Thesis for the Degree of Doctor of Oriental Medicine

Effects of SBY-Ⅰ on Weight, Plasma, and UCP mRNA Expressions in Zucker Rats

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by

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Submitted to the Department of Oriental Rehabilitation Medicine and the Faculty of Graduate School of KyungHee University in partial fulfillment of the requirements for degree of Doctor of Oriental medicine

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ABSTRACT

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Objective: SBY-Ⅰ is a herbal formula based on *Mahwangbalpyo-tang* (*Mahuangfabiao-tang*, 麻黃發表湯) which regulates the *Taeumin*(*Taiyinren*, 太陰人)'s exterior syndrome in Korea's *Sasang*(*Sixiang*, 四象) constitutional medicine. The present study was aimed to investigate whether the obesity can be improved by the oral administration of SBY-Ⅰ extracts.

Materials and Methods: The weight of the whole body, liver and adipose tissues, food consumption, uncoupling protein(UCP) mRNA expression, and plasma levels of total-cholesterol, triglyceride, HDL-cholesterol, and leptin were measured in male lean(+/+) and obese(fa/fa) Zucker rats administered SBY-Ⅰ extracts(0.125mg/g, twice daily) for 3 weeks. These were then compared with those of control groups administered physiological saline. Statistical comparisons between the two groups were done by student's

t-test.

Results: Oral administration of SBY-Ⅰ extracts for 3 weeks has been shown to exert anti-obesity and hypolipidemic effects in both lean and obese Zucker rats. The extracts not only reduced weight of body, liver, and adipose tissues but also increased plasma levels of HDL-cholesterol, induced leptin, and up-regulated UCP mRNA expressions in brown adipose tissue which contribute to mitigation of obesity.

Conclusions: This study demonstrates that SBY-Ⅰ has an effect on the treatment of obesity. Clinical studies are thought to be required.

Key Words: Obesity, Herb, Zucker rat, Leptin, UCP, Lipid

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Ⅰ. INTRODUCTION

Obesity is a disorder of energy balance in which energy intake is greater than energy expenditure, and it is associated with increased risk of chronic diseases such as type 2 diabetes and cardiovascular disease, including hypertension[1,2] as a major cause of morbidity and mortality[3].

Methods to control obesity through limiting energy intake have had modest success at best, and it is widely recognized that energy expenditure must be increased in an obese individual if long term weight loss is to be achieved. The recent discovery of several new uncoupling proteins(UCPs) provide new molecular targets for increasing energy expenditure. Thus, UCPs are potentially important in disorders of energy balance such as obesity and diabetes[4,5].

In oriental medicine, the metabolic disorders of spleen, lungs and kidneys are thought to be the main cause of obesity. Wind, phlegm, damp and heat are also can be considered[6,7]. The frequent cause of obesity, such as damp-phlegm, appears when the metabolism of water is broken. Present forms of treatment include acupuncture, herbal formula, and *Qigong*(氣功), etc[7].

According to Korea's *Sasang*(*Sixiang*, 四象) constitutional medicine, *Taeumin*(*Taiyinren*, 太陰人) takes the most part of obesity. He or she has the best physique among the four types: well built skeleton, large limbs and well developed skin and muscle[8]. That's why when we treat the

obesity by use of herb, the herb for *Taeumin* is the most frequently used one.

SBY-Ⅰ is a herbal formula based on *Mahwangbalpyo-tang* (*Mahuangfabiao-tang*, 麻黃發表湯) which regulates the *Taeumin*'s exterior syndrome.

The current study was designed to elucidate the anti-obesity and hypolipidemic effect of SBY-Ⅰ extract by measurement of weight, plasma lipid and leptin levels, and UCP mRNA expressions in Zucker rats.

Ⅱ. MATERIALS AND METHODS

A. Materials

1. Animals

Male lean(+/+) weighing 128.22±12.84g and obese(fa/fa) weighing 137.25 ±2.92g Zucker rats(SLC. Co., Hammatsu, Japan) were obtained at the age of 5 weeks and housed individually in plastic cages until the age of 10 weeks at 22±2℃ with 12hr light-dark cycle. They were allowed to free access to laboratory chow(Samyang Co., Seoul, Korea) and tap water.

2. Herbal formula

The herbs used in this study were obtained from Department of Herbal Pharmacology, Graduate School of East-West Medical Science, KyungHee University.(Seoul, Korea). The composition of a pack of SBY-Ⅰ are listed in Table Ⅰ.

Table Ⅰ. Composition of SBY-Ⅰ

Composition	Dose(g)
Platycodi Radix(root of *Platycodon grandiflorum*, 桔梗)	12.00
Ephedrae Herba(root of *Ephedra sinica*, 麻黃)	6.00
Mori Folium(leaf of *Morus alba*, 桑葉)	6.00
Liriopis Tuber(root of *Liriope platyphylla*, 麥門冬)	4.00
Scutellariae Radix(root of *Scutellaria baicalensis*, 黃芩)	4.00
Armeniacae amarum Semen (seed of *Prunus armeniaca var. ansu*, 杏仁)	4.00
Total amount	36.00

B. Methods

1. Preparation of extracts

180g of SBY-Ⅰ was decocted in a 3,000㎖ round flask with 1,000㎖ distilled water at 100℃ for 2hr and then filterated. After that, secondary 1,000㎖ distilled water was added and decocted at 100℃ for 1hr and then

filterated again. Each filterates were mixed and condenced using an evaporative system. Finally, they were powdered using a freezing dryer(EYELA CA-1500, Rikakikai, Japan). 79.46g(44.2%) of powder was obtained.

2. Experimental procedures

After an acclimation period of 2 weeks, the animals were divided into four groups consisting of 5 rats each, and fed a normal diet. Control Ⅰ and sample Ⅰ group used lean Zucker rats and control Ⅱ and sample Ⅱ group used obese Zucker rats. The sample groups were orally administered SBY-Ⅰ extracts at a dose of 0.125mg/g twice daily for 3 weeks. The control groups were administered physiological saline daily. The contents of diet(laboratory chow: Samyang Co., Seoul, Korea) are listed in Table Ⅱ.

Table Ⅱ. The Contents of Normal Diet

Contents	Weight(g)
Gross protein	248.0
Gross lipid	44.0
Gross cellulose	35.0
Gross gray flour	70.0
Water	87.0
Soluble-lacking nitrogen substance	516.0
Total amount	1,000.0

3. Measurement of weight and food consumption

Body weight and food consumption were measured with an electronic balance(Voyager 210A, Ohaus, Swizerland) daily(6:00pm) for 3 weeks.

4. Blood sampling and tissue harvesting

At the end of 3-week-administration of SBY-Ⅰ extracts or saline, animals were anesthetized, and blood was collected by cardiac puncture. Blood was then centrifuged at 1500× G for 15min at 4℃. Separated plasma had been stored at -70℃ until later biochemical measurements. After blood collection, animals were killed and tissues were collected. Liver, interscapular brown adipose tissue(BAT) as well as epididymal and retroperitoneal white adipose tissue(WAT) were removed, weighed and immediately frozen in liquid nitrogen. The tissues had been stored at -70℃ until analyses. Final weights were translated 10^{-3}g per 1g of body weight.

5. Plasma determination

Levels of total cholesterol, triglyceride and HDL-cholesterol were determined by enzymatic methods(Hitachi 736-40 autoanalyzer, Japan) as previously described[9,10]. Leptin level was determined using insulin radioimmunoassay kit(Linco Research Inc., St. Charles, U.S.A.).

6. Reverse transcription-polymerase chain reaction(RT-PCR)

Total RNA was extracted from liver and adipose tissues by a sequential

addition of 4M guanidinium thiocyanate, 2M sodium acetate, and acid phenol and chloroform as described previously[11]. Reverse transcription was carried out using a superscript(Gibco-BRL) and oligo(dT) primers. PCR amplification using specific primer sets was carried out at 60℃ annealing temperature for 30 cycles(Table Ⅲ). Nucleotide sequences of the primers specific for rat UCP1, UCP2, and rat glyceraldehyde-3-phosphate dehydrogenase(GAPDH) based on published cDNA sequences are listed in table Ⅳ. Amplified RT-PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The expected size of RT-PCR products for UCP1, UCP2, and GAPDH are 197bp, 293bp and 234bp, respectively.

Table Ⅲ. Temperature Conditions of PCR

	Temperature (℃)	Time (second)	Cycle
	95	180	1
Denature	95	30	
Annealing	60(UCP1) (UCP2)	30	30
Extension	72	30	
	72	420	1

PCR: Polymerase chain reaction

Table Ⅳ. Sequence of Primers Used for Quantitative RT-PCR

Primers		Sequences
UCP1	UCP1-sense	5′-GTG AAG GTC AGA ATG CAA GC-3′
	UCP1-antisense	5′-AGG GCC CCC TTC ATG AGG TC-3′
UCP2	UCP2-sense	5′-ACA AGA CCA TTG CAC GAG AG-3′
	UCP2-antisense	5′-CAT GGT CAG GGC ACA GTG GC-3′
GAPDH	GAPDH-sense	5′-CTG CCA CTC AGA AGA CTG TGG-3′
	GAPDH-antisense	5′-CTT GAT GTC ATC ATA CTT GGC-3′

RT-PCR: Reverse transcription-polymerase chain reaction

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

7. Statistical analysis

Sample Ⅰ,Ⅱ group were compared with control Ⅰ,Ⅱ group respectively and statistical comparisons of data between the two groups were done by student′s *t*-test. Results represent mean±S.D. of 5 animals administered with either saline or SBY-Ⅰ extracts for 3 weeks.

Ⅲ. RESULTS

A. Effect on the body weight

The increased rate of body weight in each group is shown in fig. 1.

Compared with control group, in sample Ⅰ group the increased rate of body weight was significantly lower($P<0.0001$) and in sample Ⅱ group increased rate was lower with no significance.

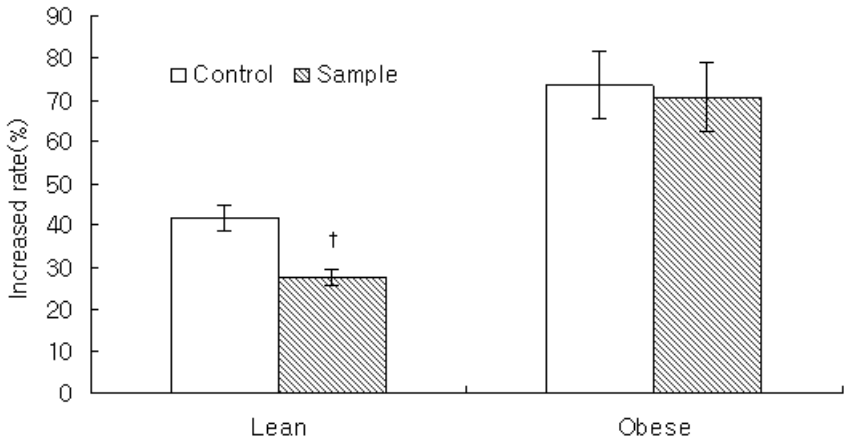

Fig. 1. Effect of SBY-Ⅰ on the body weight gain in lean and obese Zucker rats.

† $P<0.0001$ vs. their normal diet control group

Values represent mean ± standard deviation

B. Effect on food consumption

The changes of food consumption in each group are shown in table Ⅴ, fig. 2 and 3.

No significant differences were observed in each group.

Table Ⅴ. Effect of SBY-Ⅰ on Food Consumption in Zucker Rats

	Mean food consumption(g/day)	
	Pre-administration	During-administration
Control Ⅰ	16.95±0.69	19.60±0.95
Sample Ⅰ	16.69±1.24	18.11±1.33
Control Ⅱ	21.40±0.96	26.69±1.90
Sample Ⅱ	19.55±0.67	25.33±2.08

Values represent mean±standard deviation

Control Ⅰ: Lean Zucker rats administered physiological saline for 3wk.

Sample Ⅰ: Lean Zucker rats administered SBY-Ⅰ extracts for 3wk.

Control Ⅱ: Obese Zucker rats administered physiological saline for 3wk.

Sample Ⅱ: Obese Zucker rats administered SBY-Ⅰ extracts for 3wk.

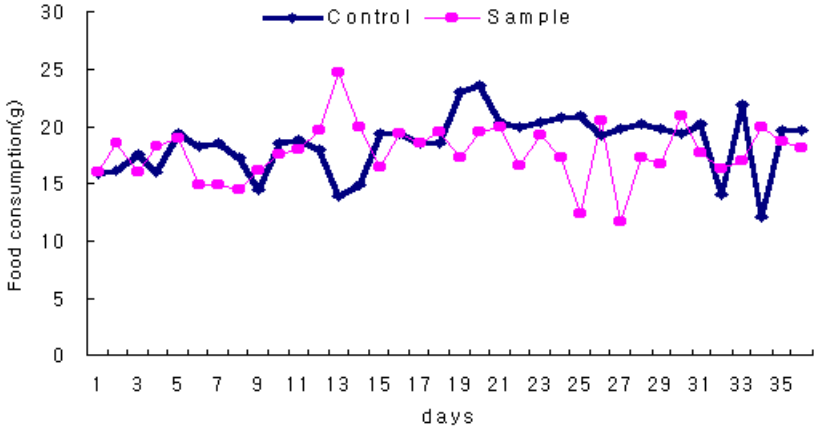

Fig. 2. Effect of SBY-Ⅰ on food consumption in lean Zucker rats.

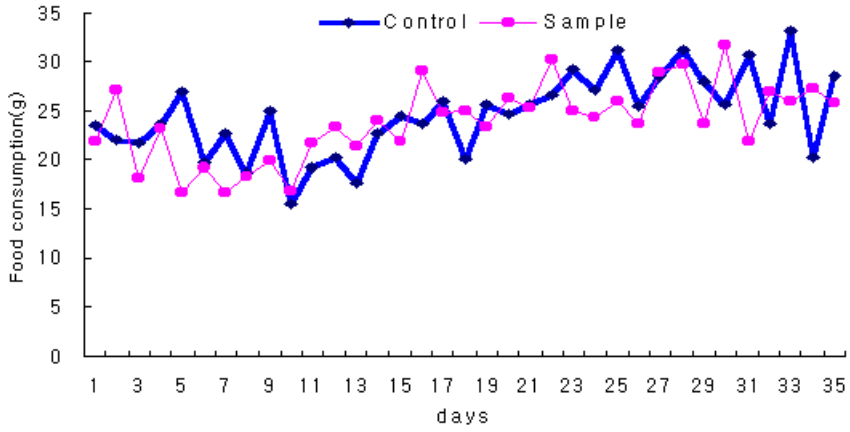

Fig. 3. Effect of SBY-Ⅰ on food consumption in obese Zucker rats.

C. Effect on the weight of the liver and adipose tissues

The weight changes of the liver, brown adipose tissue(BAT), and white adipose tissues(WAT) are shown in table Ⅵ, fig. 4 and 5.

In sample Ⅰ group, weight of interscapular BAT was significantly lower($P<0.05$) and in sample Ⅱ group, weight of liver and epididymal WAT was significantly lower($P<0.05$) as compared with control groups.

Table Ⅵ. Effect of SBY-Ⅰ on the Weight of Liver and Adipose Tissues in Lean and Obese Zucker Rats

	10^{-3}g per 1g of body weight			
	Liver	BAT	Epi	Retro
Control Ⅰ	35.79 ± 2.46	2.29 ± 0.60	5.29 ± 2.13	11.20 ± 1.58
Sample Ⅰ	35.53 ± 2.00	1.33 ± 0.27*	3.57 ± 0.57	11.40 ± 1.30
Control Ⅱ	43.72 ± 0.96	4.23 ± 0.71	21.47 ± 2.22	56.60 ± 2.07
Sample Ⅱ	41.04 ± 1.04*	2.30 ± 1.19	15.53 ± 2.52*	56.27 ± 6.94

Values represent mean ± standard deviation

BAT : Interscapular brown adipose tissue

Epi : Epididymal white adipose tissue

Retro : Retroperitoneal white adipose tissue

* $P<0.05$ vs. their normal diet control group

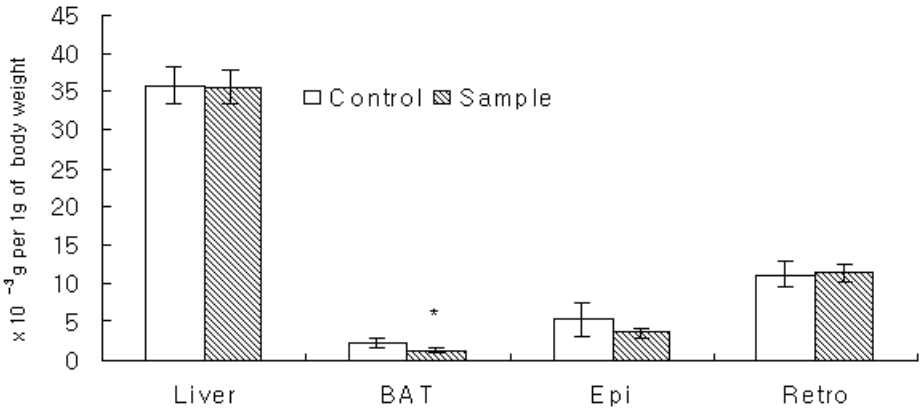

Fig. 4. Effect of SBY-Ⅰ on the weight of liver and adipose tissues in lean Zucker rats.

Values represent mean±standard deviation

＊P<0.05 vs. their normal diet control group

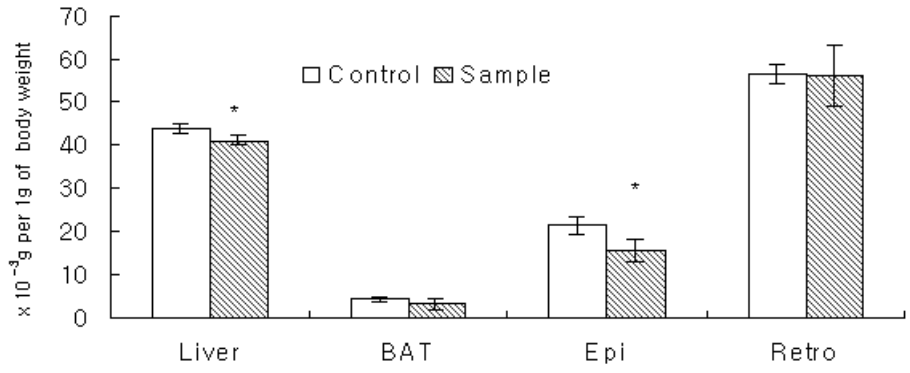

Fig. 5. Effect of SBY-Ⅰ on the weight of liver and adipose tissues in obese Zucker rats.

Values represent mean±standard deviation

＊P<0.05 vs. their normal diet control group

D. Plasmatic changes

1. Total cholesterol level

The changes of total cholesterol level are shown in table Ⅶ.

No significant differences were observed in each group.

2. Triglyceride level

The changes of triglyceride level are shown in table Ⅶ.

Compared with control groups, triglyceride level was lower in both sample Ⅰ and Ⅱ group with no significance.

3. HDL-cholesterol level

The changes of HDL-cholesterol level are shown in table Ⅶ and fig. 6.

Plasma HDL-cholesterol level in both sample Ⅰ and Ⅱ group was significantly higher($P<0.05$) compared with control groups.

4. Leptin level

The changes of leptin level are shown in table Ⅶ, fig. 7 and 8.

Compared with control groups, plasma leptin level was lower in sample Ⅰ group with no significance and significantly higher($P<0.05$) in sample Ⅱ group.

Table Ⅶ. Effect of SBY-Ⅰ on Plasmatic Changes in Lean and Obese Zucker Rats

	T-chol (mg/dl)	TG (mg/dl)	HDL-chol (mg/dl)	Leptin (ng/ml)
Control Ⅰ	71.00± 12.77	55.67± 20.98	17.67± 3.79	2.37± 0.53
Sample Ⅰ	73.25± 4.27	48.50± 4.80	27.50± 2.65*	1.81± 0.21
Control Ⅱ	102.75± 9.07	244.75± 76.40	30.50± 1.91	28.37± 6.74
Sample Ⅱ	106.40± 10.90	197.20± 28.12	46.40± 5.37*	53.71± 9.54*

Values represent mean ± standard deviation

* P<0.05 vs. their normal diet control group

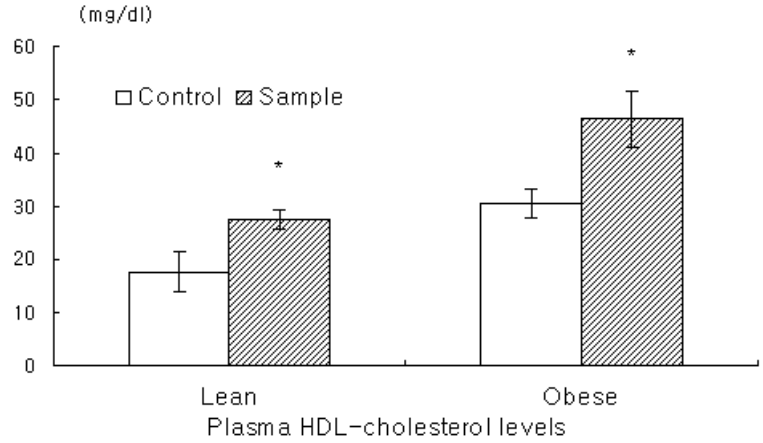

Fig. 6. Effect of SBY-Ⅰ on HDL-cholesterol levels in lean and obese Zucker rats.

Values represent mean ± standard deviation

* P<0.05 vs. their normal diet control group

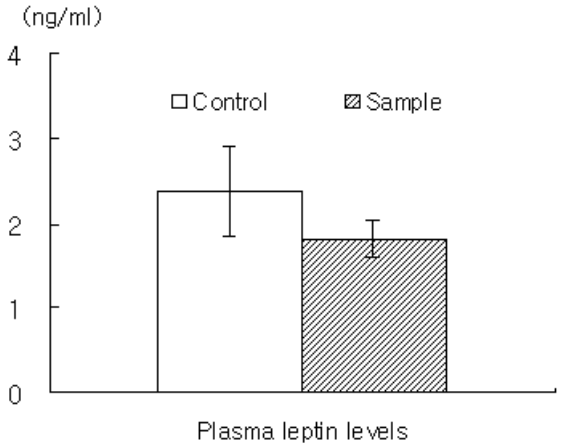

Fig. 7. Effect of SBY-Ⅰ on plasma leptin levels in lean Zucker rats.

Values represent mean±standard deviation

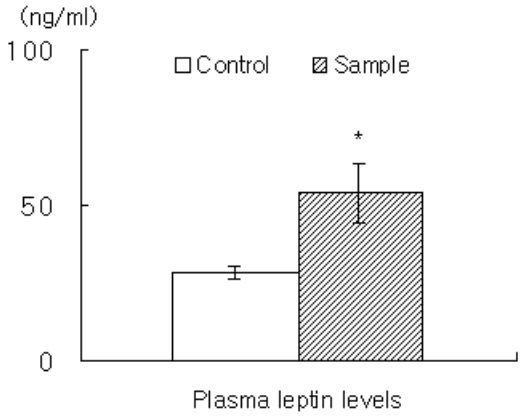

Fig. 8. Effect of SBY-Ⅰ on plasma leptin levels in obese Zucker rats.

Values represent mean±standard deviation

*P<0.05 vs. their normal diet control group

E. UCP mRNA expressions

The changes of UCP mRNA expressions are shown in fig. 9.

Compared with control groups, UCP1 mRNA expressions in interscapular BAT were up-regulated in both sample groups and in lean sample group showed a better result. In obese sample group, UCP2 mRNA expression in interscapular BAT was mild up-regulated.

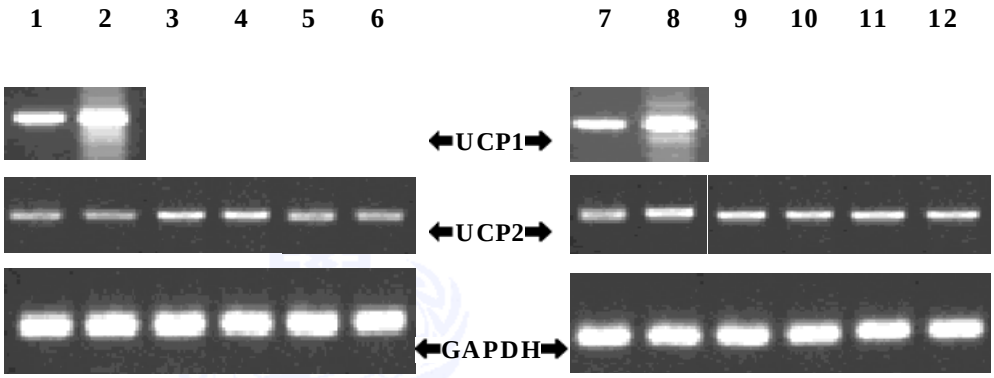

Fig. 9. Effect of SBY-Ⅰ on UCP mRNA expressions in lean and obese Zucker rats.

Lane 1-6 : Lean Zucker rats

Lane 7-10 : Obese Zucker rats

Lane 1,3,5,7,9,11 : Control group

Lane 2,4,6,8,10,12 : Sample group

Lane 1,2,7,8 : UCP mRNA expressions in interscapular BAT

Lane 3,4,9,10 : UCP mRNA expressions in epididymal WAT

Lane 5,6,11,12: UCP mRNA expressions in liver

Ⅳ. DISCUSSION

Obesity has reached epidemic proportions in the developed countries of the world. This phenomenon is frequently ascribed to the combination of excess consumption and decreased physical activity. Changes in body weight result from the difference between energy intake and energy expenditure. It is presumed that each individual has a target weight that the body tries to maintain. Since weight remains relatively constant despite large variations in energy intake, energy expenditure must be regulated. For example, people who gain weight become metabolically less efficient, whereas those who lose weight become more efficient[4,5,12].

A low rate of energy expenditure may predispose to the development of human obesity[13]. The basal metabolic rate, i.e., the obligatory metabolic cost for maintenance of physiological processes and cellular functions, accounts for ~60% of total energy expenditure and constitutes a familial trait[14], suggesting that genetic factors controlling energy expenditure might be of importance in the development of obesity[15].

It is well established that the risk and incidence of cardiovascular disease is related to alterations in the concentrations of plasma lipids and lipoproteins. The major features of this dyslipidemia shown to be associated with cardiovascular disease are elevations in total and LDL-cholesterol, higher triglycerides, and a lower HDL-cholesterol[16-19].

<*Huang-di-nei-jing*>[20] dated back to BC 3 century said "Obese and

noble person has a disease due to rich fatty diet", "Obese person often eats sweeteners". After that, etiology of obesity has been diverse. Chen[21] said "Obese person has a lot of phlegm and *Qi*(氣) deficiency syndrome". Zhu[22] said "Obese person's *Qi* deficiency causes coldness, coldness causes dampness, and dampness gives rise to phlegm". Kim[23] said "*Qi* deficiency syndrome, dampness-phlegm, emotional disorder, activity reduction, rich fatty diet and inborn constitution are the main causes of obesity". <*Zhongyi-zhengzhuang-jianbie-zhenduanzue*>[6] said "Phlegm-dampness and *Qi* deficiency are the main causes of obesity".

In short, causes of obesity can be divided into endogenous and exogenous factors: *Qi* deficiency, *Yang*(陽) deficiency, dampness, phlegm, heat, water and stagnation can be included in endogenous factors. Rich fatty diet, affection due to exogenous pathogenic dampness and reduced activity can be included in exogenous factors.

According to Korea's *Sasang*(*Sixiang*, 四象) constitutional medicine, all human beings can be divided into four types of constitutions: *Taeumin*(*Taiyinren*, 太陰人), *Taeyangin*(*Taiyangren*, 太陽人), *Soeumin*(*Shaoyinren*, 少陰人), and *Soyangin*(*Shaoyangren*, 少陽人). Among these four types, *Taeumin* has the best physique and well built skeleton, so he or she takes the most part of obesity. *Taeumin* has a strong function of the liver and weak function of the lungs. Therefore, inhale-take *Qi* of the liver is stronger than exhale-disperse *Qi* of the lungs and it can cause obesity. The weaker exhale-disperse *Qi* of the lungs or the stronger

inhale-take *Qi* of the liver, the more decrease the sea of body fluid and increase the sea of oil and blood. As the flesh is made of dense part of food essence in the sea of oil and blood, increased oil and blood sea result in obesity[8,24,25]. If we want to treat the *Taeumin*'s obesity, we should control this condition.

The obese Zucker rat has a genetically flawed leptin system and is a model of hyperphagia, obesity, and hyperlipidemia[26]. A gene defect in the obese Zucker rat causes an amino acid substitution in the leptin receptor and reduced leptin binding at the cell surface. A model of genetically obese Zucker fa/fa rats in which there is a decrease of thermogenesis[27] has been studied.

Leptin, a product of the obese(ob) gene, is secreted by adipocytes and appears to act as a hormone to regulate food intake, metabolism and body weight. It plays an important role in controlling body weight by regulating both energy intake and energy expenditure[28,29]. In humans, leptin concentration is directly proportional to the amount of adipose tissue in the body[30], and leptin resistance is thought to play a role in developing obesity[31]. When obese humans lose fat mass, leptin levels decline[30]; however, the decline in circulating leptin does not always correlate with the amount of weight loss[32]. This suggests that, in addition to the loss of body fat, other factors may affect changes in leptin levels with weight reduction. Nonshivering thermogenesis in brown adipose tissue(BAT) helps to regulate body weight after hyperphagia[27], and thermogenesis in BAT

may be one mechanism by which leptin regulates body weight.

The UCPs are integral membrane proteins of the mitochondrial inner membrane, where they function as a proton channel or shuttle. These proteins uncouple the process of mitochondrial respiration from oxidative phosphorylation, diminishing the resulting production of ATP and instead yielding dissipative heat. The action of these proteins creates a futile cycle that decreases the metabolic efficiency of the organism[4,5]. The brown fat uncoupling protein(UCP)1 was the first uncoupling protein to be described[33]. UCP1 is expressed exclusively in BAT, where its primary role appears to be thermoregulation[34], although it has also been linked to regulation of body composition[35-38]. BAT containing functional UCP1 is present in neonatal humans[37], but it is still controversial whether there are sufficient quantities of BAT in adult humans to have a role in nutrient partitioning[36]. Recently, two additional UCPs were identified that are more broadly expressed in metabolically active tissues of both humans and other animals: UCP2[4] and UCP3[35,38]. Interesting differences exist among these three UCPs in terms of both tissue distribution and physiological regulation. For example, whereas UCP1 is found exclusively in BAT, UCP2 is found in a variety of tissues including white adipose tissue(WAT), BAT, muscle tissue, and immune system tissue. UCP3 is expressed mainly in skeletal muscle but also in BAT tissue of rodents and to a lesser extent in cardiac muscle[35,38].

In oriental medicine, for the treatment of obesity, Lee et. al.[39] said, "In

deficiency syndrome we should invigorate the spleen and replenish *Qi*; in excess syndrome we should improve the relationships between the spleen, lungs, and kidneys to relieve dampness, resolve phlegm, induce diuresis, promote blood flow, and remove food stagnation. Chen[21] said, "We must replenish *Qi* and invigorate the spleen". Jiang[40] said, "We must use the eight methods when we try to treat obesity: relieve dampness, resolve phlegm, induce diuresis in order to remove food stagnation, soothe the liver and gall bladder, and invigorate the spleen and *Yang*". <*Zhongyi-zhengzhuang-jianbie-zhenduanzue*>[6] said "When we treat the obesity caused by accumulation of phlegm-dampness, we should relieve dampness and resolve phlegm, and if obesity is due to deficiency of *Qi*, we should replenish *Qi* and invigorate the spleen. We can classify all of these theories according to excess or deficiency syndrome. In deficiency syndrome we should invigorate the spleen, replenish *Qi*, tonify the kidneys, invigorate *Yang*, and nourish *Yin*(陰); in excess syndrome we should relieve dampness, resolve phlegm, induce diuresis, remove food stagnation, promote blood flow to elimate blood stasis, and remove internal heat."

When we treat the obesity, symptoms are the key factor in establishing treatment. Symptoms according to the theory of deficiency and excess syndromes[41] can be divided into two categories: Obesity due to accumulation of phlegm-dampness and obesity due to deficiency of *Qi*. When classified by pathological changes in the *Jang-Bu*(*Zang-fu*, 臟腑) organs and the state of *Qi* and blood[42], patterns can be divided as follows:

accumulation of dampness due to deficiency of the spleen *Qi*, accumulation of dampness due to heat in the stomach, liver *Qi* stagnation, *Qi* stagnation with blood stasis, accumulation of phlegm, and the spleen and kidney yang insufficiency.

In my country, there are several reports that extracts of Lycii Fructus(枸杞子)[43], Rhei Radix et Rhizoma(大黃)[44], *Banggihwanggi-tang* (*Fangjihuangshi-tang*, 防己黃芪湯)[39], *Oryung-san*(*Wuling-san*, 五苓散) and *Oryung-san*(*Wuling-san*, 五苓散) with Atractylodis Rhizoma(蒼朮)[45], *Bangpungtongsung-san*(*Fangfengtongsheng-san*, 防風通聖散)[46], *Sochangeumja*(*Xiaozhangyinzi*, 消脹飮子)[47], *Sosiho-tang*(*Xiaochaihu-tang*, 小柴胡湯)[48] and *Chungpaesagan-tang*(*Qingfeixiegan-tang*, 淸肺瀉肝湯)[49] have a significant effect on obesity treatment.

In terms of herbal formula according to the goal of treatment[50], the following studies are needed: ①activate the metabolism of fat and decrease the fat in hypoderm and organs, ②promote the catabolism and activate the dissolution of fat. In this point, some herbs can be compared with thermogenic drugs in western medicine.

SBY-Ⅰ, herbal formula, is aimed to treat the *Taeumin*'s obesity by activating thermogenesis. It's contents are as follows: Platycodi Radix(桔梗) 12g, Ephedrae Herba(麻黃) 6g, Mori Folium(桑葉) 6g, Liriopis Tuber(麥門冬) 4g, Scutellariae Radix(黃芩) 4g, and Armeniacae amarum Semen(杏仁) 4g.

Platycodi Radix has a bitter and pungent taste and its property is even. Therapeutic channel is the lung and it disperses the upper energizer.

Ephedrae Herba is pungent and warm. The pungent taste expells the pathogenic wind-cold. Therapeutic channels are the lung and urinary bladder. Mori Folium is sweet-bitter and cold. Therapeutic channels are the lung and liver. Cold property clears away heat from the lungs and clears up heat from the liver. Liriopis Tuber has a sweet with somewhat bitter taste and its property is cold. Therapeutic channels are the lung, stomach and heart. Sweet taste and cold property moisten the lungs and stomach by generating body fluid. Besides that, it clears away heat from the heart, results in tranquilization. Scutellariae Radix is bittter and cold. Therapeutic channels are the lung, gall bladder, stomach, and large intestine. Bitter taste eliminates dampness and cold property clears away heat, so it is usually used in dampness-heat syndrome. Armeniacae amarum Semen has a bitter with pungent taste and its property is warm. Therapeutic channels are the lung and large intestine. Bitter taste helps the descending of the lungs and pungent taste helps disperse. It also can be used in constipation[51].

All of these herbs act on the lung channel and help exhale-disperse *Qi* of the lungs as to regulate the exterior syndrome of *Taeumin*[8]. Obesity of *Taeumin* is due to stronger inhale-take *Qi* compared with exhale-disperse *Qi*. Therefore, invigorate the exhale-disperse *Qi* might be the valuable tool for the treatment of obesity.

When we treat the disease by use of herbal formula, properties and tastes have a great meaning. Four properties are the classification of

drug′s disposition based on *Yin* and *Yang* theory. They express physiological activities which influence body in four branches. The properties of warm and hot express *Yang*′s character, whereas cold and cool express *Yin*′s character. When we say ′Five tastes′, it′s meaning includes not only a simple sensation acquired through gustatory cell, but also their physiological functions of promoting or reducing and regulating homeostasis. Each tastes, i.e., sour, bitter, sweet, pungent, and salty, influence directly on five *Jang*(*Zang*, 臟) organs. Herbal formulas are always made in considering each herb′s properties and tastes as to be more effective and reduce each herb′s side effect. In addition, by use of herbal mixture, we can not only treat the disease itself, but also promote general condition by regulating the whole body[51].

In this experiment, administration of SBY-Ⅰ extracts reduced the body and final liver weights gain compared with the control groups. Increased rate of body weight was significantly lower in lean sample group compared with lean control group($P<0.0001$) and final liver weight was significantly lower($P<0.05$) in obese sample group compared with obese control group. Reduced liver weight suggests that SBY-Ⅰ might exert their anti-obesity action through the inhibition of intestinal absorption of dietary fat or acceleration of lipolysis in adipose tissue. No significant difference was found in the mean food consumption per day per animal during experimental period.

Both sample groups had a lower weight of interscapular BAT and

epididymal WAT compared with control groups. In lean sample group, weight of interscapular BAT was significantly lower($P<0.05$) as compared with control group. Reduction of BAT weights is not surprising and it is similar to what is observed after physiological activation of BAT thermogenesis, where the loss in BAT weight is due to metabolism of stored lipids[27]. In obese group, epididymal WAT was significantly lower($P<0.05$) as compared with control group.

The sample groups showed the reduction in plasma levels of triglyceride. Plasma HDL-cholesterol level was significantly higher in both lean and obese sample group($P<0.05$) compared with the control groups. These data provide SBY-Ⅰ might be a valuable tool for therapeutic intervention against chronic metabolic disease such as obesity, diabetes, and atherosclerosis.

Leptin is synthesized by adipose tissues[52,53] and acts on hypothalamus causing decreased food intake and increased energy expenditure[54,55], and as such may be part of feedback loop regulating body fat store. Leptin concentration increases with obesity and tends to decrease with weight loss. Plasma leptin level became lower in lean sample group suggesting fat reduction, on the other hand, in obese sample group, plasma leptin level became significantly higher compared with control group. Obese Zucker rat are a model of leptin receptor defect. The increased level of leptin in obese sample group in present study may be explained by a defect in the leptin receptor system. It is thought that in lean Zucker rats, as there was no

defect in the leptin receptor, leptin exerted its role i.e., reduced fat store resulted in decreased leptin level while in obese Zucker rats it didn't. That's why obese sample group had a increased plasma leptin level and lean sample group had a more effect on reduction of body weight gain.

Overexpression of leptin causes the rapid disapperance of all gross visible body fat, usually within 1 week[56]. As SBY-Ⅰ had an effect on inducing leptin, it might be used instead of other leptin gene therapy when we treat the obesity.

Until recently the thermogenic effects of leptin were thought to be confined to BAT, the major site of UCP1 expression. However, the recent discovery of UCP2[4], a far more ubiquitously expressed protein, raised the possibility that hyperleptinemia might up-regulate UCP2 in tissue that express the leptin receptor[57]. So, we investigated whether SBY-Ⅰ extracts administration affected the expression of UCP1 and UCP2 mRNA in adipose tissues and liver.

RT-PCR analysis revealed that SBY-Ⅰ extracts administration up-regulated the UCP1 mRNA expression in interscapular BAT of both lean and obese sample group as compared with the control groups. Lean sample group showed a better result, which is correspond to the result that in lean sample group had a significantly lower BAT weight and leptin level. It can be explained that leptin up-regulated the expression of UCP1 mRNA in lean Zucker rat, but had no effect in obese Zucker rat with mutated leptin receptors. However, though there was no role of leptin in

obese sample group with mutated leptin receptor, SBY-Ⅰ administration also up-regulated UCP1 mRNA expression which result in weight loss of body, liver and adipose tissues. Up-regulated UCP2 mRNA expression only in BAT of obese sample group was unexpected. The biological role of UCP2 is less clear, although evidence suggests that there is a role for this protein in energy balance and thermogenesis. In UCP1-deficient mice, UCP2 expression in BAT is up-regulated, possibly contributing to the surprising homeostasis. The family of uncoupling proteins is thought to be implicated in the regulation of energy metabolism and there must be another line of defense against obesity[34].

These results suggest that SBY-Ⅰ can exert its beneficial effect even though there is mutated leptin receptor, and it is thought there must be other factors which up-regulated the UCP1 and UCP2 mRNA expressions in obese Zucker rats.

In summary, the data of this study showed that SBY-Ⅰ administration in lean Zucker rat down-regulated the plasma leptin level and up-regulated UCP1 mRNA expression, while in obese Zucker rat up-regulated plasma leptin level and expression of UCP1 and UCP2 mRNA in BAT. This discrepancy might be due to the defect of leptin receptor in obese Zucker rat. In spite of the fact that leptin couldn't have any effect in obese Zucker rats, up-regulated UCP1 and UCP2 mRNA expression might be responsible for the reduction in weight of body, liver, and adipose tissues as well as plasma lipid levels.

These data suggests that SBY-Ⅰ has a contributory role in regulating energy homeostasis and can be used as an effective herbal formula for the treatment of obesity. Further clinical studies are needed to improve the obesity.

V. CONCLUSIONS

The present study was undertaken to evaluate the effects of SBY-Ⅰ on weight, plasma, and UCP mRNA expressions in Zucker rats. Based on the results, following conclusions were made:

1. Body weight gain was reduced in lean sample group with significance($P<0.0001$) and in obese sample group with no significance.
2. The weight of interscapular BAT was significantly decreased($P<0.05$) in lean sample group and weight of the liver and epididymal WAT was significantly decreased($P<0.05$) in obese sample group.
3. In both lean and obese sample group, plasma levels of triglyceride were decreased with no significance, and HDL-cholesterol levels were significantly increased($P<0.05$).
4. Plasma levels of leptin were decreased in lean sample group with no significance, while increased in obese sample group with significance($P<0.05$).
5. The expression of UCP1 mRNA was up-regulated in interscapular BAT of both lean and obese sample group and UCP2 mRNA was mild up-regulated in interscapular BAT of obese sample group.

These results show that SBY-Ⅰ has an effect on the treatment of obesity and it is recommendable for the clinical use as an effective herbal formula.

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국문초록

SBY-Ⅰ이 Zucker rat의 體重, 血液 및 UCP mRNA 發顯에 미치는 影響

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목적: SBY-Ⅰ은 四象醫學에서 太陰人表證에 사용되어지는 麻黃發表湯에 근거한 처방으로 본 연구는 SBY-Ⅰ이 비만치료제로 활용되어질 수 있는지를 보고자 하였다.

재료 및 방법: SBY-Ⅰ을 3주간(0.125mg/g, 1일 2회) 투여한 male lean(+/+) Zucker rat과 obese(fa/fa) Zucker rat의 체중, 간과 지방세포의 중량, 사료섭취량, 혈청 triglyceride, HDL-cholesterol, leptin level과 UCP mRNA의 발현도를 측정하여 생리식염수를 투여한 대조군과 비교하였다. 실험군과 대조군 사이의 결과 비교에는 student's *t*-test를 사용하였다.

결과: SBY-Ⅰ을 3주간 투여한 결과, lean(+/+) Zucker rat과 obese(fa/fa) Zucker rat 모두에서 비만억제와 지질감소의 효과를 얻었다. SBY-Ⅰ은 체중, 간과 지방조직의 중량을 감소시켰을 뿐 아니라 혈장 HDL-cholesterol을 증가시켰고 leptin을 유도하였으며 갈색지방조직의 UCP mRNA 발현도를 증가시

켰다.

결론: 본 연구로 볼 때 SBY-Ⅰ은 비만치료제로 응용될 수 있을 것으로 생각되며, 향후 이에 대한 임상적 연구가 필요할 것으로 사료된다.

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