Effects of Soy Isoflavones on the Leptin-Mediated Janus Kinase/Signal Transducer and Activator of Transcription Signaling Pathway in the Intestine of Obese Rats (Postprint)

Authors: Li Like, Luo Qihui, Tang Xiuying, Huang Chao, Xiaolin Chen, Liu Wentao, Chen Zhengli

Date: 2017-10-11T00:00:00+00:00

Abstract

This study aimed to investigate the regulatory effect of soy isoflavones on the leptin-mediated Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway in the intestine of obese rats and to explore the intervention mechanism of soy isoflavones in obese rats. Eighty 5-week-old Sprague-Dawley (SD) male rats were acclimated for 1 week and then randomly divided into a basal diet group (12 rats) and a high-fat diet group (68 rats), which were fed basal diet and high-fat diet, respectively. After 9 weeks of feeding, the high-fat diet group successfully established a diet-induced obese rat model. Forty obese rats were randomly assigned to low-, medium-, and high-dose soy isoflavone intervention groups and an obese control group, with 10 rats per group. Rats in the low-, medium-, and high-dose groups were intragastrically administered 50, 150, and 450 mg/kg BW of SIF extract, respectively, while the basal diet group and obese control group were administered vehicle without SIF extract. Soy isoflavones were continuously administered to obese rats for 5 weeks, during which body weight was monitored. Immunohistochemical SABC staining was used to investigate the expression and distribution of long-form leptin receptor (OB-Rb), Janus kinase 2 (JAK2), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), suppressor of cytokine signaling 3 (SOCS3), and neuropeptide Y (NPY) in the rat intestine. The results showed that at all experimental time points, the body weight of rats in the basal diet group was extremely significantly lower than that in the obese control group ($P < 0.01$). After 5 weeks of SIF intervention, compared with the obese control group, the body weight of rats in each SIF intervention group decreased in a dose-dependent manner, with the medium- and high-dose groups being extremely significantly lower than the obese control group

(P<0.01). OB-Rb, JAK2, p-STAT3, SOCS3, and NPY were expressed in the duodenum, jejunum, ileum, colon, and rectum of rats in all groups. Compared with the basal diet group, the expression levels of these five factors in each intestinal segment of obese control group rats were significantly decreased ($P < 0.05$). In the soy isoflavone intervention groups, the expression levels of OB-Rb, JAK2, p-STAT3, and NPY in each intestinal segment were increased compared with the obese control group, showing an overall dose-dependent pattern. It was concluded that soy isoflavones could exert a weight-reducing effect by increasing OB-Rb expression in the intestine, activating JAK, accelerating STAT phosphorylation, improving energy metabolism, and attenuating the leptin resistance state in obese rats.

Full Text

Effects of Soybean Isoflavone on the Leptin-Mediated Janus Kinase/Signal Transducer and Activator of Transcription Signaling Pathway in the Intestine of Obese Rats

LI Like[1], LUO Qihui[1,2], TANG Xiuying[1], HUANG Chao[1], CHEN Xiaolin[1], LIU Wentao[1,2], CHEN Zhengli[1,2,*]

[1]Laboratory of Animal Disease Model, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China [2]Engineering and Technology Center for Laboratory Animals, College of Veterinary Medicine, Sichuan Agricultural University, Ya' an 625014, China

Abstract: This study investigated the regulatory effects of soybean isoflavone (SIF) on the leptin-mediated Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway in the intestine of obese rats to explore the intervention mechanism of SIF on obesity. Eighty 5-week-old male SD rats were randomly divided into a basal diet group (12 rats) and a high-fat diet group (68 rats) after a one-week acclimation period. The rats were fed either a basal diet or high-fat diet for 9 weeks, after which the high-fat diet group successfully established a diet-induced obesity model. Forty obese rats were then randomly assigned to low, medium, and high-dose SIF intervention groups and an obesity control group, with 10 rats per group. The intervention groups received oral administration of SIF extract at doses of 50, 150, and 450 mg/kg body weight (BW), respectively, while the basal diet and obesity control groups received the vehicle without SIF extract. After 5 weeks of continuous SIF intervention, rat body weight was monitored, and the expression and distribution of long-form leptin receptor (OB-Rb), Janus kinase 2 (JAK2), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), suppressor of cytokine signaling 3 (SOCS3), and neuropeptide Y (NPY) in the intestine were examined using immunohistochemical SABC staining.

The results showed that at all time points, the body weight of the basal diet group was significantly lower than that of the obesity control group ($P < 0.01$).

After 5 weeks of SIF intervention, all SIF intervention groups exhibited reduced body weight compared to the obesity control group in a dose-dependent manner, with the medium and high-dose groups showing extremely significant differences ($P < 0.01$). OB-Rb, JAK2, p-STAT3, SOCS3, and NPY were expressed in the duodenum, jejunum, ileum, colon, and rectum of all groups. Compared with the basal diet group, the expression levels of these five factors were significantly decreased in the obesity control group across all intestinal segments ($P < 0.05$). In the SIF intervention groups, the expression levels of OB-Rb, JAK2, p-STAT3, and NPY were increased compared to the obesity control group, showing an overall dose-dependent trend. These findings suggest that SIF can attenuate leptin resistance and reduce body weight in obese rats by increasing intestinal OB-Rb expression, activating JAK, accelerating STAT phosphorylation, and improving energy metabolism.

Keywords: soybean isoflavones; immunohistochemical SABC staining; OB-Rb; JAK2; p-STAT3; SOCS3; NPY; obese rat

---

Obesity is a nutritional and metabolic disorder resulting from the interaction of genetic and environmental factors, which increases the risk of type 2 diabetes, atherosclerosis, hypertension, and fatty liver disease [1]. However, the incidence of obesity and related diseases is relatively low in Asian countries, possibly due to higher consumption of soybeans and soy products in Asian diets [2]. Studies have shown that daily isoflavone intake among Asians ranges from 25 to 40 mg, significantly higher than that in Americans [3-4]. Soy isoflavone (SIF) is a plant-derived estrogen with a molecular structure and weight similar to estrogen that can bind to estrogen receptors and exert weak estrogen-like effects. Through multiple signaling pathways, SIF can reduce preadipocytes and improve lipid and glucose metabolism [5-7], thereby achieving weight loss and lipid-lowering effects [8]. Previous studies have confirmed that SIF can significantly improve leptin levels and regulate energy metabolism [9-10].

Leptin is a functional polypeptide secreted by adipocytes that senses and regulates the body' s energy status [11], with primary physiological functions of regulating food intake and energy expenditure. When leptin binds to the long-form leptin receptor (OB-Rb), it phosphorylates and activates endogenous tyrosine kinase Janus kinase 2 (JAK2). Activated JAK2 phosphorylates specific tyrosine residues (Tyr985 and Tyr1138) on the receptor, with p-Tyr1138 serving as the docking site for phosphorylated signal transducer and activator of transcription 3 (p-STAT3). Upon phosphorylation, p-STAT3 forms dimers that translocate to the nucleus to regulate specific gene transcription and protein synthesis [12-13], influencing membrane potential and action potential firing frequency, altering neurotransmitter and neuropeptide Y (NPY) release, and thereby regulating obesity. In mammalian cell lines, overexpression of suppressor of cytokine signaling 3 (SOCS3) inhibits leptin action by binding to JAK2 and blocking JAK-induced autophosphorylation and receptor phosphorylation. In the peripheral system, the intestine is undoubtedly an important site for

leptin action.

Therefore, to investigate the changes in OB-Rb and JAK/STAT signaling pathway-related factors in the intestine of obese rats regulated by SIF and to explore the intervention mechanism of SIF on obesity, this study utilized immunohistochemical streptavidin-biotin-peroxidase complex (SABC) staining to detect the distribution and expression changes of OB-Rb, JAK2, p-STAT3, SOCS3, and NPY in the intestine of rats under different doses of SIF intervention, aiming to regulate lipid metabolism, attenuate leptin resistance, and improve body weight through SIF modulation.

1.1.1 Experimental Animals

Eighty 5-week-old male SD rats weighing (140 ± 10) g were purchased from the Laboratory Animal Institute of Sichuan Provincial People' s Hospital [License No. SCXK (Chuan) 2013-15].

1.1.2 Experimental Materials and Reagents

The basal diet, high-fat diet, and bedding materials for the experimental rats were purchased from Sichuan Pulaimi Biotechnology Co., Ltd. The composition and nutrient levels of the experimental diets are shown in Table 1 . SIF extract was purchased from Xi' an Tianfeng Biotechnology Co., Ltd. (Batch No. NF-20140806), with a purity of 80% as determined by high-performance liquid chromatography. Rabbit anti-mouse OB-Rb, JAK2, p-STAT3, SOCS3, and NPY antibody kits were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. Ready-to-use SABC-AP kits and diaminobenzidine (DAB) chromogenic reagents were purchased from Wuhan Boster Biological Engineering Co., Ltd.

1.1.3 Experimental Instruments and Equipment

Leica cryostat (Leica Microsystems, Germany), Nikon 50i-BF fluorescence biological digital microscope (Nikon Corporation, Japan), Jiangsu Jieda 801 morphological analysis software (Jiangsu Jieda Technology Development Co., Ltd.), and clean bench, among others.

1.2 Experimental Methods

1.2.1 Animal Treatment The 80 rats were randomly housed in cages under natural light with a 12-hour light-dark cycle, with free access to water and food. The room temperature was maintained at (20 ± 2)°C and humidity at 50%-60% throughout the experimental period. After one week of acclimation, the rats were randomly divided into a basal diet group (12 rats) and a high-fat diet group (68 rats) and fed accordingly. After 9 weeks of feeding, rats in the high-fat diet group whose body weight exceeded the average weight of the basal diet group plus 1.4 times the standard deviation were selected (40 rats total) and randomly

assigned to four groups: low, medium, and high-dose SIF intervention groups and an obesity control group, with 10 rats per group. The SIF intervention groups received oral administration of SIF extract at low, medium, and high doses (50, 150, and 450 mg/kg BW, respectively) dissolved in 0.5% sodium carboxymethyl cellulose solution. The basal diet and obesity control groups received the vehicle (0.5% sodium carboxymethyl cellulose solution without SIF extract) at a dosage of 2 mL/kg BW. SIF intervention lasted for 5 weeks, during which body weight changes were recorded weekly. All animal procedures complied with the Guidelines for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of the People' s Republic of China.

At the end of week 13, rats were euthanized by intraperitoneal injection of 4% pentobarbital sodium. The intestinal segments were rapidly isolated and fixed in 4% paraformaldehyde solution. The fixed tissues were rinsed overnight in running water, then subjected to gradient alcohol dehydration, paraffin embedding, sectioning (4-5 μm thickness), and baking for subsequent use.

1.2.3 Sample Staining and Analysis Five conventional paraffin sections were selected from each rat for hematoxylin-eosin (HE) staining to observe tissue structure under an optical microscope. Additional intestinal tissue paraffin sections from each rat were subjected to immunohistochemical SABC staining to detect the distribution and expression of OB-Rb, JAK2, p-STAT3, SOCS3, and NPY. The immunohistochemical SABC staining procedure was as follows: after conventional deparaffinization, sections were incubated in freshly prepared 3% hydrogen peroxide (H O) for 30 minutes in the dark, washed three times with phosphate-buffered saline (PBS), subjected to antigen retrieval at 95°C for 15 minutes, blocked with 10% goat serum at 37°C for 30 minutes, washed three times with PBS, incubated with primary antibody diluted in PBS (1:200) overnight at 4°C (negative controls omitted primary antibody), washed three times with PBS, incubated with secondary antibody diluted in PBS (1:100) for 1 hour, washed three times with PBS, incubated with SABC at 37°C for 20 minutes, washed four times with PBS, developed with DAB with reaction time controlled under microscope, terminated with double-distilled water, washed four times with double-distilled water, and finally dehydrated, cleared, mounted, and examined. Negative controls were included in all experiments.

1.2.4 Image Acquisition and Data Processing Tissue sections were observed under an optical microscope and photographed using a Nikon 50i-BF fluorescence biological digital microscope. Five sections were selected per tissue, with five random fields photographed per section. The positive expression area and average optical density of each intestinal factor were measured within a 200× field area (4,000 μm²) under light microscopy. Positive expression area and average optical density were compared among different groups. Data were analyzed using one-way ANOVA with SPSS 17.0 statistical software. Data in bar graphs are presented as mean ± standard deviation (±SD), with $P < 0.05$ considered statistically significant.

2 Results and Analysis

2.1 Establishment of the Obese Rat Model

From week 4 onward, the body weight of rats in the high-fat diet group began to exceed that of the basal diet group. By the end of week 8, the average body weight of the high-fat diet group exceeded the average weight of the basal diet group by ±1.4 standard deviations, meeting the criteria for obese rat classification [14], indicating successful establishment of the diet-induced obese rat model.

2.2 Effects of SIF on Body Weight of Diet-Induced Obese Rats

As shown in Table 2 , at the beginning of SIF intervention (week 9), there was no significant difference in body weight between the SIF intervention groups (low, medium, and high dose) and the obesity control group ($P > 0.05$). At all time points, the body weight of the basal diet group was significantly lower than that of the obesity control group ($P < 0.01$). Over time, the rate of body weight gain in all SIF intervention groups was markedly lower than in the obesity control group. After 5 weeks of SIF intervention (week 13), all SIF intervention groups showed reduced body weight compared to the obesity control group in a dose-dependent manner, with the medium and high-dose groups showing extremely significant differences ($P < 0.01$).

Table 2 Body weight of rats at each period of SIF administration (g)

Group	Week 9	Week 10	Week 11	Week 12	Week 13
Obesity control	363.00±6.43	385.00±8.61	405.67±11.57	420.86±12.50	437.57±14.71
Low dosage	366.11±6.11	384.25±6.36	397.56±6.06	414.33±6.60	432.33±6.64
Middle dosage	361.22±3.66	370.98±3.49**	378.71±3.93**	390.00±4.58**	399.63±5.19**
High dosage	364.90±4.41	367.29±5.14*	373.50±3.57**	370.33±9.05**	372.87±8.54**
Basal diet	273.00±5.37**	292.45±6.42**	294.89±10.33**	306.87±6.82**	322.83±6.90**

- and ** indicate significant ($P < 0.05$) and extremely significant differences ($P < 0.01$) compared with the obesity control group, respectively.

2.2 HE Staining Results

After HE staining, optical microscopy revealed clear intestinal tissue structures in all groups, with brightly stained nuclei and intact, uniform tissue sections. No obvious pathological abnormalities were observed in any group (Figure 1 [Figure 1: see original paper]).

2.3.1 Expression of OB-Rb in the Intestine

Microscopic observation revealed that OB-Rb-positive cells appeared yellowish-brown, with OB-Rb-positive substances primarily expressed in the cytoplasm (Figure 2 [Figure 2: see original paper]; as all groups showed consistent patterns, only the medium-dose group is shown). Among the intestinal layers from outer to inner, the lamina propria of the mucosal layer showed the most abundant expression, particularly in the duodenum.

As shown in Figure 3-A, the expression of OB-Rb in all intestinal segments was significantly higher in the basal diet group than in the obesity control group ($P < 0.05$). OB-Rb expression in the duodenum and rectum increased significantly with SIF dose ($P < 0.05$). In the jejunum and ileum, the high-dose group showed significantly higher expression than the medium and low-dose groups ($P < 0.05$), while in the colon, both the high and medium-dose groups showed significantly higher expression than the low-dose group ($P < 0.05$).

2.3.2 Expression of JAK2 in the Intestine

Microscopic observation revealed JAK2 expression throughout the entire intestine (Figure 4 [Figure 4: see original paper]; as all groups showed consistent patterns, only the medium-dose group is shown), with positive JAK2 staining appearing brownish-brown or brownish-yellow. JAK2 was positively expressed in intestinal glands and villi, with expression also observed in the submucosal layer.

As shown in Figure 3-B, JAK2 expression in all intestinal segments was significantly higher in the basal diet group than in the obesity control group ($P < 0.05$). In the duodenum, JAK2 expression was significantly higher in the low and medium-dose groups than in the high-dose group ($P < 0.05$). In the jejunum, the high-dose group showed significantly higher expression than the medium and low-dose groups ($P < 0.05$), while in the ileum, colon, and rectum, expression increased significantly with SIF dose ($P < 0.05$).

2.3.3 Expression of p-STAT3 in the Intestine

Microscopic observation revealed that p-STAT3-positive cells appeared brownish-brown or brownish-yellow (Figure 5 [Figure 5: see original paper]; as all groups showed consistent patterns, only the medium-dose group is shown). p-STAT3 was expressed in all intestinal segments, with positive products predominantly distributed in intestinal glands, villi, and the submucosal layer.

As shown in Figure 3-C, except for the jejunum, p-STAT3 expression in all intestinal segments was significantly higher in the basal diet group than in the obesity control group ($P < 0.05$). In the duodenum, p-STAT3 expression was significantly higher in the low-dose group than in the medium and high-dose groups ($P < 0.05$). In the jejunum, the medium-dose group showed significantly higher expression than the low and high-dose groups ($P < 0.05$), with the low-

dose group also significantly higher than the high-dose group ($P < 0.05$). In the ileum, the medium and high-dose groups showed significantly higher expression than the low-dose group ($P < 0.05$). In the colon, the high-dose group showed significantly higher expression than the low and medium-dose groups ($P < 0.05$), with the low-dose group also significantly higher than the medium-dose group ($P < 0.05$). In the rectum, expression increased significantly with SIF dose ($P < 0.05$).

2.3.4 Expression of SOCS3 in the Intestine

Microscopic observation revealed that SOCS3-positive staining appeared brownish-brown or brownish-yellow, primarily localized in the cytoplasm (Figure 6 [Figure 6: see original paper]; as all groups showed consistent patterns, only the medium-dose group is shown). SOCS3-positive expression products were present throughout the intestine, concentrated in the lamina propria of the mucosal layer.

As shown in Figure 3-D, SOCS3 expression in all intestinal segments was significantly higher in the basal diet group than in the obesity control group ($P < 0.05$). In the duodenum, SOCS3 expression was significantly higher in the high and medium-dose groups than in the low-dose group ($P < 0.05$). In the jejunum, the low and medium-dose groups showed significantly higher expression than the high-dose group ($P < 0.05$). In the ileum, the low-dose group showed significantly higher expression than the medium and high-dose groups ($P < 0.05$). In the colon, the low and medium-dose groups showed significantly higher expression than the high-dose group ($P < 0.05$). In the rectum, the high-dose group showed significantly higher expression than the low and medium-dose groups ($P < 0.05$).

2.3.5 Expression of NPY in the Intestine

Microscopic observation revealed that NPY-positive staining appeared brownish-brown or brownish-yellow (Figure 7 [Figure 7: see original paper]; as all groups showed consistent patterns, only the medium-dose group is shown). Thick NPY-positive nerve fibers were present from the serosa to the submucosal layer and in the duodenal region, while NPY-positive nerve fibers began to decrease in the ileal region. The distribution density of NPY-positive nerve fibers appeared directly related to the distribution of nerve plexuses, with NPY-positive nerve fibers accompanying almost all small blood vessels.

As shown in Figure 3-E, NPY expression in all intestinal segments was significantly higher in the basal diet group than in the obesity control group ($P < 0.05$). In the duodenum and colon, NPY expression was significantly higher in the high-dose group than in the medium and low-dose groups ($P < 0.05$). In the jejunum, ileum, and rectum, expression increased significantly with SIF dose ($P < 0.05$).

3 Discussion

3.1 Effects of SIF on Body Weight of Diet-Induced Obese Rats

In recent years, the prevalence of obesity and related diseases has increased rapidly, particularly in Western countries, making obesity a significant public health risk factor. Therefore, effective strategies for weight reduction and obesity prevention are of great importance. This study demonstrated that SIF intervention significantly reduced body weight in obese rats, consistent with findings by Ali et al. [9] and Ørgaard et al. [15]. The dose-dependent reduction in body weight indicates that SIF possesses weight-loss effects. Research has shown that SIF can significantly reduce triglycerides, total cholesterol, and low-density lipoprotein while increasing high-density lipoprotein content and the ratio of low-density to high-density lipoprotein [15-16]. Therefore, in animal husbandry applications, SIF could be used as a feed additive to improve obesity status by inhibiting adipocyte differentiation [17], reducing adipocyte size [18], decreasing fat deposition [19], and attenuating leptin resistance.

3.2 Effects of SIF on Intestinal Expression of OB-Rb, JAK2, p-STAT3, SOCS3, and NPY in Obese Rats

El-Haschimi et al. [20] reported that most obese patients exhibit significantly higher serum leptin levels than normal individuals, a condition known as hyperleptinemia. However, elevated serum leptin does not lead to weight reduction, indicating leptin resistance, which is considered a fundamental cause of obesity. Leptin resistance is currently thought to involve both central and peripheral mechanisms [21-25]. Data suggest that in diet-induced obesity, leptin resistance primarily occurs at two stages: blood-brain barrier defects and receptor signaling pathway defects [26].

This study demonstrated that OB-Rb, JAK2, p-STAT3, SOCS3, and NPY were all expressed in the intestine of SIF-treated obese rats, consistent with the findings of Cammisotto et al. [26]. Research indicates that SIF is a relatively potent estrogen receptor agonist with potential anti-adipogenic effects, and its obesity-reversing action is related to leptin [27]. Since adipose tissue is the primary source of leptin, which can cross the blood-brain barrier to provide information about body fat stores to the brain for negative feedback regulation [28], adipose tissue also interferes with leptin, OB-Rb, and NPY. As the main functional receptor for leptin, OB-Rb is distributed in both central and peripheral systems, primarily in the hypothalamus [29], where it controls satiety, energy expenditure, and other neuroendocrine functions [30]. The JAK/STAT pathway is the primary signaling pathway for leptin' s biological actions [31], and in rodents, hypothalamic STAT phosphorylation and STAT transcription binding levels are commonly used as markers of leptin signaling.

This study found that in diet-induced obese rats, the expression levels of OB-Rb, JAK2, p-STAT3, and NPY were significantly lower in all intestinal segments compared to the basal diet group, which may be closely related to increased

adiposity in obese rats. Since leptin is a functional polypeptide secreted by adipocytes that senses and regulates the body' s energy status, increased body fat content accelerates leptin secretion, thereby activating the JAK/STAT signaling pathway for negative feedback regulation of adipose tissue. After SIF intervention in obese rats, increased intestinal OB-Rb expression accelerated JAK2 activation and STAT phosphorylation, thereby inhibiting intestinal digestive juice secretion and nutrient absorption and attenuating leptin resistance, resulting in appetite suppression and weight reduction. The role of SOCS3 in inducing leptin resistance has been extensively studied, and it has been confirmed that brain-specific SOCS3 deficiency can inhibit obesity to some extent [32-34]. In this study, SOCS3 expression varied across intestinal segments with no clear dose-dependent differences, suggesting that as a negative regulator of leptin, SOCS3' s inhibitory effect on the JAK/STAT signaling pathway in the intestine was not significant, thereby attenuating leptin resistance and alleviating obesity symptoms. NPY, as an appetite-stimulating neuropeptide, plays an important role in regulating food intake and energy expenditure, and its overexpression can induce weight gain in rats [35]. In this study, increased OB-Rb expression did not suppress NPY expression; instead, NPY showed dose-dependent increases without causing weight gain. This may be because although NPY expression increased, its effect was less potent than the regulation of intestinal digestion and absorption mediated by the OB-Rb/JAK/STAT signaling pathway. However, whether OB-Rb participates in SIF' s regulatory effects and whether interactions exist among OB-Rb, JAK2, p-STAT3, SOCS3, and NPY in the intestine require more detailed and in-depth investigation.

In summary: (1) SIF can increase intestinal OB-Rb expression, activate the JAK/STAT signaling pathway, improve energy metabolism, and attenuate leptin resistance in obese rats. (2) SIF exhibits weight-reducing effects on diet-induced obese rats in a dose-dependent manner within the range of 50–450 mg/kg BW.

References

[1] MARQUES M D, SANTOS R D, PARGA J R, et al. Relation between visceral fat and coronary artery disease evaluated by multidetector computed tomography[J]. Atherosclerosis, 2010, 209(2): 481–486.

[2] LLANEZA P, GONZÁLEZ C, FERNANDEZ-IÑARREA J, et al. Soy isoflavones, diet and physical exercise modify serum cytokines in healthy obese postmenopausal women[J]. Phytomedicine, 2011, 18(4): 245–250.

[3] WAKAI K, EGAMI I, KATO K, et al. Dietary intake and sources of isoflavones among Japanese[J]. Nutrition and Cancer, 1999, 33(2): 139–145.

[4] CHEN Z, ZHENG W, CUSTER L J, et al. Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai[J]. Nutrition and Cancer, 1999, 33(1): 82–87.

[5] ZHU C F, ZHANG F, XU X Y, et al. Effects of soybean isoflavone combined with antioxidants on lipid metabolism in type 2 diabetic patients[J]. Chinese Journal of Clinical Rehabilitation, 2004, 8(9): 1674-1676.

[6] MAO G M, CHEN J, XIA Y, et al. Study on the effect of soybean isoflavone on lipid metabolism and weight loss in rats[J]. Zhejiang Journal of Preventive Medicine, 2007, 19(7): 10-11, 14.

[7] CEDERROTH C R, VINCIGUERRA M, GJINOVCI A, et al. Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism[J]. Diabetes, 2008, 57(5): 1176-1185.

[8] MEZEI O, BANZ W J, STEGER R W, et al. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese zucker rats and murine RAW 264.7 cells[J]. The Journal of Nutrition, 2003, 133(5): 1238-1243.

[9] ALI A A, VELASQUEZ M T, HANSEN C T, et al. Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes[J]. The Journal of Nutritional Biochemistry, 2004, 15(10): 583-590.

[10] GONG H F, ZHANG Y X, WANG X D, et al. Effects of soybean isoflavone on glucose and lipid metabolism and serum leptin and resistin expression in ovariectomized rats[J]. Journal of Jinan University: Natural Science Edition, 2011, 32(3): 324-327, 338.

[11] ZHANG Y Y, PROENCA R, MAFFEI M, et al. Positional cloning of the mouse obese gene and its human homologue[J]. Nature, 1994, 372(6505): 425-432.

[12] BANKS A S, DAVIS S M, BATES S H, et al. Activation of downstream signals by the long form of the leptin receptor[J]. Journal of Biological Chemistry, 2000, 275(19): 14563-14572.

[13] DARNELL J E, Jr. STATs and gene regulation[J]. Science, 1997, 277(5332): 1630-1635.

[14] HUANG C, PANG D J, LUO Q H, et al. Soy Isoflavones Regulate Lipid Metabolism through an AKT/mTORC1 Pathway in Diet-Induced Obesity (DIO) Male Rats[J]. Molecules, 2016, 21(5): 1-13.

[15] ØRGAARD A, JENSEN L. The effects of soy isoflavones on obesity[J]. Experimental Biology and Medicine, 2008, 233(9): 1066-1080.

[16] LIN Y, MEIJER G W, VERMEER M A, et al. Soy protein enhances the cholesterol-lowering effect of plant sterol esters in cholesterol-fed hamsters[J]. The Journal of Nutrition, 2004, 134(1): 143-148.

[17] TAXVIG C, SPECHT I O, BOBERG J, et al. Dietary relevant mixtures of phytoestrogens inhibit adipocyte differentiation in vitro[J]. Food and Chemical Toxicology, 2013, 55: 265-271.

[18] LUO Q H, LIU Y, TANG X Y, et al. Effects of soybean isoflavone on the expression of -MSH, POMC, and Ob-Rb in the arcuate nucleus and pituitary of obese rats[J]. Acta Agriculturae Boreali-Sinica, 2015, 30(4): 84-89.

[19] NAAZ A, YELLAY S, ZAKROCZYMSKI M A, et al. The soy isoflavone genistein decreases adipose deposition in mice[J]. Endocrinology, 2003, 114(8): 3315-3320.

[20] EL-HASCHIMI K, PIERROZ D D, HILEMAN S M, et al. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity[J]. Journal of Clinical Investigation, 2000, 105(12): 1827-1832.

[21] ZHAI L L, YAO X J, WANG B, et al. Relationship between leptin, neuropeptide Y mRNA and obesity resistance[J]. Chinese Journal of Public Health, 2005, 21(3): 259-260.

[22] QIN P J, TONG X L. Research progress on leptin resistance mechanisms in obesity[J]. Medical Recapitulate, 2010, 16(5): 662-665.

[23] LIU H, HU Y H. Research progress on genetic epidemiology of leptin receptor gene and resistin gene in relation to overweight and obesity[J]. Chinese Journal of Prevention and Control of Chronic Diseases, 2008, 16(5): 544-546.

[24] LI X H, ZOU N. Research progress on leptin signal transduction and leptin resistance[J]. Chinese Journal of Child Health Care, 2009, 17(2): 179-180, 183.

[25] CUI Z H, MA G S. Research progress on mechanisms of leptin resistance[J]. Foreign Medical Sciences: Hygiene, 2004, 31(2): 101-104.

[26] CAMMISOTTO P G, RENAUD C, GINGRAS D, et al. Endocrine and exocrine secretion of leptin by the gastric mucosa[J]. Journal of Histochemistry & Cytochemistry, 2005, 53(7): 851-860.

[27] BU L H, SETCHELL K D R, LEPHART E D. Influences of dietary soy isoflavones on metabolism but not nociception and stress hormone responses in ovariectomized female rats[J]. Reproductive Biology and Endocrinology, 2005, 3: 58.

[28] BRENNAN A M, MANTZOROS C S. Drug insight: the role of leptin in human physiology and pathophysiology-emerging clinical applications[J]. Nature Clinical Practice Endocrinology & Metabolism, 2006, 2(6): 318-327.

[29] WANG X Z, MA A Q, WANG J, et al. Distribution of leptin receptors in the central nervous system of normal rats[J]. Chinese Journal of Hypertension, 2007, 15(1): 66-69.

[30] ZIGMAN J M, ELMQUIST J K. Minireview: from anorexia to obesity-the yin and yang of body weight control[J]. Endocrinology, 2003, 144(9): 3749-3756.

[31] HEGYI K, FÜLÖP K, KOVÁCS K, et al. Leptin-induced signal transduction pathways[J]. Cell Biology International, 2004, 28(3): 159-169.

[32] HOWARD J K, CAVE B J, OKSANEN L J, et al. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3[J]. Nature Medicine, 2004, 10(7): 734–738.

[33] MORI H, HANADA R, HANADA T, et al. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity[J]. Nature Medicine, 2004, 10(7): 739–743.

[34] ZAMPIERI T T, RAMOS-LOBO A M, FURIGO I C, et al. SOCS3 deficiency in leptin receptor-expressing cells mitigates the development of pregnancy-induced metabolic changes[J]. Molecular Metabolism, 2015, 4(3): 237–245.

[35] MAHMOODI M, GARDINER J V, ASADI KARAM G R, et al. Implantation of fibre encapsulated RIN 1056a cells transfected with NPY cDNA into the lateral ventricle of rats alters body weight[J]. Regulatory Peptides, 2005, 132(1/2/3): 80–84.

Note: Figure translations are in progress. See original paper for figures.