staining. Scale bar, 50-μm. Lipids are indicated by green arrows. *$P<0.05$. PSP, *Polygonatum sibiricum F. Delaroche* polysaccharide; HFD, high-fat diet.

the PSP group was only 0.68±0.09 ng/ml, significantly lower than the HFD group ($P<0.01$; Table II).

PSP prevents HFD-induced hepatic lesions and adipose hypertrophy. To evaluate the hepatoprotective effects of PSP, mouse livers and abdominal adipose tissue were collected immediately when the mice were sacrificed. The appearance of the livers differed between the groups. As shown in Fig. 2A, the control group livers were in dark red and were the smallest in size. The HFD group livers were large in size and light in color; in addition, fat particles were visible to the naked eye on those livers. The PSP group livers were significantly smaller and the color was darker compared with those of the HFD group. The differences in liver weights were as expected; the liver weight of the control group was 1.32±0.12 g, while that of the HFD group was 1.67±0.13 g and that of the PSP group was 1.41±0.14 g, which was significantly lower compared with the HFD group (Fig. 2B). The H&E results revealed why the livers of the HFD group were large and light in color. There was a large amount of fat in the intercellular space of the livers. The PSP group livers contained much less fat (Fig. 2C).

The trend in abdominal adipose tissue was similar to that among the livers; mice in the control group had the smallest adipose tissues weighing 0.80±0.06 g, while the HFD group was 1.12±0.13 g and PSP significantly decreased the weight of the adipose tissues to 0.93±0.11 g (Fig. 3A and B). H&E results indicated that the adipose cells of the HFD group were larger compared with those of the control group. The size of the PSP group adipose cells was significantly decreased (Fig. 3C and D). Adipose secreted factors are an important index of adipose health. The adiponectin in control group was 6.39±0.72 μg/ml, while the HFD decreased it to 4.50±0.39 μg/ml and PSP effectively reversed this trend to 5.83±0.80 μg/ml ($P<0.05$). In contrast, leptin was increased from 4.07±0.58 to 17.05±2.03 ng/ml by HFD. Compared with the HFD group, the PSP group exhibited a lower leptin content, only 10.34±0.64 ng/ml ($P<0.01$). Resistin in the HFD group increased from 0.71±0.08 to 2.03±0.13 ng/ml and PSP effectively decreased this to 1.47±0.16 ng/ml (Table II).

The molecular mechanisms by which PSP prevents HFD-induced obesity. The liver is one of the key organs involved in lipid regulation (12). In this present study, mouse livers were collected to explore the potential signaling pathways of PSP in HFD-induced hepatic steatosis. Lipid
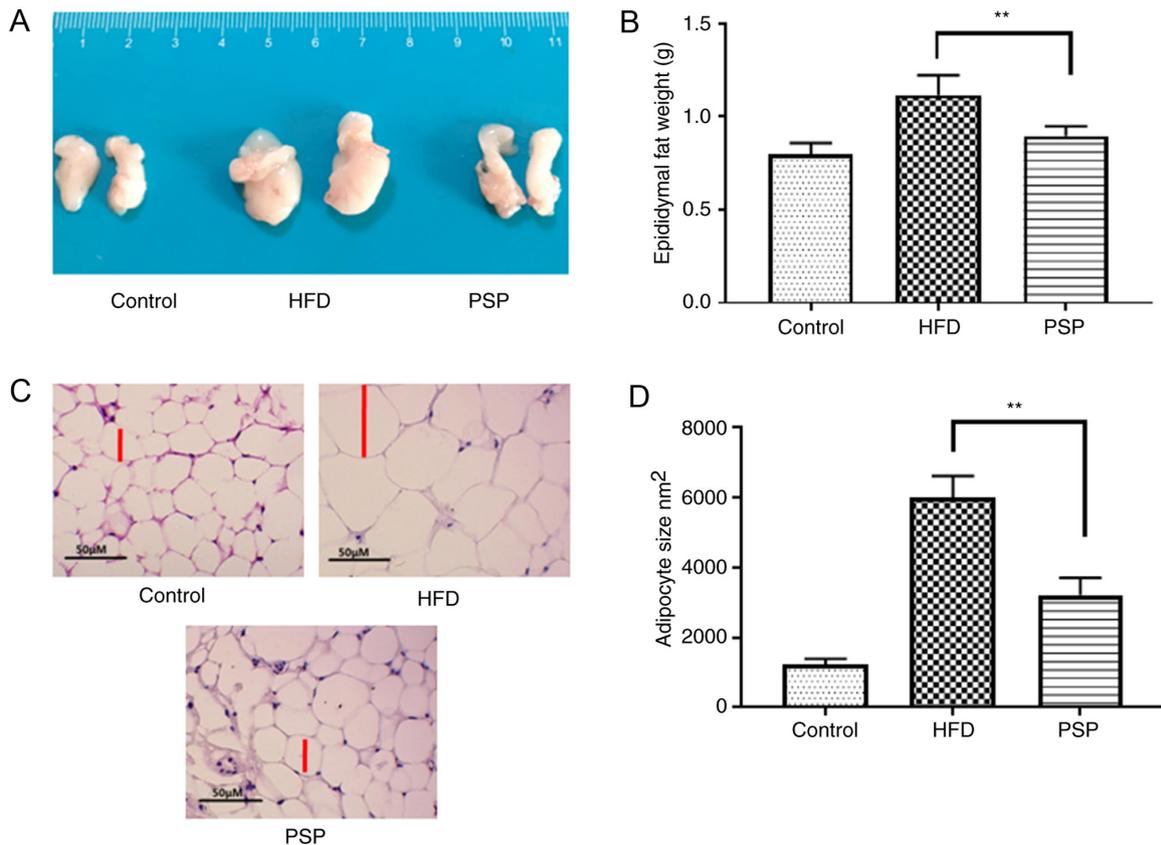
Figure 3. Effects of PSP on adipose tissue and adipocytes of mice administered HFD. The epididymal fat pads were removed immediately, weighed, images captured and then stored at -80°C. (A) The morphology of epididymal fat pads. (B) The weight change in the epididymal fat pads. (C) Adipocytes with hematoxylin and eosin staining; scale bar, 50-μm. (D) Adipocyte sizes. **P<0.01. PSP, *Polygonatum sibiricum F. Delaroche* polysaccharide; HFD, high-fat diet.

metabolic and inflammatory mRNA levels were evaluated by RT-qPCR and western blotting. The fatty acid and cholesterol synthesis-associated genes FAS and SREBP-1 and the inflammatory cytokines TNF-α, IL-6, IL-1β and iNOS were highly expressed, while PPARα and CPT-1 were inhibited in the HFD group. PSP reversed this trend, indicating that PSP inhibits lipid synthesis and inflammation (Figs. 4 and 5A).

The AMPK signaling pathway was assessed and western blotting results indicated that p-AMPK was suppressed in the HFD group, while PSP significantly activated p-AMPK. P-ACC results were similar to p-AMPK; clearly blocked in the HFD group and activated in the PSP group (Fig. 5B-D).

Discussion

Obesity is the commonest chronic metabolic disease in the modern world. Indeed, >13% of adults are overweight (29). Obesity is the main cause of a number of serious diseases, including hepatic diseases, diabetes and even cancer (30). The pathogenesis of obesity is very complex. It is widely accepted that the development of obesity is mainly determined by genetic and environmental factors (27). One of the most important environmental factors is the modern lifestyle, which involves the excessive intake of carbohydrates and fat, with a low intake of fiber (31), as well as a lack of physical activity. Although this reason is widely accepted, unhealthy lifestyles remain hard to change. Compared with the effects of lipid-lowering medicines, healthy foods provide long-term protection against

obesity and fewer side effects (32). Consequently, research on preventing the pathogenesis of obesity by eating healthy foods has become increasingly popular.

Non-starch polysaccharides are considered effective healthy foods for preventing obesity and one of the main reasons is their unique chemical structures (33). They consist of or contain a number of monosaccharides, such as fructose, galactose, mannose, ribose, rhamnose, xylose and arabinose (34). In addition, the bonds differ from those of starch; for example, oat β-glucan is β-1,3/1,4-D-glucan and mushroom and yeast glucans are β-1,3/1,6-D-glucan (27). These characteristics indicate that polysaccharides are difficult for mammals, which lack the relevant enzymes, to digest and are broken down into monosaccharides, which can be absorbed and used as an energy source (34).

P. sibiricum is a traditional Chinese herbal medicine that is effective in treating metabolic diseases such as atherosclerosis and hypertension (31). PSP is one of the main functional components of *P. sibiricum* and has been determined to be effective in enhancing immunity (35) and treating diabetes and diabetic retinal injury (17), acute heart failure (36) and cancer (24). An ethanol extract of *P. sibiricum* is effective in high-fat diet-induced obesity (37). Additionally, PSP regulates bone marrow-derived macrophages to osteoclast differentiation through the ERK/GSK-3β/β-Catenin signaling pathway (38). However, to the best of the authors' knowledge, there has been no study on the anti-obesity effect of PSP. Thus, the present study evaluated the anti-obesity effect of PSP and
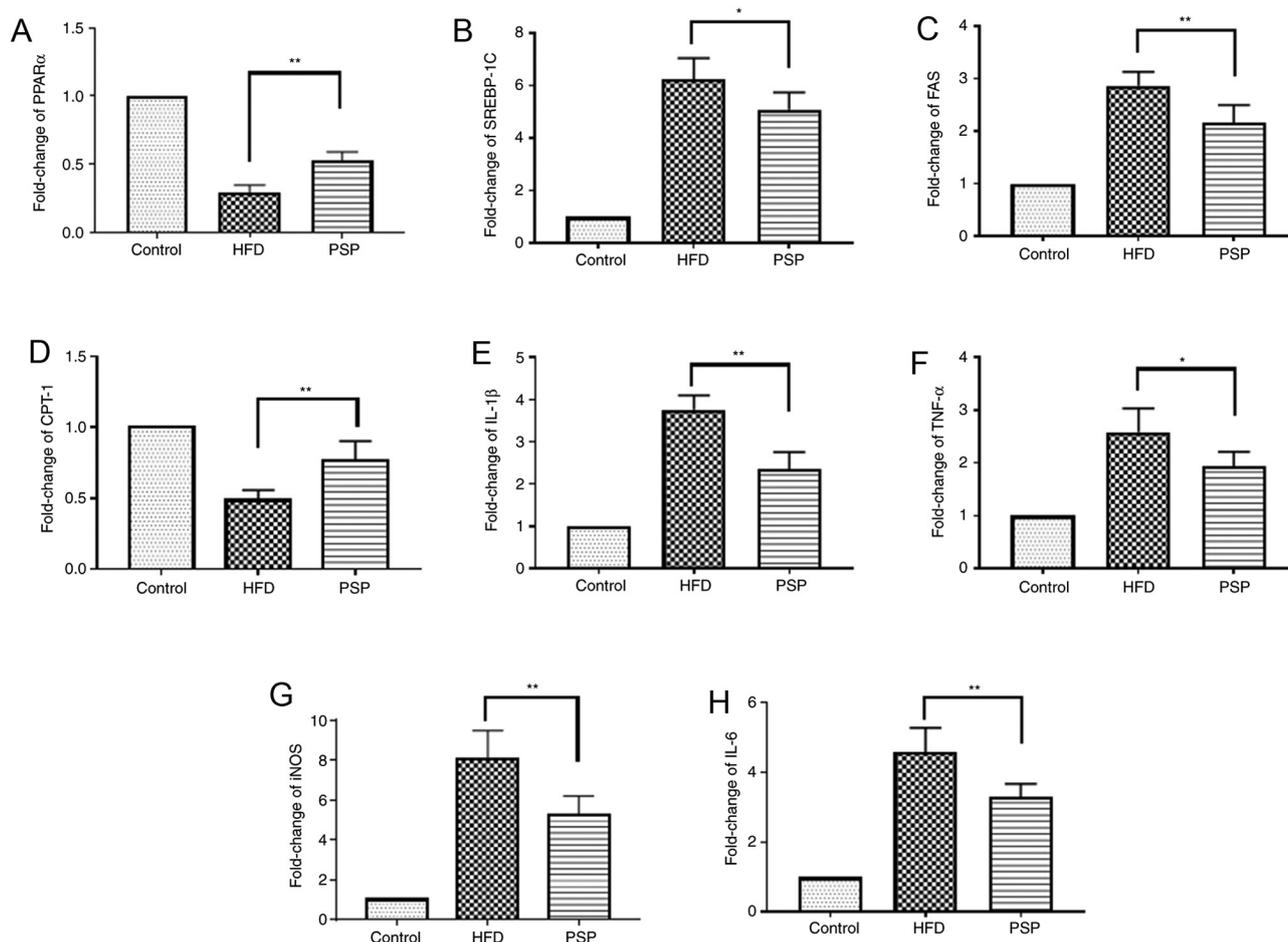
Figure 4. Lipid-associated and inflammatory cytokine mRNA changes. Results of reverse transcription-quantitative PCR. Fold change of lipid-associated (A) PPARα, (B) SREBP-1, (C) FAS and(D) CPT-1 and inflammatory (E) IL-1β, (F) TNF-α, (G) iNOS and (H) IL-6 mRNA. *P<0.05, **P<0.01.PSP, *Polygonatum sibiricum F. Delaroche* polysaccharide; HFD, high-fat diet; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory-element binding protein; FAS, fatty acid synthase; CPT-1, carnitine palmitoyltransferase I; iNOS, inducible nitric oxide synthase.

the latent molecular mechanisms. PSP groups mice had slower bodyweight increases and the livers and abdominal fat pads were smaller compared with those of the HFD group. The glucose and insulin contents of the PSP group were lower compared with those of the HFD group. PSP also increased the adiponectin and decreased the leptin and resistin levels. Adiponectin represents one potential contributor to improved insulin sensitivity, which exerts anti-inflammatory and insulin sensitizing effects in the liver and adipocytes (39). Resistin levels are positively associated with increased levels of insulin and glucose and an elevation of resistin levels induces insulin resistance in mice (40). Leptin is another important factor in obesity and inflammation. A reduced leptin level is considered a beneficial effect because leptin has pro-inflammatory effects and contributes to the development of comorbidities of obesity (41). Lipid metabolic and inflammatory cytokines, as well as the AMPK signaling pathway, were also altered by PSP.

AMPK is a serine/threonine kinase and plays critical roles in metabolism (42) and inflammation (43). AMPK activation promotes glucose metabolism and adiposis oxidation which is associated with significant upregulation of autophagy, mitophagy and mitochondrial metabolism. By contrast, it inhibits gluconeogenesis and glycogenesis, fatty acid synthesis

and cholesterol synthesis (44). Obesity is associated with chronic energy imbalance and with reduced AMPK activation, accompanied with alterations in glycolysis, hepatic lipid metabolism and inflammation (45).

AMPK downstream genes such as FAS, SREBP-1c, CPT-1 and PPAR-α are associated with lipid biosynthesis and metabolism. An activation of FAS results in lipid synthesis and deposition (44). Conversely, SREBP-1c is negatively correlated with AMPK, meaning that AMPK activity controls SREBP-1c inhibition (46). CPT-1 and PPAR-α are the main directors regulating β-oxidation which is associated with the hepatic diseases (47). In the present study, AMPK signaling was suppressed in the HFD group and the liver fatty acid and cholesterol synthesis-associated genes FAS and SREBP-1 were highly expressed, while CPT-1 and PPAR-α were inhibited by HFD. PSP significantly activated AMPK signaling, suppressed the expression of FAS and SREBP-1c and activated CPT-1 and PPAR-α. HFD-induced obesity is also associated with chronic inflammation (48). In the present study, proinflammatory cytokines, including TNF-α, IL-6, IL-1β and iNOS, were highly expressed in the HFD group but significantly inhibited in the PSP group.

In conclusion, the present study is the first, to the best of the authors' knowledge, to investigate the effect of PSP treatment in
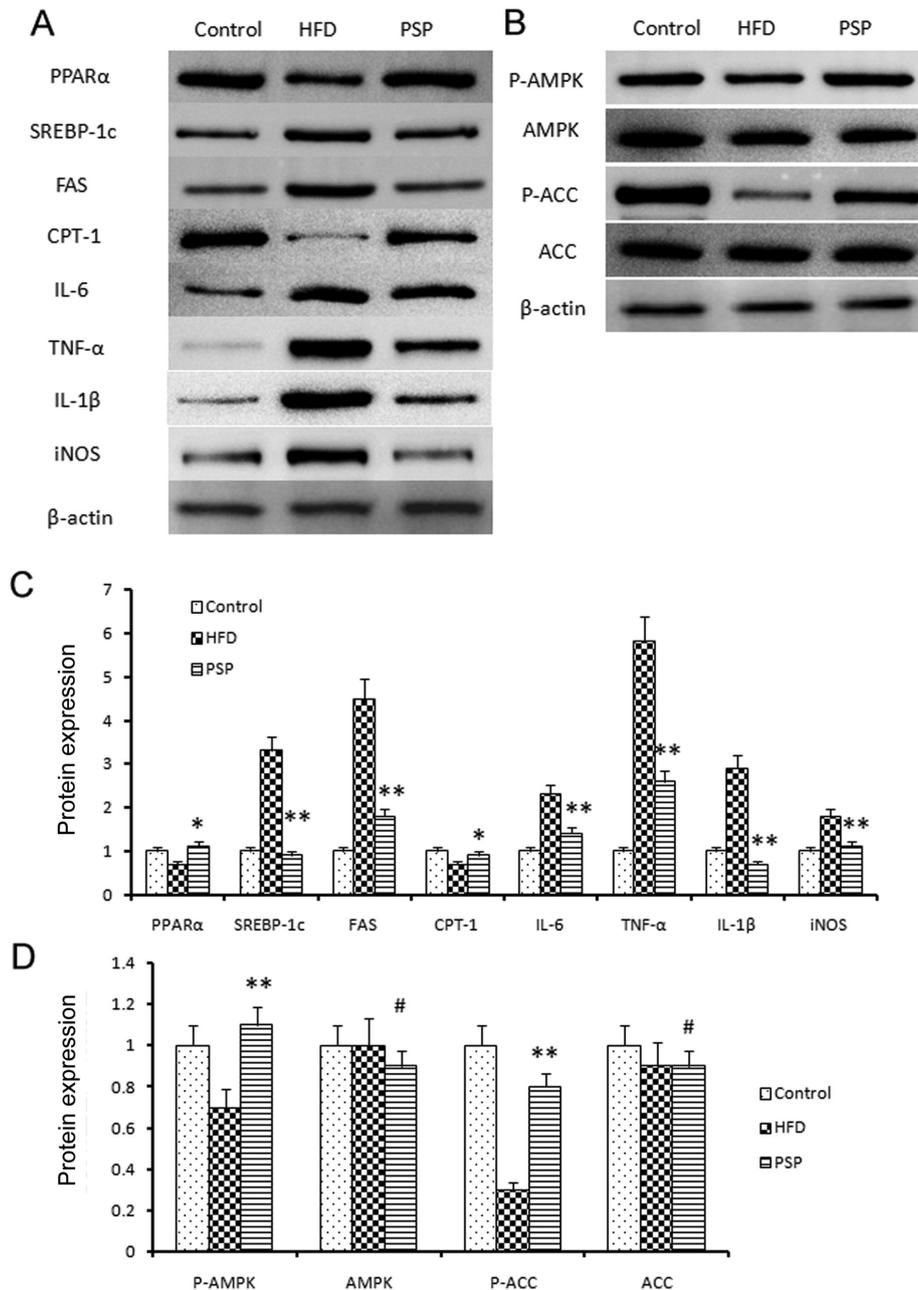
Figure 5. Signal pathway changes and lipid-associated genes and inflammatory cytokine protein changes. (A) Western blotting was performed to reveal the protein expressions of the lipid and inflammatory genes as well as the key genes of the AMPK signal pathway. Lipid synthesis-associated genes and inflammatory genes were inhibited by PSP. (B) P-AMPK and the downstream gene p-ACC was clearly activated by PSP, which demonstrated that the PSP could ameliorate HFD-induced mouse obesity through activating the AMPK signal pathway. (C) Semi-quantification of protein expression levels from part (A) (D) Semi-quantification of protein expression levels from part (B) #$P>0.05$, *$P<0.05$, **$P<0.01$. AMPK, 5' adenosine monophosphate-activated protein kinase; p-, phosphorylated; ACC, acetyl-coenzyme A carboxylase; PSP, *Polygonatum sibiricum F. Delaroche* polysaccharide; HFD, high-fat diet; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory-element binding protein; FAS, fatty acid synthase; CPT-1, carnitine palmitoyltransferase I; iNOS, inducible nitric oxide synthase.

the prevention of HFD-induced obesity. It is possible that PSP will have the beneficial property of decreasing inflammation in the liver, along with protecting against dyslipidemia, hepatic steatosis and blood glucose increase. It is hypothesized that the mechanism by which PSP supplementation protects against lipid accumulation and inflammatory stress might be due to modulation of the energy balance in the signaling pathway of AMPK.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BL and JG designed the experiments and wrote the manuscript; YT performed the animal experiments, histological examination and statistical analysis; BL and ZS performed the RT-qPCR and western blotting experiments. BL and JG confirm the authenticity of all the raw data. JG revised the manuscript and given final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The care and use of the animals and experimental protocols complied with the Guidelines for the Care and Use of Experimental Animals of Hunan University of Chinese Medicine. The project was approved by the Office of Animal Experiment Ethics of Hunan University of Chinese Medicine (approval no. 20190165).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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