Reduction of Body Weight by Dietary Garlic Is Associated with an Increase in Uncoupling Protein mRNA Expression and Activation of AMP-Activated Protein Kinase in Diet-Induced Obese Mice1–3

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Abstract

This study investigated the antiobesity effect of garlic in diet-induced obese mice. Male C57BL/6J mice were fed a high-fat diet (45% fat) for 8 wk to induce obesity. Subsequently, they were fed a high-fat control diet, high-fat diets supplemented with 2%, or 5% garlic (wt:wt) for another 7 wk. Dietary garlic reduced body weight and the mass of various white adipose tissue deposits and also ameliorated the high-fat diet-induced abnormal plasma and liver lipid profiles. Garlic supplementation significantly decreased the mRNA levels of adipogenic genes in white adipose tissues (WAT). However, consumption of garlic increased the expression of mRNA for uncoupling proteins in brown adipose tissue (BAT), liver, WAT, and skeletal muscle. Mice treated with garlic maintained a significantly higher body temperature than untreated mice during a 6-h, 4°C cold challenge and, notably, AMP-activated protein kinase (AMPK) activity was stimulated in BAT, liver, WAT, and skeletal muscle. These results suggest that the antiobesity effects of garlic were at least partially mediated via activation of AMPK, increased thermogenesis, and decreased expression of multiple genes involved in adipogenesis. J. Nutr. 141: 1947–1953, 2011.

Introduction

Obesity results from the excess storage of TG in adipose tissue and is caused by a disequilibrium between energy intake and expenditure. The prevalence of obesity has increased at an alarming rate and is now a major worldwide health epidemic, because it is highly correlated with pathological disorders such as heart disease, type 2 diabetes, hypertension, and some forms of cancer (1). In the field of food science, studies on obesity have focused on the search for food ingredients that have the potential to stimulate energy expenditure.

Garlic (Allium sativum L.) has been used as a medicinal plant in many countries for a long time. The main components of garlic are water, carbohydrates, protein, fat, and dietary fiber, and it contains essential amino acids, vitamins, and minerals (2). In addition, extracts of garlic contain various biologically active compounds such as alliin, allicin, allyl methanethiosulfinate, ajoene, diallyl disulfide, diallyl trisulfide, and S-allylcysteine (3). It is widely known that garlic produces various biological benefits, which include hypocholesterolemic (4), hypoglycemic (5), antihypertensive (6), anticancer (7), and antioxidant effects (8). UCP7 are mitochondrial inner membrane proteins that enable the dissipation of part of the proton electrochemical gradient that is generated by the electron transfer chain across the mitochondrial inner membrane. This dissipation increases heat production by uncoupling respiration from ATP synthesis. UCP1 is expressed mainly in BAT, which plays a crucial role in nonshivering heat production. Thermogenesis in BAT is activated by the sympathetic nervous system via norepinephrine, thyroid hormone, and β-adrenergic receptor signaling (9). As a consequence, the rate of lipolysis increases and the liberated fatty acids are used preferentially as substrates for mitochondrial oxidation and the activation of UCP1 without ATP synthesis.

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Abbreviations used: ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; BAT, brown adipose tissue; CON, control group; GL2, 2% garlic-fed group; GL5, 5% garlic-fed group; IRS-1, insulin receptor substrate-1; LPL, lipoprotein lipase; UCP, uncoupling protein; WAT, white adipose tissue.
 diets for a further 7 wk. Body weight and food intake were monitored different tissues such as BAT, liver, WAT, and skeletal muscle. The current study examined whether dietary supplementation (SREBP-1c), fatty acid-binding protein (aP2), and LPL in WAT. to adipogenesis, such as CCAAT/enhancer binding protein- the mechanism that is responsible for the antiobesity effects of the adrenergic pathways were involved in the regulation of AMPK activity in vivo. Hence, it is logical to hypothesize that AMPK is involved in garlic-induced metabolic regulation with effects on thermogenesis and adipogenesis. As a consequence, this study was designed to investigate the antiobesity effects of garlic in diet-induced obese mice. To verify the mechanism that is responsible for the antiobesity effects of garlic, we analyzed the expression of genes that are related to adiopogenesis, such as CCAAT/enhancer binding protein-α (C/EBPα), PPARγ, sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid-binding protein (aP2), and LPL in WAT. The current study examined whether dietary supplementation with garlic increases thermogenesis, particularly through the expression of the mRNA of UCP and activation of AMPK in different tissues such as BAT, liver, WAT, and skeletal muscle.

Materials and Methods

Reagents. Trizol reagent and M-MLV reverse transcriptase were obtained from Promega. Universal SYBR Green PCR Master Mix was obtained from QIAGEN. Kits for the analysis of AST, ALT, total cholesterol, and TG were purchased from Asan Pharmaceutical. The kit to measure FFA NEFA-C was obtained from Wako. The mouse leptin ELISA kit was obtained from R&D Systems. All other reagents were obtained from Sigma-Aldrich.

Mice and diets. Eighteen male C57BL/6J mice, 4 wk of age, were obtained from Charles River and individually housed in stainless steel wire-mesh cages in a room kept at 22 ± 1°C with a 12-h-light/dark cycle (light period: 0800–2000 h). They consumed a commercial diet, and were exposed to 4°C for up to 6 h. Their core body temperature was measured during the period of exposure to a temperature of 4°C with a digital thermometer (Testo 925). Liver lipid analysis. Liver lipid was extracted by using the method of Bligh and Dyer (21) with slight modifications as follows. A sample (200 mg) of liver tissue was homogenized in 1 mL of water, and 1.25 mL of water and 5 mL of methanolchloroform (2:1, v/v) were added. The mixture was shaken horizontally for 10 min and centrifuged at 2000 × g for 10 min. The lower chloroform phase was withdrawn and the lipid in this phase was dried and weighed. Total cholesterol and TG were determined by enzymatic colorimetric methods using commercial kits as above.

Real-time qRT-PCR. Total RNA was isolated from WAT, BAT, liver, and muscle using Trizol reagent. The corresponding cDNA was synthesized from 5 μg of RNA using M-MLV reverse transcriptase. After cDNA synthesis, real-time qRT-PCR was performed using Universal SYBR Green PCR Master Mix on a fluorometric thermal cycler (Rotor-Gene 2000). The sequences of the sense and antisense primers (Supplemental Table 1) were designed using an online program (22). The ΔΔCt method was used for relative quantification. The ΔΔCt value for each sample was determined by calculating the difference between the Ct value of the target gene and the Ct value of the β-actin reference gene. The normalized level of expression of the target gene in each sample was calculated using the formula 2−ΔΔCt. Values were expressed as fold of the control.

Statistical analysis. All data are expressed as the mean ± SEM for 6 mice in each group. Statistical analyses were performed using SPSS software (version 17; SPSS). The significance of the differences among the groups (CON, GL2, and GL5) was analyzed by 1-way ANOVA and post hoc Tukey’s multiple comparison tests. P < 0.05 was taken to indicate a significant difference.

Results

Body weight, energy intake, and fat accumulation. At wk 7, body weight was significantly less in both the GL2 (11%) and twice per week. All animal procedures conformed to NIH guidelines as stated in the Principles of Laboratory Animal Care (20). At the end of the experiment, the mice were feed deprived overnight and anesthetized with ketamine/xylazine. A central longitudinal incision was made in the abdominal wall and blood samples were collected by cardiac puncture. Blood samples were centrifuged at 1500 × g for 20 min at 4°C and the plasma was separated and stored at −20°C until analyzed. Liver, gastrocnemius skeletal muscle, WAT (epididymal, mesenteric, retroperitoneal, subcutaneous flank), and interscapular BAT were harvested, frozen immediately in liquid nitrogen, and stored at −70°C.

Measurement of body temperature. Body temperature was measured in mice that were feed deprived for 16 h after 7 wk of dietary supplementation with garlic. Mice were exposed to 4°C for up to 6 h. Their core body temperature was measured during the period of exposure to a temperature of 4°C with a digital thermometer (Testo 925). Plasma biochemical measurements. The plasma levels of AST, ALT, total cholesterol, and TG were determined by enzymatic colorimetric methods using commercial kits. The concentration of FFA was measured using an acyl-coA oxidase-based colorimetric NEFA-C kit. Plasma leptin levels were determined using a mouse leptin ELISA kit.
GL5 (20%) groups compared with the CON group (Fig. 1A; Table 1). In the GL5 group, it was also less than in the CON group at wk 4 and 5 (P < 0.05). Food and energy intakes did not differ among the groups (Fig. 1B; Table 1), but energy efficiency was significantly lower in the GL5 group than in the CON group (Table 1). The relative weights of epididymal, mesenteric, retroperitoneal, and subcutaneous flank adipose mass were 24–55% lower in the GL5 groups than in the CON group (Table 1) (P < 0.05).

Plasma and liver metabolites. The GL2 and GL5 groups had significantly lower plasma concentrations of TG (38%) and total cholesterol (31–43%) compared with the CON group (Table 2). The FFA concentration also was 45% lower in the GL5 group than in the CON group and tended to be lower in the GL2 group. The plasma leptin level was lower in the GL5 group than in the GL2 group and both were lower than in the CON group (P < 0.05). In addition, the hepatic concentrations of TG and total cholesterol were lower in the garlic-fed groups than in the CON group (P < 0.05).

Liver weight and plasma AST and ALT activities. At the doses given, garlic did not induce hepatic toxicity, because the plasma activities of AST and ALT were within the reference ranges for mice [AST, 55–251 IU/L; ALT, 18–184 IU/L (23)] and did not differ among the groups (Table 2). In addition, liver weight (Table 1) was unaffected by garlic treatment. These data indicate that the garlic was well tolerated by the mice (Table 2).

Body temperature. To assess the effects of garlic on thermogenesis, a cold test was performed after 7 wk of dietary supplementation with garlic. Core body temperature was higher in the garlic-fed groups than the CON group at 4 and 6 h of treatment (Fig. 2) (P < 0.05).

mRNA levels for adipogenesis-related genes and UCP. To elucidate the mechanism by which garlic decreases the weight of WAT, we measured the mRNA levels for genes that are related to adipogenesis in WAT. The mRNA levels of genes associated with adipocyte differentiation, which included adipogenic transcription factors such as PPARγ, C/EBPα, and SREBP-1c, were lower in the GL2 and GL5 groups than in the CON group (P < 0.05) (Table 3). The levels of aP2 and LPL expression, genes that are targets of PPARγ and C/EBPα, also were lower in the garlic-supplemented groups.

To test if there was a connection between garlic supplementation and the increase in body temperature, we measured the levels of mRNA for UCP1, 2, and 3 in different tissues (Table 4). Compared to the CON group, the GL2 and GL5 groups had greater UCP1 expression in interscapular BAT by 2.9- and 3.3-fold, respectively (P < 0.05). The levels of UCP2 mRNA were lower than in the CON group (Table 4).
also greater in the GL2 and GL5 groups in BAT (2.6- and 3.1-fold), liver (2.0- and 2.3-fold), WAT (3.4- and 8.0-fold), and skeletal muscle (1.7- and 1.9-fold), respectively, compared with the CON group \((P < 0.05)\). The UCP2 mRNA level in WAT also was greater in the GL5 group than in the GL2 group. UCP3 mRNA expression in skeletal muscle was greater in the GL5 group than in the GL2 group and both were greater than in the CON group, but there were no differences among the groups in BAT.

**AMPK activity.** In view of the role of AMPK activation in the downregulation of genes involved in adipogenesis and upregulation of genes related to thermogenesis, with the resultant antiobesity effects, the effect of garlic on AMPK activity was determined in BAT, liver, WAT, and skeletal muscle. AMPK activity in several tissues was greater in the GL5 group than in the CON group, and in WAT it also was greater in the GL2 group (Table 5) \((P < 0.05)\).

### Discussion

In this study, we investigated the effects of garlic on the development of obesity due to a high-fat diet in C57BL/6J mice. The doses of garlic (20 or 50 mg/kg diet) that were used in our study were in the nonhepatotoxic range for mice, as demonstrated by the fact that the plasma levels of AST and ALT were unaffected by garlic supplementation. In the present study, dietary garlic successfully reduced body weight and the mass of various WAT. Energy intake did not differ among the groups. These observations imply that dietary garlic did not cause an anorectic effect, which could have been responsible for the prevention or reduction of the increases in body weight and WAT that were induced by the high-fat diet. WAT is a primary site of energy storage in the form of TG and it accumulates TG during an excess of energy. It has been confirmed that the weight of visceral adipose tissue is strongly correlated with total body weight and adiposity (24). Thus, the reduced body weight, which could be attributed partially to a decrease in the mass of the fat pad in the garlic-supplemented groups, reflected a marked antiobesity effect of garlic. Antioxidant action of garlic could be focused on the fact that it enhances fecal mass and frequency of fecal excretion (25), probably due to its fiber composition, and hence would increase fecal loss of energy.

The beneficial effects of garlic can be attributed to diverse organosulfur compounds, including allyl and its derivatives (26). Allicin is a volatile compound and is highly unstable; it breaks down into a series of compounds, such as sulfides, ajene, vinylthiinins, and many others (26). Ajoene has been shown to induce apoptosis and decrease lipid accumulation in 3T3-L1 cells.
that UCP1 expression in BAT significantly increased upon garlic supplementation (27). In addition, treatment with ajoene significantly reduced the rate of body weight gain of mice without any change in the amount of food intake (28), which implies that it has an effect on the process of energy expenditure. Moreover, 1,2-vinylthiin has been reported to inhibit the differentiation of human preadipocytes (29). These antiadipogenic actions of organosulfur compounds that are derived from garlic might significantly contribute to the antiobesity effect of garlic.

Various reports suggesting beneficial effects of garlic on plasma lipid have been published to date, although some reports question its therapeutic use (30,31). We have demonstrated herein that garlic reduced the plasma concentrations of TG, FFA, total cholesterol, and leptin. Moreover, our data showed that the amounts of TG and total cholesterol in the liver were also lower in garlic-treated mice than in the controls. A previous study suggested that the cholesterol-lowering effects of garlic extract stem in part from the inhibition of hepatic cholesterol synthesis by water-soluble sulfur compounds, such as ajoene and S-allylcysteine (5).

To understand the mechanisms that underlie the antiobesity action of garlic, we investigated the mRNA levels of genes involved in adipogenesis in WAT. Adipocyte differentiation/adipogenesis is a crucial process in the expansion of adipose tissue during the human life span and, consequently, in the development of obesity (32). The preadipocytes differentiate into adipocytes under the coordinated control of several transcription factors, including C/EBPα, PPARγ, and SREBP-1c (33), which are predominantly expressed in adipose tissue and have a central role in differentiation/adipogenesis by inducing anabolic processes such as TG synthesis through the transcriptional induction of genes that encode proteins such as aP2 and LPL (33,34). A garlic-derived organosulfur, 1,2-vinylthiin, has been reported to reduce lipid accumulation by decreasing the expression of C/EBPα, PPARγ, and LPL and the activity of PPARγ in human adipocytes (29). In the present study, the mRNA levels of adipogenic genes such as C/EBPα, PPARγ, SREBP-1c, aP2, and LPL were significantly decreased in WAT in response to garlic supplementation. These results imply that garlic modulates lipid accumulation by suppressing the expression of genes that encode transcription factors and enzymes associated with adipocyte differentiation and adipogenesis in WAT.

Our data showed a decrease in body weight after garlic treatment without any significant difference in energy intake, which suggests that dietary garlic has a physiological effect on the process of energy expenditure. WAT acts as a storage organ for reserve energy in the form of TG, whereas BAT uses fatty acids to generate heat, which results from the function of UCP1. UCP1 knockout mice that are kept at room temperature show a reduced rate of body weight gain and a decreased mass of adipose tissue (35). Moreover, an increased level of UCP1 was observed in aP2-UCP1 transgenic mice, which showed reduced adiposity and an ability to prevent obesity (36). Thus, the expression of UCP1 in BAT could be an attractive target for the development of antiobesity nutraceuticals. We investigated the effect of garlic on UCP1 expression in BAT to make the molecular connection between garlic supplementation and the increase in body temperature, because BAT is strongly associated with thermogenesis and UCP1 plays a critical role in this process. We found that UCP1 expression in BAT significantly increased upon garlic treatment and this effect was consistent with the observed increase in body temperature during cold acclimation. These results suggest that garlic treatment can produce a thermogenic effect, which explains the increase in energy expenditure and resistance to weight gain of these mice. It has been reported that garlic increases the expression of UCP1 protein, oxygen consumption, and body temperature (17,18,37).

UCP2 and UCP3 are expressed in various tissues, such as BAT, liver, WAT, skeletal muscle, kidney, and lung, and also in the immune system. Although UCP2 and UCP3 were proposed to function in adaptive thermogenesis in a manner equivalent to UCP1, much evidence indicates that UCP2 and UCP3 primarily attenuate the mitochondrial production of reactive oxygen species in a number of cell types and organs, which protects against oxidative damage (13). However, fatty acids, superoxide, and alkenals activate UCP2 and UCP3 when thermogenesis is required (13,38,39), which suggests that the activation of UCP by physiological activators might increase thermogenesis under certain conditions. In support of this suggestion, UCP3 knockout mice have diminished muscle hyperthermia in response to the recreational drug ecstasy (3,4-ethyleneoxymethamphetamine) (40). For these reasons, UCP2 and UCP3 remain attractive thermogenic targets for the treatment of obesity (13).

In our study, garlic treatment significantly increased the level of expression of UCP2 in BAT, liver, WAT, and skeletal muscle. Dietary garlic also stimulated the expression of UCP3 in skeletal muscle. Considering the large mass of muscle and its important contribution to the metabolic rate, the increase of UCP3 expression in muscle in association with garlic treatment implies that the activation of UCP3 in muscle might contribute to the effect of thermogenesis in inhibiting the development of obesity.

AMPK activation can regulate energy metabolism that favors the inhibition of lipogenesis by inactivating acetyl CoA carboxylase and increasing fatty acid oxidation in WAT (41,42). These metabolic processes are accompanied by upregulation of the expression of PPARα, PPARγ, and PPARγ-coactivator-1α (43). In addition to its role in adipocyte metabolism, it has been reported that AMPK activation can inhibit the differentiation of 3T3-L1 preadipocytes and blocks the expression of late adipogenic markers, such as fatty acid synthase and the transcription factors C/EBPα and PPARγ, which promote apoptosis (44). In the present study, garlic supplementation increased AMPK activity in several tissues, including BAT, liver, WAT, and skeletal muscle. In our experiments, the activation of AMPK might have been related to the decreased levels of C/EBPα, PPARγ, SREBP-1c, aP2, and LPL mRNA in the WAT of mice treated with garlic. AMPK activation has also been reported to increase the expression of UCP2 in skeletal muscle (45). Similar effects of AMPK on UCP2 and UCP3 have been reported in liver (46), suggesting a role of AMPK activation for thermogenesis in the regulation of storage of body fat. It is likely that the garlic effect is primarily through AMPK activation, because changes in adipogenesis and thermogenesis have been shown to be mediated by AMPK. Activation of AMPK in mice results in a similar phenotype to garlic administration in WAT (43), whereas mice lacking AMPK show the opposite phenotype to garlic administration in adipose tissue (47).

In conclusion, dietary supplementation with garlic resulted in reduced body weight with a decreased mass of adipose tissue and ameliorated plasma lipid profiles in mice with high-fat diet-induced obesity. These effects were mediated at least partially by downregulation of the expression of multiple genes that are involved in adipogenesis together with upregulation of the expression of UCP. It is likely that these antiobesity effects of garlic result from its ability to activate AMPK. AMPK activation also
might be related to the enhancement of body temperature that is induced by garlic. These results suggest that the antiobesity effects of garlic were at least partially mediated via activation of AMPK, increased thermogenesis, and decreased expression of multiple genes involved in adipogenesis.

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Literature Cited


