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Garlic supplementation enhances norepinephrine secretion, growth of brown adipose tissue, and triglyceride catabolism in rats

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We investigated the effects of garlic supplementation on triglyceride metabolism, norepinephrine secretion, and growth of interscapular brown adipose tissue in rats. Rats were fed high-fat diets with or without a garlic powder (supplemented at 0.8%) for 10 or 28 days. After 28 days of feeding, body weights and the concentrations of plasma triglycerides and free fatty acids in rats fed a diet containing garlic powder were significantly lower than in rats given no garlic powder. Interscapular brown adipose tissue weights and the mitochondrial protein were significantly greater in rats given garlic powder as compared with rats fed no garlic powder for 28 days. Furthermore, plasma concentrations and urinary excretion of norepinephrine were significantly greater in rats fed a diet containing garlic powder than in those diets without garlic powder. The in situ effects of a major volatile sulfur-containing compound (diallyldisulfide) in garlic on norepinephrine secretion were also evaluated in anesthetized animals. The norepinephrine concentration of arterial blood was significantly increased by diallyldisulfide administration, and the increase was dose-dependent. These results suggest that garlic supplementation enhances the triglyceride catabolism and growth of interscapular brown adipose tissue by increasing norepinephrine secretion in rats. (J. Nutr. Biochem. 6:250–255, 1995.)

Keywords: garlic; high-fat diet; interscapular brown adipose tissue; norepinephrine secretion; triglyceride catabolism; rats

Introduction

Garlic has long been used as a spice, and it has been reported to possess medicinal and pharmacological properties.[1–8] Several studies have indicated that garlic is effective as a hypoglycemic, anticoagulative, antihypertensive, and/or hypolipidemic agent.[1,2,4,5,7,8] However, in these studies a single or short-term administration of an extremely high dose of garlic components has been used in the form of garlic oil, garlic clove, or garlic powder. There have been few reports on the nutritional effects of garlic administration at relatively low doses for extended periods.

Previous work from our laboratory showed that a pungent compound of hot red pepper, capsaicin, at a dose in daily use in Thailand, enhanced triglyceride metabolism and energy metabolism by stimulating epinephrine secretion from the adrenal medula through sympathetic activation of the central nervous system in rats.[9–14] Triglyceride metabolism is known to be stimulated by epinephrine and norepinephrine through activities of the sympathetic nervous system, and the subsequent thermogenesis.[15–17] Sympathetic nervous stimulation is considered to be the most important action in the regulation of thermogenesis by brown adipose tissue (BAT).[15,17–20] Furthermore, it is believed that

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norepinephrine, secreted in response to sympathetic nervous system stimulation, plays a major role in the regulation of thermogenesis in BAT.[15,17,19–22] Therefore, the present study was undertaken to evaluate the nutritional effects of garlic, and especially to determine the effects of garlic supplementation on norepinephrine secretion, growth of interscapular brown adipose tissue (IBAT), and triglyceride catabolism in rats.

Materials and methods

Animal care

Male Sprague–Dawley rats (Japan SLC, Inc., Shizuoka, Japan) were individually housed in stainless steel wire-bottom cages in a room maintained at 22–24°C and about 50% relative humidity. The room was lighted from 07:00 to 19:00 hr. Tap water was freely available. The rats were given a commercial diet (CE-2, Japan Clea Inc., Tokyo, Japan) for 3 days before starting the experiment. This study was approved by the Kobe Women's University, Faculty of Home Economics, Institutional Animal Care and Use Committee.

Experiment 1

The experimental diets were the high-fat diet as shown in *Table 1*. One half of the animals received the high-fat diet alone, while the others received the same diet containing 0.8% garlic powder. The garlic powder used was heat-dried at 60 to 70°C, (Riken Chemical Industry Limited Co., Kyoto, Japan). The volatile compounds in garlic powder were analyzed by gas-chromatography using diallyldisulfide as a standard, and they were determined as diallyldisulfide equivalent.[23,24] The data indicated that the garlic powder used in this study contained 5.05 mg of total diallyl sulfides/mg (containing 0.05 mg/g of monosulfide, 1.0 mg/g of disulfide, 3.4 mg/g of trisulfide and 0.6 mg/g of tetrasulfide). Rats weighing 80–90 g were separated into two groups and were given the diet with or without garlic powder for 10 or 28 days.

At the end of the experimental period, the rats were individually transferred to a metabolic cage, where urine and feces were separately collected for 1 day. During the collection, to each urine sample was added 1 ml of 50% HCl solution to maintain the stable state. After the collection, urinary excretion of epinephrine and norepinephrine was determined by the method of Davidson and Fitzpatric.[25] Urinary creatinine was measured by the method of Clark and Thompson.[26] Fecal samples were dried, weighed, and pulverized. The fecal lipids were extracted by the method of Folch et al.[27] After the collection of urine and feces, in the fed state, rats were anesthetized by intraperitoneal injection of α-chrolarose and urethan (75 mg/kg and 750 mg/kg, respectively). Blood samples were collected from the abdominal aorta, and plasma was separated after centrifugation (3,000*g*, for 15 min) and stored at −40°C until assayed. After blood sampling, the liver, kidney, perirenal fat pad, epididymal fat pad, and IBAT were immediately excised, washed in chilled saline, blotted, weighed, and stored at −40°C for further analyses. The plasma was treated with aluminum oxide for the determination of epinephrine and norepinephrine concentrations using HPLC with electrochemical detection.[11,12] Plasma triglycerides and free fatty acid concentrations were analyzed enzymatically using commercial kits (for triglycer-

Table 1 Composition of experimental diets (Experiment 1)

	High-fat diet
Casein (g/kg)*	250
Shortening (g/kg)†	300
Vitamins (g/kg)‡	17
Minerals (g/kg)§	50
Cellulose (g/kg)*	40
Sucrose (g/kg)*	300
α-Cornstarch (g/kg)*	43
Garlic (g/kg)‖	—
Energy density (MJ/kg)¶	21.21

*Oriental Yeast Co., Tokyo, Japan.
†Crisco®: Partially hydrogenated vegetable shortening from Procter and Gamble Co., Cincinnati, OH.
‡Purchased from Oriental Yeast Co., Tokyo, Japan. Vitamin mixture (mg/kg diet) contained retinyl acetate 17, cholecalciferol 0.0425, all-rac-α-tocopherol acetate 85, menadione 88.4, thiamin-HCl 20.4, riboflavin 68, pyridoxine-HCl 13.6, vitamin B_{12} 0.0085, vitamin C 510, D-biotin 0.34, folic acid 3.4, Ca-pantothenate 85, *P*-aminobenzoic acid 85, nicotinic acid 102, inositol 102, choline chloride 3,400, and cellulose powder 12,419.809.
§Purchased from Oriental Yeast Co., Tokyo, Japan. Mineral mixture (mg/kg diet) contained $CaHPO_4 \cdot H_2O$ 7,280, KH_2PO_4 12,860, $NaH_2PO_4 \cdot H_2O$ 4,675, NaCl, 330, Ca-lactate 17,545, Fe-citrate 1,590, $MgSO_4 \cdot 3H_2O$ 3,585, $ZnCO_3$ 55, $MnSO_4$ 60, $CuSO_4$ 15, and KI 5.
‖Added at 0.8% as garlic powder of high-fat diet in garlic diet. Prepared by Riken Chemical Ind. Co., Kyoto, Japan. The composition of garlic powder was as follows: moisture 5.5%, ash 3.2%, protein 17.2%, fat 0.4%, fiber 1.4%, and carbohydrate 72.3%.
¶Energy values were as follows: starch, soluble carbohydrates and protein (16.70 MJ/kg); fat (37.70 MJ/kg).

Table 2 Effect of garlic supplementation on energy intake, body weight, liver weight, fecal lipid, urinary creatinine, perirenal adipose tissue weight, and epididymal fat pad weight in rats fed a high-fat diet for 10 or 28 days (Experiment 1)

	High-fat diet	Garlic diet
Energy intake (MJ)		
10 days	1.548	1.548
28 days	6.321	6.321
Body weight (g)		
10 days	157.5 ± 1.7	153.5 ± 1.7
28 days	261.6 ± 1.6	250.3 ± 4.1*
Liver weight (g)		
10 days	6.1 ± 0.1	5.9 ± 0.2
28 days	9.9 ± 0.1	9.6 ± 0.2
Fecal lipid (mg/day)		
10 days	62.7 ± 18.9	52.7 ± 10.1
28 days	85.3 ± 15.3	82.2 ± 15.3
Apparent fat digestibility (%)		
10 days	97.91 ± 12.05	98.24 ± 18.83
28 days	98.82 ± 16.04	98.90 ± 10.14
Urinary creatinine (μmol/day)		
10 days	20.49 ± 2.34	22.10 ± 2.35
28 days	45.02 ± 1.96	43.61 ± 2.96
Perirenal adipose tissue weight (g)		
10 days	0.46 ± 0.05	0.40 ± 0.02
28 days	1.78 ± 0.14	1.54 ± 0.07
Epididymal fat pad weight (g)		
10 days	1.39 ± 0.06	1.33 ± 0.03
28 days	5.24 ± 0.31	3.49 ± 0.20†

Values are means ± SEM for 7 or 8 rats.
* and †Significantly different from high-fat diet group at $P < 0.05$, $P < 0.01$, respectively.

ides, Triglyceride G-Test Wako, Wako Chemical Ind., Osaka, Japan; for free fatty acids, NEFA C-Test Wako, Wako Chem. Ind.). Plasma total and HDL cholesterol concentrations were measured according to the method of Pearson et al.[28] IBAT mitochondrial proteins were isolated by the method of Cannon and Lindberg,[29] and the protein contents were measured by the method of Lowry et al.[30]

Experiment 2

The effects of a major volatile sulfur-containing compound of garlic diallyldisulfide on norepinephrine secretion were investigated in situ. Furthermore, the effects of phentolamine hydrochloride, a sympathetic nervous system blocking agent, on the norepinephrine secretion caused by diallyldisulfide administration were also investigated in selected animals. Rats weighing about 220 g were anesthetized as described above, and their rectal temperature was maintained between 36.5°C and 37.5°C using a direct current heating pad.[11] The rats were infused through the right femoral vein with 1 mL of either a vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 mL of the same vehicle containing diallyldisulfide solution (0.64, 1.28, or 1.93 mg/mL).

It was determined by preliminary experiment that 0.55 mg of diallyldisulfide administered was an equivalent to the average intakes of garlic powder per day per rat. Diallyldisulfide was purchased from Tokyo Chemical Industry, Co. (Tokyo, Japan) and its purity was 88.9%. The remaining components were allylsulfide 5.4% and diallyltrisulfide 5.3%. After 10 min, blood samples were collected from the abdominal aorta and transferred into heparinized tubes. Plasma samples were obtained by centrifugation. The assay of plasma norepinephrine concentrations was performed as in Experiment 2. A dose of phentolamine hydrochloride (2 mg/kg; Sigma Chemical Co., St. Louis, MO, USA) was given by intramuscular injection 15 min before the anesthesia. All other experimental procedures were the same as described above.

Statistical analysis

All data are presented as means ± SEM. Statistical analyses were performed using Student's *t*-test. Differences were considered significant when P was less than 0.05.

Results

Experiment 1

The mean values of body weight and epididymal fat pad weight were significantly lowered after 28 days of garlic supplementation, although there were no significant differences at 10 days (*Table 2*). There were no differences in liver weight, fecal lipid, apparent fat digestibility, urinary creatinine excretion, and perirenal adipose tissue weight between rats fed a high-fat diet with and without garlic powder for 10 or 28 days (*Table 2*). Body muscle mass apparently was not affected by garlic powder supplementation, since urinary excretion of creatinine was not affected significantly by garlic supplementation for 10 or 28 days. Plasma concentrations of triglycerides and free fatty acids were significantly decreased by garlic supplementation for 10 or 28 days. In contrast, there were no significant differences in plasma total cholesterol or HDL cholesterol concentrations (*Table 3*). Although the mean body weight of rats fed a diet without garlic powder was larger than that of rats fed garlic powder (*Table 2*), IBAT weights and the IBAT mitochondrial protein were significantly greater in rats given garlic powder (*Figure 1*). The excretion of epinephrine and norepinephrine in urine during 1 day are given in *Figure 2*. In our preliminary experiments, we have confirmed that the urinary epinephrine and norepinephrine lev-

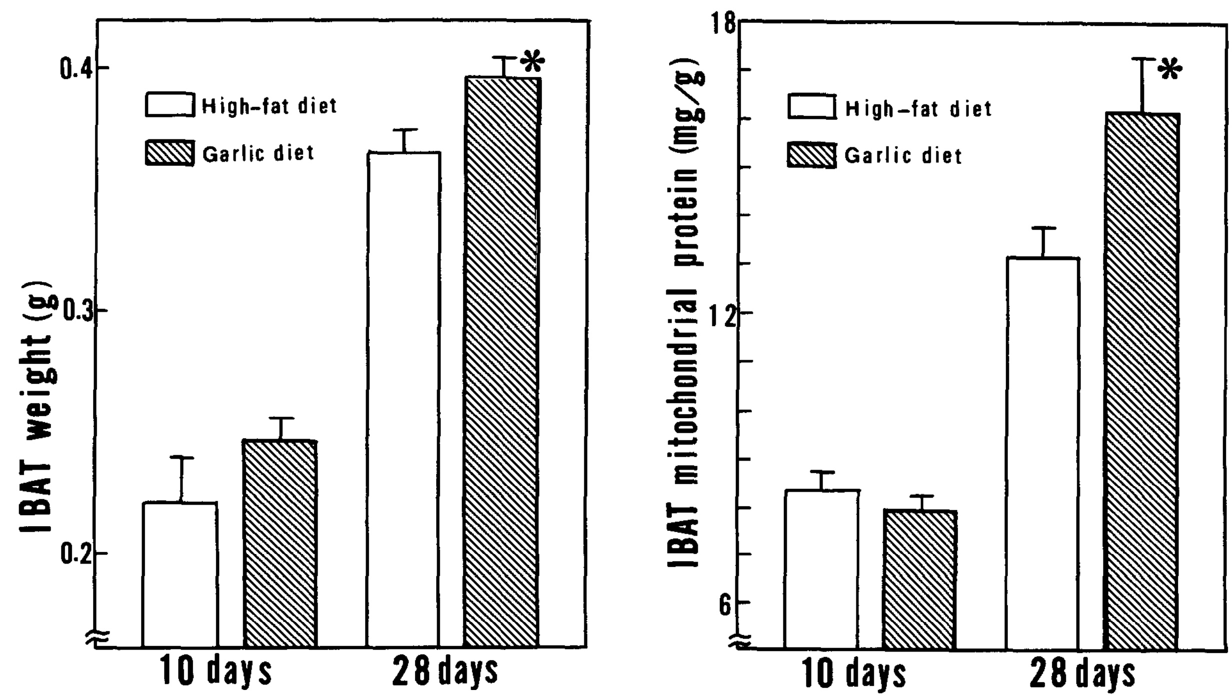

Figure 1 Effect of garlic supplementation on IBAT weight and on IBAT mitochondrial protein contents in rats fed a high-fat diet for 10 or 28 days (Experiment 1). Values are means ± SEM of 5 or 6 rats. *Significantly different from high-fat diet fed group at $P < 0.01$.

Table 3 Effect of garlic supplementation on plasma concentrations of triglycerides, free fatty acids, total cholesterol, and HDL cholesterol in rats fed a high-fat diet for 10 and 28 days (Experiment 1)

	High-fat diet	Garlic diet
Triglyceride (mmol/L)		
10 days	6.93 ± 0.80	5.25 ± 0.38*
28 days	11.23 ± 1.04	8.30 ± 0.77*
Free fatty acids (μmol/L)		
10 days	16.21 ± 2.52	13.19 ± 3.55*
28 days	19.29 ± 1.52	15.46 ± 2.16*
Total cholesterol (mmol/L)		
10 days	1.671 ± 0.112	1.674 ± 0.036
28 days	1.565 ± 0.070	1.650 ± 0.079
HDL cholesterol (mmol/L)		
10 days	0.526 ± 0.062	0.593 ± 0.054
28 days	0.819 ± 0.135	0.699 ± 0.163

Values are means ± SEM for 6 or 7 rats.
*Significantly different from high-fat diet group at $P < 0.01$.

els in rats during 1 day (24 hr) were not affected by the moving to metabolic cages for the urine collection. Urinary excretion of norepinephrine was significantly increased by garlic supplementation, while the epinephrine was not affected (*Figure 2*). Similarly, plasma norepinephrine concentrations were significantly increased by garlic supplementation, while epinephrine levels were not significantly affected (*Figure 3*).

Experiment 2

Figure 4 shows that the plasma norepinephrine secretion was significantly increased by in situ administration of 1.28 and 1.93 mg of diallyldisulfide as compared with the infusion of a vehicle or of 0.64 mg of diallyldisulfide. Furthermore, there was a significant positive correlation between the norepinephrine secretion and the diallyldisulfide dose ($P < 0.001$, $r = 0.873$). Phentolamine hydrochloride (10.0 mg/kg) given together with diallyldisulfide did not affect norepinephrine secretion (data not shown).

Discussion

It has been reported that garlic has hypoglycemic and hypolipidemic properties and a protective effect on atherosclerosis.[3–5,8,31] However, these investigators used large doses of garlic or garlic oil in a relatively short experimental period. Therefore, little is known about the nutritional effects of garlic at ordinary levels of dietary intake.

In the present study, to investigate the nutritional effects of continuous intakes of garlic in daily use, rats were given a diet containing 0.8% garlic powder. The experimental diet used in Experiment 1 (*Table 1*) was a high-fat diet containing 30% weight of the diet as vegetable shortening and 30% weight of the diet as sucrose, which was a model of palatable "cafeteria" diet with fatty and sweet taste.[20]

Recently, it has been suggested that normal rats and mice overfed a "cafeteria" diet may resist the development of obesity by exhibiting a compensatory increase in energy expenditure. This phenomenon is known as diet-induced

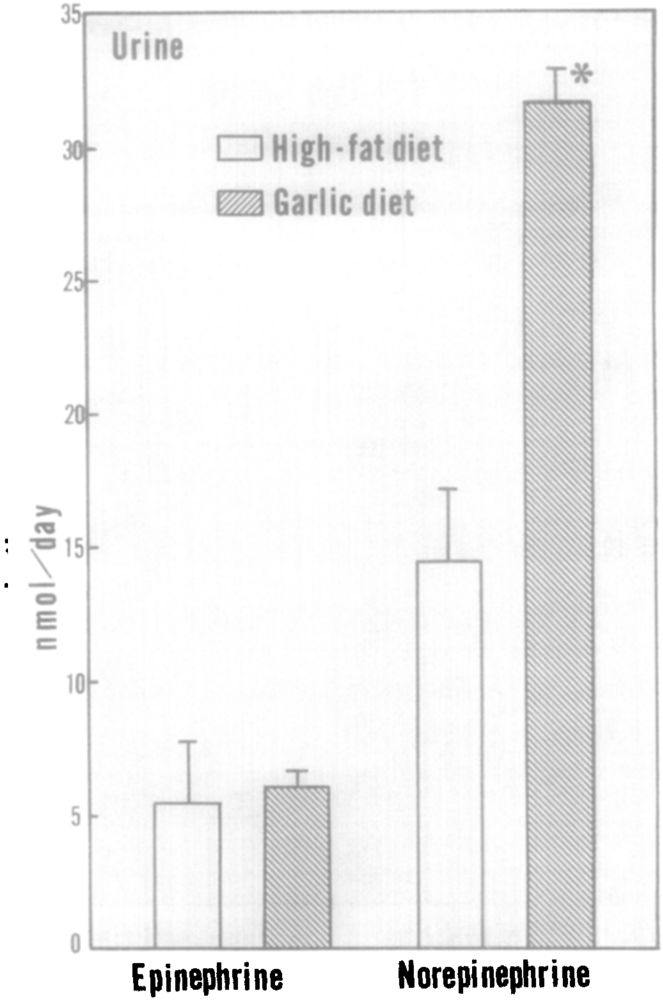

Figure 2 Effect of garlic supplementation on urinary excretion of epinephrine and norepinephrine (Experiment 1). Values are means ± SEM of 6 or 7 rats. *Significantly different from the high-fat diet-fed group at $P < 0.001$.

thermogenic activity of BAT. Long-term overeating of a palatable "cafeteria" diet can also induce hypertrophy of BAT, although the best stimulus to growth of BAT is chronic exposure to cold.[15–22,32]

Growth of IBAT was induced by the feeding of a high-fat diet in our preliminary experiment (data not shown) and this was further enhanced by garlic supplementation (*Figure 1*). This observation together with the increased norepinephrine secretion suggests that the thermogenic activity of BAT was enhanced by garlic supplementation (*Figures 2* and *3*). We have demonstrated that capsaicin, fed together with a high-fat diet, lowered the perirenal adipose tissue weight and serum triglyceride concentrations in rats as a result of enhanced energy metabolism associated with increased epinephrine secretion from the adrenal medulla.[9–13] In contrast, diallyldisulfide, a sulfur-containing, volatile, pungent compound of garlic, did not enhance epinephrine secretion in situ.[33] Plasma epinephrine level in the present study (*Figures 3* and *4*) may be affected by the use of urethan anesthesia which leads to enhanced epinephrine secretion from the adrenal medulla due to increasing sympathetic nervous system activity.[34,35]

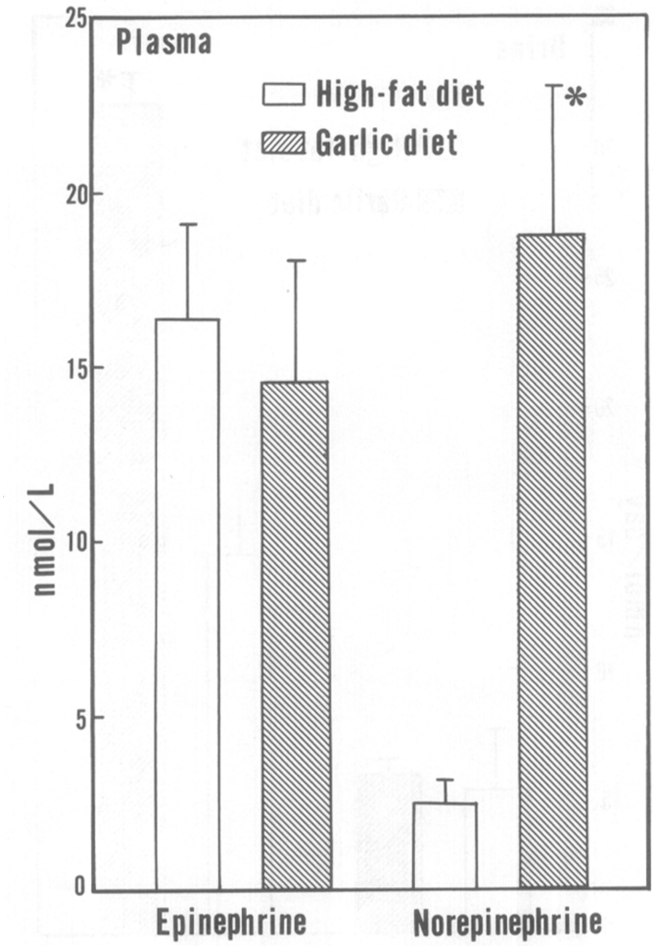

Figure 3 Effect of garlic supplementation on epinephrine and norepinephrine secretion in plasma (Experiment 1). Values are means ± SEM of 6 or 7 rats. *Significantly different from the high-fat diet-fed group at $P < 0.01$.

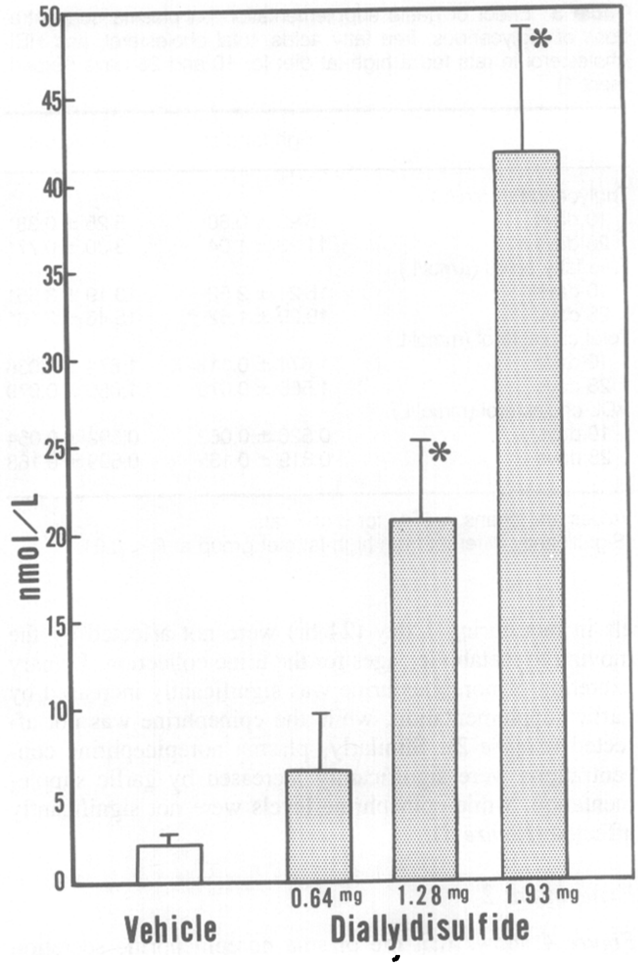

Figure 4 Effect of diallyldisulfide administration on norepinephrine secretion in rats in situ (Experiment 2). Rats were infused with 1 mL of a vehicle (0.9% NaCl solution containing 2% ethanol and 0.5% Tween 80) or 1 mL of diallyldisulfide solution containing 0.64, 1.28, or 1.93 mg into the right femoral vein. After 10 min, abdominal aortic blood was collected. Values are means ± SEM of 4 or 5 rats. *Significantly different from vehicle infusion at $P < 0.01$.

Nowadays urethan is widely used for animal experimentation, which induces profound narcosis for a long duration with little change in respiration or circulation. In the present study, we found that norepinephrine secretion, but not epinephrine secretion, into both urine and plasma was increased by the continual administration of garlic in rats fed a high-fat diet (*Figures 2* and *3*). Furthermore, the data of the present study indicate that the increase in norepinephrine secretion was proportional to the quantities of diallyldisulfide administered to femoral vein (*Figure 4*). However, the enhancement of norepinephrine secretion was not induced in the presence of an agent that blocks the sympathetic nervous system. Therefore, it may be possible to consider that garlic administration enhances norepinephrine secretion via the increasing the activity of the peripheral sympathetic nervous system. Norepinephrine secretion from the sympathetic nervous system has been found to control the thermogenesis of BAT via the regulation of uncoupling protein gene expression.[19] Sympathetic nervous stimulation has been reported to regulate thermogenesis and uncoupling protein (thermogenin) synthesis in BAT.[18–22]

Recent papers have suggested that BAT is important in the regulation of energy balance.[15,20,32] Accordingly, in view of the importance of norepinephrine in the activation and hypertrophy of BAT to regulate the energy balance, our results suggest that the lowering of plasma concentrations of triglycerides and of free fatty acids are concerned with the enhancement of triglyceride catabolism due to the hypertrophy of IBAT via the stimulation of increasing norepinephrine secretion. Therefore, the present results suggest that increased norepinephrine secretion accounts for a decrease in plasma concentrations of triglycerides and free fatty acids as a result of increased growth in IBAT by garlic supplementation.

Several investigators have demonstrated that garlic administration was effective in lowering serum cholesterol.[3–5,36] In the present study, however, plasma cholesterol levels were not affected by garlic supplementation, because plasma cholesterol concentrations were low in both high-fat diet and garlic diet-fed groups (*Table 3*). The reason for these low cholesterol concentrations may be the experimental diet using vegetable shortening which contains no cholesterol. To evaluate the hypocholesterolemic effect of garlic supplementation, the plasma cholesterol concentrations in rats must be kept at high levels by increasing cholesterol in the experimental diet. Further investigation is necessary on the hypocholesterolemic effect of garlic supplementation. These studies are now in progress.

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