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Effect of Garlic on Serum Lipids Profiles and Leptin in Rats Fed High Fat Diet

Soon Ah Kang[1], Ho Jung Shin[1,4], Ki-Hyo Jang[2], Sung Eun Choi[1], Kyung Ah Yoon[1], Jin Sook Kim[3], Hye Kyung Chun[3] and Yoongho Lim[4†]

[1]Department of Fermented Food Science, Seoul University of Venture & Information, Seoul 137-070, Korea
[2]Department of Food & Nutrition, Kangwon University, Chuncheon 245-711, Korea
[3]Department Agriproduct Processing, Rural Resources Development Institute, Suwon 441-853, Korea
[4]Department of Molecular Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 134-701, Korea

Abstract

Although garlic has been reported to have impressive effects in lowering serum lipids, there have been controversial evaluations on these effects. To find the potential fator causing the inconsistency in the previous studies, we examined the effects of two types of garlic according to the producing-area (hangihyung garlic, nangihyung garlic) on serum lipid profiles and leptin level. Thirthy six of 4 wk old Sprague Dawley male rats fed high fat diet (40% of calories as fat) for 6 wks to induce obesity, and subsequently fed 5% garlic powder supplemented (HF+H: hangihyung garlic powder, HF+N: nangihyung garlic powder) high fat diets (w/w) for further 5 wk. For the comparison, normal control group fed AIN-76A diet (11.7% of calories as fat). Supplementation with hangihyung and nangihyung garlic resulted in a significant reduction of high fat induced body weight gain, white fat (i.e., epididymal, visceral and peritoneal fat) development, adipocyte hypertrophy and the development of hyperinsulinemia and hyperliptinemia. Serum triglyceride and total cholesterol level was greatly reduced by hangihyung garlic supplementation ($p<0.05$). The HDL-cholesterol level was increased by dietary hangihyung and nangihyung garlic. There were slight non-significant decreases in triglyceride and total cholesterol of HF+N group as compared to those of HF group. Leptin level of HF+H group was found to be significantly lower than HF group ($p<0.05$). There was no significant difference among N group and HF+N group. These results suggest that hangihyung garlic may lead to the higher activity in improving lipid profiles than nangihyung garlic. Whether the hypolipidemic effect of garlic increases in a species-dependent has yet to be determined and awaits further research.

Key words: nangihyung garlic, hangihyung garlic, serum lipids, leptin, adipocyte, rat, obesity

INTRODUCTION

The favorable properties of the garlic (*Allium sativum*) utilization in human health have been known for thousands of years. The ancient Chinese consumed the garlic to achieve longevity. In the first century AD, Dioscorides, the chief physician of the Roman army, prescribed garlic to his warriors and wrote, 'garlic do cleaneth the arteries'. Use of garlic throughout the ages has continued unabated to this day (1). Garlic is widely used in preventive cardiovascular medicine. Recent studies report that garlic has beneficial effects on risk factors associated with cardiovascular disease, including modulation of plasma lipid levels (2-4). Generally, garlic (*Allium sativum* Linn) is used in conventional allopathic therapies for cancer and diabetic-related vascular diseases. Obesity is often related disturbances of lipid metabolism that lead to an increase in serum triglyceride and cholesterol levels, which are involved in the development of cardiovascular disease.

The mechanism by which garlic or garlic preparations reduce plasma lipids has not been fully investigated. Animal studies, however, have shown that garlic supplementation on the diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase (5-7) and 3-hydroxy-3-methyl-glutaryl-Co A (HMG-CoA) reductase (7-9). It is, therefore, reasonable that the hypo-cholesterolemic effect of garlic may stem in part from impaired cholesterol synthesis. In fact, we observed recently that garlic extracts that contained various sulfur compounds effectively decreased the

†Corresponding author. E-mail: yoongho@konkuk.ac.kr
Phone: +82-2-450-3760, Fax: +82-2-453-3761

plasma concentration of cholesterol, resulting possibly from an inhibition of hepatic cholesterol synthesis (10,11). Studies with cultured rat hepatocytes suggest that the inhibition of cholesterol synthesis at least partly explains the possible hypolipidemic effect of garlic (12,13).

Confirming these reports, previous studies with rabbits and rats treated with garlic also report a modulation of lipids, caused by a decrease in concentrations of plasma cholesterol and triglycerides and markers of cholesterol synthesis and absorption (14-16). In addition, human studies report a decrease in plasma cholesterol and triglyceride levels after garlic treatment (3,17,18). Conversely, numerous animal and human studies report that garlic treatment does not affect plasma lipids (2-4, 19,20). These conflicting data may be due to several factors, including a lack of consistency among studies in relation to dosage, standardization of garlic preparations, period of treatment, and a difference on spices of garlic. The objective of the present study is to demonstrate the effect of garlic on serum lipids profiles and leptin levels according to the producing-area.

MATERIALS AND METHODS

Preparations of garlic

Raw garlic samples were from the Chunnam and Chungbuk area. Garlic cloves were washed extensively with sterilized water, surface fractions were removed aseptically. The samples were cooled, crushed, freeze dried and stored at -70°C until use.

Rats and diets

Three wk old male Sprague-Dawley rats purchased from Central Experimental Animals (Samtaco, Seoul Korea) and housed individually. After adaptation for 1 wk, rats were weighed, randomly assigned and fed normal (11.7% of calories as fat) or high fat diet (40% of calories as fat). Six wk later, high fat fed rats randomly assigned to the three groups and allowed to one of the four diets; high fat with 0, 5% (wt/wt) hangihyung garlic, 5% (wt/wt) nangihyung garlic diets for 5 wk.

HF group was fed a high fat diet without garlic powder, HF+H group was fed a high fat diet with hanjihyung garlic powder, and HF+N group was fed a high fat diet with nanjihyung garlic powder for 5 weeks, respectively. The rats were kept at constant ambient conditions (24±2°C, humidity 55~60%). All rats were fed foods and water unrestrictedly. Garlic powder was added to the food before feeding. The composition of experimental diets is shown Table 1. Water and food were consumed *ad libitum*.

Collection of sample

The food intake and body weight weighed twice a week and food efficiency ratio (FER) was calculated. After 5 wk of feeding the normal, high fat or garlic supplemented high fat diets, blood was collected from portal vein under anesthesia with dietyl ether and serum was separated by centrifugation (3,000 × *g*, for 15 min at 4°C). After collecting blood samples, soleus muscle, interscapular brown adipose tissue (BAT), epididymal fat pad, visceral fat and peritoneal fat pad were immediately excised, weighed and frozen in liquid N_2. All serum and tissue samples were stored at -70°C until analysis.

Adipocyte size determination

Adipose tissue samples (0.5 g) were taken from visceral fat depots and isolated adipocytes were prepared using collagenase (19). Adipose tissue was immediately washed in 145 mmol/L NaCl-buffer containing 3% BSA, cut into small pieces, and added to 1 mL NaCl-buffer containing 1.5 mg collagenase (Sigma Chemical, St. Louis, MO), and incubated in a shaking water bath at 80 cycles/min for 1 hr at 37°C. After incubation the cells was filtered through 450 μm nylon mesh, and adipocytes were allowed to float for 3 min. The adipocytes were washed twice with 3 mL of NaCl-buffer containing 5 mM glucose and 3% BSA. Between each washing, the adipocytes were centrifuged at 470 × *g* for 1 min. Then cells were resuspended in 1~2 mL NaCl-buffer with glucose and BSA. The adipocytes were evaluated by microscopy using a calibrated grid, and the mean diameter of 30 cells from each preparation of cells was calculated.

Blood analyses

Serum cholesterol, HDL-cholesterol, and triglyceride (TG) were measured using commercial kits (Sigma Chemical, St. Louis, MO). Serum leptin was measured by

Table 1. Composition of experimental diets (g/kg diet)

Ingredients	N[1)]	HF[2)]
Casein	200	200
DL-methionine	3	3
Corn starch	150	150
Sucrose	500	345
Cellulose	50	50
Corn oil	50	-
Beef tallow	-	205
Salt mixture	35	35
Vitamin mixture	10	10
Choline bitartrate	2	2
Fat % (calories)	11.7	40.0

[1)]Normal diet: AIN-76A diet #100000.
[2)]High fat diet: AIN-76 diet #100496 (Dyets Inc., Bethlehem, PA, USA).

Linco Leptin Assay kit (Linco Research Immunoassay, St. Charles, MO).

Statistical analysis

Results are expressed as means±SE. ANOVA and Duncan's multiple range test were used to determine the significance of differences after 5 weeks garlic supplementation. Statistical analyses were carried out with the SAS program (SAS 8.0, SAS institute, Cary, NC) and statistical significance of difference was defined at a $p<0.05$.

RESULTS AND DISCUSSION

Natural remedies have been investigated for centuries for a wide variety of ailments. Among them, garlic has received special attention for its beneficial effects (21-23). However, there is no satisfactory data from randomized controlled trials linking supplementation of garlic in the diet with a reduction in the cardiovascular morbidity and mortality (24). Although significant reductions in blood cholesterol and triglyceride levels were observed in some studies when garlic extract or powder were used (24,25), no satisfactory agreement has been reached on this kind of clinical and experimental data (26,27), as many of the trials have been limited by lack of controlled methods and by the use of preparations with unknown amounts and chemical identification of active ingredient (28). Therefore this study was designed to examine the effects of standardized garlic powder which was divided into two species according to producing-area (hangihyung garlic, nangihyung garlic)

Significant differences were observed in body weight gain and daily food intake among the groups (Table 2). Garlic powder, regardless of hangihyung garlic or nangihyung garlic, added to the high fat diets (HF+H group, HF+N group) affect food intakes, and body weight gains. Weight gains and food intakes of rats fed a high fat diet and administrated a hangihyng garlic powder or nangihyung garlic powder were significantly decreased compared to those of rats fed a high fat diet without garlic powder (HF group). There was no significant difference was observed in body weight gains among the normal diet group (N group), HF+H group, and HF+N group.

In previous studies (29,30), the consumption of high cholesterol/garlic showed no changes in the total body weight and food intake during the duration of the study whereas in the present study, there was significant changes on weight gains and food intakes. Results of animal studies suggest that garlic may have an effect on the central nervous system. The concentration of labeled allicin metabolites at several organ sites including the vertebral column has been demonstrated in an animal whole body autoradiographic study after oral administration of allicin (31). It is therefore possible that satiety may change with garlic intake and operate through a central mechanism, and, in turn, this may have mediated the lipid-lowering action of garlic. It is intriguing to consider what mechanisms by which organosulfur and non-sulfur compounds in garlic might operate on food intake and/or lipid metabolism.

In this study, effects on garlic on adipocyte weight were in Table 3. Epididymal fat weight, and visceral fat weight of HF+H group were significantly lower as compared to HF ($p<0.05$). Peritoneal fat weights of HF+H group and HF+N were significantly lower than N group and HF. There were slight non-significant decreases in epididymal fat and visceral fat of HF+N group as compared to those of HF group.

As seen in Fig. 1, visceral fat isolated adipocyte size of HF+H group was significantly lower than those of HF group ($p<0.05$).

As seen in Fig. 2, serum triglyceride and total cholesterol of rats fed on a high fat diet supplemented with hangihyung garlic powder (HF+H group) were significantly lower as compared to those of rats whose diet was not supplemented with garlic powder (HF group) ($p<0.05$). HTR (HDL-cholesterol/total cholesterol ratio)

Table 2. Weight gain and food intake of the experimental animals

Group[1)]	Weight gain (g/d)	Food intake (g/d)	FER[4)]
N	4.99±0.56[2)b3)]	22.44±0.26[b]	0.23±0.03
HF	5.52±0.35[a]	24.52±0.52[a]	0.24±0.01
HF+H	4.74±0.49[b]	20.43±0.01[c]	0.23±0.02
HF+N	4.88±0.42[b]	20.37±0.34[c]	0.24±0.02

[1)]N: normal diet, HF: high fat diet, HF+H: high fat diet with 5% hanjihyung garlic powder, HF+N: high fat diet with 5% nanjihyung garlic powder.
[2)]Values are mean±SE for 9 rats.
[3)]Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test.
[4)]Food efficiency ratio=body weight gain (g/day) / food intake (g/day).

Table 3. Effects of dietary garlic on adipocyte weight (g)

Group[1)]	Epididymal fat	Visceral fat	Peritoneal fat	BAT[4)]
N	3.99±0.88[2)ab3)]	3.99±1.59[ab]	5.05±1.58[b]	0.88±0.12
HF	5.23±1.35[a]	4.43±2.19[a]	11.25±2.63[a]	1.05±0.09
HF+H	3.46±0.75[b]	2.94±1.02[b]	6.07±1.93[b]	0.99±0.11
HF+N	3.89±0.37[ab]	3.94±0.87[ab]	6.77±1.25[b]	0.98±0.20

[1)]Groups are the same as in Table 2.
[2)]Values are mean±SE for 9 rats.
[3)]Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test.
[4)]Brown adipose tissue.

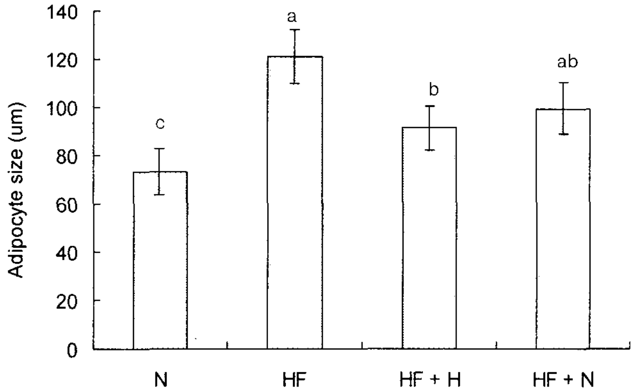

Fig. 1. Effect of dietary garlic on adipocyte size. Adiocyte was isolated by collagenase-treatment from visceral fat pad and adipocyte size was determined. Different letters indicate significant difference at $p<0.05$ by Duncan's multiple range test. Groups are the same as in Table 2.

of HF+H group was significantly higher than those of HF group. HDL-cholesterol of rats fed on a high fat diet and garlic powder, both hangihyung garlic powder and nangihyung garlic powder, were significantly higher than N group and HF group. There were slight non-significant decreases in triglyceride and total cholesterol of HF+N group as compared to those of HF group.

As seen from our results, nangihyung garlic extract treatment can improve blood lipid profile to a significant extent. The mechanism by which garlic or garlic preparation reduce plasma lipids has not been fully investigated. Animal studies, however, have shown that garlic supplementation in the diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA

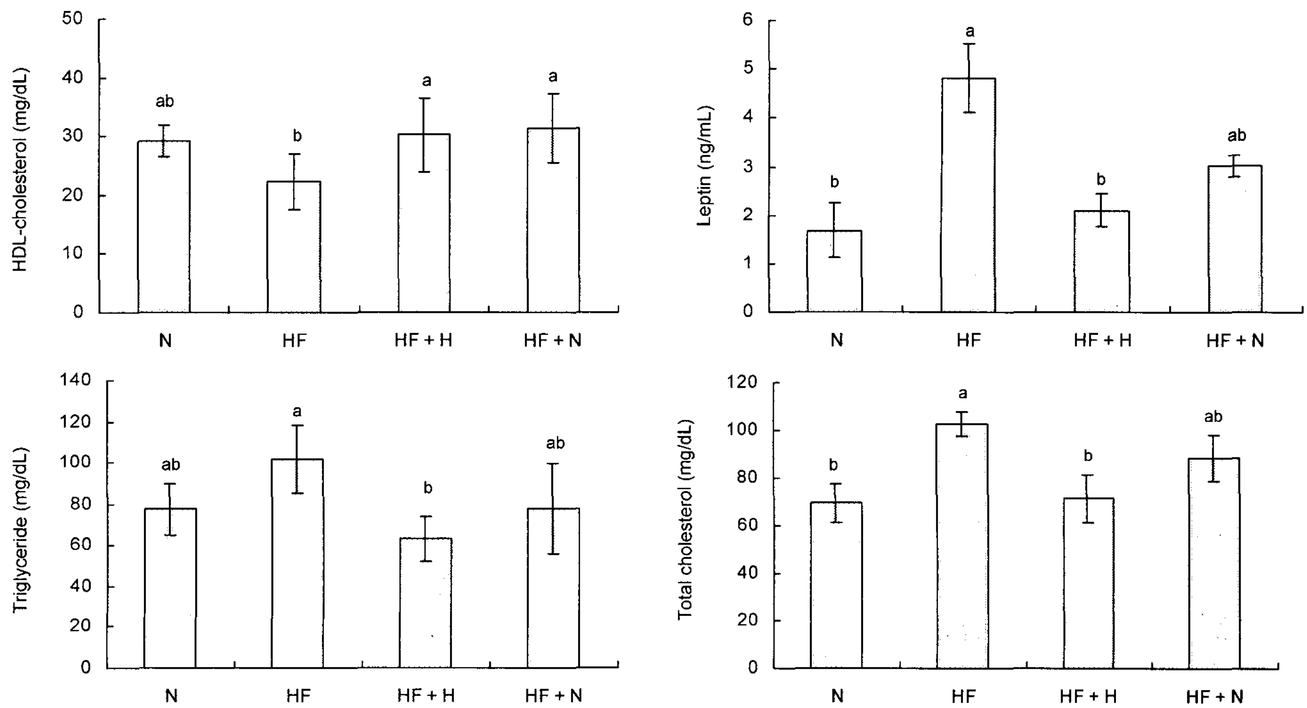

Fig. 2. Effects of garlic on lipid profiles and leptin concentration. Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test. Groups are the same as in Table 2.

(HMG-CoA) reductase (29). It is, therefore, reasonable that the hypocholesterolemic effect of garlic may stem in part from impaired cholesterol synthesis. To clarify the issues there were inconsistent results on effects of garlic on lipid profiles, this study investigated the effects of standardized garlic powder divided into two species according to producing-area (hangihyung garlic, nangihyung garlic). This study's results suggest that hangihyung garlic powder may lead to the higher activity in improvement of lipid profiles than those of nangihyung garlic powder.

Leptin, the product of the *ob* gene, has been suggested as a risk factor for body weight gain (32). It is secreted by adipose tissue and seems to be involved in signaling the level of body fat stores to the central nervous system and thereby may control appetite (33). In previous study (34-37), leptin was influenced by the amount and distribution of body fat. In this study, serum leptin of HF+H group was found to be significantly lower than HF group. There was no significant difference among N group and HF+N group. There were slight non-significant decreases in leptin of HF+N group as compared to those of HF group. As expected, there was a significant decrease in leptin in response to adipocyte weight loss of HF+H group. Adipocyte weight and size loss of HF+H group demonstrated the possibility of garlic-effect to prevent the obesity.

The present study has demonstrated the effect of garlic on lipid-profiles and leptin levels. There are some inconsistent results concerning serum lipids lowering effects of garlic have been reported as described in the discussion section above. This study suggests the possibility of inconsistency in the published results may be due to the differences in the species of the garlic according to producing-area. Whether the hypolipidemic effect of garlic increases in a species-dependent has yet to be determined and awaits further research. Moreover, this study shows the effect of garlic on leptin and adipocyte weight, it can raise the possibility that garlic may contribute to prevent the obesity. This is also needed further research.

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