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Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin-induced diabetes

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Abstract

We investigated the effects of garlic oil and diallyl disulfide (DADS) on glycemic control and renal function in rats with streptozotocin-induced diabetes. Rats received by gavage garlic oil (100 mg/kg body wt) or DADS (40 or 80 mg/kg body wt) every other day until 16 weeks after the induction of diabetes. The control rats were treated with corn oil only. Neither garlic oil nor DADS significantly affected fasting blood glucose concentrations throughout the investigation period. Garlic oil did not affect oral glucose tolerance in diabetes acutely but significantly improved oral glucose tolerance at 4, 8, 12, and 16 weeks and significantly ameliorated proteinuria at the end of 16 weeks. DADS did not significantly affect oral glucose tolerance or renal function. Diabetic rats fed 80 mg DADS/kg body wt had a significantly lower rate of body weight gain and a significantly lower ratio of muscle weight to body weight than did vehicle-treated diabetic rats. In conclusion, long-term treatment of diabetes with garlic oil can improve oral glucose tolerance and renal function in diabetes but not through the action of DADS. High doses of DADS may further complicate the metabolic disturbances in diabetes.

Keywords: Diallyl disulfide; Garlic oil; Glycemic control; Kidney; Rats; Streptozotocin

1. Introduction

Diabetes is a metabolic disturbance that gradually affects the function of various systems in the body. Poorly controlled blood glucose is believed to be the most important factor in the development of diabetic complications in both type 1 and type 2 diabetes (American Association of Diabetes Educators, 2002). The kidney has become the focus of investigation in studies of diabetic complications because many of the same factors are involved in the development of diabetic nephropathy and the development of other common diabetic complications, such as microvascular disease and retinopathy.

According to a 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. Garlic and garlic constituents prepared by various means have been shown to have diverse biological activities, including antitumorigenetic, anticarcinogenic, antiatherosclerotic, antithrombotic, and antidiabetic actions [see reviews by Agarwal (1996) and Augusti (1996)]. Although these biological activities are widely recognized, however, only certain disorders, such as cardiovascular disease and tumor growth, have been extensively investigated (Brace, 2002; Thomson and Ali, 2003). The number of studies of the hypoglycemic effect of garlic is limited, and the results of such studies are inconsistent.

Abbreviations: DADS, diallyl disulfide; iNOS, inducible nitric oxide synthase; STZ, streptozotocin.

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In the 1970s, Jain and coworkers (Jain et al., 1973; Jain and Vyas, 1975) investigated the 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 of 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). Recently, it was reported that garlic juice reversed hyperglycemia, and alleviated oxidative stress and damage in liver and kidney in alloxan-induced diabetic rats (El-Demerdash et al., 2005). In diabetic patients, it was reported that garlic oil can correct hyperglycemia (Duncan, 1999). In addition, a precursor of various allyl sulfide constituents of garlic oil, *S*-allyl-cysteine sulfoxide (alliin), was shown to have a hypoglycemic effect similar to that of glibenclamide (Sheela and Augusti, 1992). However, Swanston-Flatt and coworkers (Swanston-Flatt et al., 1990) failed to find a hypoglycemic effect of garlic powder in animals with streptozotocin (STZ)-induced diabetes. Similarly, Baluchnejadmojarad and Rohgani (2003a,b) found no hypoglycemic effect of an aqueous extract of garlic in STZ-induced diabetic rats, although 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, the duration of preparation, and the 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 (Liu et al., 1998 Sheen et al., 1999; Chen et al., 2003). Recently, we reported the hypoglycemic effect of garlic oil and its organosulfur compound, diallyl trisulfide, through increased insulin secretion and increased insulin sensitivity in STZ-induced diabetic rats (Liu et al., 2005). The aim of the present study was to investigate the short- and long-term effects of garlic oil and its major organosulfur compound, diallyl disulfide (DADS), on glycemic control and renal function in an animal model of diabetes.

2. Materials and methods

2.1. Reagents

Garlic oil was prepared as previously described (Sheen et al., 1992). Briefly, a steam distillation technique was used, and the final product contained major garlic oil essential components, including 38.6% DADS, 30.8% diallyl trisulfide, 10.0% dially sulfide, and minor amounts of many other volatile compounds. DADS was purchased from Aldrich Chemical Co. (Milwaukee, WI). Hexokinase/glucose-6-phosphate dehydrogenase was purchased from Boehringer Mannheim (E. Sussex, United Kingdom). STZ and other biochemical reagents were purchased from Sigma Chemical (St. Louis, MO). The commercial kits used to measure creatinine and blood urea nitrogen were from Wako (Tokyo, Japan). The protein assay kit used was from Bio-Rad (Hercules, CA).

2.2. Animals and treatment protocol

Six-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 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. The animals were treated in compliance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1985).

Diabetes was induced by injection of STZ (65 mg/kg body wt in citrate buffer, pH 4.5) in a lateral tail vein (Junod et al., 1969). Control rats were injected with the same volume of vehicle. Three days after the injection, diabetic animals were randomly assigned to four groups and received by gavage 100 mg/kg body wt garlic oil, 40 or 80 mg/kg body wt DADS, or vehicle (corn oil, 2 ml/kg body wt) every other day until 16 weeks after the induction of diabetes. The nondiabetic control rats received corn oil (2 ml/kg body wt). The dose of garlic oil used in the present study was in accordance with the finding by Chen et al. (2003), who carried out a study with garlic oil prepared the same way and reported dose-dependent antioxidant effects in rats fed 0–200 mg/kg three times a week for 6 weeks. A 40 mg/kg dose was used for DADS because in our garlic oil preparation this composition represent 40% of the constituents. Eighty-microgram/kg dose of DADS was also used in the present study to investigate whether there is dose-dependent antidiabetic effect of this garlic compound.

During the 16 weeks, 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). Body weight, food and water intakes, and urine excretion were measured. Fasting blood glucose concentrations were monitored at week one and week two and then every 2 weeks by sampling from a lateral tail vein.

The acute effect of the garlic compounds on oral glucose tolerance was determined 3 days after the induction of diabetes, when the rats were administered the garlic compounds for the first time. The chronic effect of the garlic compounds on oral glucose tolerance was determined at 4, 8, 12, and 16 weeks after the induction of diabetes. The animals were starved overnight before each test and before being killed by carbon dioxide euthanasia 3 days after the last oral-glucose-tolerance test. Urine collected during the last 24 h of the animal's life was used to measure concentrations of creatinine and protein. Blood collected immediately after the animals were killed was used to measure concentrations of creatinine and urea nitrogen. The creatinine clearance rate was calculated by using the standard formula. At the time the animals were killed, the kidney, soleus muscle, extensor digitorum longus muscle, and gastrocnemius muscle were isolated and weighed, and the ratio of organ tissue to body weight was calculated. Kidney weight was defined as the sum of the right and left kidney weight for each animal.

2.3. Oral-glucose-tolerance test

To measure the acute effect of the garlic compounds on oral glucose tolerance, a blood sample was withdrawn from the lateral tail vein immediately before the administration of the garlic compounds (−30 min), and glucose loading was carried out at 30 min afterward. To study the chronic effect of the garlic compounds on postprandial glycemic control, an oral-glucose-tolerance test was performed by administering by oral gavage a solution of 10% (w/v) glucose (1 g/kg body wt). Blood samples were withdrawn from a lateral tail vein immediately before (zero time) and 30, 60, 90, 120, and 180 min after the bolus glucose loading. Heparin-containing blood samples were centrifuged immediately at 500*g* for 10 min. Plasma obtained from each sample was stored at −20 °C until analyzed. Glucose concentrations in plasma were measured within one month. For the study of the chronic effect of the garlic compounds, the

area under the glucose concentration-by-time curve during the oral-glucose-tolerance test was calculated.

2.4. Biochemical analysis of blood and urine 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 with hexokinase/glucose-6-phosphate dehydrogenase as described by Bergmeyer (1974). The dilution factor during the deproteinization procedure was adjusted for the glucose concentration of each sample. Creatinine in plasma and urine and blood urea nitrogen in plasma were determined by a standardized method adapted to a Hitachi 747 autoanalyzer (Roche, Basel, Switzerland) with commercial kits. The creatinine clearance rate was calculated to determine the capacity of glomerular filtration. Total 24-h urinary protein was measured by using the Bradford assay with the use of bovine serum albumin to generate the standard curve (Bradford, 1976).

2.5. Statistical analysis

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

3. Results

3.1. Animal characteristics

Induction of diabetes with STZ was associated with the characteristic development of a slower rate of body weight gain, increased food and water intakes, and increased urine excretion. The STZ-injected animals maintained these characteristics during the 16-week investigation period (Fig. 1). Skeletal muscle weight was also lower in the diabetic animals than in the controls. However, the ratio of muscle mass to body weight was not significantly different between the control and diabetic groups (Table 1). Fasting blood glucose concentrations 1 week after the induction of diabetes were significantly higher in the diabetic animals than in the controls (266 ± 80 compared with 114 ± 49 mg/dl, respectively), and the fasting blood glucose concentration in the diabetic rats increased progressively to

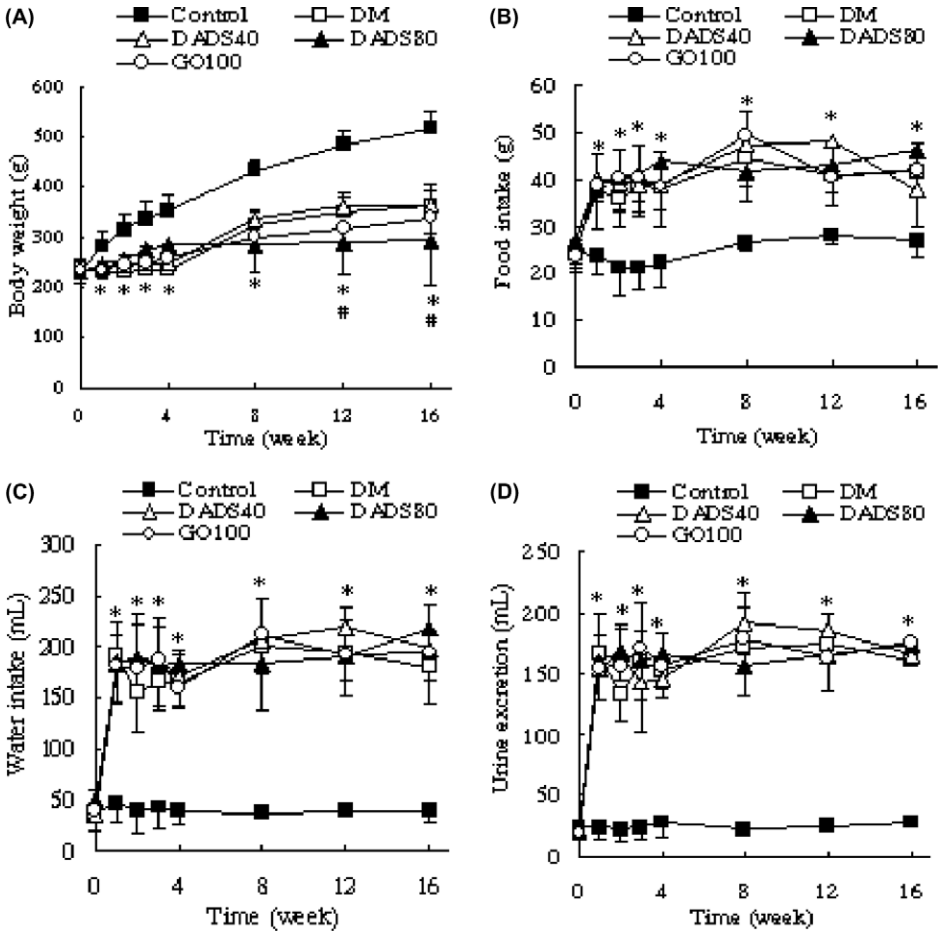

Fig. 1. Effect of treatment of control and diabetic rats with diallyl disulfide (DADS) or garlic oil (GO) on body weight (A), food intake (B), water intake (C), and urine excretion (D) during the 16-week treatment period. Data are the mean ± SD of six rats per group. DM, vehicle-treated diabetic rats; DADS40, diabetic rats treated with 40 mg DADS/kg body wt; DADS80, diabetic rats treated with 80 mg DADS/kg body wt; GO100, diabetic rats treated with 100 mg GO/kg body wt. *Significant difference between the DM group and the control group ($P < 0.05$). #Significant difference between the DADS80 group and the DM group ($P < 0.05$).

Table 1
Skeletal muscle weight of control rats and rats with streptozotocin-induced diabetes that did or did not receive diallyl disulfide (DADS) or garlic oil (GO)[a]

	Control	DM	DADS40	DADS80	GO100
Muscle weight (g)					
Soleus	0.243 ± 0.005	0.181 ± 0.048[b]	0.180 ± 0.024	0.129 ± 0.032[c]	0.150 ± 0.028
EDL	0.221 ± 0.017	0.149 ± 0.042[b]	0.123 ± 0.019	0.073 ± 0.025[c]	0.123 ± 0.019
Gastrocnemius	2.95 ± 0.12	1.99 ± 0.45[b]	1.68 ± 0.22	0.92 ± 0.33[c]	1.65 ± 0.21
Skeletal muscle weight/body weight × 100 (%)					
Soleus	0.0453 ± 0.0029	0.0462 ± 0.0047	0.0523 ± 0.0068	0.0485 ± 0.0063	0.0420 ± 0.0064
EDL	0.0410 ± 0.0020	0.0378 ± 0.0023	0.0356 ± 0.0043	0.0270 ± 0.0030[c]	0.0344 ± 0.0043
Gastrocnemius	0.547 ± 0.015	0.509 ± 0.020	0.487 ± 0.048	0.340 ± 0.055[c]	0.461 ± 0.035

[a] Values are the mean ± SD for six rats per group. DM, vehicle-treated diabetic rats; DADS40, diabetic rats treated with 40 mg DADS/kg body wt; DADS80, diabetic rats treated with 80 mg DADS/kg body wt; GO100, diabetic rats treated with 100 mg GO/kg body wt; EDL, extensor digitorum longus.
[b] Significantly different from the control group ($P < 0.05$).
[c] Significantly different from the DM group ($P < 0.05$).

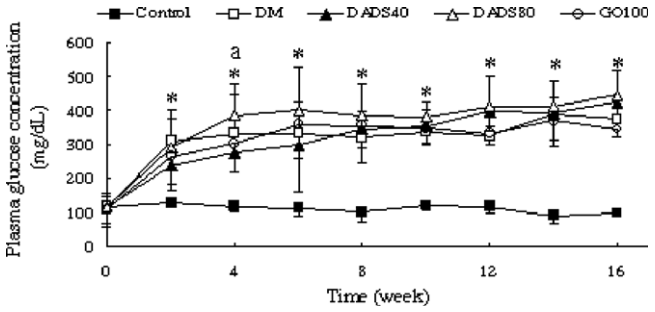

Fig. 2. Effect of treatment of control and diabetic rats with diallyl disulfide (DADS) or garlic oil (GO) on fasting blood glucose concentrations during the 16-week treatment period. Data are the mean ± SD of six rats per group. DM, vehicle-treated diabetic rats; DADS40, diabetic rats treated with 40 mg DADS/kg body wt; DADS80, diabetic rats treated with 80 mg DADS/kg body wt; GO100, diabetic rats treated with 100 mg GO/kg body wt. *Significant difference between the DM group and the control group ($P < 0.05$). [a]Significant difference between the DADS40 group and the DADS80 group ($P < 0.05$).

372 ± 68 mg/dl at the end of the investigation period (Fig. 2). Thus, the STZ-injected animals displayed characteristics typical of diabetes.

Compared with that in the vehicle-treated diabetic group, there was no significant effect of garlic oil or 40 mg DADS/kg on the rate of body weight gain, food or water intake, or urine excretion (Fig. 1). However, treatment with 80 mg DADS/kg resulted in a lower rate of body weight gain during weeks 12–16, although food and water intakes were not significantly affected. The ratio of skeletal muscle mass to body weight remained similar to that of the control and vehicle-treated diabetic rats in animals treated with garlic oil and 40 mg DADS/kg; however, this ratio was significantly lower in animals treated with 80 mg DADS/kg at the end of 16 weeks ($P < 0.05$; Table 1). Neither garlic oil nor DADS significantly affected fasting blood glucose concentrations (Fig. 2).

3.2. Oral-glucose-tolerance test

The acute effect of garlic oil and DADS on oral glucose tolerance was studied on day 3 after these compounds were administered to the animals for the first time (Fig. 3). Under fasting conditions, administration of the garlic compounds did not significantly affect blood glucose concentrations within 30 min. After oral glucose loading, blood glucose concentrations in the vehicle-, garlic oil-, and DADS-treated diabetic rats were all dramatically increased and were higher than those of the control animals (Fig. 3). Thus, there was no short-term effect of garlic oil or DADS on either fasting blood glucose concentrations or oral glucose tolerance in diabetic rats.

The chronic effects of the garlic compounds on oral glucose tolerance were studied at weeks 4, 8, 12, and 16 after the start of treatment. For the control rats and the diabetic rats with or without treatment with DADS, the plasma glucose concentration profile as a function of time during the oral-glucose-tolerance test was similar to the curves shown in Fig. 3 (data not shown). Treatment with garlic oil, however, significantly improved oral glucose tolerance in diabetic rats and brought the curve down to values between those of the diabetic and control rats.

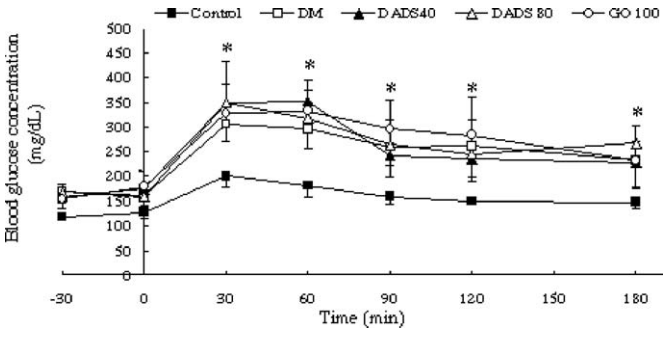

Fig. 3. Acute effect of diallyl disulfide (DADS) or garlic oil (GO) on oral glucose tolerance in control and diabetic rats. Fasting blood samples were drawn at −30 min. GO and DADS were administered orally immediately afterward. Thirty minutes after administration of the garlic compounds, blood was drawn again and the data are expressed as values for zero time. A glucose bolus (1 g/kg body wt) was administered orally immediately afterward. Data are the mean ± SD of six rats per group. DM, vehicle-treated diabetic rats; DADS40, diabetic rats treated with 40 mg DADS/kg body wt; DADS80, diabetic rats treated with 80 mg DADS/kg body wt; GO100, diabetic rats treated with 100 mg GO/kg body wt. *Significant difference between the DM group and the control group ($P < 0.05$).

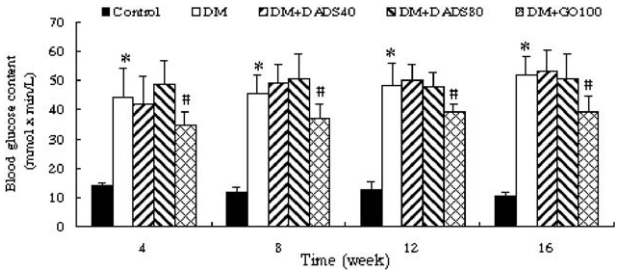

Fig. 4. Chronic effect of diallyl disulfide (DADS) or garlic oil (GO) on the plasma glucose response to an oral glucose bolus in control and diabetic rats. A glucose bolus (1 g/kg body wt) was administered orally 4, 8, 12, and 16 weeks after the induction of diabetes. Data are calculated from the area under the curve for the oral-glucose-tolerance test and are shown as the mean ± SD of six rats per group. DM, vehicle-treated diabetic rats; DADS40, diabetic rats treated with 40 mg DADS/kg body wt; DADS80, diabetic rats treated with 80 mg DADS/kg body wt; GO100, diabetic rats treated with 100 mg GO/kg body wt. *Significant difference between the DM group and the control group ($P < 0.05$). #Significant difference between the GO100 group and the DM group ($P < 0.05$).

Integral blood glucose values from the area under the curve during the oral-glucose-tolerance test were calculated and are given in Fig. 4. The area under the glucose tolerance curve was greater in the vehicle-treated diabetic group than in the control group throughout the investigation period ($P < 0.05$). Glucose tolerance in diabetes was improved by garlic oil at 4 weeks after the treatment and remained constant over the rest investigation period ($P < 0.05$) but glucose tolerance was not improved by treatment with DADS.

3.3. Renal function indexes

As shown in Table 2, the higher ratio of kidney weight to body weight in the vehicle-treated diabetic animals than in the controls indicated the development of kidney hypertrophy in the diabetic animals ($P < 0.05$). Creatinine clearance was 54.1% higher and blood urea nitrogen was 57.6% higher in the vehicle-treated diabetic animals than in the controls (Table 2). The higher creatinine clearance rate in the diabetic animals suggests hyperfiltration in these animals. Although neither garlic oil nor 40 mg DADS/kg significantly affected the elevated creatinine clearance rate in the diabetic animals, treatment with 80 mg DADS/kg did result in a rate that was significantly lower than that in the vehicle-treated diabetic animals (Table 2). Neither garlic oil nor DADS significantly affected blood urea nitrogen in diabetes. STZ injection resulted in dramatically high urinary excretion of total protein at week 16. Garlic oil but not DADS ameliorated the excretion of protein in urine ($P < 0.05$). Additionally, the ratio of urinary protein to urinary creatinine was 38.4% lower in the diabetic rats treated with garlic oil than in those treated with the vehicle (NS; Table 2).

4. Discussion

STZ is a broad-spectrum antibiotic from *Streptomyces achromogenes* (Weiss, 1982). Since the finding that STZ possesses diabetogenic properties mediated by pancreatic beta cell destruction, this compound has been widely used to induce diabetes in experimental animals (Junod et al., 1969; Like and Rossini, 1976). 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). Garlic oil was previously reported to lower fasting blood glucose concentrations in rats with STZ-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 DADS. 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 DADS throughout the investigation period, neither did we find these two garlic compounds to affect oral glucose tolerance

Table 2
Ratio of kidney weight to body weight (KW/BW), creatinine clearance rate (CCR), blood urea nitrogen (BUN), urinary protein, and ratio of urinary protein to creatinine at week 16 for control rats and rats with streptozotocin-induced diabetes that did or did not receive diallyl disulfide (DADS) or garlic oil (GO)[a]

	Control	DM	DADS40	DADS80	GO100
KW/BW (%)	0.76 ± 0.02	1.04 ± 0.15[b]	1.12 ± 0.08	1.32 ± 0.21	1.06 ± 0.16
CCR (ml/min)	2.29 ± 0.26	3.53 ± 0.18[b]	3.62 ± 0.40	2.43 ± 0.63[c]	3.24 ± 0.32
BUN (mg/dl)	16.5 ± 0.7	26.0 ± 4.5[b]	26.3 ± 2.3	27.5 ± 4.9	23.0 ± 2.6
Urinary protein (mg/24 h)	20.8 ± 12.2	125.2 ± 50.5[b]	107.0 ± 47.1	87.6 ± 31.9	62.8 ± 42.5[c]
Protein/creatinine (mg/mmol)	170.5 ± 77.1	465.8 ± 236.0[b]	383.0 ± 199.3	450.3 ± 250.2	287.0 ± 168.8

[a] Values are the mean ± SD for six rats per group. DM, vehicle-treated diabetic rats; DADS40, diabetic rats treated with 40 mg DADS/kg body wt; DADS80, diabetic rats treated with 80 mg DADS/kg body wt; GO100, diabetic rats treated with 100 mg GO/kg body wt.
[b] Significantly different from the control group ($P < 0.05$).
[c] Significantly different from the DM group ($P < 0.05$).

acutely. In contrast, the oral-glucose-tolerance test carried out 4–16 weeks after the induction of diabetes showed that long-term treatment with garlic oil significantly improved glucose tolerance. These results suggest that garlic oil could be used as an adjuvant for glycemic control in diabetes. Long-term treatment of diabetes with DADS did not have a significant effect on oral glucose tolerance, which suggests that the garlic oil did not act via this compound.

The present study showed that fasting blood glucose concentrations were dramatically elevated 3 days after STZ administration and continued to increase, but at a relatively slower rate, in vehicle-treated diabetic animals throughout the investigation period. The animals in this group also showed progressively deteriorating oral glucose tolerance during the investigation period. Garlic oil improved glucose tolerance in the diabetic animals. Although a direct stimulatory effect of a garlic compound on the pancreas for the secretion of insulin has been shown with *S*-allyl-cysteine sulfoxide, which is a precursor of garlic oil (Augusti and Sheela, 1996), it is unlikely that garlic oil stimulates insulin secretion directly, because no acute effect of garlic oil on either fasting blood glucose or glucose tolerance was found in the present study.

The results of some studies suggest that hyperglycemia may be a proinflammatory state (Pickup and Crook, 1998; Festa et al., 2000). An elevation of the inflammatory cytokines interleukin 6 and tumor necrosis factor α in plasma was shown in persons with uncontrolled diabetes, in healthy volunteers who underwent a hyperglycemic clamp procedure (Pickup et al., 2000; Esposito et al., 2002), and in isolated human monocytes challenged with a high concentration of glucose (Shanmugam et al., 2003). These cytokines may contribute to peripheral insulin resistance and to the beta cell damage in diabetes in part through the induction of expression of inducible nitric oxide synthase (iNOS) and nitric oxide production in peripheral tissue and islet beta cells (Sandler et al., 1990; Lang et al., 1992; Hotamisligil et al., 1993, 1994; Kapur et al., 1997; Sprangers et al., 1998; Kwon et al., 1999). In addition, chronic hyperglycemia causes elevated concentrations of reactive oxygen species accompanied by lowered enzymatic and nonenzymatic cell antioxidant defenses (Bonnefont-Rousselot et al., 2000; Catherwood et al., 2002). Reactive oxygen species have been suggested to be involved in beta cell dysfunction and insulin resistance (Evans et al., 2003).

Although it is not clear how garlic oil would affect the production of these inflammatory cytokines, in one of our other studies, we found that garlic oil can suppress the synthesis of nitric oxide via the inhibition of iNOS expression in LPS-activated Raw 264.7 macrophages in vitro (unpublished observations, 2004). In addition, with garlic oil prepared in the same manner as in the present study, Wu et al. (2001) showed an improved enzymatic antioxidant defense system in liver and red blood cells from normal rats. Recently, garlic oil was shown to reduce oxidative stress in STZ-induced diabetes (Anwar and Meki, 2003). Thus, we propose that the beneficial effect of long-term treatment with garlic oil on glycemic control was at least partly due to an inhibitory effect on iNOS expression and an antioxidative effect. Whether garlic oil has any effect on the production of inflammatory cytokines in diabetes is a topic for further study.

The clinical course of diabetic nephropathy includes an initial increase in the glomerular filtration rate, a thickening of the glomerular basement membrane, an expansion of the mesangium, microalbuminuria, proteinuria, and eventually a decline in glomerular filtration (Viberti et al., 1996). In the present study, renal hypertrophy was indicated by higher kidney weights in the diabetic rats. Our results also showed hyperfiltration and higher urinary protein excretion in the vehicle-treated diabetic rats than in the controls at the end of 16 weeks. These findings are consistent with those reported in STZ-injected rats and in diabetes (Ding et al., 2003; Viberti et al., 1996). The pathogenesis of diabetic nephropathy has been shown to be associated with an elevated accumulation of glycosylation products, elevated inflammatory cytokines, elevated iNOS expression, and elevated oxidative stress in the kidney (Forbes et al., 2003; Mora and Navarro, 2004; Trachtman et al., 2002; Ha and Kim, 1999). The present study found that treatment with garlic oil partially ameliorated renal function in diabetic rats at 16 weeks: garlic oil significantly reduced urinary protein excretion and marginally improved renal function in terms of creatinine clearance, blood urea nitrogen and protein/creatinine ratio. The above-mentioned effects of garlic oil on glycemic control combined with the improved blood glucose tolerance may at least partially explain the ameliorated renal function in the garlic oil-treated diabetic rats. Because systemic hypertension also contributes to the development of diabetic nephropathy via associated glomerular hypertension (Parving et al., 1999), it is also possible that garlic oil may benefit renal function in diabetes through the known regulatory effect of garlic on blood pressure (Brace, 2002).

The present study found no preventive effect of DADS on hyperglycemia or the development of nephropathy in STZ-injected rats. Furthermore, our results suggest that high doses of DADS are toxic in diabetic animals. This is noted by the finding that the rats treated with 80 mg DADS/kg had a significantly lower rate of body weight gain and a significantly lower ratio of muscle mass to body weight than did the vehicle-treated animals and the highest fasting blood glucose concentration of all the diabetic groups. Thus, not only did DADS fail to act as a hypoglycemic compound, but the toxic effect of long-term treatment with high doses of DADS may even complicate the metabolic problems in diabetes. The adverse effect of excessive consumption of the organosulfur compounds of garlic has been addressed (Amagase et al., 2001); however, systematic study of the underlying mechanisms of the cytotoxicity of DADS has been carried out only with certain tumor cell lines (Hong et al., 2000; Kwon et al., 2002; Bottone et al., 2002; Sakamoto et al., 1997). How DADS affects

metabolism in diabetes remains unclear and will require further investigation.

In conclusion, the results of the present study show that long-term treatment with garlic oil can improve oral glucose tolerance and renal function and suggest that garlic oil may be a useful supplemental remedy in diabetes. DADS was shown to not be the compound responsible for garlic oil's effects on diabetes, and our results suggest that high doses of DADS may further exacerbate the metabolic disturbances in diabetes.

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