

Original Article

Taeyeumjoweetang Affects Body Weight and Obesity-related Genes in Mice

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Taeyeumjoweetang (TYJWT) is a herbal medication that was mentioned in Jema Lee's *Donguisusebowon*, which is a book about Sasang constitutional medicine. Tae-eumnis, one of the four constitutions, tend to suffer from metabolic diseases such as obesity and diabetes. It is widely used to treat the digestive problems and obesity of Tae-eumins. We divided mice that were fed a normal diet for 48 days into control, TYJWT 250 mg kg⁻¹ and TYJWT 500 mg kg⁻¹ groups. After carrying out the experiments, the serum levels of leptin, adiponectin, ghrelin and resistin were measured. The results showed that TYJWT significantly reduced the weights of mice that were fed a normal diet, and that this was due to a decrease in food intake. Also, the two TYJWT groups had lower serum levels of leptin compared to the control group, and the ghrelin levels were proportionately increased by the dosage of TYJWT given. These results show that TYJWT has obesity-suppressing effects similar to those previously reported using high fat diets. In addition, these results also provide evidence that TYJWT has anti-obesity effects.

Keywords: ghrelin – leptin – Sasang constitutional medicine

Introduction

Taeyeumjoweetang (TWJWT) was first mentioned by Jema Lee (1837–1900) in *Donguisusebowon* as a herbal medication consisting of eight herbs, which is used to treat 'exterior-cold disease by cold in the esophagus' of Tae-eumin (1). To date, research on the anti-obesity effects of TYJWT showed that it suppressed the weights and serum lipid profiles of obese mice induced by a high-fat diet (2), its herbal acupuncture form improved the high-fat-diet-induced lipid profiles of serum and the liver (3) and that it suppressed leptin gene expression in obese mice (4).

Obesity is a chronic metabolic disorder caused by an imbalance between energy intake and usage, and is characterized by weight gain due to an increase in body fat and serum lipid content (5,6). Recent research shows that several substances are secreted by adipose cells that either cause or improve obesity. Among these, leptin and resistin are important factors that control weight, although their exact roles have not yet been defined (7). Ghrelin is secreted by the stomach (8) and its synthesis and secretion are primarily controlled by changes in body energy. Its secretion is increased when the energy level is low and secretion decreases when the energy level is high (9). Ghrelin expression is also controlled by leptin (10).

To date, research into the anti-obesity effects of TYJWT have used obese animal models that were induced by a high-fat diet (2–4). TYJWT has been widely used for treatment of Tae-eumins' obesity in Korea. In order to closely approach the clinical

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condition, we explored the effects of TYJWT on mice that were fed a normal diet. For 48 days, we measured the weights and food intakes of mice that were given 250 and 500 mg kg⁻¹ doses of TYJWT, as well as the levels of serum leptin, resistin, ghrelin and adiponectin.

Methods

Test Animals

We purchased 30 C57BL/6J male mice (20–25 g) that were 3 weeks old from Central Lab. Animal Inc. The mice were adapted to the test environment by being fed standard experiment diet for 1 week. The mice were raised in a controlled environment (temperature at 20°C, 50% humidity, light cycle from 06:00 to 18:00 hours and dark cycle from 18:00 to 06:00 hours), and during the experiment they were allowed to drink water freely. All these procedures involving the use of animals have been conducted in agreement with NIH Guidelines and approved by the Animal Use and Care Committee of Wonkwang University.

TYJWT Extraction Method

After purchasing TYJWT (Table 1) from Wonkwang Oriental Medical Hospital, it was certified by the Department of Herbology, College of Oriental Medicine, Wonkwang University before use. TYJWT (200 g) was placed in a round bottom flask with 1.8 l of triple distilled water. After attaching a freezer, it was boiled for 3 h, centrifuged at 3000 rpm for 20 min, decompressed and concentrated using a rotating vacuum concentrator. Using a freeze drying machine, 31.3 g of powder was obtained. This was stored at -70°C until used in experiments.

Test Groups

The mice were divided into three groups and were fed for 48 days as follows. The control group was fed a normal

diet and was given saline twice daily. The TYJWT groups were given oral doses of 250 and 500 mg kg⁻¹ twice daily, at 09:00 and 21:00 hours. Weights and food intakes were measured every 4 days. After this period, the mice were decapitated, serum was separated, and separated epididymal fat and stomach were stored at -70°C until assayed.

ELISA

Serum concentrations for leptin, adiponectin, ghrelin and resistin were measured using ELISA kits (Roche Diagnostics, CA, USA) according to the manufacturer's protocols.

RNA Extraction

The extraction of total RNA from the stomach and fat tissues used Trizol reagent (Life Technologies, UK) according to the manufacturer's protocols. Tissue (100 mg) was cut into pieces, dissolved in 500 µl RNAzol B, 50 µl chloroform was added, and the reaction was run for 5 min on ice. After the reaction, samples were centrifuged at 15 700g at 4°C for 20 min, and then the upper layer was put in a new tube. An equal volume of isopropanol was added to the upper layer, and the reaction was run for 30 min. After the reaction, samples were centrifuged at 15 700g at 4°C for 20 min, and then the sediments were cleansed with 80% EtOH. The cleansed RNA was dried, melted with 20 of DEPC-treated water and the optical density was quantified using a spectrophotometer.

Real-Time Reverse Transcription Polymerase Chain Reaction

Reverse transcription reactions used 3–5 µg total RNA and reverse transcriptase (MMLV; Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The reaction used a reaction solution of total RNA (3–5 µg), oligo d (T) 12–18 (1 µg), 2 µl dNTP (10 mM), MMLV reverse transcriptase (200 U), DTT (10 mM), RNase inhibitor (1 µl; Promega, USA) and a 20 µl buffer solution (50 mM Tris-Cl, pH 8.3, 75 mM KCl, 3 mM MgCl₂) at 42°C for 60 min, after which cDNA was synthesized. Real-time reverse transcription polymerase chain reaction was done by mixing cDNA (diluted 10 times) with 2× SYBR-Green buffer solution (Roche Diagnostics Ltd, UK), which contained reverse transcription enzymes, using a LightCycler rapid thermal cycler system (Roche). In summary, after letting the reaction mixture react at 95°C for 10 min, 45 cycles of denaturation (95°C 10 s), annealing (58°C for leptin, ghrelin or 60°C for β-actin, 5 s) and elongation (72°C 10 s) were done. The expressed leptin and ghrelin concentrations were calculated relative to the amount of β-actin using

Table 1. Composition of TYJWT

Herbal name	Scientific name	Amount (g)
薏苡仁	Semen Coicis	11.25
乾栗	Castanea Mollisima	11.25
蘿菔子	Semen Raphani	7.5
五味子	Fructus Schizandrae	3.75
麥門冬	Radix Ophiopogonis	3.75
石菖蒲	Rhizoma Acori Graminei	3.75
桔梗	Radix Platycodi	3.75
麻黃	Heraba Ephedrae	3.75
	Total amount	48.75

Table 2. Sequences and accession numbers for primers used for real-time PCR

Gene	Sequence for Primers (5'→3')	Accession no.
β-Actin	Forward: gtgctatgttgcctctagact Reverse: cacaggattccataccaag	NM 007393
Leptin	Forward: caggatcaatgacatttcacaca Reverse: gctggtgaggacctgtgat	NM 008493
Ghrelin	Forward: ccagaggacagaggacaagc Reverse: catcgaaggagcattgaac	NM 021488

LightCycler System software (Roche). The primers for leptin, ghrelin and β-actin are shown in Table 2.

Statistical Analysis

Test results were expressed as mean ± SEM. Group comparisons used a one-way ANOVA test (Microcal Origin; version 6.0; Microsoft, USA), with $P < 0.05$ considered statistically significant.

Results

Decrease of Body Weight

In order to explore the effects of TYJWT on weight changes of normal mice, 250 and 500 mg kg⁻¹ oral doses of TYJWT were given for 48 days, and weight changes were measured every 4 days. As shown in Fig. 1, the control group mice, which were fed a normal diet, showed significant weight gains as time passed. However, the TYJWT groups showed significant weight losses compared to the control group starting at Day 36, and the weight loss effects in the 250 mg kg⁻¹ group was more pronounced than the 500 mg kg⁻¹ group (Fig. 1). Regarding food intake, starting at Day 36, the TYJWT groups ate less compared to the control group (Fig. 2).

Changes in Leptin and Ghrelin Expression

In order to find a relationship between the weight loss effects of TYJWT and appetite, we examined the serum levels of leptin and ghrelin, as well as their mRNA expressions in the fat and stomach tissues (Fig. 3). The results showed that the 500 mg kg⁻¹ TYJWT group secreted significantly less leptin into the bloodstream compared to the control group, and the expression of leptin mRNA in fat tissue also coincided with this result. By comparison, the levels of ghrelin secreted into the serum as well as the expression of mRNA in the stomach tissue were proportionate to the dosage of TYJWT given.

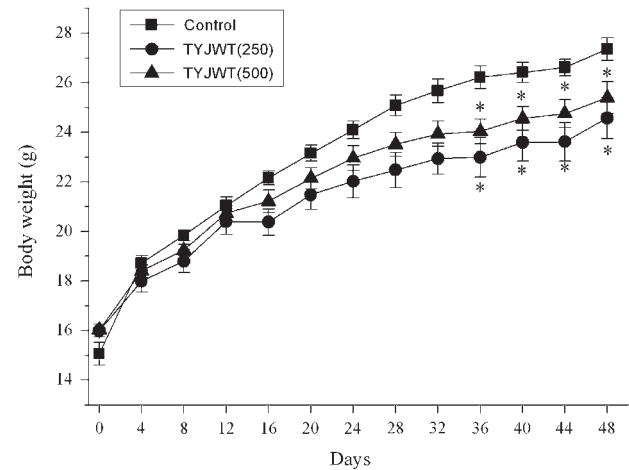


Figure 1. Effects of TYJWT on body weight changes in mice. Control: normal diet group, $n=10$; TYJWT (250): normal diet + 250 mg kg⁻¹ TYJWT extract; TYJWT (500): normal diet + 500 mg kg⁻¹ TYJWT extracts. Values are mean ± SEM. * $P < 0.05$ versus control.

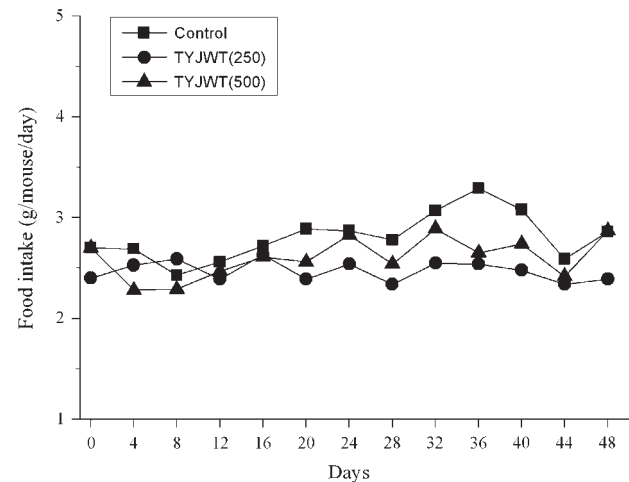


Figure 2. Effects of TYJWT on food intake in mice.

Reduction in Secretions of Adiponectin and Resistin

In order to examine a weight loss mechanism for TYJWT, the levels of adiponectin and resistin secreted into the serum were measured (Fig. 4). The adiponectin levels of the two TYJWT groups were not significantly different from one another, but TYJWT reduced the amounts of resistin secreted into the bloodstream in a dose-dependent manner.

Discussion

Obesity is a metabolic disorder caused by excess energy intake relative to energy usage and is a state in which the supply of energy is so excessive that the remainder of energy accumulates in the body (11,12). Because obesity

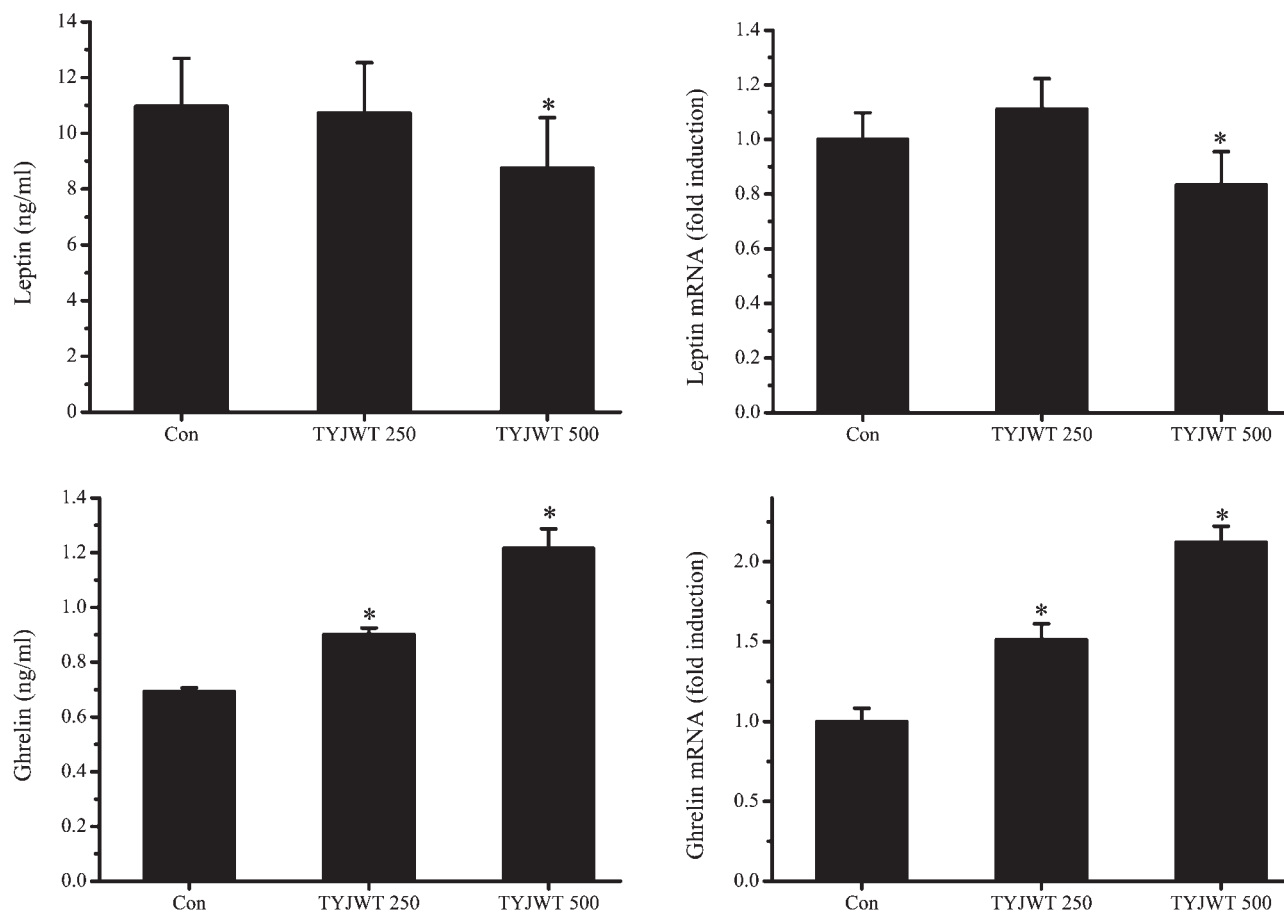


Figure 3. Effects of TYJWT on leptin and ghrelin expressions in mice. Leptin and ghrelin concentrations in plasma were measured by ELISA; mRNA levels were measured by the real-time PCR assay as described in the 'Methods' section. Values are mean \pm SEM. * $P < 0.05$ versus control.

is strongly associated with depression, diabetes, hypertension, hyperlipidemia, coronary heart disease and arthritis, there have been numerous efforts to prevent and treat obesity. However, most medications used have significant side effects as well as hepatotoxicity, so their long-term use is impossible. And long-term medications that have been approved by the FDA, such as orlistat (Xenical) and sibutramine (Reductil), have been shown to suppress fat accumulation and induce weight loss, but have side effects like oily stool, phisconia, undurable stool, headache, constipation, thirst, nausea, dizziness and insomnia (13,14). Therefore, research into herbal medications, which have been proven to have fewer side effects than these medications, is needed.

TYJWT is the most widely used clinical anti-obesity herbal medication. Although it has been reported on its positive effects for obesity many times (2–4), its anti-obesity mechanism has not yet been clarified. Tests that examined the anti-obesity effects of TYJWT have mainly used obese animal models that were induced by a high fat diet (2–4). In contrast, our research examined the effects of TYJWT on mice that were fed a normal diet. TYJWT extract significantly reduced the weight gains caused by

a normal diet (Figs 1 and 2). Furthermore, TYJWT suppressed slightly fat accumulation in the epididymal fat pad (data not shown) without suppressing feed intake, which led to a decrease in the body weight gain. These results indirectly suggest that TYJWT decreases weight gain not only by reducing fat mass but also by reducing lean body mass. Therefore, further study is required to clarify the possible metabolic effect of TYJWT on lean body mass.

Generally, leptin is secreted by fat tissues and controlled by neuropeptide Y from the thalamus, which reduces food intake and suppresses weight gain (15). But, when its secretion is excessive, or when leptin exposure is prolonged, it accelerates lipolysis and increases energy use (16). The lower leptin concentration observed in the serum of TYJWT-treated mice could not be related to the improvement of metabolic changes, such as the lipid-lowering effect of TYJWT. Considering the current state of research into the role of leptin in obesity and obesity-related genes, more research is needed.

In contrast to the appetite-suppressing effects of leptin, ghrelin, which is secreted by the stomach, increases appetite. Ghrelin has been shown to be controlled by

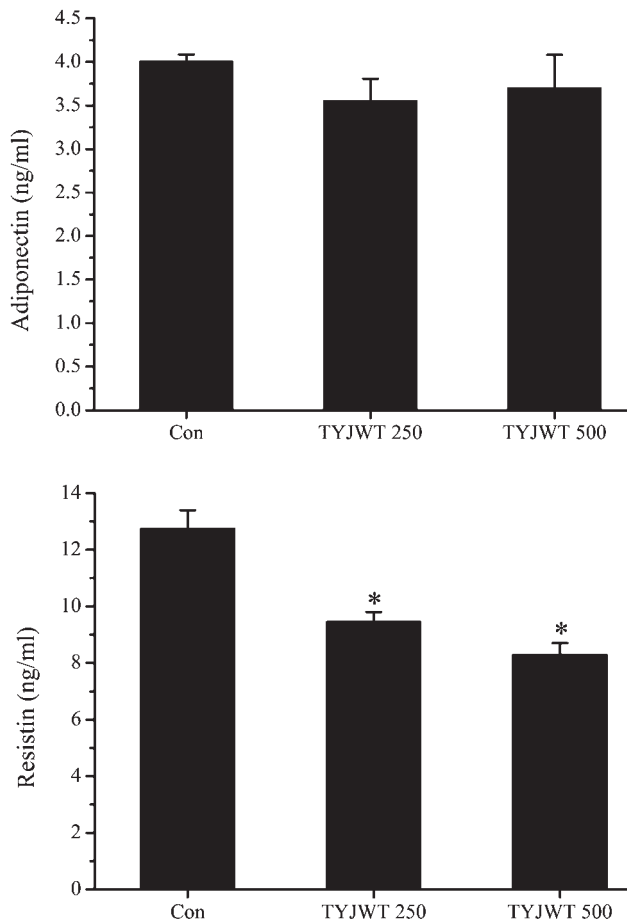


Figure 4. Effects of TYJWT on plasma adiponectin and resistin in mice. Adiponectin and resistin levels in plasma were measured by ELISA. Values are mean \pm SEM. * $P < 0.05$ versus control.

the body's energy levels, as its levels increase during hunger and decrease when food is eaten (9). Leptin, insulin and somatostatin have been reported to be affected by the secretion of ghrelin in the stomach (9,10,17). In our tests, the 500 mg kg⁻¹ TYJWT group showed reduced leptin expression compared to the control group. But, the levels of ghrelin increased proportionately to the dosage of TYJWT given. According to research on the effects of leptin and ghrelin on appetite and weight (9,17), TYJWT extract increased appetite and weight, yet in our tests, TYJWT extract resulted in reduced weight. According to Toshinai (18), when leptin was given to mice for 5 days, the ghrelin mRNA levels increased. However, Asakawa *et al.* (9) reported that leptin decreased the expression of ghrelin. Initial reports showed that leptin is secreted by fat tissues (19), but it is now known that it is also secreted from various tissues, including the stomach (20). The possibility that the leptin-reducing effect of TYJWT is due to its influence on leptin expression and secretion by other tissues besides fat tissue cannot be excluded, and further research

is necessary. Also, we conclude that leptin influences the expression of ghrelin in the stomach.

Recently, fat tissue has been found not only to store fat but also to secrete adipocytokines, such as adiponectin, leptin and TNF- α . Recent research shows that serum adiponectin levels are lower in obesity that is accompanied by type 2 diabetes (21). Resistin increases insulin resistance and fat synthesis. Thus, in resistin knock-out mice, glucose intolerance was improved and fat synthesis was suppressed in the fat tissues (22). As shown in Fig. 4, TYJWT extract did not influence the serum adiponectin levels, but it did reduce the resistin levels inversely according to the dosage. We can analogize that TYJWT extract lowers weight by suppressing fat synthesis due to decreased food intake, but the lipolysis effects of TYJWT cannot be excluded.

In our tests, TYJWT reduced the weights of mice that were fed a normal diet. Along with previous results showing that TYJWT extract suppressed obesity in high-fat-diet-fed mice (2-4), these results will be a basis for the development of TYJWT as an anti-obesity medication. For the future, more detailed research into the mechanisms of TYJWT's anti-obesity effects must be done.

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