Bofutsushosan ameliorates obesity in mice through modulating PGC-1α expression in brown adipose tissues and inhibiting inflammation in white adipose tissues

CHEN Ying-Ying 1, 2, YAN Yan 1, ZHAO Zheng 3, SHI Mei-Jing 1, ZHANG Yu-Bin 1*

1 State Key Laboratory of Natural Medicines, Department of Biochemistry, China Pharmaceutical University, Nanjing 210009, China;
2 Shanghai Key Laboratory of Complex Prescription, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China;
3 Department of Pharmacy, Nanjing First Hospital, Nanjing Medical University, Nanjing 210009, China

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[ABSTRACT] The inducible co-activator PGC-1α plays a crucial role in adaptive thermogenesis and increases energy expenditure in brown adipose tissue (BAT). Meanwhile, chronic inflammation caused by infiltrated-macrophage in the white adipose tissue (WAT) is a target for the treatment of obesity. Bofutsushosan (BF), a traditional Chinese medicine composed of 17 crude drugs, has been widely used to treat obesity in China, Japan, and other Asia countries. However, the mechanism underlying anti-obesity remains to be elucidated. In the present study, we demonstrated that BF oral administration reduced the body weight of obese mice induced by high-fat diet (HFD) and alleviated the level of biochemical markers \( (P < 0.05) \), including blood glucose (Glu), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C) and insulin. Our further results also indicated that oral BF administration increased the expression of PGC-1α and UCP1 in BAT. Moreover, BF also reduced the expression of inflammatory cytokines in WAT, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). These findings suggested that the mechanism of BF against obesity was at least partially through increasing gene expression of PGC-1α and UCP1 for energy consumption in BAT and inhibiting inflammation in WAT.

[KEY WORDS] Bofutsushosan; Obesity; PGC-1α; UCP1; Inflammation

[CLC Number] R965

Introduction

Obesity has reached an epidemic status worldwide and is correlated with various comorbidities, such as dyslipidemia, diabetes mellitus, and cardiovascular (CV) diseases \(^{[1-3]}\). It is well known that obesity develops when food intake exceeds energy expenditure. Now the treatment options for obesity is either reduction of energy intake, increase in energy expenditure, or both at the same time \(^{[4]}\). Considering that physical activity to increase energy expenditure is relatively limited in modern life, it is a critical alternative strategy to modify the metabolic efficiency and increase the energy consumption in key metabolic organs, such as adipose tissues \(^{[5]}\). Two types of adipose tissues, brown adipose tissue (BAT) and white adipose tissue (WAT), have been found in mammals. Recently, researchers have increasingly suggested that beige fat, the third type adipose tissue, shares features of BAT \(^{[6]}\). BAT is a thermogenic tissue to maintain core temperature through regulating energy expenditure. When BAT is activated, it requires the uptake of substrate from the circulation, mostly free fatty acids from WAT. Therefore, BAT represents a natural target for the modulation of energy...

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[Corresponding author] Email: ybzhang@cpu.edu.cn

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expenditure [7-8]. Peroxisome proliferator activator receptor-γ coactivator 1α (PGC-1α) is first identified as a co-activator of peroxisome proliferator activator receptor γ (PPARγ) in BAT with marked and rapid induction upon exposure of mice to cold due to sympathetic nervous system input through β3-adrenergic receptor (β3-AR) and cyclic adenosine monophosphate (cAMP) signaling pathway and doubles oxygen consumption [9-10]. CL316243, an agonist of β3-AR adrenergoreceptor, stimulates uncoupling protein 1 (UCP1) expression through cAMP-protein kinase A (PKA) signaling pathway and doubles oxygen consumption [11]. As the adverse effects of CL316243 for obesity treatment in clinical application have been reported, there is a need for finding novel drugs for obesity therapy. During β3-AR activation in adipose tissue, PGC-1α plays a crucial role in adaptive thermogenesis by activating thermogenic gene and mitochondrial release of chemical energy in the form of heat by UCP1 [12-14]. Therefore, they have been suggested as new targets for obesity treatment [14].

WAT is responsible for triglyceride (TG) storage and researchers have indicated that obesity is associated with chronic activation of inflammatory pathways in both adipocytes and macrophages residing in or infiltrating the adipose tissue in recent years [15]. The study of epididymal adipose tissue reveals that the percentage of cells expressing the macrophage marker F4/80 is significantly correlated with both adipocyte size and body mass [16]. Meanwhile, increasing evidence suggests the presence of an overall, low-grade inflammation in obesity, with increased levels of several circulating factors such as TNF-α, IL-6, and other biological markers of inflammation [17-18]. The growing body of research results suggests that chronic silent inflammation is a key feature in abdominal obesity. Though the observed metabolic effects remain rather modest in most clinical trials, and the available data supports the concept that targeting inflammation ameliorates metabolic syndrome in patients [19].

Bofutsushosan (also called Fangfengtongshengsan/pills in China), a traditional Chinese herbal medicine composed of 17 crude drugs (Table 1), has been proven to be an effective treatment for obesity [20], diabetes mellitus [21], hypertension [22], and hepatic steatosis in animals with high-fat diet [23]. In particular, BF reduces body weight and improves impaired glucose tolerance, as shown in an clinical trial [24]. However, the mechanism of BF to treat obesity remains to be elucidated. It has been reported that ephedrine promotes the gene expression of UCP1 and emodin inhibits the release of TNF-α and IL-6, and they are two of major chemical composition of BF [25-26]. Therefore, we hypothesized that BF could treat obesity through promotion of PGC-1α expression in BAT and inhibition of inflammation in WAT. In the present study, we investigated the molecular mechanism of anti-obesity of BF by detecting the expression of PGC-1α and UCP1 in the mouse BAT and primary brown adipocytes, and measuring inflammatory cytokines (TNF-α, IL-6) in WAT. We found that BF promoted energy consumption in BAT and had anti-inflammatory action in WAT.

### Materials and Methods

#### Preparation of BF extracts

BF was obtained from Tongren Drugstore (Beijing, China), which was consisted of 17 crude drugs as shown in Table 1. BF pills (30 g) were extracted with 600 mL of 75% ethanol or 600 mL of water at room temperature for 24 h to acquire ethanol fraction or aqueous fraction, respectively. The ethanol fraction or aqueous fraction was pooled separately and evaporated at 55 °C or 78 °C under reduced pressure. Finally, the dried powders of ethanol and aqueous extracts were obtained by lyophilisation, and the yields of both extracts were 36.8% and 28.5%, respectively.

#### Animals and treatment

Female ICR mice (6-week old, weighing 16–18 g) were purchased from Comparative Medicine Centre of Yangzhou University (SUXR 2012-0004; Yangzhou, China) and housed in an environment with temperature (22–25 °C) and humidity (40%–60%) under 12 h light/12 h dark cycle, with access to

<table>
<thead>
<tr>
<th>Table 1 Components of Bofutsushosan</th>
<th>Dry weight of crude drugs in BF (%)</th>
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</thead>
<tbody>
<tr>
<td>1) Ephedrae Herba (麻黄, mà huáng)</td>
<td>3.8</td>
</tr>
<tr>
<td>2) Saposhnikoviae Radix (防風, fáng fēng)</td>
<td>3.8</td>
</tr>
<tr>
<td>3) Schizonepetae Spica (荆芥, jīng jiè)</td>
<td>1.9</td>
</tr>
<tr>
<td>4) Rhei Rhizoma (大黄, dà huáng)</td>
<td>3.8</td>
</tr>
<tr>
<td>5) Natrium Sulfricum (芒硝, máng xiāo)</td>
<td>3.8</td>
</tr>
<tr>
<td>6) Glycyrrhizae Radix (甘草, gān cǎo)</td>
<td>15.1</td>
</tr>
<tr>
<td>7) Forsythiae Fructus (连翘, lián qiào)</td>
<td>3.8</td>
</tr>
<tr>
<td>8) Platycodi Radix (桔梗, jí jiè gěng)</td>
<td>7.5</td>
</tr>
<tr>
<td>9) Cnidii Rhizoma (川芎, chuān xiōng)</td>
<td>3.8</td>
</tr>
<tr>
<td>10) Scutellariae Radix (黄芩, huáng qín)</td>
<td>7.5</td>
</tr>
<tr>
<td>11) Gardeniae Fructus (山桅子, Shān wéi zǐ)</td>
<td>1.9</td>
</tr>
<tr>
<td>12) Gypsum Fibrosum (石膏, shí gù)</td>
<td>7.5</td>
</tr>
<tr>
<td>13) Talcum (滑石, huá shí)</td>
<td>22.6</td>
</tr>
<tr>
<td>14) Angelicae Radix (当归, dāng guī)</td>
<td>3.8</td>
</tr>
<tr>
<td>15) Paonieae Radix (芍药, sháo yào)</td>
<td>3.8</td>
</tr>
<tr>
<td>16) Atractylodis Lanceae Rhizoma (苍术, cāng zhú)</td>
<td>1.9</td>
</tr>
<tr>
<td>17) Menthae Herba (薄荷, bó hé)</td>
<td>3.8</td>
</tr>
</tbody>
</table>
water and food ad libitum. All the procedures were reviewed and approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University, Nanjing, China, and adhered to the Jiangsu Provincial Guidelines for the use of experimental animals. After acclimation for 1 week by feeding with a basal diet, 8-week-old mice were randomly divided into two groups: the mice in the control group (SD) were given commercial rodent chow, and the mice in the HFD group were given commercial HFD (58% fat diet) for 12 weeks. Then the mice in the HFD group were further divided into two groups and 6 mice of each (HFD control group, HFD + BF group: 7.2 g·kg–1·d–1) at the age of 20 weeks. Dosage of BF pill in this experiment was calculated according to clinical dosage of humans [25]. To prepare the suspension of BF, the BF pills were grinded and then suspended in sodium carboxymethyl cellulose (CMC-Na) to the final concentration of 0.36 g mL−1. The HFD + BF group mice were given BF by gavage once a day (0.2 mL/10 g body weight) for 7 weeks with HFD simultaneously. Meanwhile, the mice received gavage in a same dosage of CMC-Na in the SD group and HFD group. Body weight and food intake were measured twice a week.

**Tissue weight measurement**

The mice were weighed and then sacrificed by cervical dislocation and the BAT and epididymal WAT were removed and weighed. The ratio of organ weight (% of body weight) was calculated for each mouse.

**Blood biochemical tests**

The mice were fasted overnight prior to necropsy and blood sampling for serum biochemistry was drawn from the posterior vena cava. Then blood samples were centrifuged 5 000 × g for 10 min at 4 °C, and sera were stored at −70 °C until analysis. Serum glucose level was measured by the glucose oxidase method with the glucose kit (BioSino Bio-technology and Science Inc, Beijing, China). The concentration of Glu (mg·dL−1) was calculated as follows: \[
\Delta A_{505} \left( A_{\text{sample}} - A_{\text{control}} \right) / \Delta A_{505} \left( A_{\text{standard}} - A_{\text{control}} \right) \times C
\] (glucose standard), total cholesterol (TC), TG, low density lipoprotein (LDL-C) levels were measured by the COD-PAP method, GPO-PAP method and PVS method with a CHO, TG and LDL-C kit (BioSino Bio-technology and Science Inc, China), following the manufacturer’s instructions. The concentration of TC and TG were calculated as follows: \[
\Delta A_{505} \left( A_{\text{sample}} - A_{\text{control}} \right) \times C
\] (TC/TG standard), and the concentration of LDL-C was calculated as: total TC concentration – TC concentration of liquid supernatant. Serum insulin level was measured with a mouse ELISA kit ((Beijing 4A Biotech Co., Ltd., Beijing, China), and the insulin resistance index was calculated using Homeostasis model assessment (HOMA) formula: \[
(\text{fasting glucose levels (mmol·L}^{-1} \times \text{fasting insulin levels (mIU·L}^{-1}) / 22.5
\]

**Histopathological examination**

After blood collection, the organs (BAT and WAT) were removed and fixed with 10% neutral formalin solution for one day and embedded in paraffin. Tissue fragments were cut to a thickness of 5-μm, then deparaffinized, hydrated and stained with hematoxylin and eosin (H&E). To assess the sizes of the adipocytes, the area of 10 adipocytes was measured in representative sections by light microscopy and histological assessment was provided by an expert pathologist, with an image analysis program (Olympus BX45-DP72, Olympus Optical Co., Tokyo, Japan).

**Immunohistochemical analysis**

For immunohistochemistry, formalin-fixed paraffin embedded tissues were cut into 5-μm section and the sections were mounted, deparaffinized, and incubated in 3% H2O2 for 10 min to quench endogenous peroxidase activity on glass slides. After blocking with normal goat serum for 20 min, the sections were stained with polyclonal rabbit antibody raised against F4/80 (1 : 100, Bioworld Technology, Inc, St. Louis, USA), TNF-α (1 : 100, Abcam Inc, Cambridge, UK), and IL-6 (1 : 100, Bioworld Technology, Inc, St. Louis, USA) at 4 °C overnight respectively, followed by washing with PBS and incubation with goat anti-rabbit antibody at 37 °C for 30 min. The antibody binding sites were visualized by incubation with diaminobenzene (DAB) at room temperature for 10 min. Images were obtained under a light microscope (ZNISS Vert1, Germany). The settings for image acquisition were identical for control and experimental tissues. For counting the number and color intensity of positive cells in each individual mouse adipose depot, ten different high-power fields were analyzed using image analysis software (Image pro-plus 6.0, Media Cybernetics, Sliver Spring, MD, USA).

**Primary brown adipocyte culture and treatment**

New-born mice (5-day) were sacrificed, and the interscapular BAT depots were dissected out. The tissues were pooled and incubated in the HEPES-buffered solution (pH 7.4) containing 0.2% (W/V) crude collagenase type I (Sigma-Aldrich (Shanghai) Trading Co., Ltd, Shanghai, China). The incubations containing 12%–15% BAT (W/V) were digested for 30 min at 37 °C with 10 s vortexing every 5 min and then filtered by nylon screen (100 μm) on 6-well plate in hood. The collected filtrate was transferred to a 1.5-mL EP tube and centrifuged at 2 000 × g for 5 min at room temperature. The remaining pellet was re-suspended in 2 mL of culture medium (DMEM-H, 20% fetal bovine serum, 20 mmol·L−1 hepes, and 100 U·mL−1 penicillin/streptomycin). The cells were seeded into 6-well-plates and refed with culture medium after 24-h incubation and every other day to reach 80% confluence. Primary brown adipocytes were treated with BF extract (600 μg·mL−1) and CL316243 (10−8 mol·L−1) for 16 h to examine the gene expression of PGC-1α and UCP1.

**RT-PCR for analysis of gene expression**

Total RNA was extracted from tissues or cells using TRizol reagent (Invitrogen). According to the manufacturer’s protocol, the cells in a well of 6-well plate or 50–100 mg of BAT was mixed with 1ml of TRizol and tissues were
homogenized using Homogenizer (IKA-T-10, IKA® Werke GmbH & Co. KG, Staufen, Germany). RNA was separated from protein and DNA and RT-PCR reactions were carried out according to Wen et al [27]. Gene-special primers (Table 2) were designed using oligo-6 software based on gene sequences from the GenBank database.

**Table 2 Primer sequences for PCR analysis**

<table>
<thead>
<tr>
<th>Gene Primer</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>PGC-1α</td>
<td>5'-TGCCATTTGTAAGGAGCG-3'</td>
<td>5'-GGTCATTTGGACTCTGG-3'</td>
</tr>
<tr>
<td>Cyclophilin</td>
<td>5'-CCATCGTGTCATCAAGGACTCTCAT-3'</td>
<td>5'-CTTGCCATCCAGCAGGAGGTCTCTTTG-3'</td>
</tr>
<tr>
<td>UCP1</td>
<td>5'-GATCCAAGGTGAAGGCAGG-3'</td>
<td>5'-GTTGACAAAGCTCTTCTGGTGG-3'</td>
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</table>

PGC-1α: peroxisome proliferators-activated receptor-γ coactivator-1α; Cyclophilin: Peptidylprolyl isomerase; UCP1: uncoupling protein1

**Western blotting analysis**

Primary brown adipocytes were homogenized and sonicated in ice-cold lysis buffer (RIPA) containing 1% protease inhibitor cocktail (Roche, Basel, Switzerland). The lysate was centrifuged at 12 000 × g at 4 °C for 15 min, and protein concentration was determined with bicinchoninic acid (BCA) method by the kit (Vazyme Biotech Co., Ltd., Nanjing, China). Equal amounts of total protein (50–100 μg) were subjected to electrophoresis on 8%–10% SDS-PAGE and then transferred to PVDF membranes (Millipore, USA). Primary anti-PGC-1α and anti-UCP1 polyclonal antibody (Santa Cruz, USA) were used to detect PGC-1α and UCP1. Immunoblots were hybridized with antibody raised against β-Actin (Santa Cruz, USA) as loading control. The membrane was then washed thrice with TBST, incubated for 1 h with HRP-conjugated secondary antibody and then detected by chemiluminescence (ECL) detection system (Merck Millipore, Shanghai, China). ImageJ64 software was used to analyze the relative density of each band.

**Statistical analysis**

Data were expressed as means ± SEM. One-way ANOVA or t-test was used for determining the statistically significant differences among various experimental groups. *P* < 0.05 was considered statistically significant.

**Results**

**BF reduces body weight and improves serum biochemical indices**

Adiposity, the fraction of total body mass, is comprised of neutral lipid stored in adipose tissues and closely correlated with important physiological parameters, such as systemic insulin sensitivity and serum triglyceride [28]. The body weight decreased significantly in the HFD + BF group compared with HFD group (Fig. 1A). In the HFD group, serum biochemical indices (TC, TG, Glu, LDL-C, insulin, and insulin resistance index) increased, compared with those in the SD group. Our results showed that the levels of TC, TG, Glu, LDL-C, and insulin decreased dramatically with the treatment of BF (Figs. 1B and 1C), while the insulin resistance index decreased compared with that of the HFD group (Fig. 1C).

**BF promotes the expression of genes associated with energy consumption in mouse BAT**

The gene expression of UCP1 is co-activated by PGC-1α, PGC-1α: peroxisome proliferators-activated receptor-γ coactivator-1α; Cyclophilin: Peptidylprolyl isomerase; UCP1: uncoupling protein1

**Fig. 1 Bofutsushosan (BF) reduces body weight and serum biochemical index. (A) The body weight of mice was measured per week during treatment of BF. (B) Serum from 27-week-old female mice was acquired and the TC (Total cholesterol), triglycerides (TG), glucose (Glu) and Low density lipoprotein (LDL-C) were determined with commercial assay kit. (C) The insulin level was measured with a mouse ELISA kit and insulin resistance index was calculated as the Homa formula (**n** = 6). *P* < 0.05 vs high fat diet group (HFD) mice, **P** < 0.01 vs standard diet group (SD) mice
and UCP1 accelerates energy expenditure through uncoupled oxidative phosphorylation in BAT [12]. Our results indicated that the ratio of BAT/body increased in the HFD + BF group compared with the HFD group (Fig. 2B). In BAT, the cell size decreased while the accumulation of macrophages increased in the HFD group (Fig. 2A). Furthermore, BF induced the mRNA expression of PGC-1α by about 4-fold (Fig. 2C), and the expression of UCP1 increased by about 12-fold in the BAT of the BF-treated mice, compared with that of the HFD group (Fig. 2D).

**BF enhances gene expression of PGC-1α and UCP1 in primary brown adipocytes**

Primary brown adipocytes were used to investigate the energy consumption by BF in vitro, and CL316243 was selected as a positive control for β3-AR agonist [13]. Our data illustrated that the expression of PGC-1α and UCP1 mRNAs in primary brown adipocyte increased by about 4-fold and 4.5-fold with 600 μg·mL⁻¹ aqueous extract and 600 μg·mL⁻¹ ethanol extract of BF (Figs. 3A and 3B). Western blotting showed that PGC-1α and UCP1 increased by 1.7-fold and 1.5-fold (Figs. 3C and 3D), meanwhile PGC-1α protein were comparably similar to the effect of CL316243 (Figs. 3C and 3E).

**BF reduced the infiltration of macrophage in WAT**

Adipose tissue mass is associated with the endocrine and metabolic functions of adipose tissue that link adiposity in systemic physiology. Our results suggested that the WAT/body ratio decreased dramatically by BF (Fig. 4A). It was reported that white adipocyte volume and proinflammatory cytokines (TNF-α and IL-6) are highly correlated with indicators of systemic insulin resistance, dyslipidemia, and risk for developing type II diabetes [29]. As shown in Fig. 4B, the average size of WAT increased by almost 3-fold in the HFD group. However the adipocyte size and the accumulation of macrophage in WAT were significantly reduced in the HFD + BF group (Figs. 4A and 4B). Immunohistochemical test showed the macrophage marker F4/80 and proinflammatory cytokines, including TNF-α, IL-6 decreased significantly by the treatment of BF (Figs. 4C, 4D and 4E). The present study indicated that BF reduced the volume of white adipocytes, while the number of macrophages and the expression of pro-inflammatory cytokines in WAT were decreased dramatically.

**Discussion**

Targeting energy expenditure and chronic inflammation represents an attractive concept for combating obesity [30-31]. Promoting thermogenic capacity in BAT and inhibiting inflammation in WAT may be a therapeutic strategy for treating obesity and its complications [21]. The beneficial effects of BF on metabolic improvement have evoked a substantial interest in the formula as a potential treatment for obesity [22]. Our data from the present study supported a clear function of BF in promotion of energy expenditure by initiating a thermogenic program and anti-inflammatory action by reducing pro-inflammatory cytokines and the number of macrophages infiltrating in WAT.

BF was first recorded as a traditional Chinese medicine in XuanMingLunFang by LIU Wan-Su in Jin dynasty, and it

Fig. 3  BF enhances gene expression of PGC-1α and UCP1 in primary brown adipocyte. (A-B) The gene expression of PGC-1α and UCP1 by RT-PCR analysis in the primary brown adipocytes. Relative expression was normalized to expression of cyclophilin. Data represent mean ± SEM of at least three independent experiments. (C) Western blotting analysis of UCP1 and PGC-1α protein expression. β-actin was blotted as a loading control. (D-E) The relative density of UCP1 and PGC-1α band were compared with the β-actin band in each group. *P<0.05, **P<0.01, ***P<0.001 vs control group

was used to reduce fever after bouts of influenza and promote bowel movements for the first time [32]. In the recent years, Nakayama et al have shown that BF activates thermogenesis in BAT [33]. However, the role of PGC-1α in the molecular mechanism of BF to the treatment of obesity has not been mentioned. Some of the components of BF, such as Ephedrae herba, Glycyrrhizae radix, Forsythiae fructus, and Schizonepetae spica extracts, have been reported to activate the thermogenesis in BAT and inhibit cyclic adenosine monophosphate (cAMP) phosphodieterase as shown with caffeine [25]. The coactivated PGC-1α is induced markedly and rapidly in BAT upon exposure of mice to cold due to sympathetic nervous system input through β3-AR and cAMP action [9-10]. Thereafter, mitochondria release chemical energy in the form of heat by UCP1 [12-13]. CL316243 stimulates UCP1 expression through cAMP-PKA signaling pathway and doubles oxygen consumption [11]. So CL316243 was chosen as a positive control in the present study. The results indicated that BF activated the gene expression of PGC-1α and UCP1 as seen with CL316243. Consistent with results from the in vitro experiments, the results from the present in vivo study also showed that the oral administration of BF for 7 weeks dramatically induced the gene expression of PGC-1α and UCP1, compared with the mice in the HFD group.
Obesity is also associated with a heightened inflammatory state in adipose tissues and growing evidences suggest that inflammatory signaling pathways contribute to obesity \cite{34-35}. The close relationship between adipocyte size and the abundance of macrophages in adipose tissue suggests that the influence of adipocyte size on adipocyte function may be conveyed through a paracrine pathway involving adipose tissue macrophages \cite{29}. Increased macrophage infiltration could represent the cause or the consequence of the low-grade inflammation state associated with obesity \cite{36-37}. Emodin, one chemical component of BF, inhibits the release of TNF-\(\alpha\) and IL-6 \cite{26}. Shimada has shown that BF suppresses the level of biochemical parameters such as TNF-\(\alpha\) in plasma from Tsumura Suzuki Obese Diabetes (TSOD) mice \cite{22}. However, the molecular mechanism for the anti-inflammation effects of BF in the obese mice has not reported. According to our hypothesis, the macrophage marker F4/80 was detected in the WAT and the results showed BF reduced the infiltrated macrophage significantly. Furthermore, the levels of pro-inflammatory cytokines (TNF-\(\alpha\) and IL-6) were suppressed by BF dramatically.

In summary, the gene expression of PGC-1\(\alpha\) and UCP1 was increased in \(\beta\)-adrenergic signaling in BAT and primary brown adipocyte by BF treatment. BF treatment reduced the percentages of microphage cells expressing F4/80, TNF-\(\alpha\) and IL-6 in WAT. The present study provided new insights into the therapeutic effects of BF on obesity, with the promotion of energy consumption and inhibition of pro-inflammatory cytokines being the most important determinants for its anti-obesity property. 

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