

Anti-obesity Effects of Black Soybean *Doenjang* in C57BL/6 Mice

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Doenjang is a traditional Korean fermented soybean paste made from meju (fermented soybean), which are fermented by diverse microorganisms including *Bacillus subtilis* and molds such as *Rizopus*, *Mucor*, and *Aspergillus* species. The purpose of this study was to investigate the anti-obesity effect of the black soybean *doenjang* (Korean fermented soybean pastes) in C57BL/6 mice. The anti-obesity effect was determined by measuring the release of adiponectin, leptin and adipogenic transcription factors by using reverse transcription-polymerase chain reaction and western blot. Weight gain was significantly reduced in the mice fed high fat diets (HFD) plus black soybean *doenjang* (HBD) compared to HFD mice. The HBD were also effective in improving the lipid profile. They significantly decreased the levels of serum triglyceride and cholesterol. In addition, they had a significantly down regulated impact on antiobesity factors; leptin level and increased adiponectin level. Also, mRNA and protein expression of two adipogenic transcription factors, SREBP-1c and PPAR- γ , in high fat with black soybean fed mice were markedly down regulated. These results indicate that the black soybean *doenjang* potentiates an anti-obesity effect by modulating lipid metabolism, thereby inhibiting adipogenic transcriptional activation.

Key words : Anthocyanin, antiobesity, black soybean, *Doenjang*, isoflavone

Introduction

Rapidly rising obesity rates have become a world-wide problem. With this growing trend and concern, obesity has become an important health issue. Obesity is a key element that increases the risk of serious diseases such as hypertension, heart disease, diabetes, stroke, cancer, and osteoarthritis [7, 14, 15]. There have been many reports regarding the effects of soybean on obesity [11, 16]. It has been reported that soy protein slows down the low-density lipoprotein (LDL) oxidation and lipid peroxidation [17, 25, 33]. In addition, the extract from the black soybean has a longer LDL oxidation lag time than that from the yellow soybean because of the high total polyphenols contained in its seed coat [35]. Soybean seed is diverse in its color of seed coat. Soybean have variant color such as yellow, brown, green, and black. Black soybean contains considerable amount of dietary fiber and functional ingredients such as anthocya-

nins. The black soybean's seed coat contains 0.87-3.52 mg/g anthocyanins, in which the highest ingredient concentration is cyanidin-3-glucoside (80.9% of total content [11]). Anthocyanins are glycosides or acylglycosides of polyhydroxy 2-phenylbenzopyrylium cations belonging to a larger group of compounds known as flavonoids, a subgroup of polyphenols [1, 10]. Anthocyanins play critical roles as antioxidant, chemo preventive, and anti-inflammatory agents [1, 16]. Some recent studies [9, 26, 33, 35] have shown that anthocyanins have an anti-obesity effect. For example, dietary cyanidin 3-glucoside-rich purple-colored corn pigments effectively suppressed the high fat diet (HFD)-induced increase in body weight, and hampered the increase in adipose tissue weights [35]. Cornelian cherry of anthocyanins is also reported to improve obesity and insulin resistance in high fat-fed mice [9]. Anthocyanin showed antihyperglycemic effect because it slows down the digestion of carbohydrates via *glucosidase* inhibition [26]. Anthocyanin from blueberries and strawberries reduces obesity in the C57BL/6 mice fed a high-fat diet [33].

Doenjang is a traditional Korean fermented soybean paste made from meju (fermented soybean), which are fermented by diverse microorganisms including *Bacillus subtilis* and molds such as *Rizopus*, *Mucor*, and *Aspergillus* species [20]. *Doenjang* has strong anti-mutagenicities against various carcinogens and anticancer activities [21-24]. As already in-

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licated, anthocyanins showed antiobestic effects [9, 26, 33, 35]. To evaluate the beneficial effect of black soybean *doenjang* against obesity in male C57BL/6 mice, subjects were divided into four groups and maintained on a normal diet (ND), high fat diet (HFD), high fat diet with black soybean *doenjang* (HBD), high fat diet with yellow soybean *doenjang* (HYD) for 8 weeks. Thus, the objective of this study was to investigate the anti-obesity effect and lipid-regulating effects of black soybean *doenjang* in C57BL/6j mice.

In this study, we determined whether the black soybean *doenjang* improves the lipid profiles, gene and protein expression levels of obesity-related genes of PPAR- γ and SREBP-1c in the liver of the mice. Given this clinical relevance of black soybean *doenjang* to obesity, this study will provide evidence for lipid-lowering effects of dietary supplementation of black soybean *doenjang* for inducing weight loss in diet-induced obese mice.

Materials and Methods

Animals

Fifty 5-week-old male C57BL/6 mice, weighing approximately 14-16 g each, were purchased from the Korean Experimental Animal Center (Orient Co., Seoul, Korea). Mice were acclimated to the experimental facility for 1 week before they were divided into four groups of eight and placed in polycarbonate cages in a room maintained at 22±1°C with 55±5% relative humidity. The room was exposed to alternating 12 hr periods of light and dark. All mice were allowed free access to their respective diets and drinking water for eight weeks. This work supported by the Technology Development Program for Agriculture and Forestry and conducted in 2007 before the committee for animal care was launched; so that there is no animal care approve number.

Diets

Mice were randomized by weight and assigned to one of five dietary treatments.

Normal diet (D12450B) and high fat diets (D12451) used in this experiment were purchased from Research Diets Inc (New Brunswick, New Jersey, USA) [37]. The experimental diets consisted of; (1) normal diet (ND) [containing 4% (wt/wt) soybean oil], based on the DC diet; (2) HFD [ND supplemented with 23%(wt/wt) lard] (3); HFD with 10% freeze-dried black soybean *doenjang* powder (HBD); (5) HFD with freeze-dried yellow soybean *doenjang* powder (HYD).

The compositions of diets are listed in Table 1.

Preparation of Doenjang

The process for making *doenjang* was as follows: soybeans were sorted, washed, soaked in water for 12 hr and then are cooked in an autoclave at 121°C for 2 hr. The cooked soybeans were cooled to 40°C, then inoculation with *Aspergillus oryzae* (0.2%), and fermented at 30°C for 48 hr. After drying at 40°C for 24 hr, meju was prepared. The meju was placed in a jar and water and salt were added (meju: salt: water =33:12:55, wt/wt/wt). The mixture was then fermented for 2 months.

Measurement of body weight and food consumption

Body weight was measured every week and rounded to the second decimal place. Feed consumption was measured every second day. Feed efficiency ratio (FER) was calculated as weight gain in grams divided by dietary intake in grams.

Preparation of blood for lipid analysis

After 8 weeks on the experimental diets, the mice were sacrificed with ethyl ether. Blood samples were collected into heparin treated tubes. Plasma was separated by centrifugation at 3,000 rpm for 15 min (VS-15CFU refrigerated centrifuge, Vision, Gyeonggi, Republic of Korea).

Quantitation of plasma triglyceride, total cholesterol and glucose

Concentration for total cholesterol (AM202K, Asan Pharm, Seoul, Korea) and triglyceride (AM1575K, Asan Pharm, Seoul, Korea) in the plasma were determined using commercially available kits.

Quantitation of plasma leptin and adiponectin

Determination of leptin and adiponectin levels in the plasma was performed with sandwich enzyme-linked immunosorbent assay (ELISA). For the leptin analysis, anti-mouse leptin, recombinant mouse leptin, biotinylated anti-mouse leptin antibodies (MOB00), and adiponectin analysis, a kit (MRP300) was purchased from R&D Systems (R&D Systems, Minneapolis, USA).

Reverse transcription-PCR analysis of mRNA expression

Gene expression was measured by RT-PCR in an Exi-Cycler (Bioneer, Daejeon, Korea). Briefly, total RNA was iso-

Table 1. Composition of experimental diets

g/kg	Normal diet (ND)	High fat diet (HFD)	HFD+ black soybean (HBD) <i>Doenjang</i>	HFD+ yellow soybean (HYD) <i>Doenjang</i>
Casein	189.6	233.1	219.2	219.4
L-cystine	2.8	3.5	3.5	3.5
Cornstarch	298.6	84.8	81.2	81.2
Dextrose	33.2	116.5	116.5	116.5
Sucrose	331.7	201.4	201.4	201.4
Cellulose	47.4	58.3	54.9	47.2
Soybean oil	23.7	29.1	21.1	21.8
Vitamin mix (V10001)	9.5	11.7	11.7	11.7
Mineral mix (S10026)	9.5	11.7	11.7	11.7
Choline bitartrate	1.9	2.3	2.3	2.3
Lard	19.0	206.9	206.9	206.9
yellow soybean <i>Doenjang</i>			100.0	
black soybean <i>Doenjang</i>				100.0
Dicalcium phosphate	12.3	15.1	15.1	15.1
Calcium carbonate	5.2	6.4	6.4	6.4
Potassiumcitrate, H ₂ O ₂	15.6	19.2	19.2	19.2
SUM	1,000.0	1,000.0	1,071.1	1,064.3
Calories	3,845.9	4,728.0	4,728.4	4,728.4
Energy, KJ/g	1,6099.1	19,791.6	19,793.3	19,793.3
Protein, % of energy		20.0		
Carbohydrate, % of energy		35.0		
Fat, % of energy		45.0		

lated from the liver in C57BL/6J mice using Trizol reagent (Invitrogen, Carlsbad, CA, USA). One microgram of total RNA was used for first-strand cDNA synthesis using Superscript II reverse transcriptase (BD Bioscience, Palo Alto, CA, USA). Reverse transcription was performed at 30°C for 10 min, 42°C for 30 min, and 99°C for 5 min to inactivate the avian myeloblastosis virus RTXL. Amplification was performed in a master-cycler (Eppendorf, Hamburg, Germany) with denaturing at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 30 sec for 25 cycles and finally 72°C for 7 min. The amplified PCR products were run in 1.0% agarose gels and stained with ethidium bromide (EtBr), and visualized under UV light. The intensities of the bands were estimated by densitometry (Multi Gauge V3.0 software, Fujifilm Life Science, Tokyo, Japan). The primer sequences specific for the genes examined were: GAPDH, forward primer, 5'-TGTGTCCGTCGTGGATCTGA-3', reverse primer, 5'-CTTGCTTACCACCTTCTTGAT-3'; SREBP-1c, forward primer, 5'-AGCAGCCCCTAGAACAACAC-3', reverse primer, 5'-CAGCAGTGAGTCTGCCTTGAT-3'; PPAR- γ , forward primer, 5'-TCGCTGATGCACTGCCTATG-3', reverse primer, 5'-GAGAGGTCCACAGAGCTGATT-3'.

Measurement of protein expression

Hepatic tissue lysates were lysed in an RIPA buffer (25 mM Tris · HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, PIERCE, Rockford, IL, USA) as previously described. Protein concentration was determined with Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). For western blot analysis, proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and then electrotransferred to a nitrocellulose where subjected to immunoblot analysis with desired antibodies and protein visualized by the enhanced chemiluminescence (ECL) method (Abcam, MA, USA). Antibodies use SREBP-1 (Abcam), PPAR- γ (Cell signaling), and β -actin (Santa Cruz Biotechnology). Peroxidase-labeled secondary antibodies were purchased from Abcam.

Statistical analysis

All statistical analyses were performed using SAS (Cary, NC) version 8.0. Significant differences among the treatment means were determined using analysis of variance (ANOVA) and Duncan's multiple range test at $p < 0.05$.

Results

Weight gain, food intake and FER

C57BL/6 mice were fed high fat diets and other experimental diets plus high fat diets for 8 weeks. The body weights and food intakes levels of the mice are shown in Table 2. In the beginning of the raising, there was not a significant difference in the average weight between each group. However, after four weeks, the weight in every group increased. The average weight gains (g/day) by mice fed normal diets, high-fat diets, high-fat diets plus black soybean *doenjang* and high-fat diets plus yellow soybean *doenjang* were 0.16 ± 0.01 , 0.26 ± 0.03 , 0.15 ± 0.01 and 0.17 ± 0.05 , respectively ($p < 0.05$). Weight gains of the HFD mice were significantly higher than the other groups. Weight gains of HBD mice were similar with ND mice. There was no significant alteration in the food intake of ND, HFD, and HBD groups.

Organ and fat tissue weight

Organ and fat weight are shown in Table 3. The HFD group differs on liver, kidney, epididymal fat pad, and perirenal fat pad weights when compared to other groups. Liver, epididymal fat pad and perirenal fat pad weights were significantly higher in the HFD groups. The HBD (26%) and HYD (23%) group had liver weights that decrease significantly more than the HFD group ($p < 0.05$). The epididymal fat pad weight of ND, HBD and HYD groups were significantly decreased by 45, 43 and 38%, respectively, compared to that of the HFD group ($p < 0.05$). The perirenal fat

pad weight of ND, HBD, and HYD groups were significantly reduced by approximately 42, 42, and 38% respectively, compared to that of the HFD group ($p < 0.05$). Adipose tissue weights of the HBD group were the same as those of ND group. Liver weights of HBD group were significantly lower than the other groups. Kidney, epididymal fat pad, and perirenal fat pad weights of the HBD group were similar to the ND group.

Plasma lipid parameter

Plasma triglyceride (TG) and total cholesterol (TC) were measured by enzymatic assay. Concentration of plasma TG and TC are shown in Table 4. TG levels were significantly higher in the HFD mice. The TG level in the ND, HBD and HYD group were significantly lower than the HFD group by 30, 40 and 24% respectively ($p < 0.05$). TG level of HBD group was lower than the other group. The TC level of ND, HBD and HYD group were significantly lower than the HFD group by approximately 31, 40 and 24% respectively ($p < 0.05$). TC levels in the HBD mice were similar to the ND mice. The levels of plasma leptin and adiponectin were determined in C57BL/6 mice (Table 5). After 8 weeks feeding, the leptin levels of HBD group was significantly lower than the HFD group by 31% ($p < 0.05$). The secretion of plasma adiponectin of ND and HBD group were significantly higher than the HFD group by 41 and 18% respectively ($p < 0.05$). Interestingly, there was no significant alteration in adiponectin secretion of the HYD group compared to the HFD group. There has been reported that adiponectin has certain

Table 2. Changes of body weight, food intake and food efficiency ratio(FER) of mice fed experimental diets for 8 weeks

	ND	HFD	HBD	HYD
Body weight				
Initial weight (g)	21.51 ± 1.36^b	23.78 ± 1.27^a	23.24 ± 1.06^a	23.56 ± 1.35^a
Final weight	31.32 ± 2.28^b	40.41 ± 3.33^a	32.94 ± 1.99^b	34.29 ± 4.53^{ab}
Weight gain (g/day)	0.16 ± 0.01^b	0.26 ± 0.03^a	0.15 ± 0.01^b	0.17 ± 0.05^b
Food intake (g/day)	3.2 ± 0.3^a	3.1 ± 0.3^{ab}	3.0 ± 0.2^{ab}	2.8 ± 0.3^b
Food efficiency ratio	0.05 ± 0.003^b	0.08 ± 0.006^a	0.05 ± 0.005^b	0.06 ± 0.005^{ab}

Data are expressed as mean \pm SD. Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 3. The weight of tissue and Fat pad weight in mice fed with experimental diets for 8 weeks

Organ weight (g)	ND	HFD	HBD	HYD
Liver	1.53 ± 0.03^b	1.79 ± 0.06^a	1.33 ± 0.04^c	1.38 ± 0.09^c
Kidney	0.67 ± 0.03^b	0.76 ± 0.05^a	0.65 ± 0.04^b	0.75 ± 0.06^a
Epididymal fat pad	1.52 ± 0.12^b	2.76 ± 0.13^a	1.57 ± 0.32^b	1.70 ± 0.64^b
Perirenal fat pad	0.87 ± 0.07^b	1.49 ± 0.16^a	0.87 ± 0.1^b	0.92 ± 0.35^b

Data are expressed as mean \pm SD. Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 4. The effect of Balck soybean *Doenjang* diets on serum TG, TC, leptin and adiponectin concentrations in mice fed experimental diets for 8 weeks

	ND	HFD	HBD	HYD
Triglyceride (mg/dl)	105.5±22.9 ^b	150±12.8 ^a	90.1±5 ^b	114.4±25.2 ^{ab}
Total cholesterol (mg/dl)	102.6±1.5 ^{bc}	161.5±13.5 ^a	97±7.4 ^c	122.7±14.7 ^b
Leptin (pg/ml)	100.9±11.9 ^d	233.8±9.7 ^a	161.3±7.0 ^c	181±9.2 ^b
Adiponectin (ug/ml)	8.5±0.3 ^a	6±0.5 ^c	7.1±0.2 ^b	6.5±0.2 ^c

Data are expressed as mean ± SD. Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

effect on insulin sensitivity the regulation of glucose [27]. Even though we did not measure glucose and insulin levels in this study which might be a limitation of this study, further studies are warranted to confirm the possible association between adiponectin and glucose homeostasis levels.

Expression of PPAR- γ and SREBP1c mRNA in liver

To investigate how lipogenesis are regulated in black soybean *doenjang*, we examined expression of PPAR- γ and SREBP-1 in the liver (Fig. 1, Fig. 2). PPAR- γ levels in the HFD group were the highest. Its level decreased in the HYD group and more so in the black soybean *doenjang*. The ND group showed the lowest level of PPAR- γ . The mRNA levels of PPAR- γ on ND, HBD, and HYD groups were significantly lower than the HFD group by 61, 54, and 44%, respectively ($p < 0.05$). The level of SREBP-1 mRNA dramatically increased in HFD group whereas it demonstrated lower-than-average value in the yellow and black soybean *doenjang*. SREBP-1 mRNA levels of the HBD group were lower than other groups. The mRNA level of SREBP-1c on ND, HBD, HYD and HE groups were significantly lower than the HFD group by 53, 67, 55, and 49%, respectively ($p < 0.05$).

Expression of PPAR- γ and SREBP1c Protein in liver

The protein levels of PPAR- γ and SREBP-1 in the liver

of mice that fed with black soybean *doenjang* were analyzed by western blot. Protein levels showed trends similar to mRNA levels. The PPAR- γ and SREBP-1 protein levels were highest in the HFD group. Expressions of PPAR- γ and SREBP-1 protein levels from liver demonstrated decreases in the HBD group. Interestingly, the HYD group markedly higher than HBD group (Fig. 2).

Discussion

A previous study on the anthocyanins extract of the black soybean showed that anthocyanins suppressed hypolipidemic effects [12, 17, 25, 33]. There was a report that indicated a protective effect of fermented soybean paste against the development of overweight and oxidative stress in adipose tissue of diet-induced obese mice [4, 8]. Fermented black soybean paste is a viable candidate to improve physiological function due to its high anthocyanin content compared to yellow soybean paste. The purpose of the present study was to verify the anti-obesity effects of dietary black soybean *doenjang* with in a population of high fat diet fed C57BL/6 mice. In this study, a significant reduction in body weight gain with black soybean *doenjang* supplementation indicates that the black soybean *doenjang* suppresses the HFD-induced increase in body weight gain and fat weight

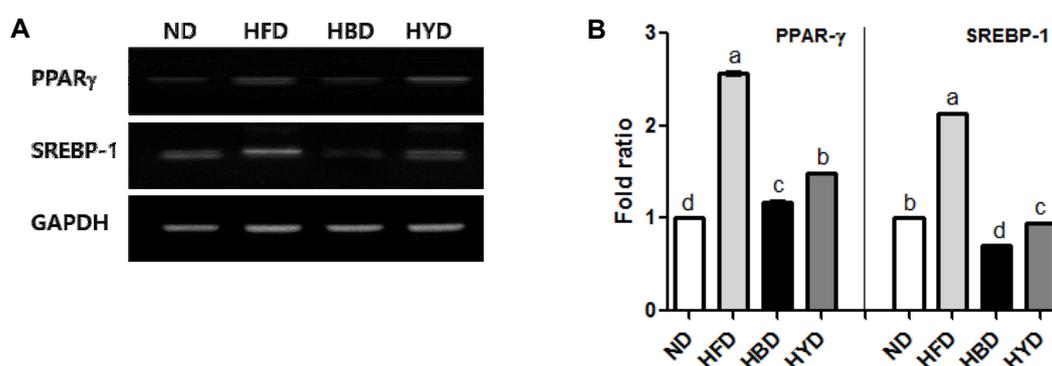


Fig. 1. Effect of lipogenesis-related mRNA expressions level in the liver. Data are expressed as mean ± SD. Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

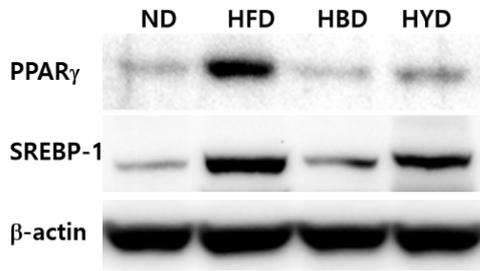


Fig. 2. Effect of lipogenesis-related protein expressions level in the liver.

(Table 2, Table 3). Despite similar food intakes between the HFD and the HBD groups, weight gain trends were significantly different. There has been a report that a high-fat/cholesterol diet leads to hyperlipidemia, characterized as elevated levels of triglyceride and total cholesterol [36]. We observed the levels of TG and TC in the plasma. Plasma triglyceride and total cholesterol levels were significantly higher in the HFD mice than the HBD mice (Table 4). It is likely due to increase of liver and fat weight in HFD group. Whereas the reduction of TG and TC in HBD group is decreased which is comparable with ND group. There have been some reports that plasma levels of TG and TC were significantly increased in HFD-fed mice. In the present study, it was shown that supplementation of black soybean *doenjang* in mice under an 8 weeks high fat diet yielded beneficial effects in triglycerides and total cholesterol which are associated with leptin, adiponectin, and adipogenic transcription factors as well as more body weight loss. Leptin levels of the HFD group were significantly lower than the other group ($p < 0.05$). The levels of leptin in adipocytes is transcriptionally regulated. It is also determined mainly by the status of triglyceride stores in white adipose tissue and the size of adipocytes [6]. Leptin is a fat-derived key regulator of appetite and energy expenditure, and leptin concentration is usually positively correlated with general adiposity [30]. In this study, leptin levels in the HBD group were significantly down regulated, and there was a correlation between leptin concentration and fat mass in Table 2. In addition, in the HBD group, plasma adiponectin levels up regulated by 27% compared to the HFD group ($p < 0.05$). Adiponectin is secreted by fat cells and circulates in the blood. The hormone has antiatherosclerosis and insulin-sensitizing properties that suppress hepatic glucose production and enhance glucose uptake into skeletal muscle [31]. The increase of plasma adiponectin may have been caused by decreased visceral fat mass. This result suggested

by the fact that lipid metabolism changes occurred in the HBD group. This finding was further supported by the hepatic lipogenesis studies that showed reductions in hepatic lipid accumulation with black soybean *doenjang*. To examine the basis for the difference in hepatic fat accumulation between groups, we observed the mRNA and protein levels of PPAR- γ , SREBP-1c in the liver of the mice. Transcription factors, such as PPAR- γ , SREBP-1 were related with lipogenesis. Lipid metabolism is regulated by the activity of peroxisome proliferator-activated receptors (PPARs) [38]. It is reported that PPAR- γ enhances the activity of some adipogenic genes despite the very small amount of manifestation in liver tissue [5, 13]. The PPAR- γ signaling pathway might be involved in the high fat diet induced fatty liver, indicating that PPAR- γ expression plays a key role in the development of fat accumulation in hepatocytes [2]. Sterol regulatory element binding proteins (SREBPs) regulate lipid metabolism [3]. SREBP-1c controls the transcription of genes involved in fatty acid and TG syntheses [29]. Therefore, to find out the mechanism that influences weight change in a body and fat tissue as well as TC and TG level in the blood, we measured the level of mRNA and protein in PPAR- γ and SREBP-1. The mRNA levels in PPAR- γ and SREBP-1 dramatically increased in the HFD group, whereas it decreased in the HBD group. The expression level of transcription factors that regulate fatty acid metabolism in mRNA was measured which was followed by the measurement of expression level in protein. Along with mRNA, the protein level also demonstrated similar tendencies. The protein level in PPAR- γ increased the most in the HFD group whereas it decreased in the HBD group. Protein level in SREBP-1 increased the most in the HFD group, whereas it decreased in the HBD group. It was possible to confirm that the high fat diet of black soybean *doenjang* led to the decreased manifestation of transcription factors which take part in fat accumulation and lipid metabolism. In the initial measurement of weight change, there was similar level of decrease in the HBD group, whereas the level of TG, TC, leptin, and adiponectin in plasma measured lower in the HBD group than in the HFD group. The manifestation level of lipogenesis-related transcription factor such as mRNA in PPAR- γ and SREBP-1 along with protein was the lowest in the HBD group. From these results, it can be concluded that black soybean *doenjang* can contribute to an effective diet, by regulating the lipid metabolism and to bringing down the level of transcription factors related to fatty acid metabo-

lism to normal levels. Therefore, black soybean *doenjang* can regulate the various complications caused by a high fat diet including weight increase, obesity of organ and fat tissue, lipid dysfunction, and lastly the manifestation level of transcription factors related to fatty acid metabolism. Our studies demonstrate that consumption of black soybean *doenjang* ameliorates obesity in diet induced obesity.

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초록 : 고지방식으로 유도된 비만 마우스에서 검정콩 된장의 항비만 효과

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된장은 콩 발효식품으로 주원료인 콩류에 *Bacillus subtilis*, *Rizopus*, *Mucor*와 *Aspergillus* species를 접종하여 발효시킨 메주를 소금물과 혼합하여 숙성 시킨 한국의 전통적인 발효식품이다. 본 연구에서는 동물실험을 통하여 검정콩 된장의 항비만 효과를 확인하였다. 항비만 효과의 확인은 혈중 TG, TC, 아디포넥틴과 렙틴의 레벨을 측정함과 동시에 지방합성에 관여하는 전사인자인 SREBP-1c과 PPAR-g의 mRNA와 단백질 발현 정도를 측정하였다. 고지방 식이에 검정콩 된장을 첨가한 그룹에서는 고지방식으로 인해 증가된 체중을 유의적으로 감소시킴을 확인하였다. 혈중 중성지방, 콜레스테롤과 렙틴의 레벨은 고지방식을 섭취한 마우스에 비하여 검정콩 된장을 섭취한 마우스에서 감소하였으며 아디포넥틴의 분비량은 유의적으로 증가하였다. 이러한 결과가 지방 생성의 억제로부터 유도되는지를 조사하기 위하여 지방 합성에 관여하는 전사인자인 SREBP-1c과 PPAR-g의 mRNA양과 단백질 발현을 측정한 결과 검정콩 된장을 섭취한 마우스에서 현저하게 감소하는 것을 확인하였다. 이러한 결과는 검정콩 된장의 섭취가 지방대사와 지방 전사 인자의 활성을 감소시킨다는 것을 확인함으로써 검정콩 된장이 비만의 예방과 진행을 개선시킬 수 있음을 증명하였다.