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Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats

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Abstract

We investigated the effects of garlic oil and diallyl trisulfide on glycemic control in rats with streptozotocin-induced diabetes. Diabetic rats received by gavage garlic oil (100 mg/kg body weight), diallyl trisulfide (40 mg/kg body weight), or corn oil every other day for 3 weeks. Control rats received corn oil only. Both garlic compounds significantly raised the basal insulin concentration. The insulin resistance index as assessed by homeostasis model assessment and the first-order rate constant for glucose disappearance were significantly improved by both garlic compounds ($P<0.05$). Oral glucose tolerance was also improved by both garlic compounds and was accompanied by a significantly increased rate of insulin secretion ($P<0.05$). Glycogen formation (but not that of lactate or carbon dioxide) from glucose by the soleus muscle in the presence of 10 or 100 μU/ml of insulin was significantly better after treatment with both garlic compounds. Both garlic oil and diallyl trisulfide improve glycemic control in diabetic rats through increased insulin secretion and increased insulin sensitivity.

Keywords: Diabetes; Diallyl trisulfide; Garlic oil; Glycemic control; Streptozotocin; (Rat)

1. Introduction

Garlic and garlic constituents prepared by various means have been shown to have diverse biological activities, including antitumorigenetic, anticarcinogenic, antiatherosclerotic, antithrombotic, antidiabetic, and various other biological actions [see reviews by Agarwal (1996) and Augusti (1996)]. According to the report by Ryan et al. (2001), one-third of diabetic patients take alternative medications that they consider efficacious, of which garlic is the most commonly used. The number of studies of the hypoglycemic effect of garlic is limited, however, and the results of such studies are inconsistent.

In the 1970s, Jain et al. (1973) and Jain and Vyas (1975) investigated the hypoglycemic effect of extracts of garlic with water or several different organic solvents on oral glucose tolerance in normal and alloxan-induced diabetic rabbits. Those authors found all the garlic preparations to possess an acute hypoglycemic effect, for which the effect of the ethyl ether extract was competitive with that of tolbutamide. Later, other researchers also reported a hypoglycemic effect of garlic oil in diabetic animals (Begum and Bari, 1985; Farva et al., 1986; Anwar and Meki, 2003). In diabetic patients, it was reported that garlic oil can correct hyperglycemia (Duncan, 1999). In addition, a precursor of garlic oil, *S*-allyl-cysteine sulfoxide, was shown to have a hypoglycemic effect similar to that of glibenclamide (Sheela and Augusti, 1992). The hypoglycemic effect of garlic has also been shown in other nondiabetic hyperglycemia animal models (Kasuga et al., 1999; Tahiliani and Kar, 2003). However, Swanston-Flatt et al. (1990) failed to find a hypoglycemic effect of garlic powder in animals with streptozotocin-induced diabetes. Similarly, Baluchnejadmojarad and Rohgani (2003a,b) found no hypoglycemic effect of an aqueous extract of garlic in rats with streptozotocin-induced diabetes, although

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they did find a significant effect of garlic on vascular reactivity. We speculate that these inconsistent results are at least partly because different preparations or derivatives of garlic were used in the different studies. The chemicals present in a garlic product are largely dependent on the processing conditions, such as temperature, duration of preparation, and extraction solvents used (Staba et al., 2001).

According to the method described by Sheen et al. (1992), we previously prepared stable garlic oil preparations and investigated some of their biological characteristics (Chen et al., 2003; Sheen et al., 1999; Liu et al., 1998). The aim of the present study was to investigate the effects of garlic oil and one of its major organosulfur compounds, diallyl trisulfide, on glycemic control and skeletal muscle glucose utilization in an animal model of diabetes.

2. Materials and methods

2.1. Garlic oil preparation

Garlic oil was prepared as previously described (Sheen et al., 1992). Briefly, a steam distillation technique was used, and the final product contained the major essential components of garlic oil, including 38.6% diallyl disulfide, 30.8% diallyl trisulfide, 10.0% diallyl sulfide, and minor amounts of many other volatile compounds.

2.2. Animals and treatments

Four-week-old weanling male Wistar rats were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). The animals were kept under a 12-h light–dark cycle at an ambient temperature of 23 °C and were given free access to water and standard rat feed (Rodent Diet 5001; Purina Mills, Richmond, IN). All animals were allowed to adapt to the environment for 1 week after their arrival before the experiment started. Diabetes was induced by the injection of streptozotocin (65 mg/kg body weight in citrate buffer, pH 4.5) into a lateral tail vein (Junod et al., 1969). The control rats were injected with the same volume of vehicle. Three days after the injection, the diabetic animals were randomly assigned to three groups and received by gavage garlic oil (100 mg/kg body weight), diallyl trisulfide (40 mg/kg body weight; LKT Laboratories, Inc., St. Paul, MN), or the vehicle (corn oil; 2 ml/kg body weight) every other day for 3 weeks. The control rats received corn oil by gavage (2 ml/kg body weight). During the 3 weeks of treatment, the animals were housed in metabolic cages and were given free access to water and a powdered diet (Rat Diet 5012; Purina Mills, Richmond, IN). Food and water intakes and urine excretion were measured.

An insulin-tolerance test was carried out on day 10 after the injection. After 1-week recovery from the insulin-tolerance test, the rats were starved overnight and an oral-glucose-tolerance test was carried out on day 17. The animals were then starved overnight before they were killed on day 21 for blood collection and soleus muscle isolation. Blood samples were collected, treated with heparin, and centrifuged at 500×g for 10 min. The plasma obtained was stored at −20 °C until analyzed. Measurements of insulin, fructosamine, and nonesterified fatty acids in plasma were carried out within 2 weeks. Housing conditions and experimental procedures were in strict accordance with the European Community ethical regulation on the care and use of animals for scientific research; the present research was moreover approved by the ethical committee for animal experimentation of Chung Shan Medical University.

2.3. Insulin-tolerance test and oral-glucose-tolerance test

To test insulin tolerance, an insulin bolus (1 U/kg body weight human regular insulin; Eli Lilly, Indianapolis, IN) was administered by intraperitoneal injection. Samples of blood were withdrawn from the lateral tail vein immediately before and 5, 10, 15, 30, 60, 90, and 120 min after the injection. Heparin-containing blood samples were immediately centrifuged, and the plasma was separated and frozen at −20 °C until analyzed for glucose. The first-order rate constant for the disappearance rate of glucose (K_{ITT}) estimated from the slope of the regression line of the logarithm of blood glucose against time was calculated as described by Akinmokun et al. (1992).

The oral-glucose-tolerance test was performed by orally administering by gavage a solution of 10% (w/v) glucose (1 g/kg body weight). Blood samples were withdrawn from the lateral tail vein immediately before and 5, 10, 15, 30, 60, 90, 120, and 180 min after the bolus glucose loading. Heparin-containing blood samples were immediately centrifuged, and the plasma was separated and frozen at −20 °C until analyzed for insulin or glucose.

2.4. Biochemical analysis of blood samples

For the analysis of glucose, plasma was deproteinized with 5% (v/v) $HClO_4$ and then neutralized with 0.5 M triethanolamine/2 M KOH. Universal pH indicator was added to ensure that the deproteinized samples were properly neutralized. Glucose concentrations were analyzed enzymatically by the method of Bergmeyer (1974). The dilution factor during the deproteinization procedure was adjusted for the concentration of glucose in each sample. Plasma concentrations of insulin, fructosamine, and nonesterified fatty acids were determined spectrophotometrically with a rat insulin enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden), a fructosamine kit (Sigma Chemical Company, St. Louis, MO), and a nonesterified fatty acid kit (Randox Laboratories, Crumlin, United Kingdom), respectively, according to the manufacturer's instructions and were analyzed with a micro-plate reader (VersaMax; Molecular Devices Ltd., Sunnyvale, CA). The insulin resistance index as assessed by homeostasis model assessment (HOMA-IR) was calculated to estimate peripheral insulin resistance after treatment according to the following formula, as described by Matthews et al. (1985): fasting plasma glucose (mg/dl) × fasting plasma insulin (μU/ml)/405.

2.5. Isolation and incubation of the soleus muscles

Stripped soleus muscles were prepared and incubated as described by Crettaz et al. (1980), with the modifications given by Challiss et al. (1983). Briefly, the rats were starved overnight and were then killed between 0900 and 1000 h by

cervical dislocation. The diabetic rats weighed approximately 180 g at the time of death, and the weight of their soleus muscles was suitable for performing the muscle incubation study (Newsholme et al., 1986). The control rats, however, all weighed more than 200 g at the time of death. Therefore, for the study of glucose metabolism in skeletal muscle, normal, untreated rats weighing 180±10 g were used instead of the control rats. The soleus muscles were isolated and dissected longitudinally and were rapidly weighed and tied at the resting length in situ on stainless steel clips. Mounted muscles were then placed immediately into 25-ml Erlenmeyer flasks containing 3.2 ml of preincubation medium (5 mM pyruvate-supplemented Krebs–Ringer bicarbonate buffer, pH 7.4) at 37 °C. The muscle strips were preincubated in a shaking (100 rpm) water bath at 37 °C for 30 min and were then transferred to separate flasks containing oxygenated Krebs–Ringer bicarbonate buffer (3.5 ml, pH 7.4) containing 5.5 mM glucose, 1.5% (w/v) defatted bovine serum albumin, and 10 or 100 μU/ml of insulin. Both the preincubation and the incubation media were gassed for 30 min with O_2/CO_2 (95:5) before use. To the incubation medium, 0.4 μCi D-[U-^{14}C]glucose/ml was added. At the end of 60 min of incubation at 37 °C, the muscles were removed from the incubation flasks and were quickly frozen in liquid nitrogen and then stored at −70 °C for the assessment of glycogen synthesis. The medium in the flasks was used to assess the formation of lactate and carbon dioxide.

2.6. Determination of glucose metabolism

The formation of glycogen from D-[U-^{14}C]glucose was estimated by alkaline hydrolysis of muscle according to the method described by Guendet et al. (1976), with the modification given by Dimitriadis et al. (1988). Briefly, after carrier glycogen (5 mg) was added to the hydrolysate, glycogen was precipitated with 66% ethanol. The glycogen precipitate was washed twice with ethanol and dissolved in 0.5 ml of water, and radioactivity was counted. For measurements of glucose oxidation, at the end of the incubation and after the muscles were removed, the flasks were rapidly resealed and the incubation was acidified with perchloric acid (final concentration 4%, w/v); the flasks were left on ice for 60 min. The $^{14}CO_2$ was trapped in pieces of filter paper moistened with 200 μL of 2-phenethylamine:methanol (1:1, v/v) as described by Leighton et al. (1985). To measure the rate of lactate production, [^{14}C]lactate formed from D-[U-^{14}C]glucose was separated from other labeled compounds in the incubation medium by using ion-exchange chromatography as described by Hammerstedt (1980). In brief, 100 μl of incubation medium was applied to a column of Dowex AG1 anion exchange resin (Sigma Chemical Company, St. Louis, MO). The column was washed with 20 ml of distilled water containing 3 mM D-glucose to elute the D-[U-^{14}C]glucose and then with 10 ml of 0.5 M formic acid to elute the [^{14}C]lactate. The radioactivity of the eluate containing lactate was counted.

2.7. Statistical analysis

The data are expressed as means±S.D. and were analyzed by one-way analysis of variance. Student's *t*-test was used to detect differences in means between the control group and the group of diabetic rats. Duncan's multiple-comparison test was used to detect differences among the means of the streptozotocin-injected groups. *P* values <0.05 were considered significant. All statistical analyses were performed with commercially available software (SPSS 12 for WINDOWS; SPSS Inc., Chicago, IL).

3. Results

3.1. Animal characteristics

Induction of diabetes with streptozotocin was associated with the characteristic development of a slower rate of body weight gain, greater food and water intakes, greater urine excretion, higher fasting glucose concentrations, and lower fasting insulin concentrations in plasma (Table 1). In addition, concentrations of fructosamine and nonesterified fatty acids in plasma were significantly higher in the diabetic group than in the control rats. Compared with that in the vehicle-treated diabetic group, the rate of body weight gain, food and water intakes, and urine excretion were not significantly affected by treatment with garlic oil or diallyl trisulfide (Table 1). The fasting blood glucose concentration of the diabetic rats was also not significantly affected by treatment with garlic oil or diallyl trisulfide. The fructosamine concentration in plasma was marginally lower in the diabetic rats treated with

Table 1
Growth characteristics; plasma concentrations of insulin, nonesterified fatty acids, glucose, and fructosamine; and the HOMA-IR[a] of control rats or streptozotocin-induced diabetic rats who did or did not receive garlic oil or diallyl trisulfide[b]

	Control	DM	DM+diallyl trisulfide	DM+garlic oil
Body weight gain (g)	104±19	70±20[c]	61±17	70±15
Food intake (g/24 h)	24.3±2.8	33.8±7.3[c]	32.0±6.0	32.8±6.0
Water intake (ml/24 h)	37±8	130±53[c]	121±25	121±48
Urine excretion (ml/24 h)	19±7	107±46[c]	110±26	103±45
Insulin (ng/ml)	1.519±0.387	0.156±0.038[c]	0.422±0.180[d]	0.459±0.178[d]
Nonesterified fatty acids (μmol/l)	515±43	617±53[c]	612±61	576±69
Glucose (mg/dl)	109±19	181±21[c]	175±13	172±14
Fructosamine (μmol/l)	96±23	175±29[c]	148±13	152±14
HOMA-IR	9.77±1.02	1.70±0.41[c]	4.16±0.67[d]	4.76±0.38[d]

[a] The insulin resistance index as assessed by homeostasis model assessment.
[b] Values are the mean±S.D. for six rats per group.
[c] Significant difference between the control group and the DM group ($P<0.05$).
[d] Significant difference between the garlic oil-treated or diallyl trisulfide-treated group and the DM group ($P<0.05$).

diallyl trisulfide and garlic oil- than in the vehicle-treated diabetic rats but not significantly so. The basal plasma nonesterified fatty acid concentration was lowered by garlic oil, but not by diallyl trisulfide, to a value similar to that of the controls. Treatment with garlic oil and diallyl trisulfide significantly raised the basal insulin concentration in plasma. To estimate the effect of garlic oil and diallyl trisulfide on insulin resistance in the diabetic rats, we calculated the HOMA-IR. Both garlic compounds were able to ameliorate the insulin resistance that developed in the diabetic rats ($P<0.05$).

3.2. Insulin-tolerance test

As shown in Fig. 1A, the difference in blood glucose response to insulin administration between the control and the vehicle-treated diabetic animals was dramatic. The greatest decrease in blood glucose, which occurred 90 min after the insulin bolus, was 16.0±4.1% for the vehicle-treated diabetic group compared with 29.4±10.5% for the control group ($P<0.05$). In addition, K_{ITT} was significantly slower in the diabetic rats, which confirmed the development of insulin resistance in these animals (Fig. 1B). At 90 min after the insulin bolus, the drop in blood glucose was improved by treatment with garlic oil (decrease in blood glucose of 30.4±10.4%) and diallyl trisulfide (decrease of 31.5±12.0%) ($P<0.05$ for the difference between the vehicle-treated diabetic group and the other treated groups; Fig. 1A). In addition, the K_{ITT} for the insulin-induced plasma glucose decay was significantly improved in the diallyl trisulfide- and garlic oil-treated diabetic groups compared with the vehicle-treated diabetic group (Fig. 1B).

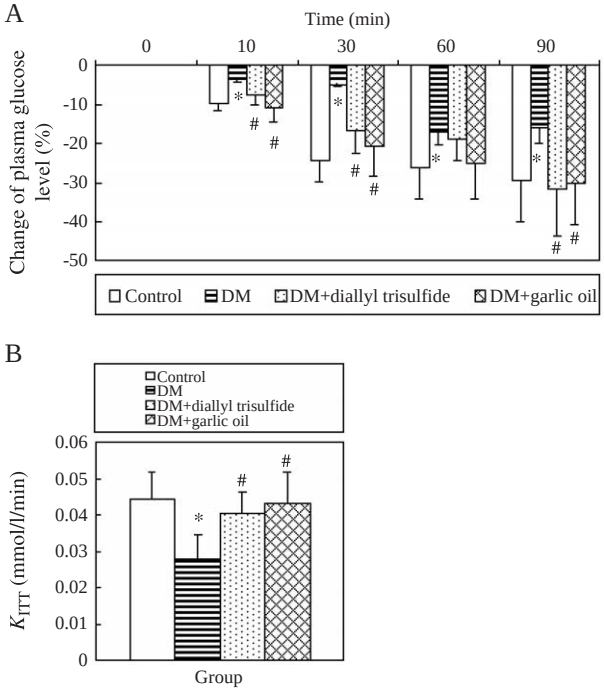

Fig. 1. Effect of treatment with garlic oil or diallyl trisulfide on the decay in plasma glucose concentrations in response to a bolus injection of insulin as a function of time (A) and the first-order rate constant for the disappearance rate of glucose in plasma (K_{ITT}) after the insulin bolus (B). An insulin bolus (1 U/kg body weight) was administered intraperitoneally on day 10 after the induction of diabetes. The change in plasma glucose concentrations is presented as the percentage of the glucose value at time zero. Data are means±S.D. for six rats per group. *Significant difference between the control group and the DM group ($P<0.05$). #Significant difference between the garlic oil- or diallyl trisulfide-treated group and the DM group ($P<0.05$).

3.3. Oral-glucose-tolerance test

The results of the glucose-tolerance test performed on day 17 after the induction of diabetes are shown in Fig. 2. After oral glucose loading, blood glucose concentrations in the vehicle-treated diabetic rats were dramatically increased, were significantly higher than the concentrations of the controls, and remained high between 30 and 180 min after glucose loading (Fig. 2A). At 30 min after glucose loading, diallyl trisulfide attenuated the increase in blood glucose compared with that in the vehicle-treated diabetic rats (Fig. 2A), and this effect was maintained throughout the investigation period. Garlic oil also showed an effect on blood glucose similar to that of diallyl trisulfide (significant from 60 min through 180 min after glucose loading).

The integral values of blood glucose from Fig. 2A were recalculated and are given in Fig. 2B. Glucose tolerance deteriorated in the vehicle-treated diabetic animals: the area under the glucose tolerance curve was significantly greater in the diabetic group than in the control group. However, this deteriorated glucose tolerance was attenuated by treatment with diallyl trisulfide and garlic oil.

Plasma insulin concentrations were measured during the first 60 min of the oral-glucose-tolerance test (Fig. 2C). When compared with concentrations in the vehicle-treated diabetic group, plasma insulin concentrations in the control group were higher at all time points. The peak insulin concentration in the control group appeared at 10 min after glucose loading. The vehicle-treated diabetic rats showed a slower insulin response to glucose loading; the peak plasma insulin concentration in these rats was at 30 min. Treatment with diallyl trisulfide and garlic oil attenuated the delayed insulin response to glucose loading; the peak plasma insulin concentrations in the treated groups was at 10 min (Fig. 2C).

The integral values of plasma insulin from Fig. 2C are calculated and are given in Fig. 2D. The integral values of plasma insulin during the oral-glucose-tolerance test for the vehicle-treated diabetic rats were only 7.56% of the value for the controls ($P<0.05$). However, treatment with diallyl trisulfide and garlic oil significantly improved the insulin response to oral glucose loading.

3.4. Skeletal muscle glucose metabolism

The utilization of glucose by skeletal muscle in response to insulin (10 or 100 μU/ml) stimulation is shown in Fig. 3. In the presence of 10 μU/ml of insulin, the rate of conversion of glucose to lactate, glycogen, and carbon dioxide was significantly lower by 31.9%, 38.6%, and 35.1%, respectively, in the vehicle-treated diabetic rats than in the control group. In the presence of 100 μU/ml of insulin, the rate of the conversion of glucose to lactate, glycogen, and carbon dioxide was significantly lower by 38.6%, 49.1%, and 36.6%, respectively. The rate of lactate formation in the diabetic rats, in the presence of 10 and 100 μU/ml of insulin, was not significantly changed by diallyl trisulfide or garlic oil (Fig.

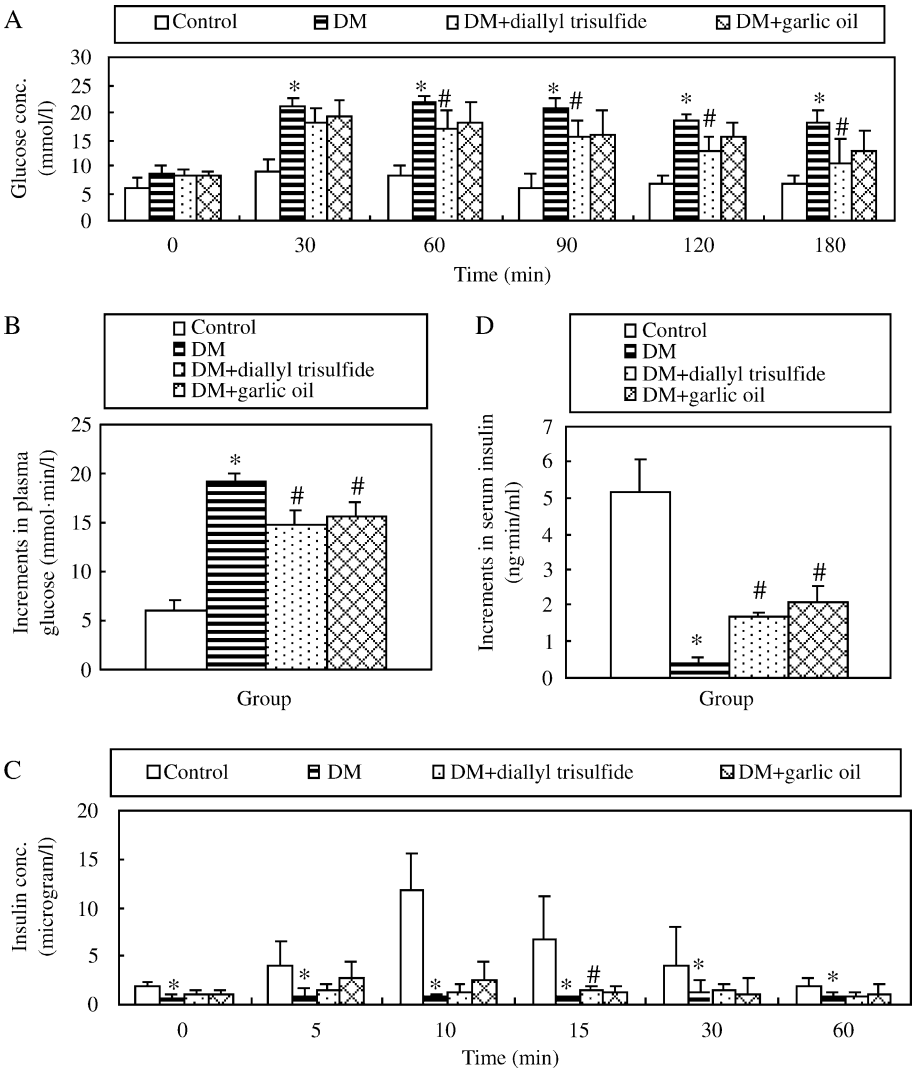

Fig. 2. Effect of treatment of diabetic rats with garlic oil or diallyl trisulfide on the concentration of plasma glucose as a function of time (A), the increment in plasma glucose (B), the concentration of plasma insulin as a function of time (C), and the increment in plasma insulin (D) in response to an oral glucose bolus. A glucose bolus (1 g/kg body weight) was administered orally on day 17 after the induction of diabetes. Glucose was measured in plasma samples from the tail vein. The data in (B) and (D) are calculated from the areas under the curve of (A) and (C), respectively. Data are means ± S.D. for six rats per group. *Significant difference between the control group and the DM group ($P<0.05$). #Significant difference between the garlic oil- or diallyl trisulfide-treated group and the DM group ($P<0.05$).

3A). However, the rate of conversion of glucose to glycogen in the presence of 100 μU/ml of insulin was significantly higher in rats treated with garlic oil and diallyl trisulfide than in the vehicle-treated rats (Fig. 3B). Garlic oil significantly lowered the oxidation rate of exogenous glucose in the presence of both 10 and 100 μU/ml of insulin (Fig. 3C).

4. Discussion

Garlic has long been believed to possess a hypoglycemic effect (Agarwal, 1996; Augusti, 1996); however, both effectiveness and ineffectiveness of garlic preparations on decreasing blood glucose have been reported (Jain et al., 1973; Jain and Vyas, 1975; Begum and Bari, 1985; Farva et al., 1986; Anwar and Meki, 2003; Duncan, 1999; Swanston-Flatt et al., 1990; Baluchnejadmojarad and Rohgani, 2003a,b). In the present study, we investigated the hypoglycemic effect of garlic oil and one of its major organosulfur compounds, diallyl trisulfide, in a widely used animal model of diabetes (Junod et al., 1969; Like and Rossini, 1976). We showed that streptozotocin injection caused a slower rate of body weight gain, hyperphagia, polydipsia, polyuria, hyperglycemia, and insulinopenia, which were accompanied by elevated plasma concentrations of glycosylated proteins and non-esterified fatty acids (Table 1). The lower hypoglycemic response of the streptozotocin-injected rats to a bolus intraperitoneal insulin injection and the lower HOMA-IR suggest insulin insensitivity in these diabetic animals (Fig.

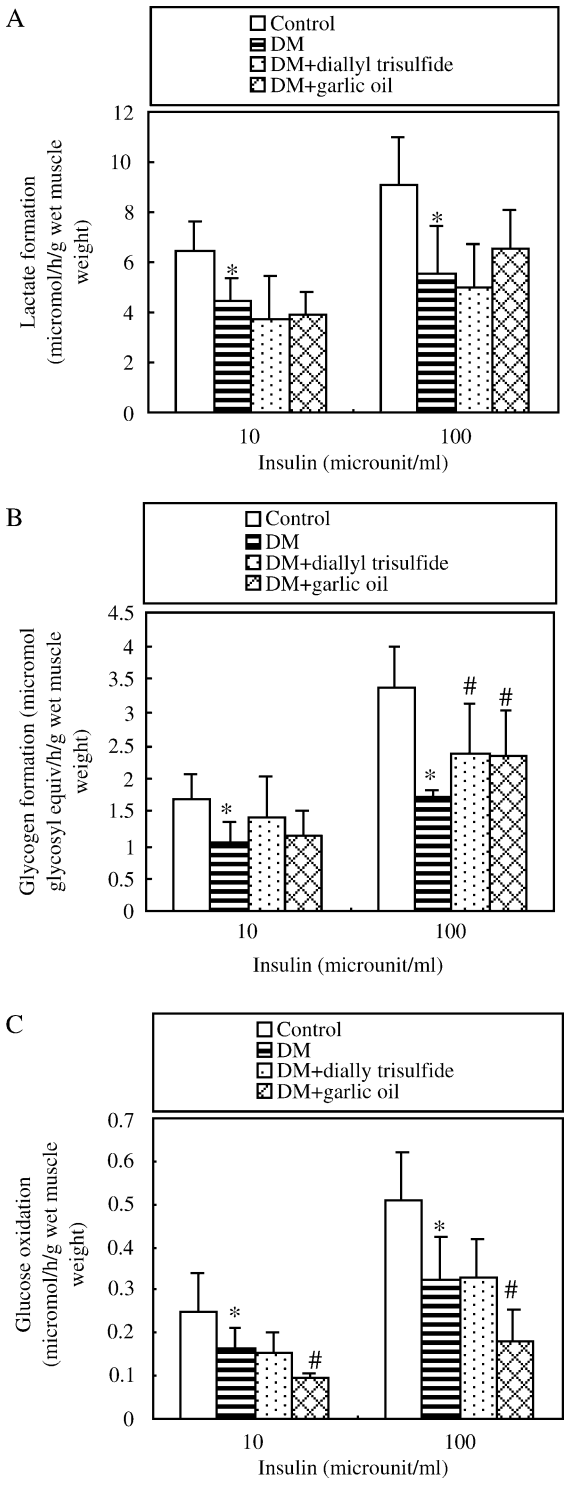

Fig. 3. Effect of treatment of diabetic rats with garlic oil or diallyl trisulfide on rates of glucose conversion to lactate (A), glycogen (B), and carbon dioxide (C) in the presence of 10 or 100 μU/ml of insulin. Muscles were isolated from diabetic rats or normal control rats. Data are means ± S.D. for six rats per group. *Significant difference between the control group and the DM group ($P<0.05$). #Significant difference between the garlic oil-treated or diallyl trisulfide-treated group and the DM group ($P<0.05$).

1). In addition, the impairment in glucose tolerance and the lowered insulin secretory capacity (Fig. 2) of the streptozotocin-injected rats indicate that the characteristics of our animals are comparable with those previously reported for streptozotocin-induced diabetes and with the characteristics of diabetes (Like and Rossini, 1976; Dall'Aglio et al., 1983; Rossetti et al., 1990).

Garlic oil was previously reported to lower fasting blood glucose concentrations in rats with streptozotocin-induced diabetes and in patients with diabetes (Begum and Bari, 1985; Farva et al., 1986; Anwar and Meki, 2003; Duncan, 1999). In the present study, however, we were unable to show the same effect with either garlic oil or diallyl trisulfide. We interpret the different results of our study as being partly due to differences in the way the garlic oil was prepared, in the route of administration, in the dose given, and in the duration of treatment. Although we did not find the fasting blood glucose concentration to be affected by either garlic oil or diallyl trisulfide, these two garlic compounds improved insulin sensitivity in the fasting state and after an intraperitoneal insulin bolus and improved the tolerance to an oral glucose challenge in the diabetic rats (Table 1; Figs. 1 and 2). Because insulin secretion during the oral-glucose-tolerance test was also higher with treatment with garlic oil and diallyl trisulfide, however, we cannot know whether the improved glucose tolerance was because the garlic oil and diallyl trisulfide increased insulin sensitivity and subsequently attenuated the deterioration of β-cell function or vice versa. A direct stimulatory effect of a garlic compound on the pancreas for the secretion of insulin has been shown only with *S*-allyl-cysteine sulfoxide, a precursor of garlic oil (Augusti and Sheela, 1996). The ability of garlic oil and diallyl trisulfide to act as secretagogues should be investigated further.

Raised nonesterified fatty acid concentrations in plasma have been proposed as a major cause of insulin resistance and may reduce the secretion of insulin in diabetes (Randle, 1998). In incubated pancreas islet cells from Zucker rats, it was reported that raised blood nonesterified fatty acid concentrations lowered the insulin secretion stimulated by high concentrations of glucose (Lee et al., 1994). In addition, raised blood nonesterified fatty acid concentrations were shown to be associated with reduced insulin sensitivity in rats (Kruszynska et al., 1991). Therefore, the improved in vivo glucose tolerance and hypoglycemic response to the insulin bolus in the garlic oil-treated diabetic rats may be partly explained by lowered nonesterified fatty acid concentrations in blood.

An additional beneficial effect of garlic oil and diallyl trisulfide on insulin secretion and insulin sensitivity may be via the antioxidant capacity of these compounds. Chronic hyperglycemia causes increased concentrations of reactive oxygen species and decreased enzymatic and nonenzymatic

cell antioxidant defenses (Catherwood et al., 2002; Bonnefont-Rousselot et al., 2000). Reactive oxygen species have been suggested to be involved in β-cell dysfunction and insulin resistance (Evans et al., 2003). With garlic oil prepared in the same manner as in the present study and with its three major organosulfur compounds (diallyl sulfide, diallyl disulfide, and diallyl trisulfide), Wu et al. (2001) showed an improved enzymatic antioxidant defense system in liver and red blood cells from normal rats. The greatest antioxidant effect among the three major organosulfur compounds was shown by diallyl trisulfide. Recently, garlic oil was also shown to reduce oxidative stress in streptozotocin-induced diabetes (Anwar and Meki, 2003).

Because garlic oil and diallyl trisulfide improve insulin sensitivity and oral glucose tolerance, one would expect the glycemic control of diabetic animals treated with these two compounds to improve. Such improvement may be reflected in the level of glycosylation of plasma proteins, which could reflect overall hyperglycemia during the elapsed 2 weeks, and is usually measured as the fructosamine concentration in plasma (Baker et al., 1985). A trend for improved glycemic control was shown in the concentration of plasma fructosamine in both treated diabetic groups; however, the decreased concentration found was not significant (Table 1). Our interpretation of this unexpected result is that the sample for fructosamine measurement was collected at the time the animals were killed, which was after the animals had experienced a bolus insulin injection, glucose loading, and three occurrences of overnight starvation over 2 weeks. This handling of the animals may have altered the glycation of protein.

Under the action of insulin, skeletal muscle mass is the main consumer of glucose in the body, and therefore skeletal muscle is the primary site responsible for the reduced insulin-induced glucose utilization in diabetic states (DeFronzo et al., 1982). In the presence of insulin, glucose utilized by skeletal muscle is oxidized and converted to glycogen and lactate. Streptozotocin-induced diabetes in rats has been reported to reduce basal and insulin-stimulated glucose utilization and lactate production in skeletal muscle in vitro, at least partly because of a lower glucose transporter-4 content and glucose transport rate and lower glycogen synthase activity in skeletal muscle (Maegawa et al., 1986; Munoz et al., 1996; Oku et al., 2000). As noted in the present study, streptozotocin-induced diabetes resulted in a lower conversion of exogenous glucose to lactate and glycogen in the soleus muscles. In addition, the conversion of glucose to carbon dioxide in insulin-stimulated states was suppressed.

In the presence of physiologic concentrations of insulin, the lactate production rate in skeletal muscles from diabetic rats treated with garlic oil or diallyl trisulfide was not significantly affected; however, the glycogen synthesis rate was significantly increased in both groups (Fig. 3). Because of the major contribution of muscle glycogen synthase activity on the regulation of insulin action (Cohen, 1987), the results of the present study suggest that garlic oil and diallyl trisulfide may have a regulatory effect on the activity of this enzyme. On the other hand, we found that treatment with diallyl trisulfide did not significantly affect the glucose oxidation rate in skeletal muscle in vitro, but garlic oil significantly lowered the rate in the presence of physiologic concentrations of insulin. This effect of garlic oil may be explained by data from George and Eapen (1974), who reported that garlic oil inhibits phosphorylation in mitochondria from the liver of mice, thus suppressing oxidative metabolism in the cells. Because the oxidation of glucose contributes relatively little to overall glucose utilization in skeletal muscle in the presence of physiologic concentrations of insulin, the improvement in the conversion of glucose to glycogen with garlic oil could play a major role in the insulin-stimulated glucose clearance in streptozotocin-induced diabetes found in the present study.

In conclusion, with the use of an streptozotocin-induced diabetes model in rats, we showed that treatment with garlic oil can improve glycemic control via significantly improved insulin sensitivity, glucose tolerance, insulin secretion, and improved glucose utilization by skeletal muscle in the presence of insulin. Diallyl trisulfide is an important functional component of the hypoglycemic effect of garlic oil.

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References

Agarwal, K.C., 1996. Therapeutic actions of garlic constituents. Med. Res. Rev. 16, 111–124.

Akinmokun, A., Selby, P.L., Ramaiya, K., Alberti, K.G.M.M., 1992. The short insulin tolerance test for determination of insulin sensitivity: a comparison with the euglycaemic clamp. Diabet. Med. 9, 432–437.

Anwar, M.M., Meki, A.-R., 2003. Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. Comp. Biochem. Physiol., A Mol. Integr. Physiol. 135, 539–547.

Augusti, K.T., 1996. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Indian J. Exp. Biol. 34, 634–640.

Augusti, K.T., Sheela, C.G., 1996. Antiperoxide effect of *S*-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia 52, 115–120.

Baker, J.R., Metcalf, P.A., Johnson, R.N., Newman, D., Rietz, P., 1985. Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. Clin. Chem. 31, 1550–1554.

Baluchnejadmojarad, T., Rohgani, M., 2003a. Endothelium-dependent and -independent effect of aqueous extract of garlic on vascular reactivity on diabetic rats. Fitoterapia 74, 630–637.

Baluchnejadmojarad, T., Rohgani, M., 2003b. Garlic extract attenuates time-dependent changes in the reactivity of isolated aorta in streptozotocin-diabetic rats. Life Sci. 73, 2281–2289.

Begum, H., Bari, M.A., 1985. Effect of garlic oil on the pancreas of experimental diabetes in guineapigs. Bangladesh Med. Res. Counc. Bull. 11, 64–68.

Bergmeyer, H.U., 1974. Enzymatic assay of glucose. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, New York, NY, pp. 1196–1201.

Bonnefont-Rousselot, D., Bastard, J.P., Jaudon, M.C., Delattre, J., 2000. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab. 26, 163–176.

Catherwood, M.A., Powell, L.A., Anderson, P., McMaster, D., Sharpe, P.C., Trimble, E.R., 2002. Glucose-induced oxidative stress in mesangial cells. Kidney Int. 61, 599–608.

Challiss, R.A., Espinal, J., Newsholme, E.A., 1983. Insulin sensitivity of rates of glycolysis and glycogen synthesis in soleus, stripped soleus, epitrochlearis, and hemi-diaphragm muscles isolated from sedentary rats. Biosci. Rep. 3, 675–679.

Chen, H.W., Tsai, C.W., Yang, J.J., Liu, C.T., Kuo, W.W., Lii, C.K., 2003. The combined effects of garlic oil and fish oil on the hepatic antioxidant and drug-metabolizing enzyme of rats. Br. J. Nutr. 89, 189–200.

Cohen, P., 1987. Role of multisite phosphorylation in the hormonal control of glycogen synthase from mammalian muscle. In: Drebs, E.G., Boyer, P.D. (Eds.), The Enzyme, vol. 18. Academic Press, Orlando, FL, pp. 462–499.

Crettaz, M., Prentki, M., Zaninetti, D., Jeanrenaud, B., 1980. Insulin resistance in soleus muscle from obese Zucker rats. Involvement of several defective sties. Biochem. J. 186, 525–534.

Dall'Aglio, E., Chang, F., Chang, H., Wright, D., Reaven, G.M., 1983. Effect of exercise training and sucrose feeding on insulin-stimulated glucose uptake in rats with streptozotocin-induced insulin-deficient diabetes. Diabetes 32, 165–168.

DeFronzo, R.A., Hendler, R., Simonson, D., 1982. Insulin resistance is a prominent feature of insulin-dependent diabetes. Diabetes 31, 781–795.

Dimitriadis, G., Leighton, B., Parry-Billings, M., Newsholme, E.A., 1988. Effects of the diuretic furosemide on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat. Diabetologia 31, 58–61.

Duncan, M.G., 1999. The effects of nutritional supplements on the treatment of depression, diabetes, and hypercholesterolemia in the renal patient. J. Renal Nutr. 9, 58–62.

Evans, J.L., Goldfine, I.D., Maddux, B.A., Grodsky, G.M., 2003. Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 52, 1–8.

Farva, D., Goji, I.A., Joseph, P.K., Augusti, K.T., 1986. Effects of garlic oil on streptozotocin-diabetic rats maintained on normal and high fat diets. Indian J. Biochem. Biophys. 23, 24–27.

George, K.C., Eapen, J., 1974. Mode of action of garlic oil-effect on oxidative phosphorylation in hepatic mitochondria of mice. Biochem. Pharmacol. 23, 931–936.

Guendet, G.S., Loten, E.G., Jeanrenaud, B., Renold, A.E., 1976. Decreased basal, noninsulin-stimulated glucose uptake and metabolism by skeletal soleus muscle isolated from obese-hyperglycemic (ob/ob) mice. J. Clin. Invest. 58, 1078–1088.

Hammerstedt, R.H., 1980. A rapid method for isolating glucose metabolites involved in substrate cycling. Anal. Biochem. 109, 443–448.

Jain, R.C., Vyas, C.R., 1975. Garlic in alloxan-induced diabetic rabbits. Am. J. Clin. Nutr. 28, 684–685.

Jain, R.C., Vyas, C.R., Mahatma, O.P., 1973. Hypoglycemic action of onion and garlic. Lancet 2, 1491 (letter).

Junod, A., Lambert, A.E., Stauffacher, W., Renold, A.E., 1969. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J. Clin. Invest. 48, 2129–2139.

Kasuga, S., Ushijima, M., Morihara, N., Itakura, Y., Nakata, Y., 1999. Effect of aged garlic extract (AGE) on hyperglycemia induced by immobilization stress in mice. Nippon Yakurigaku Zasshi 114, 191–197.

Kruszynska, Y.T., McCormack, J.G., McIntyre, N., 1991. Effect of glycogen stores and non-esterified fatty acid availability on insulin-stimulated glucose metabolism and tissue pyruvate dehydrogenase activity in the rats. Diabetologia 34, 205–211.

Lee, Y., Hirose, H., Ohneda, M., Johnson, J.H., McGarry, J.D., Unger, R.H., 1994. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte–beta-cell relationships. Proc. Natl. Acad. Sci. U. S. A. 91, 10878–10882.

Leighton, B., Budohoski, L., Lozeman, F.J., Challiss, R.A., Newsholme, E.A., 1985. The effect of prostaglandins E1, E2 and F2 alpha and indomethacin on the sensitivity of glycolysis and glycogen synthesis to insulin in stripped soleus muscles of the rat. Biochem. J. 227, 337–340.

Like, A.A., Rossini, A.A., 1976. Streptozotocin-induced pancreatic insulitis: a new model of diabetes mellitus. Science 139, 415–417.

Liu, C.T., Chen, H.W., Sheen, L.Y., Kung, Y.L., Chen, PC.H., Lii, C.K., 1998. Effect of garlic oil on hepatic arachidonic acid content and immune response in rats. J. Agric. Food Chem. 46, 4642–4647.

Maegawa, H., Kobayashi, M., Watanabe, N., Ishibashi, O., Takata, Y., Kitamura, E., Shigeta, Y., 1986. Effect of duration of diabetic state on insulin action in isolated rat soleus muscles. Metabolism 35, 499–504.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419.

Munoz, P., Chillaron, J., Camps, M., Castello, A., Furriols, M., Testar, X., Palacin, M., Zorzano, A., 1996. Evidence for posttranscriptional regulation of glucose transporter 4 expression in muscle and adipose tissue from streptozotocin-induced diabetic and benfluorex-treated rats. Biochem. Pharmacol. 52, 1665–1673.

Newsholme, E., Leighton, B., Challiss, J., Lozeman, F., 1986. Assessment of biochemical viability of isolated incubated muscle preparations. Biochem. J. 238, 621–622.

Oku, A., Ueta, K., Arakawa, K., Kano-Ishihara, T., Matsumoto, T., Adachi, T., Yasuda, K., Tsuda, K., Ikezawa, K., Saito, A., 2000. Correction of hyperglycemia and insulin sensitivity by T-1095, an inhibitor of renal Na^+-glucose cotransporters, in streptozotocin-induced diabetic rats. Jpn. J. Pharmacol. 84, 351–354.

Randle, P.J., 1998. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab. Rev. 14, 263–283.

Rossetti, L., Giaccari, A., DeFronzo, R.A., 1990. Glucose toxicity. Diabetes Care 13, 610–630.

Ryan, E.A., Pick, M.E., Marceau, C., 2001. Use of alternative medicines in diabetes mellitus. Diabet. Med. 18, 242–245.

Sheela, C.G., Augusti, K.T., 1992. Antidiabetic effects of *S*-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J. Exp. Biol. 30, 523–526.

Sheen, L.Y., Lin, S.Y., Tsi, S.J., 1992. Odor assessments for volatile compounds of garlic and ginger essential oils by sniffing method of gas chromatography. J. Chin. Agric. Chem. Soc. 30, 14–24.

Sheen, L.Y., Chen, H.W., Kung, Y.L., Liu, C.T., Lii, C.K., 1999. Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets. Nutr. Cancer 35, 160–166.

Staba, E.J., Lash, L., Staba, J.E., 2001. A commentary on the effects of garlic extraction and formulation on product composition. J. Nutr. 131, 1118S–1119S.

Swanston-Flatt, S.K., Day, C., Bailey, C.J., Flatt, P.R., 1990. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia 33, 462–464.

Tahiliani, P., Kar, A., 2003. Mitigation of thyroxine-induced hyperglycaemia by two plant extracts. Phytother. Res. 17, 294–296.

Wu, C.C., Sheen, L.Y., Chen, H.W., Tsai, S.J., Lii, C.K., 2001. Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. Food Chem. Toxicol. 39, 563–569.