

Effect of *Salicornia herbacea* L. Supplementation on Tissue Triglyceride Concentrations and PGC-1 α & PPAR- γ Expression of Skeletal Muscle of Rats Fed a High-fat Diet

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This study examined whether the supplementation of *Salicornia herbacea* L. (SH), a member of the Chenopodiaceae subfamily, affects tissue specific triglyceride (TG) accumulation and the peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) and peroxisome proliferator-activated receptor- γ (PPAR- γ) protein expressions of skeletal muscle in rats with a high-fat diet. Sprague-Dawley male rats were randomly divided into three groups: control normal diet group (CD), high-fat diet group (HD), and 5.0% SH supplemented high-fat diet group (SD). The weights of fat tissue of the SD group were reduced by approximately 25% ($p < 0.01$), while the skeletal muscle weight of the SD group increased approximately 5% compared to those in the HD group ($p < 0.01$). The serum and hepatic TG of the SD group decreased approximately 20% compared to those of the HD group ($p < 0.05$). In the protein expression levels in the skeletal muscle, the PGC-1 α and PPAR- γ expressions of the SD group were 1.5-folds higher than those of the HD group ($p < 0.01$). From these results, SH supplementation contributes to the improvement of the serum and hepatic TG concentrations, and the PGC-1 α and PPAR- γ protein expression levels in the skeletal muscle of fed a high-fat diet. Thus, SH supplementation was effective in reducing fat mass and increasing muscle mass.

Key words : Anti-obesity, PGC-1 α , PPAR- γ , *Salicornia herbacea* L., triglyceride

Introduction

Obesity is becoming a major public health problem in affluent societies [8]. In recent years, some natural sources have been reported to reduce body weight effectively without significant side effects and may be an excellent alternative strategy for developing effective and safe anti-obesity drugs [13, 24, 28]. The inhibitory effect of flavonoid compounds on adipocyte differentiation has been reported, and plant extracts and compounds have been intensely studied in order to fully understand their mechanisms [10, 27, 30]. *Salicornia herbacea* L. (SH), a halophyte, is a member of the Chenopodiaceae subfamily which grows mostly on mudflats with high salinity on the western and southern coasts of South Korea. SH consists of ~50% essential fatty acids, ~40%

essential amino acids, and natural minerals such as K⁺, Ca⁺, and Mg⁺ [1, 4, 29]. Reported studies of SH have focused on the improvement of physiologic functions such as osteoblastogenesis [15], antioxidative activity [2, 17], and metabolism disorders [11, 20].

Most tissues that are involved in lipid metabolism, such as adipose tissue, skeletal muscle, and liver tissue, are quantitatively more important than other tissues [23]. Each of these types of tissues has a store of triacylglycerol that can be mobilized in a regulated way to release fatty acids. In adipose tissue, fatty acids may be released into the circulation system for delivery to other tissues, while in skeletal muscle, fatty acids are a substrate for oxidation, and in the liver, fatty acids are a substrate for re-esterification within the endoplasmic reticulum to make triglycerol [3]. In skeletal muscle, peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) is abundant and plays an important role in the regulation of mitochondrial biogenesis, an insulin sensitivity [14]. PGC-1 α acts as a transcription factor that has been shown to participate in pathways that control the promotion of fatty acid oxidation via increasing mitochondrial function and activity [5]. In the physiological role of PGC-1 α

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gene, the peroxisome proliferator-activated receptor- γ (PPAR- γ) regulates the development of fat cells and their capacity to store lipids [31, 32].

Considering the above, the present investigation was carried out to evaluate the effect of SH supplementation on changes in weight, lipid accumulation according to specific tissue, and changes in protein expression regulating lipid metabolism such as PGC-1 α and PPAR- γ in rats fed a high-fat diet.

Materials and Methods

Experimental animals and diets

All of the experimental protocols were approved by the Animal Study Committee of Sunmoon University (2014-04-01). Six-week-old male Sprague-Dawley rats were obtained (Samtako Co., Osan, Korea) and individually housed in a controlled environment at 23 \pm 1 $^{\circ}$ C at 50 \pm 5% relative humidity, under a 12 hr light-dark cycle. All animals were given free access to tap water and food. After an acclimatization period of 1 week, all rats were randomly divided into 3 groups: control normal diet group (CD, n=8), high-fat diet group (HD, n=8) [33], and *Salicornia herbacea* supplemented high-fat diet group (SD, n=8). Each group was then monitored for 8 weeks. During this period, dietary intake was measured daily, and the change in the body weight of each animal was noted weekly.

Preparation of experimental diet

As shown in Table 1, the HD consisted of 20% protein, 48% carbohydrate, and 20% fat, as modified from a previous study [6] and based on AIN-76G. Dried SH powder was purchased from Buan Hamcho (Buan, Cheonranam-do, S. Korea). The SD was prepared by using 5% SH and substituting a portion of the fiber component with the HD.

Collection of serum and tissue samples

After 12 hr (20:00-08:00) of overnight fasting, the rats were sacrificed by exsanguination, and blood was drawn from the left ventricle under light diethyl ether anesthesia. Serum was obtained by centrifuging the blood at 700 \times g for 20 min at 4 $^{\circ}$ C. The tissue samples such as liver and perirenal fat pad were dissected and immediately snap-frozen in liquid nitrogen. The triglyceride (TG) level of the tissue samples was analyzed, and 350~400 mg of the minced tissues were homogenized in 2 ml of the diluted standard diluents con-

Table 1. Composition of experimental diets

Variable	Control normal diet ¹⁾	High-fat diet ²⁾	<i>Salicornia herbacea</i> L. diet
Ingredients (g/kg diet)			
Casein	200.0	200.0	200.0
Starch	500.0	111.0	111.0
Sucrose	150.0	370.0	370.0
Lard	-	170.0	170.0
Corn oil	50.0	30.0	30.0
Cellulose	50.0	50.0	-
Vitamin mix	10.0	12.0	12.0
Mineral mix	35.0	42.0	42.0
Cholesterol	-	10.0	10.0
D,L-methionine	3.0	3.0	3.0
Choline barbiturate	2.0	2.0	2.0
Tert-butylhydroquinone	0.01	0.04	0.04
<i>Salicornia herbacea</i> L.	-	-	50.0 ³⁾

¹⁾The control normal diet group (CD) was based on AIN-76G

²⁾The high-fat diet (HD and SD) were consisted of 20% protein, 48% carbohydrate, and 20% fat of total diet weight as modified diet from a previous study

³⁾The *Salicornia herbacea* L. supplemented group (SD) was received by substituting 5.0% of the fiber component of the high-fat diet

taining protease inhibitors. The tissues were then centrifuged at 10,000 \times g for 10 min at 4 $^{\circ}$ C. The serum and tissue samples were stored at -80 $^{\circ}$ C until needed for analysis.

Analysis of triglycerides in serum, liver, and skeletal muscle

The serum TG level was analyzed using enzyme kits (Asan Pharmaceutical Co., Yongin, S. Korea). Hepatic TG and muscle TG were extracted using the following method. Briefly, liver and gastrocnemius muscle were homogenized in 5% Triton X-100 solution and heated in an 80~100 $^{\circ}$ C water bath for 2~5 min to solubilize the TG. The samples were then centrifuged at 10,000 g for 10 min, and the resulting supernatant was used to determine the TG level following the manufacturer's protocol (Cayman chemical, MI, USA).

Western blot analysis

In order to analyze the PGC-1 α and PPAR- γ protein expression levels, the gastrocnemius muscle was homogenized on ice with a polytron homogenizer in a 20 mmol/l Tris-HCl buffer (pH 7.5) containing 5 mmol/l ethylenediaminetetraacetic acid, 2 mmol/l phenylmethylsulfonyl fluoride, and 1:200 protease inhibitor cocktail (Sigma, St Louis, MO, USA).

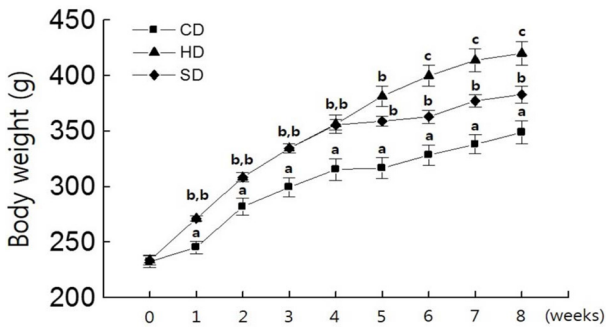


Fig. 1. Changes of body weight during total experimental period. $p < 0.05$. CD: control normal diet group, HD: high-fat diet group, SD: *Salicornia herbacea* supplemented high-fat diet group.

The protein concentrations were determined using the Bradford method (Bio-Rad, Hercules, CA, USA) with bovine serum albumin (BSA) as the standard. An aliquot of tissue extract containing 20 ug (PGC-1 α and PPAR- γ) of protein was separated on a 10% SDS-PAGE gel. After electrophoresis, the proteins were transferred to a PVDF membrane (Millipore, Bedford, MA, USA) in a semi-dry blotting apparatus (Bio-Rad, Hercules, CA, USA). After treating with a blocking buffer (phosphate-buffered saline (PBS) containing 10% skim milk) for 90 min, the membrane was incubated with primary polyclonal antibodies for 2 hr, followed by five 10-minute washes with PBS (5% tween 20). The membranes were washed then incubated with horseradish peroxidase (HRP)-conjugated anti-goat IgG or anti-rabbit IgG (Santa Cruz, CA, USA) for 1 hr, followed by five 10-min washes with PBS (5% Tween 20). The target proteins were detected using an enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The films were photographed, and the protein bands of interest were quantified with band analyzer software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

The data were expressed as the means \pm the standard error of the mean (SE) using the SPSS/PC program (version 18.0 for Windows; SPSS Inc., USA). All statistical analyses were conducted using one-way ANOVA followed by the Least Significant Difference (LSD) post hoc test. Statistical significance was set at $p < 0.05$.

Results and Discussion

It is well known that the lifestyle habits of humans in modern society, such as a high-fat diet and physical inactivity, promote weight gain, dyslipidemia, and profound systemic perturbations in lipid metabolism. In response to the small number of investigations carried out thus far to evaluate the effect of SH on lipid accumulation, in this study, we examined whether SH supplementation affects tissue-specific lipid metabolism characteristics such as weight and TG accumulation in rats with a high-fat diet.

The changes of body weight during the 8 week period, as the total experiment period, are presented in Fig. 1. During the obesity-inducing period, regardless of SH supplementation, the body weights of the high-fat diet groups such as SD and HD groups significantly increased compared to those in the CD group ($p < 0.01$). After the obesity-inducing period, the body weight of the SD group was significantly lower than that of the HD group, whereas the body weight of the HD group was significantly higher than that of the CD group ($p < 0.05$). The differences of weight in the fat pad, liver, and skeletal muscle among the groups are illustrated in Fig. 2. The weights of the fat pad and liver in the HD group were significantly higher than those in the CD group ($p < 0.01$), while the fat and liver weights in the SD group significantly reduced compared to those in the HD group ($p < 0.01$, $p < 0.05$). For skeletal muscle weight, the gastro-

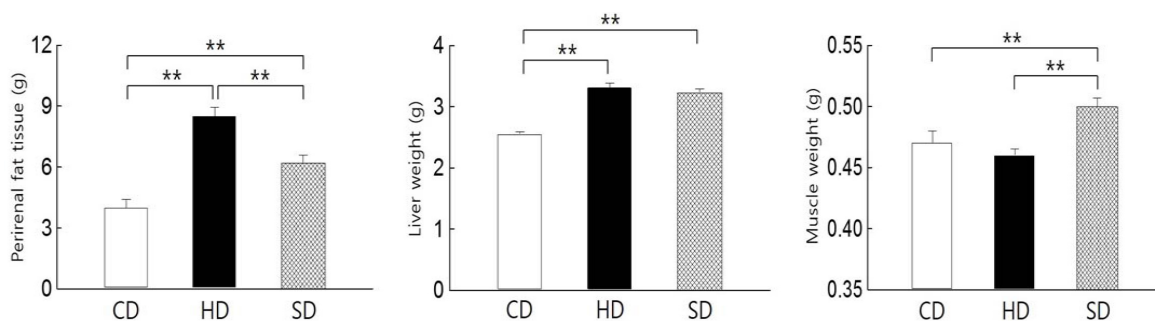


Fig. 2. Weight difference of tissues among groups. $**p < 0.01$. CD: control normal diet group, HD: high-fat diet group, SD: *Salicornia herbacea* supplemented high-fat diet group.

cnemius weight in the SD group significantly increased compared to that in the CD and HD groups ($p<0.01$), whereas no significant difference was observed between the CD and HD groups.

Our results show the ability of SH supplementation to reduce the weight increase of the whole body and fat tissue while enhancing skeletal muscle mass. In other words, although the body and fat tissue weight increased, gastrocnemius muscle mass reduced on the high-fat diet compared to the normal diet. These results indicate that, in the case of SH supplementation, even a high-fat diet with physical inactivity, body and fat tissue weights were reduced while muscle mass was enhanced. Although the inhibitory effect of flavonoid compounds on adipocyte differentiation has been reported, many studies of SH have focused on the improvement of the metabolism of metabolites [1, 2, 11, 15, 17, 20, 29], but have not considered the effects of SH on TG levels and related lipid metabolism in specific tissues such as serum, fat, and skeletal muscle. Thus, for the first time, we attempted to investigate the possibility of SH supplementation to enhance muscle hypertrophy and improve lipid metabolism in skeletal muscle. We assume that SH might be useful in the utilization of fat as a substrate during supplementation and that SH feeding stimulated protein synthesis, which enhanced skeletal muscle mass. Therefore, even on a high-fat diet, SH supplementation could be recommended for the improvement of fat tissue with muscle hypertrophy.

The levels of TG in serum ($p<0.05$), liver ($p<0.01$), and skeletal muscle ($p<0.05$) in the HD group were significantly higher than those in the CD group. The serum ($p<0.05$) and hepatic TG ($p<0.01$) levels were found to have significantly reduced in the SD group compared to those in the HD group. However, a significant difference was not observed in the TG level of gastrocnemius muscle between the HD

and SD groups (Fig. 3). Our results showed strong inhibition on lipid accumulation in serum and liver by the SH supplementation. Regarding lipid metabolism, the adipose tissue, liver, and skeletal muscle are the main components that store TG in a regulated way and use hydrolysis to release fatty acids, either for export or for internal consumption [7, 12, 22]. Dyslipidemia and intramuscular accumulation of triglyceride are increasingly recognized as an important feature of obesity and type 2 diabetes [16, 21]. Similar to the trends of tissue weights, the serum and hepatic TG levels also improved by the SH supplementation. SH supplementation has a positive role for the improvement of TG level in blood and liver. Interestingly, while muscle hypertrophy was shown in the SD group, muscle TG did not show significant improvement in the SD group compared to the HD group. From these results, it could be assumed that the TG utilization within muscles was limited by only SH supplementation. Thus, further studies, considering the muscle TG metabolism and catabolism, are needed to better elucidate the relationship between muscle weight and TG concentration.

As shown in Fig. 4, the PGC-1 α protein expression level of the SD group was significantly higher than that in the HS group ($p<0.01$) and CD group ($p<0.05$), respectively. In addition, the PPAR- γ protein expression level in the SD group was significantly higher than that in the HD group ($p<0.01$), while the PPAR- γ protein expression level in the HD group was significantly lower than that in the CD group ($p<0.01$).

PPAR- γ protein is reported to play an important role in the regulation of PGC-1 α gene expression in skeletal muscle tissues [14]. Our study also showed that the PPAR- γ and PGC-1 α protein expressions in the muscle of the SD group were significantly higher than those in the HD group. Previous studies reported that adipogenesis induces a series

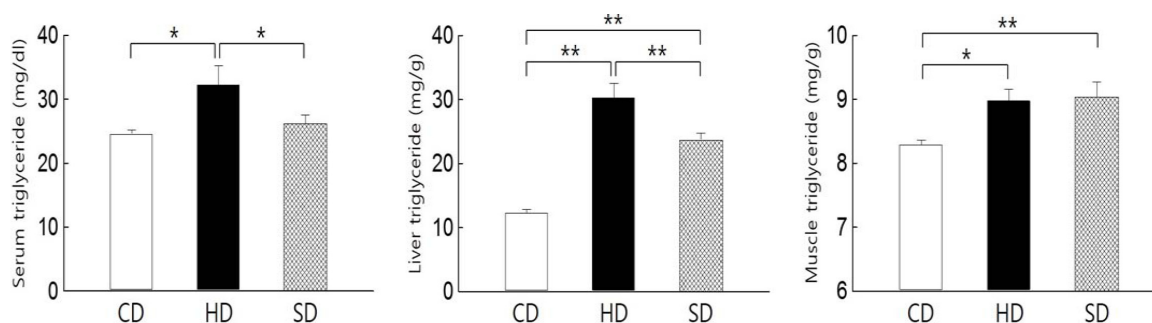


Fig. 3. Levels of triglyceride in serum, liver, and muscle. * $p<0.05$, ** $p<0.01$. CD: control normal diet group, HD: high-fat diet group, SD: *Salicornia herbacea* supplemented high-fat diet group.

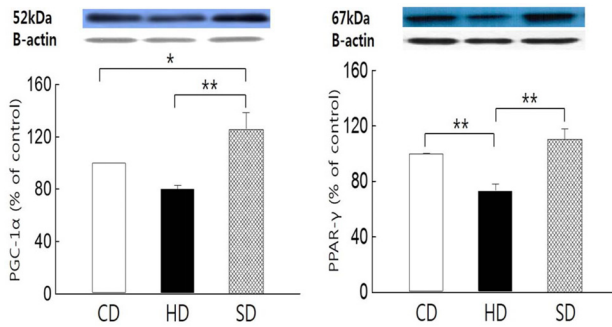


Fig. 4. Difference of PGC-1 α and PPAR- γ protein expression in skeletal muscle. * $p < 0.05$, ** $p < 0.01$ CD: control normal diet group, HD: high-fat diet group, SD: *Salicornia herbacea* supplemented high-fat diet group.

of programmed changes in adipogenic gene expression that is activated by the transcription factors such as PGC-1 α and PPAR- γ [18, 19]. Our finding is in accordance with previous studies which show that the PGC-1 α and PPAR- γ protein expression in the SD group was significantly higher than that of the HD group. Skeletal muscle comprises a relatively large mass in the body and is an important target tissue for glucose metabolism by insulin, as reported in the previous studies [9, 25, 26]. Additionally, as mentioned previously, skeletal muscles have a store of triacylglycerol that can be mobilized in a regulated way to release fatty acids, while muscle fatty acids are a substrate for oxidation. Therefore, although we could not precisely elucidate the mechanism of the PGC-1 α and PPAR- γ protein expressions associated with SH supplementation in the present study, we can assume that the significant changes in the SH supplementation might have significant physiological effects because the skeletal muscle is one of the major lipid metabolism target tissues.

Taken together, even on a high-fat diet, *Salicornia herbacea* L. supplementation plays a role in the suppression of lipid accumulation in serum and liver with muscle hypertrophy. When *Salicornia herbacea* L. supplementation was accompanied, the PGC-1 α and PPAR- γ protein expressions in the skeletal muscle were enhanced compared to the high-fat diet group without S *Salicornia herbacea* L. supplementation.

In conclusion, SH supplementation may play a role in enhancing muscle hypertrophy and improving the weights of fat tissue and liver in rats fed a high-fat diet. In addition, *Salicornia herbacea* L. supplementation was shown to significantly suppress lipid accumulation, which is mediated via PGC-1 α and PPAR- γ activation. These results suggest that *Salicornia herbacea* L. supplementation may be useful as

an effective source for preventing lipid accumulation related disease.

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초록 : 함초의 보충식이 고지방식이 흰쥐의 혈청 및 조직의 중성지방 농도와 골격근 내 PGC-1 α 및 PPAR- γ 단백질 발현에 미치는 영향

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본 연구는 함초의 보충식이 고지방식이 흰쥐의 혈청 및 조직의 중성지방 농도와 골격근 내 PGC-1 α 와 PPAR- γ 단백질 발현에 미치는 영향에 대하여 연구하였다. SD계 수컷 흰쥐를 대조군(CD, n=8), 고지방식이군(HD, n=8), 고지방식이+ 5% 함초 보충식이군(SD, n=8)으로 분류하여 8주간 사육하였다. 그 결과 SD군의 지방조직 중량은 HD군에 비해 약 25%정도 유의하게 감소한 반면 골격근의 중량은 SD군이 HD군에 비해 약 5%정도 유의하게 증가하였다($p<0.01$). SD군의 혈청과 간장 내 중성지방은 HD군에 비해 약 20% 유의하게 감소하였다($p<0.05$). SD군의 골격근 내 PGC-1 α 와 PPAR- γ 단백질 발현은 HD군에 비해 1.5배 유의하게 높게 나타났다($p<0.01$). 이상의 결과로부터 함초 보충은 골격근 내 PGC-1 α 와 PPAR- γ 단백질 발현의 증가를 통하여 지방감소 및 근육량 증가에 효과적이었음이 시사되었다.