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Cardiac Contractile Dysfunction and Apoptosis in Streptozotocin-Induced Diabetic Rats Are Ameliorated by Garlic Oil Supplementation

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Previous studies have suggested that garlic oil could protect the cardiovascular system. However, the mechanism by which garlic oil protects diabetes-induced cardiomyopathy is unclear. In this study, streptozotocin (STZ)-induced diabetic rats received garlic oil (0, 10, 50, or 100 mg/kg of body weight) by gastric gavage every 2 days for 16 days. Normal rats without diabetes were used as control. Cardiac contractile dysfunction examined by echocardiography and apoptosis evaluated by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay were observed in diabetic rat hearts. Additionally, a shift in cardiac myosin heavy chain (MHC) gene expression from α- to β-MHC isoform, decreased levels of superoxide dismutase-1 (SOD-1) and cardiac α-actin, and elevated cardiac thiobarbituric acid reactive substances (TBARS) and caspase- and p38-NFκB-leading apoptosis signaling activities were demonstrated in diabetic hearts. However, these diabetes-related cardiac dysfunctions were almost dose-dependently ameliorated by garlic oil administration. In conclusion, garlic oil possesses significant potential for protecting hearts from diabetes-induced cardiomyopathy.

KEYWORDS: Diabetes-induced cardiomyopathy; echocardiography; oxidative stress; superoxide dismutase; garlic oil

INTRODUCTION

Diabetes mellitus is one of the major risk factors for the development of cardiovascular disease, accounting for 80% of all diabetic mortality. The mortality of cardiac disease in patients with diabetes is 2−4-fold higher than that in subjects without diabetes. The destruction of cardiac function has been well documented in diabetes. Several pathologic processes may initiate myocyte injury and dysfunction in patients with diabetes (*1*).

Hyperglycemia, resulting from either insulin deficiency in type 1 diabetes or insulin resistance in type 2 diabetes, induces the production of reactive oxygen species (ROS), which is the major cause of diabetic myocardial injury. Due to low content of free radical scavengers, the heart is susceptible to being damaged by ROS. The harmful effects of oxidative stress on the diabetic heart include abnormal gene expression, altered signal transduction, and activation of pathways leading to programmed myocardial cell death (*2*). Several antioxidant enzymes were identified to be decreased in the diabetic heart, due to hyperglycemia, by an oxidative mechanism found in both rats and humans. Interestingly, the exogenous or insulin-mediated antioxidant ability can inhibit this rise in oxidative stress, indicating a possible beneficial effect of antioxidants on preventing diabetic cardiomyopathy (*3*,*4*).

Although apoptosis has long been considered a mechanism for the elimination of redundant cells, it has only recently been recognized as a process of inhibition of cell proliferation. In fact, apoptosis is involved in mechanisms of many human diseases,

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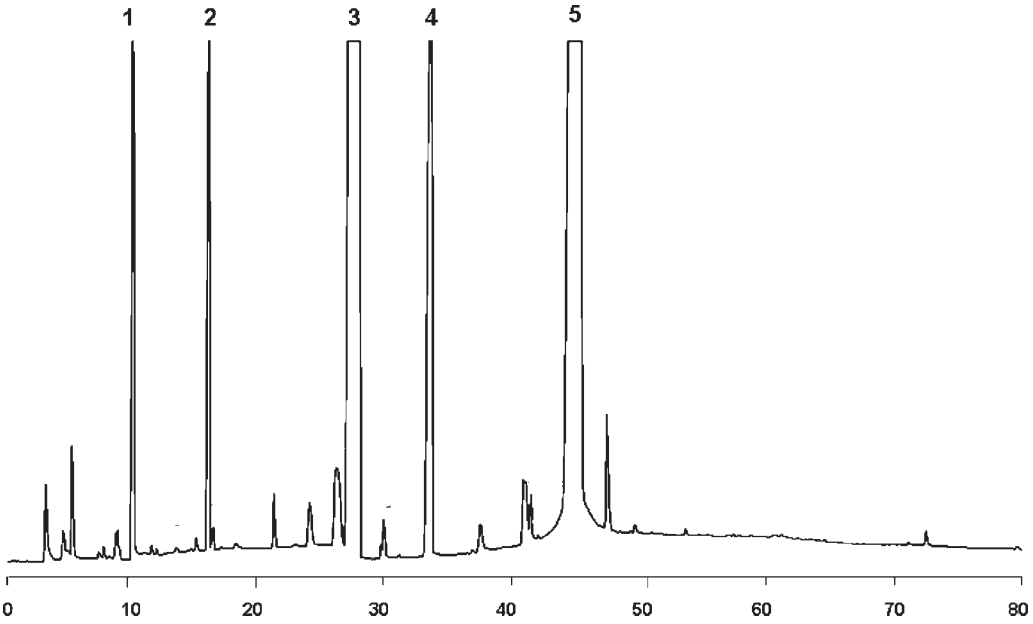

Figure 1. Analysis profile of garlic oil by gas chromatography. Diallyl sulfide (peak 1), methyl allyl disulfide (peak 2), diallyl disulfide (peak 3), methyl allyl trisulfide (peak 4), and diallyl trisulfide (peak 5) were quantified at 3.77, 2.75, 40.83, 7.17, and 38.93% in garlic oil, respectively.

Table 1. Physiological and Echocardiographic Parameters in Rat Hearts[a]

		DM			
	control	GO-0	GO-10	GO-50	GO-100
At Basal Level					
BW, g	187.1 ± 14.4	155.0 ± 14.1*	137.5 ± 17.5*	165.0 ± 15.0*	152.5 ± 12.5*
blood glucose, mg/dL	74.6 ± 7.5	266.2 ± 59.8*	205.0 ± 42.2*	266.8 ± 47.5*	220.3 ± 49.7*
HR, beats/min	414.7 ± 10.7	403.0 ± 22.8	399.4 ± 30.0	417.8 ± 39.4	394.4 ± 21.2
LVEDD, mm	5.72 ± 0.55	5.27 ± 0.40	6.12 ± 0.78	5.25 ± 0.40	6.01 ± 0.63
LVESD, mm	2.63 ± 0.27	2.63 ± 0.05	2.93 ± 0.54	2.70 ± 0.30	3.07 ± 0.31
FS, %	51.0 ± 2.2	49.6 ± 3.8	49.9 ± 4.2	49.0 ± 2.0	49.8 ± 4.1
EF, %	87.7 ± 1.0	85.7 ± 2.8	85.7 ± 3.6	85.4 ± 1.7	85.6 ± 3.4
CO, L/min	0.20 ± 0.03	0.19 ± 0.05	0.20 ± 0.04	0.19 ± 0.05	0.19 ± 0.02
After 16 Days of Feeding of Different Doses of Garlic Oil					
BW, g	300.0 ± 13.1	205.0 ± 15.0*	162.5 ± 27.5*	200.0 ± 30.0*	210.0 ± 20.0*
blood glucose, mg/dL	68.9 ± 9.10	425.0 ± 35.4*	367.0 ± 52.0*	406.0 ± 13.0*	425.0 ± 35.0*
HR, beats/min	407.0 ± 13.3	291.0 ± 14.2*	288.0 ± 6.1*	320 ± 0.9*	335.0 ± 3.1*,†
LVEDD, mm	6.58 ± 0.18	6.83 ± 0.38	5.47 ± 0.15*,†	6.87 ± 0.32	6.56 ± 0.15
LVESD, mm	2.78 ± 0.14	3.83 ± 0.29*	2.60 ± 0.12†	3.80 ± 0.06*	3.05 ± 0.03†
FS, %	58.1 ± 1.40	44.0 ± 3.3*	43.7 ± 1.8*	52.2 ± 1.3†	53.4 ± 1.5†
EF, %	91.5 ± 0.90	80.2 ± 3.5*	80.1 ± 1.8*	87.8 ± 1.0†	88.5 ± 1.0†
CO, L/min	0.24 ± 0.19	0.10 ± 0.04*	0.10 ± 0.03*	0.19 ± 0.03†	0.20 ± 0.01†

[a] GO, garlic oil; HR, heart rate; BW, body weight; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; FS, fractional shortening; EF, ejection fraction; CO, cardiac output; LVM, left ventricular mass; TL, tibia length. The percentage of LV fractional shortening (FS, %) was calculated as [(LVEDD − LVESD)/LVEDD] × 100 (%). Left ventricular end diastolic volume (LVESV) and left ventricular end systolic volume (LVESV) were used to calculate ejection fraction (EF, %) = [(LVEDV − LVESV)/LVEDV] × 100 (%). Cardiac output (CO) was calculated as stroke volume × heart rate (L/min). Results are mean ± SE of six rats per group. GO-0, -10, -50, and -100 represent doses of 0, 10, 50, and 100 mg of garlic oil/kg of body weight, respectively. *, $P < 0.05$ compared with control rats; †, $P < 0.05$ compared with GO-0 group rats.

including cardiovascular disorders, such as diabetic cardiomyopathy (*5*). Apoptosis contributes to loss of cardiomyocytes in cardiomyopathy, or progressive dysfunctions in the left ventricle, and is recognized as a predictor of adverse outcomes in subjects with failing hearts (*6*). Therefore, the assessment of the apoptosis process could be a good way to predict the development of heart failure induced by diabetes, and the specificity of the related signaling pathways involved in apoptosis must be evaluated.

Garlic (*Allium sativum*) has played an important dietary as well as medicinal role for centuries. Due to its exhibition of inhibiting enzymes involved in lipid synthesis, decreasing platelet aggregation, preventing lipid peroxidation of oxidized erythrocytes and LDL, increasing antioxidant status, and inhibiting angiotensin-converting enzyme (*7*), even today the medicinal use of garlic is widespread and growing. The main property of garlic for therapeutic effects is from the effective antioxidant activity against oxidative damage in cardiovascular diseases (*8*). In addition, according to Ryan's paper (*9*), garlic is also the most commonly used alternative medication of diabetic patients, and it was also reported garlic can improve hyperglycemia in diabetic patients (*10*, *11*). However, information about the effect of garlic on diabetic heart functions and related mechanisms is very limited.

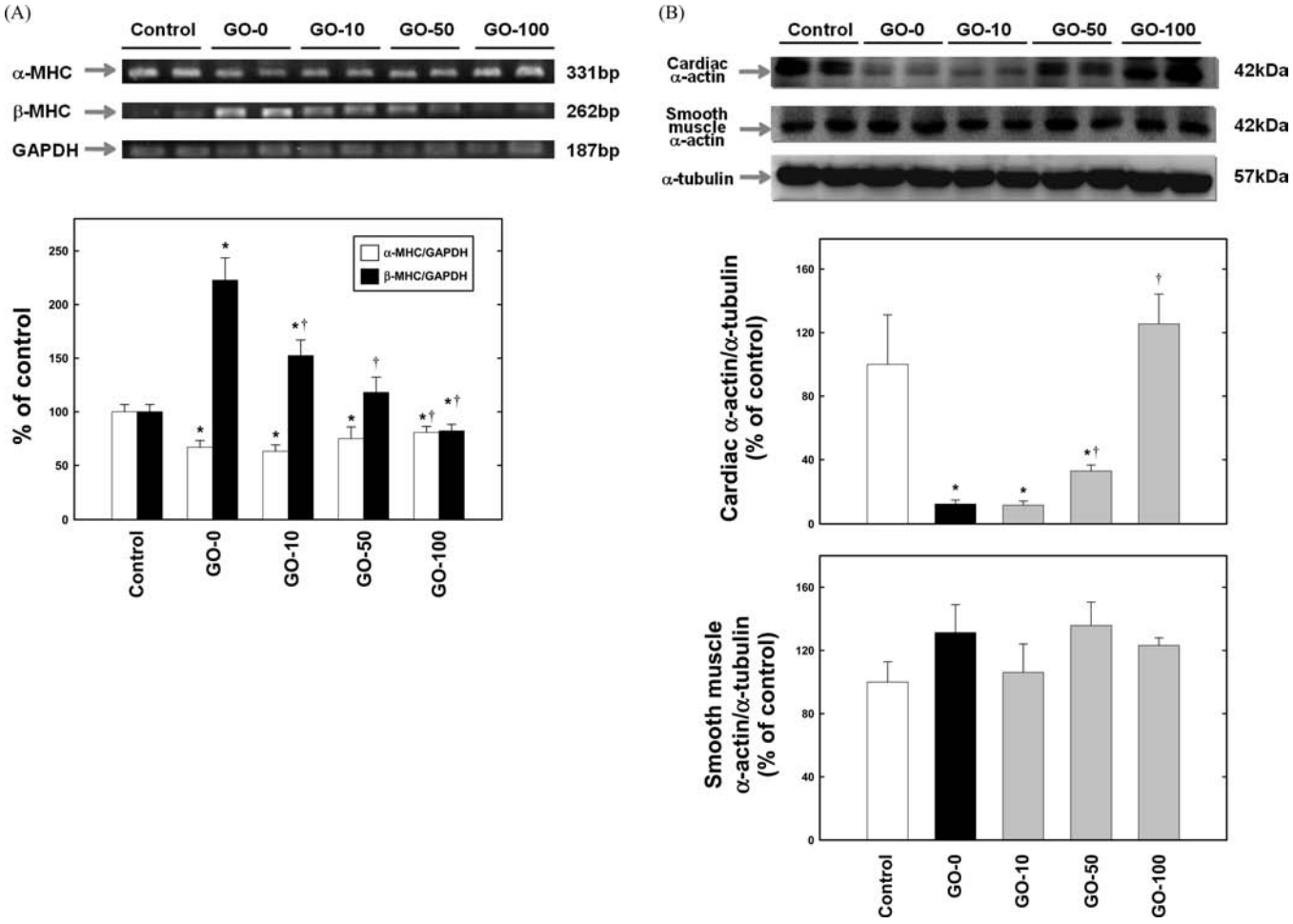

Figure 2. (**A**) Expression of α-MHC and β-MHC, analyzed by semiquantitative RT-PCR. GAPDH was used as a loading control. (**B**) Expression of α-actin of cardiac and smooth muscle analyzed by Western blotting. α-Tubulin was used as a loading control. Signal intensity was quantitated using a PhosphoImager. Values are expressed as a percentage of the control group. The average result ± SE of three independent experiments is shown. GO-0, -10, -50, and -100 represent doses of 0, 10, 50, and 100 mg of garlic oil/kg of body weight, respectively. ∗, $P < 0.05$ compared with control rats; †, $P < 0.05$ compared with GO-0 group rats.

Therefore, in the present study, we hypothesize that cardiac contractile dysfunction and apoptosis induced by diabetes can be alleviated by garlic oil supplementation. This study examined the cardiac contractile function, oxidative stress-related proteins, myofibrillar formation, apoptosis, and related signaling activities in rats with streptozotocin (STZ)-induced diabetes and the effects of garlic oil treatment on this diabetes-related cardiac dysfunction.

MATERIALS AND METHODS

Materials. Fresh garlic (*A. sativum*) was purchased from the local market, and garlic oil was prepared by steam distillation (*12*). The final product was analyzed and identified using a GC-MS system (G1800 GCD, Hewlett-Packard, Palo Alto, CA), which was processed as described (*13*). The constituent profile of the garlic oil is shown in **Figure 1**. The major essential components include diallyl disulfide (DADS), diallyl trisulfide (DATS), diallyl sulfide (DAS), and minor amounts of many other volatile compounds. The monoclonal antibody against caspase 3 was purchased from Cell Signaling Technology Inc. (Beverly, MA), and polyclonal antibodies against cytochrome *c*, superoxide dismutase (SOD)-1, α-actin, caspases 8 and 9, and truncated BH3 interacting domain death agonist (tBid) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

Animal Model and Treatments. Male Wistar rats (4 weeks old) were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). The animals were kept under a 12 h light−dark cycle, and ambient temperature was maintained at 25 °C. Animals were given free access to water and standard laboratory chow (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO). Housing conditions and experimental procedures were performed according to the *NIH Guide for the Care and Use of Laboratory Animals*, and all protocols were approved by the Institutional Animal Care and Use Committee of China Medical University, Taichung, Taiwan. After 1 week of acclimatization, diabetes was induced by injection of STZ (65 mg/kg of body weight (BW) in citrate buffer, pH 4.5) into a lateral tail vein. At 3 days after injection, glycemia was measured with the Accu-Check Compack kit (Roche Diagnostics Gmbh, Mannheim, Germany). Only animals in which hyperglycemia had been successfully induced were randomly separated into four groups ($n = 6$) and fed 10, 50, and 100 mg/kg of BW garlic oil or vehicle (corn oil, 2 mL/kg of BW) every other day for 16 days. The other normoglycemic control animals ($n = 6$) were fed corn oil (2 mL/kg oF BW). After 16 days of treatment, all animals were anesthetized and echocardiography was performed. Then, they were sacrificed, and their hearts were removed for further analysis.

In Vivo Cardiac Function. Transthoracic echocardiograms were performed at heart rates of 300−450 beats per minute in rats anaesthetized with isoflurane mixed with O_2 at flow rate of 5 psi before and 16 days after the garlic oil feeding by an echo machine (Vivid *i*, 10S transducer, GE Medical Systems, Milwaukee, WI) using a 4−11 MHz phase-array transducer. M-mode images were obtained in the parasternal long- and short-axis views of the left ventricle.

TUNEL Assay. All of the procedure was described in our previous study (*14*). The 3 μm thick paraffin sections were deparaffinized. The number

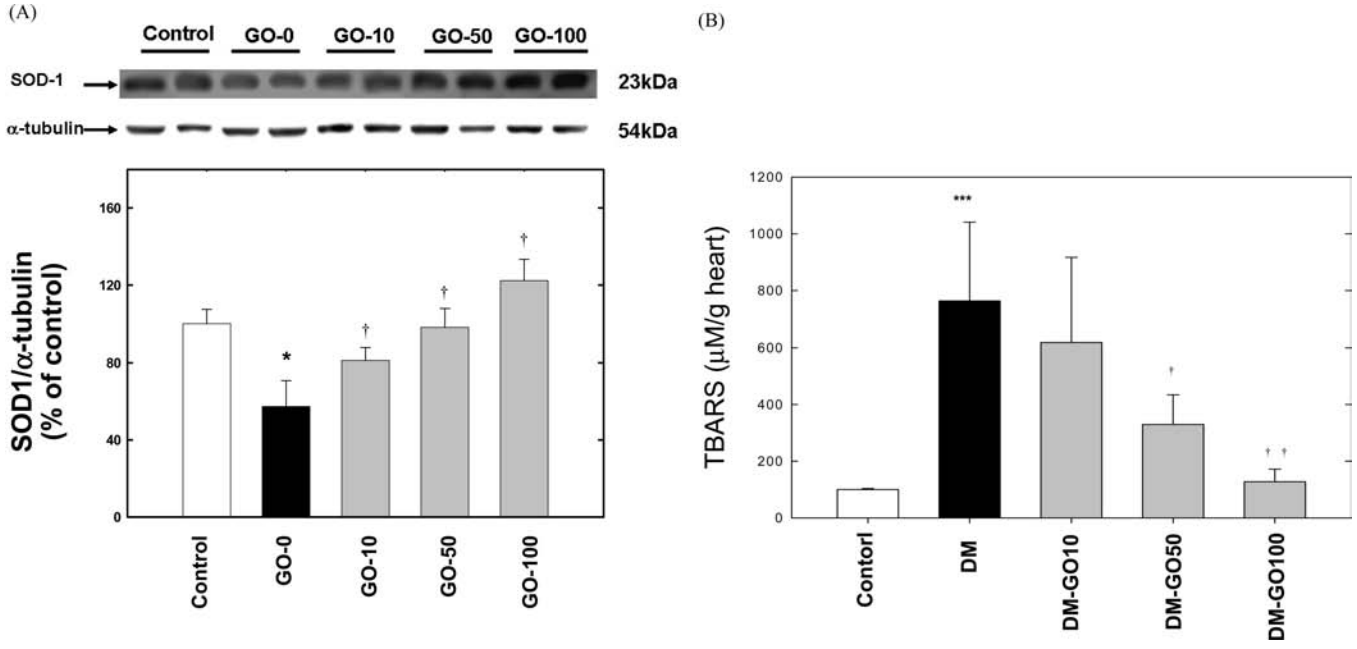

Figure 3. (**A**) Expression of superoxide dismutase-1 (SOD-1) of cardiac muscle analyzed by Western blotting. Signal intensity was quantitated using a PhosphoImager. α-Tubulin was used as a loading control. Values are expressed as a percentage of control group. (**B**) Effect of garlic oil feeding on TBARS in diabetic rat hearts. The average result ± SE of three independent experiments is shown. GO-0, -10, -50, and -100 represent doses of 0, 10, 50, and 100 mg of garlic oil/kg of body weight, respectively. ∗, $P < 0.05$ compared with control rats; †, $P < 0.05$ compared with GO-0 group rats.

of TUNEL-positive cardiac myocytes was determined. All morphometric measurements were performed by at least three independent individuals in a blinded manner.

Determination of the Thiobarbituric Acid Reactive Substance (TBARS). One milliliter of the tisuue homogenates was mixed with 1 mL of 10% trichloroacetic acid, centrifuged at 10000*g* for 10 min. One milliliter of the supernatant was mixed with 1 mL of 0.4% thiobarbituric acid (TBA) reagent in 0.2 N HC1 and 0.1 mL of 0.2% BHT in 95% ethanol. After an incubation at 50 °C for 1 h, the mixtures were cooled, the TBA−malondialdehyde (MDA) adduct was extracted with 3 mL of isobutanol, and the fluorescence was measured with excitation at 515 nm and emission at 550 nm. 1,1,3,3-Tetramethoxypropane (Sigma Chemical, St. Louis, MO) was used as the standard for the determination.

Tissue Extraction. The left ventricle samples were homogenized for protein extract in a PBS buffer (0.14 M NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 14 mM K_2HPO_4) at a concentration of 1 mg of tissue/10 μL of PBS for 5 min. The homogenates were centrifuged at 12000 rpm for 30 min. Then, supernatant was collected for further analysis.

Electrophoresis and Western Blot. The protein content of cardiac tissue extract was analyzed using the Bradford protein assay. Extracted proteins were then separated in 12% gradient SDS-PAGE and transferred to nitrocellulose membranes. All of the procedure was described in our previous study (*15*). Nonspecific protein binding was stopped in blocking buffer (5% milk, 20 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) and blotted with specific first antibodies in the blocking buffer at 4 °C overnight. For repeated blotting, nitrocellulose membranes were stripped with Restore Western blot stripping buffer (Pierce Biotechnology, Inc., Rockford, IL) at room temperature for 30 min. Signal intensity was quantitated using a PhosphoImager. α-Tubulin was used as a loading control.

RT-PCR. Total RNA was extracted using the Ultraspec RNA Isolation System (Biotecx Laboratories, Inc.) according to the instructions of the manufacturer. All of the procedure was described in our previous study (*15*). The cDNA was amplified by PCR with α-MHC primers, forward primer (5′-GGCAG ATATG AAGGG AAGAT-3′) and reverse primer (5′-CGAAC ATGTG GTGGT TGAAG-3′); β-MHC primers, forward primer (5′-CTTCA ACCAC CACAT GTTCG-3′) and reverse primer (5′-TATTG TAGTC CACGG TGCCA-3′); and GAPDH primers, forward primer (5′-TCCCT CAAGA TTGTC AGCAA-3′) and reverse primer (5′-AGATC CACAA CGGAT ACATT-3′).

Statistical Analysis. Statistical differences were examined by one way-ANOVA. $P < 0.05$ was considered to be statistically significant. Data were expressed as the mean ± SE.

RESULTS

Improved Cardiac Contractile Dysfunction in Diabetic Rats As Response to Garlic Oil Feeding. To assess cardiac function and dimension in vivo, we performed echocardiography on rats. In addition to significantly high blood glucose induced in diabetic rats, all of the animals showed a normal appearance and had a generally normal cardiac function at day 0 of induction (**1**). At 16 days after induction, diabetes decreased body weight and increased blood glucose in all diabetic animal groups. However, diabetes significantly decreased the heart rate (HR), which was dose-dependently reversed by garlic oil (GO) feeding. The percentages of fractional shortening (FS) and ejection fraction (EF), typically representing cardiac contractile function of rat hearts, were significantly decreased in diabetic rat hearts and dose-dependently reversed back to the control level at GO doses of 50 and 100 mg/kg of BW. The cardiac output (CO) shows similar results to HR, FS, and EF. Collectively, diabetic rats receiving GO showed a better cardiac output and contractile function.

Shift in Myosin Heavy Chain Gene Expression from α to β Isoforms and Decreased Levels of Cardiac α-Actin in Diabetic Rat Hearts Were Reversed by Garlic Oil Feeding. To evaluate the contractile function of cardiac muscle, we examined MHC gene expressions in rat heart, using RT-PCR analysis. The result is shown in **Figure 2A**. Compared with the control group, the diabetic rats showed a significantly decreased level of α-MHC and an increased level of β-MHC isoforms, which were dose-dependently reversed by the administration of GO. Even the β-MHC level of the group treated with GO at a dose of 100 mg/kg of BW is similar to the control group. The levels of cardiac α-actin sarcomeric contractile protein are shown in **Figure 2B**. This myofibrillar protein in diabetic hearts was drastically reduced and dose-dependently restored by treatment with GO. In the

group at the dose of 100 mg/kg of BW, the increased α-actin level reached control level. On the contrary, the levels of nonsarcomeric smooth muscle α-actin did not alter at all.

Decreased Antioxidant Enzyme Expression and Elevated Lipid Peroxidation in Diabetic Rat Hearts Were Reversed by Garlic Oil Supplementation. Intracellular ROS levels are regulated by antioxidant enzymes. We next turned our attention to the expressions of SOD-1 in diabetic hearts in response to GO administration. The result is shown in **Figure 3A**. Diabetes significantly decreased SOD-1 levels in hearts. In addition, the cardiac lipid peroxidation resulting from the oxidative damage was assessed by measuring TBARS, and this result is shown in **Figure 3B**. Diabetes enhanced the TBARS level in hearts. Interestingly, GO treatment dose-dependently attenuated the SOD-1 reduction and TBARS elevation, suggesting that the reduced SOD-1 activity for ROS scavenger and the increased lipid peroxidation were reversed by the GO.

Development of Apoptosis in Diabetic Rat Hearts Was Ameliorated by Garlic Oil Feeding. To clarify whether cardiac apoptosis was induced by diabetes and improved by GO supplementation in rats, DAPI staining and TUNEL assay were examined, and the results are shown in **Figure 4**. Compared with control group, a 5-fold increase in apoptosis in the left ventricles of rats was induced by diabetes, and this increase was significantly decreased by GO in a dose-dependent manner. This indicates that GO feeding can improve diabetes-induced cardiac damages.

Increased Activities of Caspase-Leading Apoptotic Signalings and p38-NFκB Signaling in Diabetic Rat Hearts Were Decreased by Garlic Oil Feeding. To further understand the signaling pathways involved in the cardiac apoptosis development induced by diabetes and improved by GO, we examined the related apoptotic protein levels in control and diabetic rat hearts. Compared with the control group, a 2.2-fold increase of active caspase 3 was observed in diabetic rat hearts. This induction was inhibited by the administration of GO and reached significantly low levels at doses of 50 and 100 mg/kg of BW (**Figure 5A**). These data are consistent with the results of cardiac apoptosis in **Figure 3**. Furthermore, compared with the control group, both caspase 8 and caspase 9 were elevated 2.5- and 3.4-fold, respectively, in diabetic rat hearts. This elevation was decreased and reached significantly decreased levels at a dose as low as 50 mg/kg of BW (**Figure 5B**,**C**). The levels of released cytochrome *c* and tBid showed similar results in **Figure 5B**,**C**. Collectively, both the receptor-dependent and mitochondrion-dependent apoptotic pathways are involved in diabetes-induced cardiac apoptosis, which is inhibited by the administration of GO.

Protein p38 and its downstream nuclear factor κB (NFκB) are known as regulators of pathophysiological gene expressions to cause cell death, leading to left ventricle dysfunction (*16*). Hence, the cardiac p-p38 and NFκB levels were evaluated, and the results are shown in **Figure 5D**. The significant increase of cardiac p-p38 level in diabetic hearts was significantly decreased at all three doses of GO. The cardiac NFκB in diabetes was more activated and as significantly attenuated after GO treatment at doses of 50 and 100 mg/kg of BW.

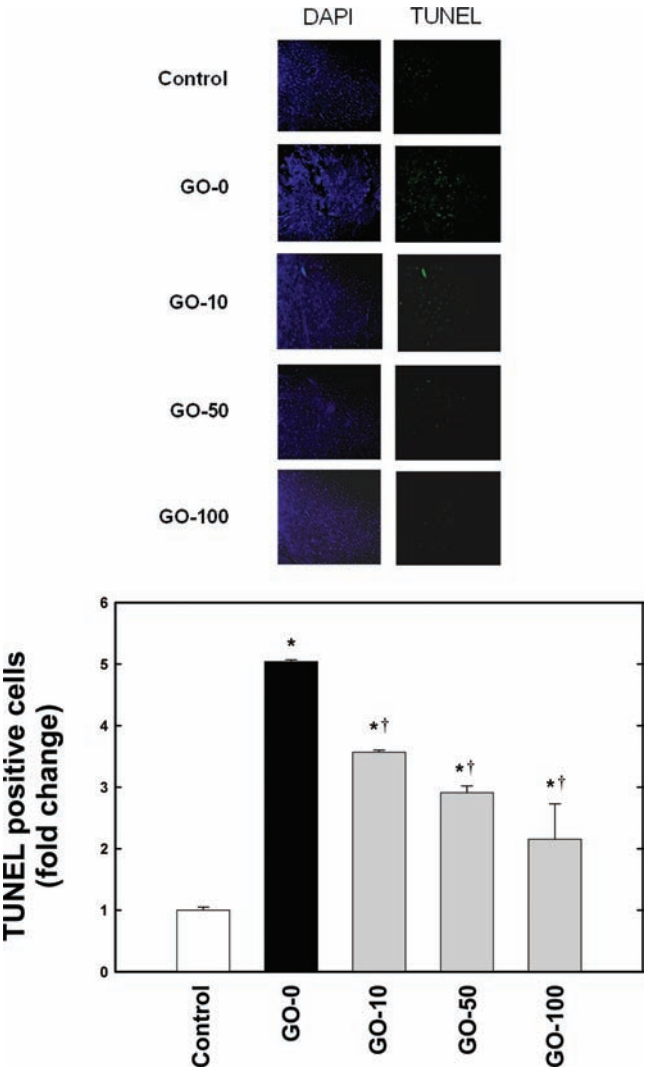

Figure 4. Apoptotic cell death in the hearts of control and diabetic rats fed different doses of garlic oil. The degree of apoptosis in rat hearts was measured by TdT-mediated deoxynucleotidyl UTP nick-end labeling (TUNEL) assay. The percentage of TUNEL-positive cells was determined on the basis of total stained cells by DAPI. Values are expressed as folds of control group. Data are means ± SE of six rats per group. GO-0, -10, -50, and -100 represent doses of 0, 10, 50, and 100 mg of garlic oil/kg of body weight, respectively. ∗, $P < 0.05$ compared with control rats; †, $P < 0.05$ compared with GO-0 group rats.

DISCUSSION

We here show that STZ-induced diabetes leads to a decrease in heart rate, cardiac contractile function, cardiac output, impaired contractile velocity of cardiac muscle, and myofibril formation. Interestingly, all of these cardiac abnormalities induced by diabetes are improved by GO treatment. We also show that diabetes develops cardiac apoptosis and oxidative stress and increases caspase-leading and p38-NFκB apoptotic signaling activities. We suggest that these oxidative stress- and apoptosis-promoting events might be associated with cardiac dysfunction in diabetic cardiomyopathy. GO treatment, which reduces oxidative stress, alleviates apoptosis development and counteracts activations of up-regulated apoptotic signalings that might be considered to possess potentials in protecting hearts from diabetic cardiomyopathy.

GO, a commonly used cooking spice and folk remedy, has been shown to exhibit beneficial effects on treatments of diabetes. This effect was suggested to be associated with the antioxidant properties of garlic. Interestingly, Anwar's (*10*) study indicates that GO effectively normalized the unbalanced antioxidant status by decreasing lipid peroxide and increasing SOD activity and total thiol in the blood and liver of rats with STZ-induced diabetes. These results provide an explanation for our experimental data demonstrating that GO treatment ameliorates cardiac dysfunction, myofibril malformation, and apoptosis with related signaling activities in STZ diabetic rat hearts through decreasing oxidative stress. Additionally, in agreement with our hypothesis

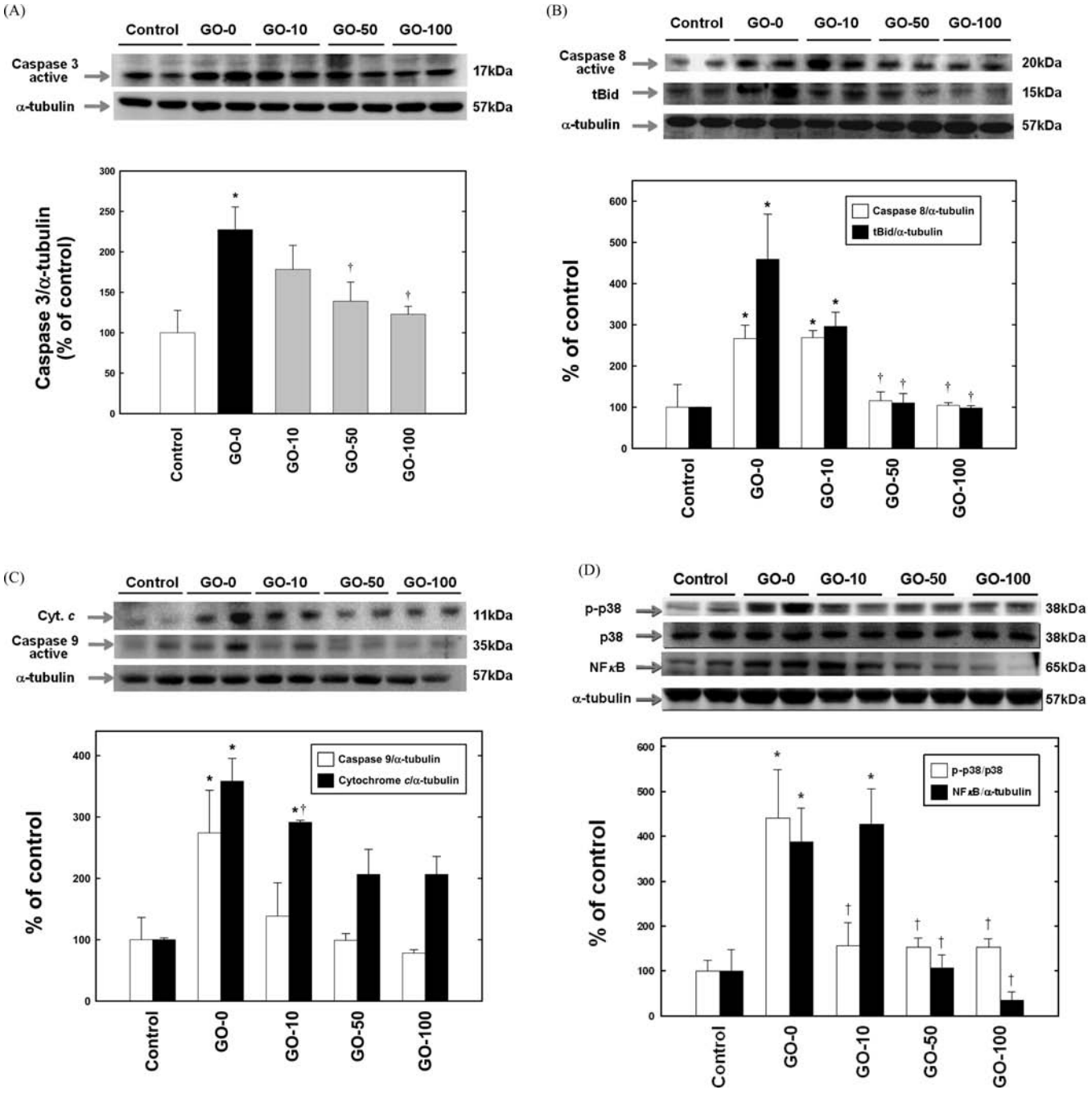

Figure 5. Cardiac pro-apoptotic proteins (**A**) caspase 3, (**B**) caspase 8 and tBid, (**C**) caspase 9 and released cytochrome *c*, and (**D**) reactive oxygen species (ROS) downstream proteins, phosphorylated p38, and NFκB in control and diabetic rats fed different doses of garlic oil. Protein levels in left ventricles of rat hearts were determined by Western blotting analysis. Signal intensity was quantitated using a PhosphoImager. α-Tubulin was used as a loading control. The average result $\pm$ SE of three independent experiments is shown. GO-0, -10, -50, and -100 represent doses of 0, 10, 50, and 100 mg of garlic oil/kg of body weight, respectively. $*$, $P < 0.05$ compared with control rats; †, $P < 0.05$ compared with GO-0 group rats.

(Fgure **6**), the study of Banerjee et al. (*17*) indicates that garlic with powerful antioxidant ability can increase glutathione content and SOD and glutathione *S*-transferase (GST) activities in cardiac muscles. In our analysis of results by using gas chromatography, more than 20 organosulfur compounds have been found in GO. Among them, DADS and DATS are the major constituents. Most of them have been identified to possess powerful antioxidant potential (*18*, *19*). They may play important roles in improving diabetic cardiac disease with GO treatment. In addition, although the treatment with GO failed to improve the glucose intolerance caused by STZ, the oxidative stress, downstream of hyperglycemia, was inhibited by GO treatment.

Contractile failure resulting in the decline in ventricular performance is another characteristic of diabetic cardiomyopathy. Several studies suggest that ventricular dysfunction associated with diabetes mellitus is also linked to the production of ROS (*20*), resulting from hyperglycemia. Although the model of STZ-induced type I DM was used in the present study, we believe that no matter which type of diabetes was examined, hyperglycemia, as the common characteristic of both types of diabetes, is the major cause of cardiac damage. The evidence of

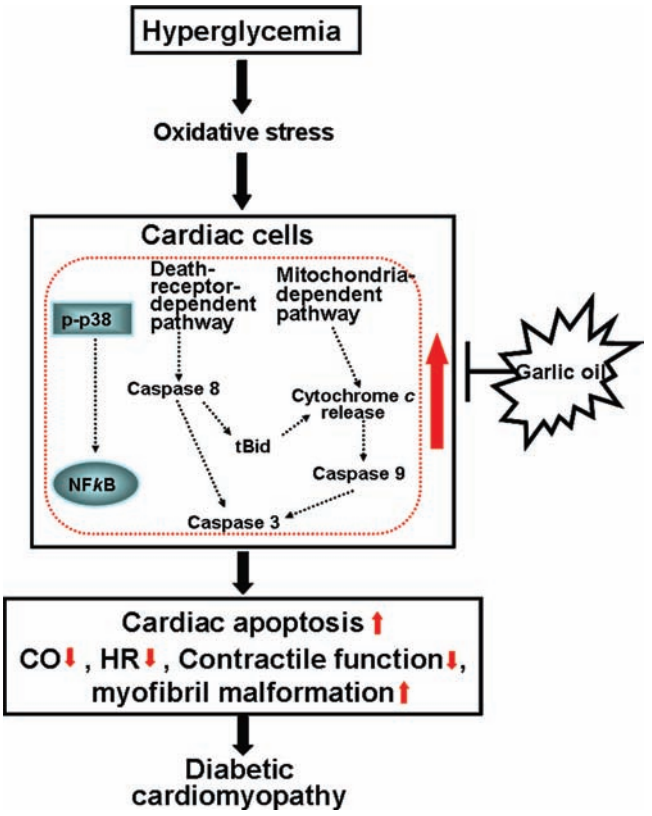

Figure 6. Proposed hypothesis that garlic oil ameliorates cardiac dysfunction in rats with diabetes by down-regulating apoptotic signaling activities. We speculate that garlic oil protects hearts by inhibiting oxidative stress in diabetic rats. Each up arrow and down arrow represent increases and decreases, respectively. These STZ-induced mitochondrion-, death receptor-, and p38-NFκB-dependent apoptotic signaling activities were significantly improved after garlic oil supplementation. Through these mechanisms, garlic oil treatment ameliorates cardiomyopathy in rats with diabetes by inhibiting apoptosis development and improving cardiac output, heart rate, and contractile function as well as myofibril formation.

diabetic cardiomyopathy (DCM) detected clinically by echocardiography is an early increase in diastolic myocardial stiffness indicated as left ventricular diastolic dysfunction and a later occurrence of left ventricular systolic dysfunction (*21*). Using an animal model, Schaffer and his colleagues observed that STZ-induced diabetic rats demonstrated glucose intolerance and developed a cardiomyopathy characterized by reduced heart rate, contractility, and cardiac output (*22*), which are very consistent with our results.

MHCs are the molecular motors of cardiac muscle contraction. The α and β isoforms differ in contractile properties on the basis of their relative adenosine triphosphatase (ATPase) activity and velocity of shortening. A switch in MHC isoform from α to β in ventricle may explain the systolic dysfunction in the left ventricle during heart failure (*23*, *24*). We show here a reduced α-MHC expression and an increased β-MHC expression in STZ-induced diabetic rat hearts were dose-dependently reversed by GO supplementation. A similar result in Aragno's study (*25*) demonstrates that reduced myocardial contractility caused by oxidative stress in the diabetic heart evidenced by the shift of MHC isoforms was reversed by the administration of dehydroepiandrosterone (DHAE), a physiological steroid with multitargeted antioxidant properties. Similarly, our observation also demonstrated that the decreased cardiac α-actin level in the STZ diabetic heart was alleviated by GO treatment. In addition, Dyntar's study (*26*) demonstrates that normal rebuilding of myofibrillar structures evaluated by the altered cardiac α-actin level were disrupted in cultured adult cardiomyocyte exposed to high glucose concentration. Interestingly, this impairment can be fully improved by treatment of antioxidant NAC. These findings indicated that oxidative stress in diabetic rat hearts may contribute to the change of myosin chain gene expressions and myofibrillar formation, leading to myofibril remodeling, and it is likely that improved redox balance could be the underlying mechanism of garlic's beneficial effects on the diabetic heart.

In addition to the death-receptor and mitochondria-dependent apoptotic signalings, p38-NFkB signaling was also involved in the development of cardiac apoptosis, which was prevented and dropped to levels observed in control animals by GO in this study. This finding is consistent with Riad's results (*16*), indicating that treatment of chronic p38 inhibition reduces the development of cardiac and endothelial dysfunction in diabetic rats. We conjecture that GO is likely to play another role, as a p38 inhibitor, in protecting hearts from DCM. In addition, the phosphorylation of p38 leads to the activation of transcription factor, nuclear factor κB (NFκB) to induce many pathophysiologic developments such as left ventricular remodeling (*27*), and this process was decreased by antioxidant supplementation. Another interesting study (*19*) related the inhibition of lipopolysaccharide (LPS)-induced oxidative stress, iNOS expression, and NFκB activation by DATS, a GO component, supporting the therapeutic potential of GO on oxidative stress-related diseases.

Recently, the endogenously generated hydrogen sulfide (H_2S) has been reported to demonstrate potent cardioprotective effects. Several mechanisms of cardiovascular actions of H_2S are investigated. The ATP-sensitive K^+ (K_{ATP}) channel is the characterized cellular target of H_2S, which is associated with the H_2S-induced vasodilation (*28*) and infarct-limiting effect in hearts (*29*). Furthermore, downstream of the K_{ATP} channel opening is the PKCε, which plays a role in cardioprotective signaling following injury stimuli (*30*). Anti-apoptotic properties of H_2S are also evidenced by the activation of several survival kinases, including ERK and PI3K/Akt (*31*), which further inactivate the pro-apoptogen Bad (*32*). In addition, H_2S may up-regulate endogenous antioxidant enzyme activities, through the nuclear factor E2-related factor-2 (Nrf-2)-dependent pathway (*32*). Interestingly, a recent study by Benavides's group indicated that GO and its components DADS and DATS can induce H_2S production (*33*), and GO was identified to promote Nrf-2 activation (*34*). Using the H_2S inhibitor propargylglycine, the main bioactive constituent of garlic, *S*-allylcysteine, was identified to exert cardioprotective action through the H_2S-mediated pathway (*35*). On the basis of the observations of low levels of H_2S in the blood of STZ-treated rats (*36*), all of these results provide another explanation of the possible role of H_2S on antidiabetic cardiomyopathy of garlic oil.

In conclusion, our results show that GO supplementation for diabetic rats leads to several alterations at multiple levels in hearts including cardiac contractile functions and structures, myosin chain gene expressions, oxidative stress, and apoptosis and related signaling activities. All of these phenomena might be associated with the antioxidant potential of GO, which is attributed to the presence of organosulfur compounds that modulate the cardiac antioxidant activity. A future study to investigate the individual GO constituent compounds on improving diabetic cardiac dysfunction is needed.

ABBREVIATIONS USED

BW, body weight; CO, cardiac output; Cyt *c*, cytochrome *c*; DATS, diallyl trisulfide; DAPI, 4′,6-diamidino-2-phenylindole;

DCM, diabetic cardiomyopathy; DHAE, dehydroepiandrosterone; EF, ejection fraction; FS, fractional shortening; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GO, garlic oil; GST, glutathione *S*-transferase; H&E, hematoxylin and eosin; HR, heart rate; IGF-I(R), insulin-like growth factor-I (receptor); iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; LVESV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; LVM, left ventricular mass; MHC, myosin heavy-chain; NAC, *N*-acetylcysteine; NFκB, nuclear factor-κB; OGTT, oral glucose tolerance test; PI3K, phosphadidylinositol 3′-kinase; PWT, posterior wall thickness; ROS, reactive oxygen species; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; tBid, truncated BH3 interacting domain death agonist; TL, tibia length.

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