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The effects of black garlic (*Allium satvium*) extracts on lipid metabolism in rats fed a high fat diet

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BACKGROUD/OBEJECTIVES: The mechanism of how black garlic effects lipid metabolism remains unsolved. Therefore, the objectives of this study were to determine the effects of black garlic on lipid profiles and the expression of related genes in rats fed a high fat diet.

MATERIALS/METHODS: Thirty-two male Sqrague-Dawley rats aged 4 weeks were randomly divided into four groups (n=8) and fed the following diets for 5 weeks: normal food diet, (NF); a high-fat diet (HF); and a high-fat diet + 0.5% or 1.5% black garlic extract (HFBG0.5 or HFBG1.5). Body weights and blood biochemical parameters, including lipid profiles, and expressions of genes related to lipid metabolism were determined.

RESULTS: Significant differences were observed in the final weights between the HFBG1.5 and HF groups. All blood biochemical parameters measured in the HFBG1.5 group showed significantly lower values than those in the HF group. Significant improvements of the plasama lipid profiles as well as fecal excretions of total lipids and triglyceride (TG) were also observed in the HFBG1.5 group, when compared to the HF diet group. There were significant differences in the levels of mRNA of sterol regulatory element binding protein-1c (SREBP-1c), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and glucose-6-phosphate dehydrogenase (G6PDH) in the HFBG1.5 group compared to the HF group. In addition, the hepatic expression of (HMG-CoA) reductase and Acyl-CoA cholesterol acyltransferase (ACAT) mRNA was also significantly lower than the HF group.

CONCLUSIONS: Consumption of black garlic extract lowers SREBP-1C mRNA expression, which causes downregulation of lipid and cholestrol metahbolism. As a result, the blood levels of total lipids, TG, and cholesterol were decreased.

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INTRODUCTION

Garlic *(Allium sativum)* has been used as a medicinal ingredient in folk remedies since the old times. It contains various bio-functional properties affecting health [1-5], but was not enjoyed by many due to its peculiar smell. Various food processing methods have been employed to get rid of the smell of garlic. Black garlic is made by ripening raw garlic at high temperature and humidity, which removes the peculiar pungent odor [6]. Black garlic gets its name from the black hue of the cloves, but retains the same shape as raw garlic. During the ripening process, the glyco-component and amino acids of the garlic undergo a non-enzymatic browning reaction, producing melanoidins and water-soluble components such as S-allylcysteine (SAC) and S-allyl melcaptocystein (SAMC), and nearly removing all volatile substances [7]. In black garlic extracts, the contents of water soluble components, such as SAC and SAMC, are known to increase significantly during the aging process, unlike raw garlic, which may play a key role in antilipidemic action as well as conferring potent antioxidant activity [8,9].

Not all garlic preparations can be assumed equivalent in composition. Studies on garlic powder and garlic oil failed to show any lipid lowering or hypoglycemia effects [11,12]. The inconsistent results of animal studies may be due at least in part to differences in the types of garlic studied (i.e. raw garlic, cooked garlic or black garlic).

Excessive intake of fat alters lipid metabolism, which can act as a risk factor for various diseases including fatty liver disease, hypertension, hyperlipidemia, and arteriosclerosis [10]. Elevation of blood lipids levels due to excessive fat intake stimulates SREBP-1c, the key transcription factor involved in fat metabolism, which leads to the activation of lipogenetic enzymes for triglyceride and cholesterol synthesis, as well as inhibition of lipolytic enzymes [13-15]. SREBP-1c also mediates the action of insulin on hepatic gene expression, while its transcriptional activity is also regulated by insulin [16].

Several studies have shown that the lipid-lowering mechanisms of fresh garlic, not black garlic, involve many gene factors such as AMP-activated protein kinase (AMPK) and enzymes such as ACAT and FAS [17,18]. Limited study has been carried out

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regarding the possible mechanism of the action of black garlic on lipid metabolism [19]. Thus, the objectives of this study were to determine the effects of black garlic on blood lipids and cholesterol profiles, and the expression of related hepatic genes in rats fed a high fat diet.

MATERIALS AND METHODS

Animals and Diets

Thirty-two male Sqrague-Dawley rats aged 4 weeks were randomly divided into four groups (n = 8) by weight, and fed the following diets: normal-fat (NF, 7% fat); high-fat (HF, 20% fat); high-fat with 0.5% black garlic extract (HFBG0.5); or high-fat with 1.5% black garlic extract (HFBG1.5). The compositions of the experimental diet were based on the AIN-93G diet (Table 1).

Black garlic extract was generously provided by the Korea Euisung Agricultural Association (Gyeongsangbuk-do, Republic of Korea). The black garlic extract used in this study was 60 brix concentrate. In the experimental group, the HFBG0.5 group was fed 0.5% black garlic extract (5 g extract/kg diet) and the HFBG1.5 group was fed 1.5% black garlic extract (15 g extract/kg diet). All experiments were approved by the guidelines of the Animal Testing Ethics Committee of Dankook Univeristy (approval code: 12-004).

Sample Preparation

After 5 weeks of feeding, the experimental animals were fasted for 12 hr and then anesthetized with ethylether. Blood samples were drawn from the hearts and centrifuged for 30 minutes at 3,000 rpm, after which the plasma was stored at -70˚C until use. After scarification of the rats, the liver, kidney, thymus, spleen, internal fat, kidney fat, and epididymal fat were removed and rinsed with 0.9% NaCl solution, then dried with filter paper. After measuring the weights of the organs and adipose tissue, all samples were stored at -70˚C until further analysis. Stool samples were individually collected twice daily and completely dried by heating at 105˚C.

Analysis of plasma biochemical parameters

Plasma glucose concentration was measured with an assay kit using the glucose oxidase method (Asan Pharmaceutical Co., Seoul, Korea). Plasma insulin concentrations were measured via a rat insulin ELIZA kit (Shibayagi Co., Shibukawa , Japan). Plasma AST (aspartate aminotransferase) and ALT (alanine aminotransferase) were measured with commercial kits (Asan Pharmaceutical Co., Seoul, Korea), and the absorbance at 505 nm was determined with a spectrophotometer.

Lipid measurements in plasma, liver and feces

Plasma total lipids were determined as described by Frings and Dunn [20], with slight modifications. Plasma total cholesterol, TG, and HDL were measured enzymatically using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). Total lipids in the liver and feces were extracted with chloroform: methanol (2 : 1, v/v) using a modified method described by Folch *et al.* [21]. After extraction, the total lipid, TG, and cholesterol were measured using the same methods as for plasma lipid determination.

Table 1. Composition of experiment diets (g/kg)

Ingredients	NF[1)]	HF	HFBG0.5	HFBG1.5
Corn starch	529.486	389.486	384.486	374.486
Casein	200.0	200.0	200.0	200.0
L-Cystine	3.0	3.0	3.0	3.0
Sucrose	100.0	100.0	100.0	100.0
Cellulose	50.0	50.0	50.0	50.0
Soybean oil	70.0	70.0	70.0	70.0
Lard	0	130.0	130.0	130.0
Mineral mix[2)]	35.0	35.0	35.0	35.0
Vitamin mix[3)]	10.0	10.0	10.0	10.0
Choline bitartrate	2.5	2.5	2.5	2.5
tert-Butylhydroquinone	0.014	0.014	0.014	0.014
Cholesterol	-	10.0	10.0	10.0
black garlic extract	-	-	5.0	15.0
Total	1,000	1,000	1,000	1,000

[1)] NF: Normal AIN-93G diet, HF: High fat diet, HFBG0.5: High fat diet + 0.5% 60Brix Aged black garlic extract, HFBG1.5: High fat diet + 1.5% 60Brix Aged black garlic extract
[2)] Mineral mixture: AIN-93G mineral mixture
[3)] Vitamin mixture: AIN-93 vitamin mixture

Table 2. Primer sequences used for realtime PCR

Protein	Primer	Sequence
β-actin	Sense	5-AGG TCA TCA CTA TCG GCA AT-3
	Antisense	5-ACT CAT CGT ACT CCT GCT TG-3
ACC	Sense	5-CAA CGC CTT CAC ACC ACC TT-3
	Antisense	5-AGC CCA TTA CTT CAT CAA AGA TCC T-3
FAS	Sense	5'-TGC TCC CAG CTG CAG -3'
	Antisense	5'-GCC CGG TAG CTC TGG GTG TA-3'
G6PDH	Sense	5-GTT TGG CAG CGG CAA CTA A-3
	Antisense	5-GGC ATC ACC CTG GTA CAA CTC-3
SREBP-1C	Sense	5- GGAG CCAT GGAT TGCA CATT-3
	Antisense	5- AGGA AGGC TTCC AGAG AGGA-3
CPT1	Sense	5'-GTGC TGGA GGTG GCTT TGGT-3'
	Antisense	5'-TGCT TGAC GGAT GTGG TTCC-3'
ACAT	Sense	5-GCT GAA GTG AAC TAC CCC TT-3
	Antisense	5-GAG CCA TGC CTC TAG TAC CT-3
HMG-CoA	Sense	5-CTT GAC GCT CTG GTG GAA TG-3
	Antisense	5-AGT TGG AAG CAC GGA CATA -3

Total RNA isolation

Total liver RNA was isolated using TRI-reagent (Sigma aldrich, MO, USA) according to the manufacturer's protocol. Liver samples (0.2 g) were homogenized in 1 mL TRI Reagent and then kept at room temperature for 5 min. The homogenate was then mixed with 200 μL chloroform (Sigma aldrich, MO, USA), shaken vigorously, left for 10 min, and then centrifuged at 12,000 g for 10 min. After transferring the supernatant, 500 μL of isopropanol was added and kept at room temperature for 5 min, after which the sample was centrifuged at 12,000 g for 10 min at 4˚C.

After removing the supernatant, the RNA pellet was washed with 75% ethanol followed by centrifugation at 7,500 g for 5 min at 4˚C. After removing the ethanol wash, samples were dried thoroughly and then the RNA was dissolved in 50 μL

RNase-free dH_2O/0.1 mmol/L EDTA through a pipette tip. The purity of each RNA preparation was evaluated by measuring the ratio of the absorbance at 260 nm to that at 280 nm. The final preparations of total RNA were essentially free of DNA and proteins, and had 260/280 ratios of 1.7-2.0.

Reverse Transcription (RT)

cDNA was synthesized using 3 μg of total RNA with Super-Script II reverse transcriptase (Invitrogen, CA, USA). In 1.5 mL tubes, 3 μg total RNA from the samples and 1 μL of oligo DT (0.5 μg/μL, Invitrogen, CA, USA) were added and the final volume was brought to 12 μL with DEPC water, followed by incubation at 70˚C for 10 min [15]. A reaction buffer (4 μL 5 X first standard buffer, 2 μL 0.1 mol/L DTT, 1 μL 10 mmol/L NTP) was added to the mixture and incubated at 42˚C for 5 min. After incubation, 0.5 μL superscript II reverse transcriptase (Invitrogen, CA, USA) was added and incubated at 42˚C for 100 min, and then 70˚C for 15 min. Finally, 80 μL Rnase DEPC water was added to the mixture, which was stored at -20˚C until subsequent analysis.

Realtime PCR

Real-time PCR was performed using the modified method recently described in detail [15]. Aliquots of 2 μL of the prepared cDNA samples were mixed with 10 μL SYBR green Master mix (Applied biosystems, CA, USA), 1 μL of each sense/antisense primer (Table 2), and 6 μL DEPC water. The initial activation of the primer at 95˚C for 10 min was followed by 40 cycles of 95˚C for 15 min and 60˚C for 1 min. Expression of mRNA was analyzed with an Applied Biosystems StepOne Plus RT-PCR system (Applied biosystems, CA, USA). Fold difference of gene expression was calculated using the $2^{-\Delta\Delta CT}$ method with the endogenous control gene.

Statistical analysis

Statistical analysis was performed using the Statistical Analysis System software (SAS Institute, Cary, NC, USA). Data were expressed as means with standard error, and statistically significant differences among groups were evaluated using one way-ANOVA (analysis of variance). Statistically significant differences among the means of groups were tested at $\alpha = 0.05$ using Duncan's multiple range tests.

RESULTS

Body weight, food intake, and organ weight in rats fed black garlic extract

Final body weight and weight gain in the HFBG1.5 group were significantly lower than the HF and HFBG0.5 groups. Significant differences in the food efficiency ratios (FER) were not observed among the groups (Table 3).

No significant differences in liver, kidney, spleen, and thymus weights were found between the HF group and the HFBG0.5 or HFBG1.5 groups (Table 4). Among adipose tissues, kidney fat and epididymal fat were significantly decreased in the HFBG1.5 group compared to both the HF and HFBG0.5 groups. Total fat of the adipose tissues weighed 9.47 g in the HFBG1.5 group, which was lower than the 11.04 g measured for the HF group, though the difference was not significant.

Plasma biochemical parameters in rats fed black garlic extract

The blood biochemical values are shown in Table 5. Both the HFBG0.5 and HFBG1.5 groups showed significantly lower blood glucose levels than the NF or HF groups. In addition, the insulin level in the HFBG1.5 group was significantly decreased compared to the HF and HFBG0.5 groups. The AST levels in the HF group were significantly elevated due to administration of the HF diet, but the levels were significantly decreased in the

Table 4. Effect of black garlic extracts on weights of various organs and adipose tissues (g)

Parameter	NF[1)]	HF	HFBG0.5	HFBG1.5
Liver	10.43 ± 0.25[a2)3)]	15.16 ± 0.77[b]	15.50 ± 0.62[b]	13.89 ± 0.46[b]
Kidney	2.35 ± 0.06[NS 4)]	2.45 ± 0.09	2.49 ± 0.04	2.31 ± 0.05
Spleen	0.69 ± 0.03[a]	0.77 ± 0.03[a]	0.90 ± 0.07[b]	0.71 ± 0.04[a]
Thymus	0.66 ± 0.03[NS]	0.70 ± 0.05	0.71 ± 0.07	0.69 ± 0.07
Adipose tissues				
Internal fat	1.81 ± 0.14[NS]	2.14 ± 0.20	2.07 ± 0.31	1.94 ± 0.20
Kidney fat	4.09 ± 0.46[a]	4.20 ± 0.45[a]	4.10 ± 0.59[a]	3.53 ± 0.60[b]
Epididymal fat	4.67 ± 0.45[a]	4.70 ± 0.30[a]	4.63 ± 0.42[a]	4.00 ± 0.27[b]
Total fat	10.57 ± 0.96[NS]	11.04 ± 0.72	10.8 ± 1.12	9.47 ± 0.91

[1)] NF: Normal AIN-93G diet, HF: High fat diet, HFBG0.5: High fat diet + 0.5% 60Brix Aged black garlic concentrate, HFBG1.5: High fat diet + 1.5% 60Brix Aged black garlic concentrate
[2)] Values are mean ± SE (n = 8)
[3)] Different letters indicate significant differences among groups at $\alpha = 0.05$ as determined by Duncan's multiple range tests.
[4)] NS: not significant

Table 3. Effect of black garlic extracts on body weight and food intakes of rats

Parameter	NF[1)]	HF	HFBG0.5	HFBG1.5
Initial body weight (g)	117.6 ± 2.7[NS 2)3)]	117.7 ± 2.7	117.6 ± 2.6	117.4 ± 2.3
Final body weight (g)	317.5 ± 7.3[a4)]	337.9 ± 8.6[b]	346.2 ± 3.9[b]	319.8 ± 8.4[a]
Weight gain (g/5weeks)	205.9 ± 5.2[a]	219.2 ± 9.8[b]	230.1 ± 3.2[b]	203.6 ± 8.4[a]
Total food intake (g/5weeks)	654.2 ± 25.1[a]	603.7 ± 15.9[b]	605.2 ± 13.4[b]	588.3 ± 18.6[b]
FER[5)]	0.37 ± 0.03[NS]	0.36 ± 0.02	0.38 ± 0.01	0.35 ± 0.02

[1)] NF: Normal AIN-93G diet, HF: High fat diet, HFBG0.5: High fat diet + 0.5% 60Brix Aged black garlic concentrate, HFBG1.5: High fat diet + 1.5% 60Brix Aged black garlic concentrate
[2)] Values are mean ± SE (n = 8)
[3)] NS: not significant
[4)] Different letters indicate significant differences among groups at $\alpha = 0.05$ as determined by Duncan's multiple range tests.
[5)] FER (food efficiency ratio) = Body weight gain for experimental period / Food intake for experimental period

Table 5. Effect of black garlic extracts on the levels of blood biochemical parameters

Parameter	NF[1)]	HF	HFBG0.5	HFBG1.5
Glucose (mg/dL)	136.4 ± 6.4[a2)3)]	145.9 ± 6.2[b]	130.3 ± 2.9[a]	123.0 ± 5.0[a]
Insulin (ng/mL)	0.634 ± 0.0023[a]	0.675 ± 0.0012[b]	0.657 ± 0.0034[b]	0.633 ± 0.0049[c]
AST (IU/L)[4)]	81.34 ± 1.87[a]	97.71 ± 1.14[b]	89.42 ± 1.88[b]	82.19 ± 3.53[a]
ALT (IU/L)[5)]	62.06 ± 0.60[a]	78.00 ± 3.18[b]	65.03 ± 1.36[a]	63.52 ± 0.95[a]

[1)] NF: Normal AIN-93G diet, HF: High fat diet, HFBG0.5: High fat diet + 0.5% 60Brix Aged black garlic concentrate, HFBG1.5: High fat diet + 1.5% 60Brix Aged black garlic concentrate
[2)] Values are mean ± SE (n = 8)
[3)] Different letters indicate significant differences among groups at α = 0.05 as determined by Duncan's multiple range tests.
[4)] AST (aspartate aminotransferase)
[5)] ALT (alanine aminotransferase)

HFBG1.5 group in comparison. Significant decrease in the ALT levels was observed in both the HFBG0.5 and HFBG1.5 groups, compared to the HF group.

Lipid profiles of the plasma, liver, and feces in rats fed black garlic extract

The levels of total lipids, cholesterol, and HDL-cholesterol in the plasma of the HFBG0.5 group were significantly lower than in the HF group, while HDL-cholesterol was significantly higher. Plasma total lipids, TG, and HDL-cholesterol in the HFBG1.5 group were also significantly improved. Total hepatic lipids in the HFBG1.5 group were significantly decreased compared to the HF group. Significant increases of total fecal lipids and TG were also observed in the HFBG1.5 group compared to the HF group (Table 6).

The levels of mRNA expression of transcription factors in rats fed black garlic extract

The mRNA expressions of ACC, FAS, and SREBP-1C were

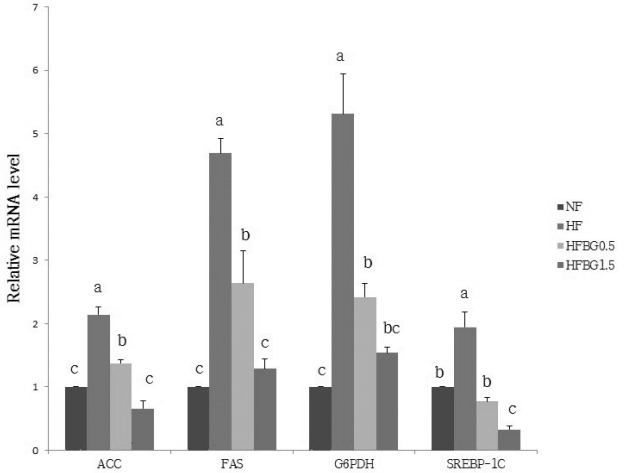

Fig. 1. Effect of black garlic extracts on mRNA expression of transcription factor and enzymes related to fat synthesis in liver of rats. Total RNA was isolated using TRI-reagenet and cDNA was synthesized using 3 μg of total RNA with SuperScript II reverse transcriptase. Realtime PCR was performed using SYBR green and standard procedures to assess the mRNA expression of primer in liver samples obtained from each group. An Applied Biosystem StepOne softwere v2.1 was used. Each bar represents the mean ± SE of three independent experiments. Different letters above each bar indicate significant differences among groups at α = 0.05 as determined by Duncan's multiple range test. ACC; Acetyl-CoA carboxylase, FAS; Fatty acid synthase, G6PDH; Glucose-6-phosphate dehydrogenase, SREBP-1C; Sterol regulatory element binding protein

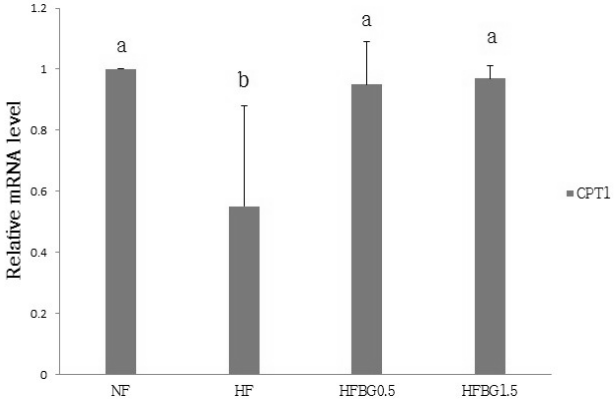

Fig. 2. Effect of black garlic extracts on mRNA expression of CPT1 in liver of rats. Total RNA was isolated using TRI-reagenet and cDNA was synthesized using 3 μg of total RNA with SuperScript II reverse transcriptase. Realtime PCR was performed using SYBR green and standard procedures to assess the mRNA expression of primer in liver samples obtained from each group. An Applied Biosystem StepOne softwere v2.1 was used. Each bar represents the mean ± SE of three independent experiments. Different letters above each bar indicate significant differences among groups at α = 0.05 as determined by Duncan's multiple range test. CPT1; Carnitine palmitoyltransferase-1

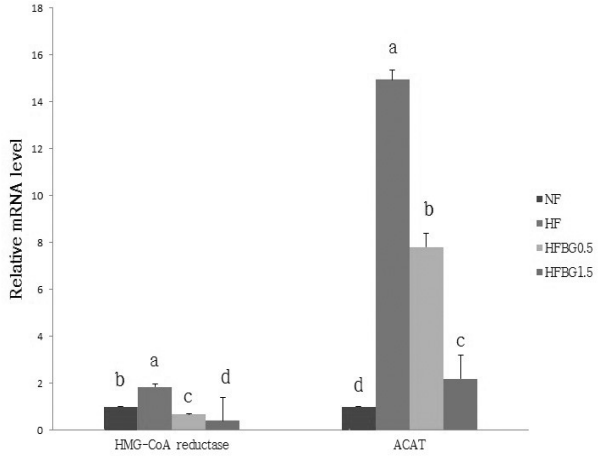

Fig. 3. Effect of black garlic extracts on mRNA expression of protein related to cholesterol synthesis in liver of rats. Total RNA was isolated using TRI-reagenet and cDNA was synthesized using 3 μg of total RNA with SuperScript II reverse transcriptase. Realtime PCR was performed using SYBR green and standard procedures to assess the mRNA expression of primer in liver samples obtained from each group. An Applied Biosystem StepOne softwere v2.1 was used. Each bar represents the mean ± SE of three independent experiments. Different letters above each bar indicate significant differences among groups at α = 0.05 as determined by Duncan's multiple range tests. ACAT; Acyl-CoA cholesterol acyltransferase, HMG-CoA; Hydroxy-3-methylglutaryl coenzyme A

significantly lower in both the HFBG1.5 and HFBG0.5 group, compared to the HF group, with higher reduction in the HFBG1.5 group than in the HFBG0.5 group (Fig. 1). The mRNA expression of G6PDH was significantly lower in the HFBG1.5 group, not in the HFBG0.5 group, compared to the HF group. The expression of CPT1 mRNA in the HF group was significantly decreased compared to the NF group (Fig. 2); however, the expressions were significantly higher than HF in both the HFBG1.5 and HFBG0.5 groups. The hepatic mRNA expressions of HMG-CoA reductase and ACAT were significantly lower than HF in both the HFBG1.5 and HFBG0.5 groups, with much greater reduction observed in the HFBG1.5 group (Fig. 3).

Table 6. The lipid profiles in plasma, liver and feces of rats fed with black garlic extracts

Parameter	NF[1)]	HF	HFBG0.5	HFBG1.5
Plasma lipids (mg/dL)				
Total lipid	194.1 ± 10.5[a2)3)]	296.3 ± 7.5[b]	233.8 ± 22.5[c]	244.1 ± 20.1[c]
Cholesterol	66.2 ± 3.1[a]	79.1 ± 4.7[b]	63.8 ± 5.0[a]	68.5 ± 5.9[ab]
Triglyceride	87.3 ± 9.1[a]	93.6 ± 7.6[a]	91.4 ± 9.3[a]	74.7 ± 6.3[b]
HDL-Cholesterol	21.2 ± 1.5[a]	13.0 ± 1.3[b]	24.3 ± 2.5[a]	23.9 ± 2.0[a]
Hepatic lipids (mg/g wet liver)				
Total lipid	134.7 ± 3.7[a]	139.4 ± 9.3[b]	140.5 ± 11.6[b]	129.4 ± 8.3[a]
Cholesterol	8.0 ± 0.047[a]	49.0 ± 2.7[b]	43.8 ± 2.8[b]	48.5 ± 3.6[b]
Triglyceride	36.0 ± 3.3[a]	72.2 ± 4.7[b]	53.4 ± 3.2[b]	64.1 ± 5.1[b]
Fecal lipids (mg/g dry feces)				
Total lipid	12.7 ± 0.7[a]	40.9 ± 1.7[b]	40.3 ± 2[b]	48.3 ± 1.2[c]
Cholesterol	3.7 ± 0.2[a]	33.4 ± 1.1[b]	30.6 ± 1.9[b]	36.2 ± 1.2[b]
Triglyceride	2.5 ± 0.3[a]	4.5 ± 1.0[b]	4.9 ± 1.1[b]	10.1 ± 1.7[c]

[1)] NF: Normal AIN-93G diet, HF: High fat diet, HFBG0.5: High fat diet + 0.5% 60Brix Aged black garlic concentrate, HFBG1.5: High fat diet + 1.5% 60Brix Aged black garlic concentrate
[2)] Values are mean ± SE (n = 8).
[3)] Different letters indicate significant differences among groups at $\alpha = 0.05$ as determined by Duncan's multiple range tests.

DISCUSSION

Based on previous animal studies regarding the effects of garlic on lipid metabolism, the hypolipidemic actions of fresh garlic were encouraging when less than 2% of garlic were administrated to rats [4-6,22,23]. In our prelimary study, similar effectiveness was observed for 0.5% and 1.0% black garlic extract. Therefore, 0.5% (5 g/kg diet) and 1.5% (15 g/kg diet) black garlic extract were selected as the applicable high and low dosage for supplementation in rats fed a high fat diet in this study.

Final body weights in the HFBG1.5 group were significantly lower than that in both the HF and HFBG0.5 groups. It could be suspected that the unique flavor and smell of the black garlic had a higher effect on the appetites of the rats in the HFBG1.5 group, resulting in a decrease in food intake and weight reduction. However, there were no significant differences in the total dietary intakes or food efficiency ratio between the HFBG1.5 group and the HF or HFBG0.5 groups. Therefore, the observed weight reduction may be mediated by other variables in the black garlic extract, rather than food intake.

Like the previous reports [9,24-26], this study showed the hypoglycemic effects of black garlic extract in rats fed a high fat diet. The effect was more potent with the addition of 1.5% black garlic extract than 0.5%, demonstrated by decreases in blood glucose levels and increases in plasma insulin level herein.

The changes of serum AST and ALT levels, which are serum markers of liver damage, are generally monitored in high fat diets [9]. Several studies reported that the alterations in AST and ALT due to liver toxicity were significantly prevented by prior administration of 0.2-1.0% fresh garlic extract for a short period of time [4,22,27]. In the present study, the AST levels in the HF group were significantly elevated compared to the NF group, while both 0.5% and 1.5% black garlic extract showed favorable hepatoprotective effects. Therefore, the present study suggests that the doses of 0.5-1.5% are safe for black garlic extract usage in animal study.

The importance of plasma TG and cholesterol levels in the pathogenesis of atherosclerosis has generally been accepted. It was clearly demonstrated herein that black garlic, dose-dependently, has hypolipidemic effects. These results were consistent with many previous studies [18,20,23-26]. However, the amounts of garlic supplemented in previous animal studies were quite different from those used herein. Some studies reported that 3-5% fresh garlic or black garlic extract significantly decreased blood cholesterol and triglyceride (TG) levels in diabetic or hypercholestemic mice [23-26]. The amounts of 3-5% garlic in the rat's diet are quite high, and are not applicable for the normal diet of humans [28]. Therefore, the results of this study are meaningful in that even low dosage, such as 0.5 or 1.5% black garlic extract, is beneficial to improve hyperlipidemia caused by high fat diet.

Allicin in fresh garlic was initially identified as the active compound responsible for the anti-atherosclerotic effect. Unfortunately, this study did not measure the bio-active compound content of the black garlic extract used; however, studies reported that the main constituents of black garlic are water soluble compounds, such as SAC and SAMC, which are produced during the ripening process of black garlic [6-9]. These water soluble compounds, which are rare in raw garlic, are thought to be responsible for black garlic's anti-hyperlipidemic effects [9,22,29].

In evaluating the mechanism of how black garlic lowers the lipid profiles in plasma and liver in rats, we measured the expressions of hepatic SREBP-1c mRNA, a key transcription factor of lipid metabolism, and decreased expression of SREBP-1c was observed in the rats treated with black garlic extract. As a consequence of the fall in hepatic SREBP-1c, the levels of ACC, FAS, and G-6PDH mRNA, target genes of SREBP-1c, were strongly reduced to the levels observed in the normal fat mice, suggesting that inhibition of the expression of these genes causes decrease of fat synthesis in the liver. This was associated with a decrease in the plasma TG concetration. Similar results were reported, in that the methanol extract of black garlic in

rats fed a high-cholesterol diet inhibited lipid accumulation via the up-regulation of AMPK and the down-regulation of SREBP-1c, ACC, and FAS [16-18,30,31].

Increased high fat diet results in the production of plenty of acetyl-CoA and malonyl CoA in the liver. The increased level of malonyl CoA, as a fuel for TG synthesis, will inhibit the expression of CPT-1, a key enzyme for lipolysis, thus decreasing fatty acid oxidation [32]. As shown in this study, the supplementation of high fat diet with black garlic extract led to increased expression of CPT-1 mRNA, causing more TG to break down (degrade) in order to supply free fatty acid as an energy source.

The extent of hepatic SREBP-1c levels is also believed to activate the enzymes, HMG-CoA reductase and ACAT, which regulate cholesterol synthesis and catalyzes esterification of cholesterol, respectively [15,29]. In rats fed black garlic extract, the plasma cholesterol concentration was markedly decrased which was related to the decreased expressions of HMG-CoA reductase and ACAT.

In this study, the supplementation of black garlic extract significantly increased the fecal excretion of lipids, both TG and cholesterol, which suggests that the mechanism of black garlic extract is not only related to the mechanisms of fat synthesis, but also could be related to another mechanisms leading to increased fecal excretion or the absorption rate of dietary fat, resulting in a significant reduction in the plasma concentration of lipid profiles in rats fed black garlic extract. Therefore, further studies regarding the mechanisms related to fecal excretion of lipids are needed to clarify this matter.

Garlic and its preparations have been widely recognized as agents for the prevention and treatment of cardiovascular diseases. However, it is commonly known that excessive consumption of garlic can cause problems such as odor of the breath and skin, occasional allergic reactions, and toxicity. The main constituents of black garlic extract are water soluble compounds, thus it lacks the toxicity and has no peculiar garlic smell. Therefore, it can be taken by humans for a long time without presenting toxic side effects or contraindications with medications to prevent or reduce hyperlipidermia.

In conclusion, administration of 0.5% or 1.5% black garlic extract was effective, dose-dependently, in improving blood lipid profiles, especially TG, and blood glucose levels in rats fed a high fat diet, as evidenced by decreased expressions of SREBP-1c and its related enzymes, along with increased expression of CPT-1. Thus, appropriate intake of black garlic will be beneficial in the prevention of hyperlipidemia and hyperglycemia caused by a high fat diet.

REFERENCES

1. Rahman K, Lowe GM. Garlic and cardiovascular disease: a critical review. J Nutr 2006;136:736S-740S.
2. Ried K, Toben C, Fakler P. Effect of garlic on serum lipids: an updated meta-analysis. Nutr Rev 2013;71:282-99.
3. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutr 2001;131:955S-962S.
4. Jo HJ, Choi MJ. Effect of 1% garlic powder on serum and liver lipid and plasma amino acid concentration in rats fed cholesterol diet. J Korean Soc Food Sci Nutr 2002;31:98-103.
5. Sheo HJ. Effects of garlic on the blood lipids and other serum components in rats. J Korean Soc Food Sci Nutr 1999;28:1339-48.
6. Jang EK, Seo JH, Lee SP. Physiological activity and antioxidative effects of aged black garlic (Allium sativum L.) extract. Korean J Food Sci Technol 2008;40:443-8.
7. Kim JH, Nam SH, Rico CW, Kang MY. A comparative study on the antioxidative and anti-allergic activities of fresh and aged black garlic extracts. Int J Food Sci Technol 2012;47:1176-82.
8. Lee YM, Gweon OC, Seo YJ, Im J, Kang MJ, Kim MJ, Kim JI. Antioxidant effect of garlic and aged black garlic in animal model of type 2 diabetes mellitus. Nutr Res Pract 2009;3:156-61.
9. Shin JH, Lee CW, Oh SJ, Yun J, Kang MR, Han SB, Park H, Jung JC, Chung YH, Kang JS. Hepatoprotective effect of aged black garlic extract in rodents. Toxicol Res 2014;30:49-54.
10. Larsson SC. Dietary fats and other nutrients on stroke. Curr Opin Lipidol 2013;24:41-8.
11. Amagase H. Clarifying the real bioactive constituents of garlic. J Nutr 2006;136:716S-725S.
12. Kwon MJ, Song YS, Choi MS, Park SJ, Jeong KS, Song YO. Cholesteryl ester transfer protein activity and atherogenic parameters in rabbits supplemented with cholesterol and garlic powder. Life Sci 2003;72:2953-64.
13. Foretz M, Guichard C, Ferré P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci U S A 1999;96:12737-42.
14. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331-40.
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-8.
16. Kim SY, Kim HI, Kim TH, Im SS, Park SK, Lee IK, Kim KS, Ahn YH. SREBP-1c mediates the insulin-dependent hepatic glucokinase expression. J Biol Chem 2004;279:30823-9.
17. Qureshi AA, Din ZZ, Abuirmeileh N, Burger WC, Ahmad Y, Elson CE. Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: impact on serum lipids. J Nutr 1983;113:1746-55.
18. Lin MC, Wang EJ, Lee C, Chin KT, Liu D, Chiu JF, Kung HF. Garlic inhibits microsomal triglyceride transfer protein gene expression in human liver and intestinal cell lines and in rat intestine. J Nutr 2002;132:1165-8.
19. Kang MJ, Lee SJ, Shin JH, Kang SK, Kim JG, Sung NJ. Effect of garlic with different processing on lipid metabolism in 1% cholesterol fed rats. J Korean Soc Food Sci Nutr 2008;37:162-9.
20. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497-509.
21. Frings CS, Dunn RT. A colorimetric method for determination of total serum lipids based on the sulfo-phospho-vanillin reaction. Am J Clin Pathol 1970;53:89-91.
22. Yeh YY, Liu L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. J Nutr 2001;131:989S-993S.
23. Lai YS, Chen WC, Ho CT, Lu KH, Lin SH, Tseng HC, Lin SY, Sheen LY. Garlic essential oil protects against obesity-triggered nonalcoholic fatty liver disease through modulation of lipid metabolism and

oxidative stress. J Agric Food Chem 2014;62:5897-906.
24. Jung YM, Lee SH, Lee DS, You MJ, Chung IK, Cheon WH, Kwon YS, Lee YJ, Ku SK. Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects. Nutr Res 2011; 31:387-96.
25. Seo YJ, Gweon OC, Im J, Lee YM, Kang MJ, Kim JI. Effect of garlic and aged black garlic on hyperglycemia and dyslipidemia in animal model of type 2 diabetes mellitus. J Food Sci Nutr 2009;14:1-7.
26. Shin SH, Kim MK. Effect of dried powders or ethanol extracts of garlic flesh and peel on lipid metabolism and antithrombogenic capacity in 16-month-old rats. Korean J Nutr 2004;37:515-24.
27. Chen L, Hong JY, So E, Hussin AH, Cheng WF, Yang CS. Decrease of hepatic catalase level by treatment with diallyl sulfide and garlic homogenates in rats and mice. J Biochem Mol Toxicol 1999;13: 127-34.
28. Gardner CD, Chatterjee LM, Carlson JJ. The effect of a garlic preparation on plasma lipid levels in moderately hypercholesterolemic adults. Atherosclerosis 2001;154:213-20.
29. Gebhardt R, Beck H. Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids 1996;31:1269-76.
30. Kamanna VS, Chandrasekhara N. Effect of garlic (Allium sativum linn) on serum lipoproteins and lipoprotein cholesterol levelsin albino rats rendered hypercholesteremic by feeding cholesterol. Lipids 1982;17:483-8.
31. Chen YC, Kao TH, Tseng CY, Chang WT, Hsu CL. Methanolic extract of black garlic ameliorates diet-induced obesity via regulating adipogenesis, adipokine biosynthesis, and lipolysis. J Funct Foods 2014;9:98-108.
32. Ryu MH, Cha YS. The effects of a high-fat or high-sucrose diet on serum lipid profiles, hepatic acyl-CoA synthetase, carnitine palmitoyltransferase-I, and the acetyl-CoA carboxylase mRNA levels in rats. J Biochem Mol Biol 2003;36:312-8.