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Anti-Obesity Effect of Garlic-added *Kochujang* in 3T3-L1 Adipocytes

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Abstract

In order to develop a functionally improved *kochujang* with antiobesity effects, garlic-added *kochujang* was prepared with freeze-dried garlic powder and followed by fermentation for 60 days at 30°C. Antiobesity effect of the garlic-added *kochujang* was investigated by measuring the leptin secretions and mRNA expression levels of obesity-related gene such as TNFα, PPARγ, C/EBPα, and SREBP1c, in cultured 3T3-L1 adipocytes. Fermentation of garlic-added *kochujang* led to decreased levels of leptin secretions and reduced the mRNA expression levels of TNFα, PPARγ, C/EBPα, and SREBP1c in the 3T3-L1 adipocytes. Accordingly, these results suggest that the addition of garlic to *kochujang* has a potential as a valuable functional food for controlling obesity.

Key words: garlic, *kochujang*, 3T3-L1 adipocytes, anti-obesity, leptin

INTRODUCTION

Recently, the obesity rate has been increasing noticeably worldwide, so that obesity has been important heath issue. Obesity is a major factor in increasing the risk of serious diseases such as heart disease, hypertension, stroke, cancer, diabetes, and osteoarthritis (1,2). That is why numerous studies have attempted to find functional foods or agents from such oriental foods or medicines, including *Korean* traditional fermented foods for weight control. The ob-protein, leptin, is secreted from adipose tissue and may be important in the development of obesity (3-5). Leptin concentration in the serum is directly related to the amount of body fat and the amount of energy stored in adipose tissue (6,7). Adipocyte reserve energy as well as the secretion of various transcription factors such as leptin, aP2, TNFα, PPARγ, C/EBPα, and SREBP1c are major transcription factors for adipogenic response.

Kochujang, a fermented red pepper soybean paste, is one of the most famous traditional Korean fermented foods. Generally, traditional *kochujang* is prepared with glutinous rice, *meju* (fermented soybean blocks), red pepper powder and salt. The unique hot, sweet, salty and savory tastes as well as color and flavors of *kochujang* are produced by the actions of microorganisms such as *koji* mold, bacteria, and yeasts during the fermentation process (8,9).

Garlic (*Allium sativum*) is a member of lily family that has been cultivated by humans as a food plant for over 10,000 years. Since ancient times, numerous medicinal properties of garlic have been discovered. It has been reported that garlic and its associated sulfur compounds suppress weight gain, and TG and cholesterol concentrations. They also affect the normalization of plasma lipids, reduction of blood pressure and glucose, and inhibition of platelet aggregation (10-14). Sulfur compounds from garlic are known as alliin, allicin (diallyl thiosulfinate), diallyl sulfinate (DAS), diallyl disulfinate (DADS), etc.

In this study, in order to develop a functionally improved *kochujang* with an antiobesity effects, garlic-added *kochujang* was made with freeze-dried garlic powder. Antiobesity effect of the garlic-added *kochujang* was investigated by measuring the leptin secretion levels and the mRNA expression levels of obesity-related genes such as TNFα, PPARγ, C/EBPα, and SREBP1c in cultured 3T3-L1 adipocytes.

MATERIALS AND METHODS

Ingredients and preparations of *kochujang*

Glutinous flour, malt flour, meju flour, and salt were purchased at a local market in Busan, Korea. Red pepper powder and garlic were purchased from Uiseong, Gyeongsangbuk-do, Korea. *Kochujang* was prepared by the standardized method (15) and fermented for 60 days at 30°C. Garlic was freeze-dried, powdered, and added in ratios of 0% and 3% during the preparation of *kochujang* and the prepared garlic-added *kochujang* was fer-

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mented for 60 days at 30°C. The prepared *kochujang* was freeze-dried, powdered, and extracted 3 times with 20-fold methanol. The methanol extract was concentrated using a vacuum rotary evaporator and followed by dissolution in dimethylsulfoxide (DMSO).

Cell culture and adipocyte differentiation

3T3-L1 mouse cells were purchased from the American Type Culture Collection (ATCC, USA). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco Service Co. (USA). Methylisobutylxanthine (IBMX), dexamethasone (DEX), and insulin (INS) were purchased from sigma Chemicals Co. (USA). The mouse 3T3-L1 preadipocytes were grown to confluence in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO_2. At 1 day post-confluence (designated "day 0"), cell differentiation was induced with a mixture of methylisobutylxanthine (0.5 mM), dexamethasone (0.25 μM), and insulin (5 μg/mL) in DMEM containing 10% FBS. On day 2 and day 4, the medium was replaced with DMEM containing 10% FBS and insulin (5 μg/mL) only. On day 6, thereafter the medium consisted of only DMEM plus 10% FBS, which was subsequently replaced every 2 days. The garlic-added *kochujang* extracts were used to treat adipocytes at the concentration of 1 mg/mL at day 8 after inducing differentiation. After the 24 hr, the medium was removed for analysis of leptin.

Measurement of leptin levels

Measurement of leptin levels was performed with a sandwich enzyme-linked immunosorbent assay (ELISA). Anti-mouse leptin, recombinant mouse leptin, and biotinylated anti-mouse leptin antibodies were purchased from R&D Systems (MN, USA) (16).

RNA isolation, RNA extraction and reverse transcription-polymerase chain reaction

Total RNA was isolated from differentiated 3T3-L1 adipocytes using a Trizol reagent (Invitrogen Co., Carlsbad, CA, USA). One μg of total RNA was used for first-strand cDNA synthesis using Superscript II reverse transcriptase (BD Bioscience, Palo Alto, CA). 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. Primers to specifically amplify the genes of interest are shown as Table 1. 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).

Statistical analysis

Data were expressed as mean±standard error values (n=3). Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range tests. Each experiment was replicated at least 3 times.

RESULTS AND DISCUSSION

Effect of garlic-added *kochujang* on leptin secretions during fermentation

In order to determine whether adding garlic affects the antiobesity properties of *kochujang*, garlic-added *kochujang* was prepared and fermented for 60 days. Since garlic includes a lot of active sulfur-compounds, one would expect that the addition of garlic powder to *kochujang* would induce a suppressive effect on lipid accumulation. The circulating leptin levels are correlated with adipose tissue mass (17,18). Therefore, the adipogenic response of garlic-added *kochujang* in differentiated adipocytes was determined by measuring the amount of leptin released in the medium by treatment

Table 1. Gene-specific primers used for the RT-PCR

Gene name	Direction	Sequence
TNFα	Forward	5'-AGG CCT TGT GTT GTG TTT CCA-3'
	Reverse	5'-TGG GGG ACA GCT TCC TTC TT-3'
PPARγ	Forward	5'-GAG ATG CCA TTC TGG CCC ACC AAC TTC GG-3'
	Reverse	5'-TAT CAT AAA TAA GCT TCA ATC GGA TGG TTC-3'
C/EBPα	Forward	5'-TGC TGG AGT TGA CCA GTG ACA A-3'
	Reverse	5'-AAA CCA TCC TCT GGG TCT CC-3'
SREBP1c	Forward	5'-ATC GGC GCG GAA GCT GTC GGG GTA GCG TC-3'
	Reverse	5'-ACT GTC TTG GTT GTT GAT GAG CTG GAG CAT-3'
β-actin	Forward	5'-AGC CAT GTA CGT AGC CAT CC-3'
	Reverse	5'-TCC CTC TCA GCT GTG GTG GTG AA-3'

with garlic-added *kochujang* (Fig. 1). Fermentation of the garlic-added *kochujang* reduced leptin secretion compared to that of the control adipocyte.

Effect of *kochujang* and garlic-added *kochujang* on leptin secretion

To determine whether the addition of garlic affects the leptin secretion of *kochujang* in 3T3-L1 adipocytes, leptin level secreted in cultured media treated with garlic-added *kochujang* was compared to that of control *kochujang* (Fig. 2). Here, traditional *kochujang* prepared without garlic was used as a control *kochujang*. Also the control *kochujang* and garlic-added *kochujang* fermented 60 days were used for comparative analysis. Leptin secretions of control *kochujang* and garlic-added *kochujang* were decreased by 34% and 48%, respectively, compared to that of the control adipocytes. The addition of garlic powder into the *kochujang* reduced leptin secretion in the medium. This result demonstrated that making garlic-added *kochujang* has the potential to improve its antiobesity effects. It might be due to a synergistic effect between garlic and the products created by fermentation of *kochujang*. As possible active components responsible for the antiobesity effect of *kochujang*, capsaicin in red pepper powder, isoflavonoide produced from *meju*, and some glycoside products caused by fermentation could be included. Several studies have reported on antiobesity effects of fermented traditional *kochujang* as well as on the decrease of inbody weight, serum lipids, and body fat gain (19-22). Similar to traditional *kochujang*, commercial *kochujang* also decreased leptin secretion and adipocytes size in 3T3-L1 adipocytes by modulating adipogenesis and lipolysis (23). Also, various biological activities of garlic and its associated sulfur compounds such as alliin, allicin (diallyl thiosulfinate), DAS, DADS, have been reported (24-31). The effects of garlic powder on reducing total lipids, TG, and cholesterol contents were studied (11-14). In *vivo*, reducing effects of garlic on weight gain, TG and cholesterol contents, and lipid values of adipose tissue were reported (30).

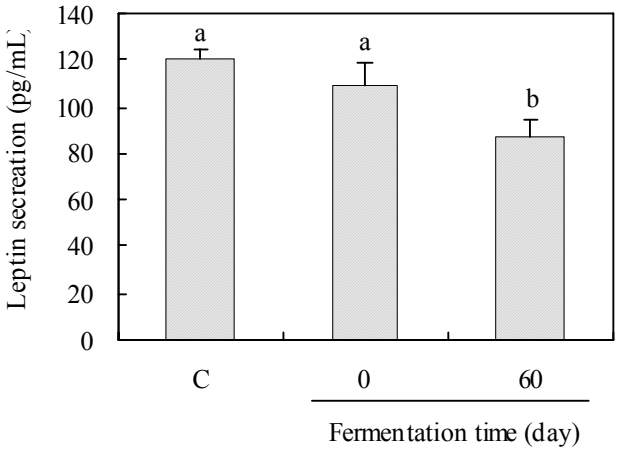

Fig. 1. Changes in leptin secretion of garlic-added *kochujang* during fermentation. Adipocytes were treated for 24 hr at "day 8" after inducing differentiation with vehicle alone (control: 5 mM methylisobutyxanthine, 0.25 μM dexamethanesone, 10 μg/mL insulin) or 1 mg/mL of *kochujang* fermented for 60 days. Data are expressed as mean±standard error values (n=3). Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

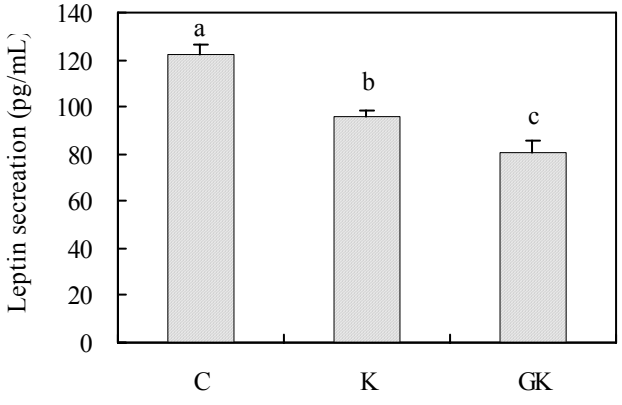

Fig. 2. Additional effect of garlic powder on leptin secretion of *kochujang*. Data are expressed as mean±standard error values (n=3). Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range test. K: control *kochujang* fermented for 60 days, GK: garlic-added *kochujang* (3%) fermented for 60 days.

Effect of garlic-added *kochujang* on obesity related gene expressions

TNFα is produced by adipocyte tissue and its mRNA level increases with the increasing adiposity. TNFα increases not only both leptin gene expression and leptin secretion in 3T3-L1 adipocytes but also lipolysis and released free fatty acid (32). Therefore, the mRNA expression levels of TNFα in the differentiated adipocytes treated with the control *kochujang* and garlic-added *kochujang* were compared (Fig. 3). Treatment with garlic-added *kochujang* in matured adipocytes markedly suppressed their TNFα expression compared with that of the control adipocytes. That is, adding of garlic during *kochujang* preparation might be associated with the reduced cellular lipid accumulation mediated by TNFα.

PPARγ is a member of the nuclear receptor superfamily of transcription factors and is predominantly expressed in adipose tissue. These transcription factors appear to function as dominant activators of adipocyte differentiation (33). PPARγ is a major coordinator of adipocyte gene expression and differentiation (34). PPARγ is induced prior to the transcriptional activation of most adipo-cyte-specific genes, and the expression of PPARγ

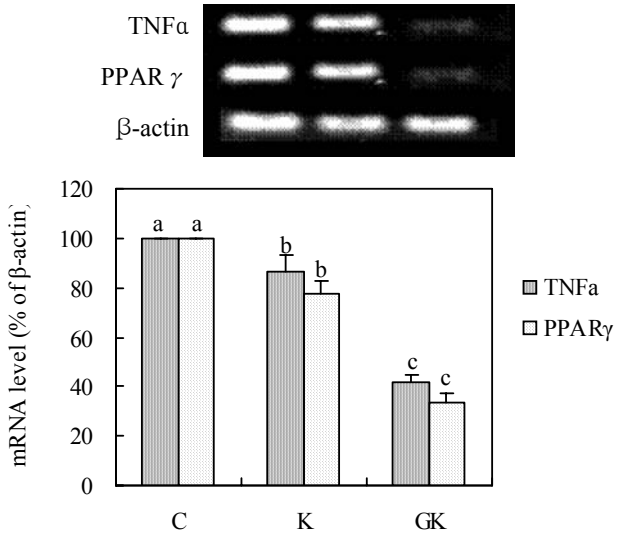

Fig. 3. Effects of garlic-added *kochujang* on TNFα and PPARγ mRNA expression levels in 3T3-L1 adipocytes. Starting at day 8 after inducing differentiation, adipocytes were cultured with or without 1 mg/mL of control *kochujang* and garlic-added *kochujang* for 24 hr. Data are expressed as mean±standard error values (n=3). Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range test. C: untreated control adipocyte (0.5 mM methylisobutyxanthine, 0.25 μM dexamethanesone, 10 μg/mL insulin), K: control *kochujang* fermented for 60 days, GK: garlic-added *kochujang* (3%) fermented for 60 days.

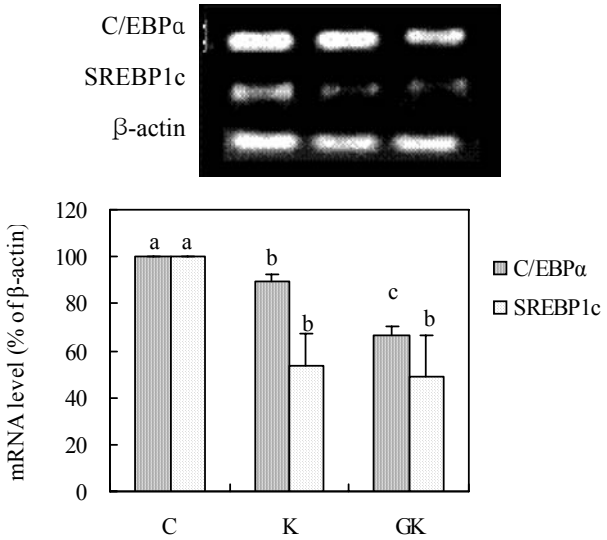

Fig. 4. Effects of garlic-added *kochujang* on C/EBPα and SREBP1c mRNA expression levels in 3T3-L1 adipocytes. Starting at day 8 after inducing differentiation, adipocytes were cultured with or without 1 mg/mL of control *kochujang* and garlic-added *kochujang* for 24 hr. Data are expressed as mean±standard error values (n=3). Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range test. C: untreated control adipocyte (0.5 mM methylisobutyxanthine, 0.25 μM dexamethanesone, 10 μg/mL insulin), K: control *kochujang* fermented for 60 days, GK: garlic-added *kochujang* (3%) fermented for 60 days.

is sufficient to induce growth arrest and to initiate adipogenesis in exponentially growing fibroblast cell lines (35). Effect of garlic-added *kochujang* on the mRNA expression level of PPARγ was evaluated by using RT-PCR analysis (Fig. 3). The mRNA expression of PPARγ of the control *kochujang* and garlic-added *kochujang* was decreased by 19% and 69%, respectively, compared to that of the control adipocytes.

PPARγ is expressed early in the differentiation of 3T3-L1 adipocytes and prior to C/EBPα (36). Overexpression of C/EBPα as well as PPARγ can induce adipocyte differentiation (37). In the present study, fermentation of garlic-added *kochujang* as well as control *kochujang* reduced the mRNA expression level of C/EBPα (Fig. 4). SREBP1c is also one of important transcription factors for adipogenesis. The expression of SREBP1c can induce endogenous PPARγ mRNA expression in 3T3-L1 adipocytes. Treatment with control *kochujang* and garlic-added *kochujang* led to down-regulation of SREBP1c mRNA (Fig. 4). However, there were a little significant difference between the adipocytes treated with control *kochujang* and garlic-added *kochujang*. In conclusion, fermentation of *kochujang* reduced expression levels of adipogenic genes such as PPARγ, C/EBPα and SREBP1c and theirs levels were more inhibited by garlic-added *kochujang*. Therefore, anti-obesity effect of garlic-added *kochujang* might be due to inhibiting regulation promoters of several adipogenic genes such as leptin though PPARγ, C/EBPα and SREBP1c transcription factors, resulting in inhibition of lipid accumulation by blocking adipogenesis. Further studies will be needed to explore the regulation and function at the *in vivo* level.

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REFERENCES

1. Kopelman PG. 1994. Causes and consequences of obesity. *Med Int* 22: 385-388.
2. Grundy SH. 1998. Muti-fratorial causation of obesity: implication for prevention. *Am J Cli Nutr* 67: 563-572.
3. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432.
4. Haals JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543-546.
5. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P.

1995. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269: 546-549.
6. Haffiner SM, Gingerich RL, Miettinen H, Stern MP. 1996. Leptin concentrations in relations to overall adiposity and regional body fat distribution in Mexican Americans. *Int J Obes Relat Metab Disord* 20: 904-908.
7. Considine RV, Sinha MK, Heiman ML, Aidas K, Thomas WS, Mark RN, Joanna PO, Cheryl CM, Linda JM, Thomas LB, Jose FC. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292-295.
8. Lee TS, Chun MS, Choi JY, Noh BS. 1993. Changes of free sugars and free amino acids in *kochujang* with different mashing method. *Food Biotechnol* 2: 102-108.
9. Chun MS, Lee TS, Noh BS. 1994. The changes in capsaicin, dihydrocapsaicin and cansanthin in *kochujang* with different mashing methods. *Food Biotechnol* 3: 104-108.
10. Yoon JY, Jung KO, Kil JH, Park KY. 2005. Antiobesity effect of major Korean spices (red pepper powder, garlic and ginger) in rats fed high fat diet. *J Food Sci Nutr* 10: 58-63.
11. Oi Y, Kawada T, Shishido C, Wada K, Kominato Y, Nishimura S, Ariga T, Iwai K. 1999. Ally-containing sulfides in garlic increase uncoupling content in brown adipose tissue, and noradrenaline and adrenaline secretion in rats. *J Nutr* 129: 336-342.
12. McMahon FG, Vargas R. 1993. Can garlic lower blood pressure? A pilot study. *Pharmacotherapy* 13: 406-407.
13. Chang MW, Johnson MA. 1980. Effects of garlic on carbohydrate metabolism and lipid synthesis in rats. *J Nutr* 110: 931-936.
14. Legnani C, Frascaro M, Guazzaloca G, Ludovici S, Cesarano G, Coccheri S. 1993. Effects of dried garlic preparation on fibrinolysis and platelet aggregation in health subject. *Arzneimittelforschung* 43: 119-122.
15. Lee HY. 2002. Preparation and fermentation characteristics of traditional *kochujang* and their cancer preventive and antiobesity effects. *MS Thesis*. Pusan National University, Korea.
16. Hardie L, Trayhurn P, Abramovich D, Fowler P. 1997. Circulating leptin in women; a longitudinal study in the menstrual cycle and during pregnancy. *Clin Endocrinol* 47: 101-106.
17. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zang Y, Fei H, Kim HS, Lallone R, Ranganathan S. 1995. Leptin levels in human and rodent: measurement of plasma leptin and RNA in obese and weigh-reduced subjects. *Nat Med* 1: 1155-1161.
18. Meister B. 2000. Control of food intake via leptin receptors in the hypothalamus. *Vitam Horm* 59: 265-304.
19. Rhee SH, Kong KR, Jung KO, Park KY. 2003. Decreasing effect of *Kochujang* on body weight and lipid levels of adipose tissues and serum in rats fed a high-fat diet. *J Korean Soc Food Sci Nutr* 32: 882-886.
20. Choo JJ. 2000. Anti-obesity effects of *Kochujang* in rats fed on a high-fat diet. *Korean J Nutr* 33: 787-793.
21. Ly SY, Lee MR, Lee KA. 1999. Effects of cakes containing sponge oligosaccharides on blood lipids and intestinal physiology in rats. *J Korean Soc Food Sci Nutr* 28: 619-624.
22. Lee HM, Chang UJ. 2001. Effect of corn peptide on the lipid metabolism in rats. *Korean J Dietary Culture* 16: 416-422.
23. Ahn IS, Do MS, Kim SO, Jung HS, Kim YI, Kim HJ, Park KY. 2006. Antiobesity effect of *Kochujang* (Korean fermented red pepper paste) extract in 3T3-L1 adipocytes. *J Med Food* 9: 15-21.
24. Sivam GP. 2001. Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J Nutr* 131: 1106-1108.
25. Tsao SM, Yin MC. 2001. *In vitro* activity of garlic oil and four diallyl sulphides against antibiotic-resistant Pseudomonas aeruginosa and Klebsiella pneumoniae. *J Antimicrob Chemother* 47: 665-670.
26. Avato P, Tursil E, Vitali C, Miccolis V, Candido V. 2000. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. *Phytomedicine* 7: 239-243.
27. Chen GW, Chung JG, Ho HC, Lin JG. 1999. Effects of the garlic compounds diallyl sulphide and diallyl disulphide on arylamine N-acetyltransferase activity in Klebsiella pneumoniae. *J Appl Toxicol* 19: 75-81.
28. Chung JG. 1999. Effects of garlic components diallyl sulfide and diallyl disulfide on arylamine N-acetyltransferase activity in human bladder tumor cells. *Drug Chem Toxicol* 22: 343-348.
29. Shenoy NR, Choughuley AS. 1992. Inhibitionary effect of diet related sulphydryl compounds in the formation of carcinogenic nitrosamine. *Cancer Lett* 31: 227-232.
30. Ahman K. 2001. Historical perspective on garlic and cardiovascular disease. *J Nutr* 131: 977-979.
31. Kwon SK. 2003. Organosulfur compounds from *Allium sativum* and physiological activities. *J Appl Pharm* 11: 8-32.
32. Nascimento CO, Hunter L, Trayhurn P. 2004. Regulation of haptoglobin gene expression in 3T3-L1 adipocytes by cytokines, catecholamines, and PPARγ. *Biochem Biophys Res Commun* 209: 250-256.
33. Cornelius P, MacDougald OA, Lane MD. 1994. Regulation of adipocyte development. *Annu Rev Nutr* 14: 99-129.
34. Guo X, Liao K. 2000. Analysis of gene expression profile during 3T3-L1 preadipocyte differentiation. *Gene* 251: 45-53.
35. Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS. 1988. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. *J Biol Chem* 263: 9402-9408.
36. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegeman BM. 1994. mPPAR γ2: tissue-specific regulator of an adipocyte enhancer. *Gene Dev* 8: 1224-1234.
37. Rosen ED, Spiegelman H. 2000. Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 16: 145-171.

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