Dimethylthiourea normalizes velocity-dependent, but not force-dependent, index of ventricular performance in diabetic rats: role of myosin heavy chain isozyme

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Yeih DF, Yeh HI, Hsin HT, Lin LY, Chiang FT, Tseng CD, Chu SH, Tseng YZ. Dimethylthiourea normalizes velocity-dependent, but not force-dependent, index of ventricular performance in diabetic rats: role of myosin heavy chain isozyme. Am J Physiol Heart Circ Physiol 297:H1411–H1420, 2009. First published July 24, 2009; doi:10.1152/ajpheart.01269.2008.—Hydroxyl radicals and hydrogen peroxide are involved in the pathogenesis of systolic dysfunction in diabetic rats, but the precise mechanisms and the effect of antioxidant therapy in diabetic subjects have not been elucidated. We aimed to evaluate the effects of dimethylthiourea (DMTU), a potent hydroxyl radical scavenger, on both force-dependent and velocity-dependent indexes of cardiac contractility in streptozotocin (STZ)-induced early and chronic diabetic rats. Seventy-two hours and 8 wk after STZ (55 mg/kg) injection, diabetic rats were randomized to either DMTU (50 mg kg−1 day−1 ip) or vehicle treatment for 6 and 12 wk, respectively. All rats were then subjected to invasive hemodynamic studies. Maximal systolic elastance (Emax) and maximum theoretical flow (Qmax) were assessed by curve-fitting techniques in terms of the elastance-resistance model. Both normalized Emax (Emaxn) and maximum theoretical flow (Qmax) were assessed by curve-fitting techniques in terms of the elastance-resistance model. Both normalized Emax (Emaxn) and afterload-adjusted Qmax (Qmaxad) were depressed in diabetic rats, concomitant with altered myosin heavy chain (MHC) isoform composition and its upstream regulators, such as myocyte enhancer factor-2 (MEF-2) and heart autonomic nervous system and neural crest derivatives (HAND). In chronic diabetic rats, DMTU markedly attenuated the impairment in Qmaxad and normalized the expression of MEF-2 and eHAND and MHC isoform composition but exerted an insignificant benefit on Emax. Regarding preventive treatment, DMTU significantly ameliorated both Emaxn and Qmaxad in early diabetic rats. In conclusion, our study shows that DMTU has disparate effects on Qmaxad and Emaxn in chronic diabetic rats. The advantage of DMTU in chronic diabetic rats might involve normalization of MEF-2 and eHAND, as well as reversal of MHC isoform switch.

myocyte enhancer factor-2; transcription factor; contractile function; oxidative stress; antioxidant

HEART FAILURE IS ONE OF THE leading causes of morbidity and mortality in the diabetic population (54). Depressed myocardial contractility may occur in both insulin-dependent and non-insulin-dependent diabetes mellitus (DM) (23, 36). Although the pathogenesis of diabetic cardiomyopathy has not been precisely described, several mechanisms have been proposed, including mitochondrial dysfunction and reduced energy production, accumulation of free radicals, glucose toxicity-induced oxidative stress and malfunction of cardiac contractile and intra-cellular calcium handling proteins such as a switch of isoforms of the myosin heavy chain (MHC), sarcoplasmic reticulum Ca2+ ATPase (SERCA), and Na+–Ca2+ exchanger (11, 35). Several lines of evidence have shown that reactive oxygen species (ROS) might play an essential role in the pathogenesis of cardiac dysfunction in diabetic rats (13, 57, 60). However, the precise mechanisms by which the ROS leads to compromised cardiac contractility and the effect of antioxidant therapy in diabetic subjects have not been elucidated. Moreover the conventional antioxidants used to prevent oxidative damage in diabetes have failed to achieve satisfactory results (12, 50, 55).

Most antioxidant studies in diabetic animals use maximal rate of left ventricular (LV) pressure rise (dP/dt max) (37, 42) or use an isolated working heart (30, 57) to assess cardiac contractility. However, dP/dt max is a load-dependent parameter and might underestimate cardiac contractility especially in diabetic animals, which are frequently associated with reduced afterload (15, 26). On the other hand, isolated working hearts are sensitive to trauma and ischemia associated with the procedures of isolation and perfusion (49). Furthermore, diabetic hearts are associated with altered myocardial substrate metabolism (3). In most studies, identical perfusates are applied to hearts from both control and diabetic animals (40, 47), which might lead to altered cardiac contractility and efficiency of energy transfer in diabetic hearts. Therefore, precise in vivo measurements are necessary to characterize the therapeutic effects of drugs on cardiac contractility and performance in diabetic animals.

In contrast to time-varying elastance theory, accumulated evidence has shown that instantaneous ventricular pressure is dependent on both instantaneous ventricular volume and flow (14, 38, 48). The ejecting flow decreases LV pressure, and the inverse linear relationship between LV pressure and flow has been conceptualized as an expression of the internal resistance of the LV. Two independent parameters generated in the elastance-resistance LV pump model to characterize systolic functions of the LV are the maximal systolic elastance (Emax) and the theoretical maximal flow (Qmax). Similar to force-velocity relation in isolated muscle, Emax and Qmax quantify, respectively, two separate facets of cardiac contractility: force-dependent and velocity-dependent indexes of myocardial contractility. Emax is sensitive to changes in contractile state and is independent of loading conditions and heart rate (HR) (21). On the other hand, Qmax is conceptually equivalent to the maximal unloaded velocity of shortening in the muscle and is inversely related to the magnitude of afterload (48, 59). We recently demonstrated that Emax and afterload-adjusted Qmax (Qmaxad)
are two major determinants in the evolution of cardiac dysfunction in streptozotocin (STZ)-induced diabetic rats, with $E_{\text{max}}$ being decreased early after the onset of diabetes and $Q_{\text{max,ad}}$ being enhanced with time initially but attenuated preceding overt systolic dysfunction (59).

Most ROS scavengers do not readily cross cell membranes and have serum half-lives on the order of minutes (24). Apart from being effective in scavenging hydrogen peroxide and hydroxyl radicals (24, 53), DMTU has also been shown to readily enter the myocardium, to have a long serum half-life of 43 h, and have no cardiac toxicity in vivo (16, 24). In this study, we aimed to evaluate the preventive and therapeutic effects of dimethylthiourea (DMTU) on cardiac dysfunction in STZ-diabetic rats. We sought to do this in terms of force-dependent ($E_{\text{max}}$) and velocity-dependent ($Q_{\text{max}}$) indexes of myocardial contractility by using the elastance-resistance LV model. Furthermore, we planned to carry out experiments to examine downstream signaling activated by oxidative stress in the LV and the effects of DMTU treatment on markers of oxidative stress and the expression of isoforms of MHC in STZ-diabetic rats.

**MATERIALS AND METHODS**

**Diabetic Rat Model**

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publications No. 85-23, revised 1996), and all procedures were approved by the Animal Care and Use Committee of the Far Eastern Memorial Hospital. Eight-week-old male Wistar rats (weighing 250–300 g) were housed at 22°C with a 12-h light-dark cycle and free access to rodent chow and tap water. Rats were injected with STZ at a dose of 55 mg/kg or vehicle (0.9% saline) via the penile vein under light ketamine anesthesia (46). Three days after STZ injection, whole blood glucose was measured with the Accu-Check Compact kit (Roche Diagnostics, Mannheim, Germany). STZ-treated rats with whole-blood glucose levels less than 15 mmol/l or body weight (BW) exceeding 380 g were excluded from the present study.

**Experimental Designs**

**Animal protocol 1: the therapeutic effect of DMTU on cardiac dysfunction in chronic STZ-diabetic rats.** Eight weeks after successful induction (blood glucose > 16.7 mM/l), STZ-diabetic and age-matched controls were randomized to either DMTU (50 mg·kg$^{-1}$·day$^{-1}$ ip), as previously described (24, 34), or vehicle for another 12 wk and then followed by invasive hemodynamic studies. In all subsequent molecular and biochemistry analysis, comparisons were made between controls and chronic diabetic rats.

**Animal protocol 2: the preventive effect of DMTU on cardiac dysfunction in early diabetic rats.** Seventy-two hours after injection, the diabetic rats were randomly divided into three groups: STZ+vehicle, STZ+DMTU (50 mg·kg$^{-1}$·day$^{-1}$), and STZ+insulin (2–10 U twice daily). After a 6-wk treatment, all diabetic and nondiabetic rats were subjected to invasive hemodynamic studies.

**Assessment of Left Ventricular Performance**

The experimental procedures for recording LV pressure and pulsatile aortic flow were done as previously described (59). After hemodynamics were stabilized, LV pressure, aortic flow, and ECG signals were simultaneously recorded and the resulting LV pressure and ascending aortic flow signals were subjected to further analysis as previously described (59). Briefly, we obtained the estimated peak isovolumic pressure ($P_{\text{iso, max}}$) by the curve-fitting technique proposed by Sunagawa and coworkers (41, 59). The optimal values of $Q_{\text{max}}$ and effective end-diastolic volume ($V_{\text{ed, max}}$) were derived from the elastance-resistance model that minimized the difference between the measured and model-derived LV pressures at the time interval between the onset of ventricular ejection and the time of $P_{\text{iso, max}}$ as previously described (14, 59). The goodness of fit of the model-derived LV pressure was determined by the standard error of the estimate (SEE). We looked for SEE to be minimal when expressed relative to the observed mean LV pressure. The $E_{\text{max}}$ was calculated by the formula $E_{\text{max}} = P_{\text{iso, max}}/V_{\text{ed, max}}$. Considering the effect of heart size on determination of cardiac systolic elastance, $E_{\text{max}}$ was normalized by dividing by 1/LV weight (i.e., $E_{\text{max}} = E_{\text{max}} \times V_{\text{ed, max}}$) for comparisons between control and diabetic rats (7, 22).

**Estimation of End-Systolic Elastance and Effective Arterial Elastance**

We used a single-beat estimation technique to evaluate the effective arterial elastance ($E_a$), as previously described (59). The $E_a$, the ratio of end-systolic pressure ($P_{\text{es}}$) to stroke volume ($SV$), was used to characterize the afterload of the LV (40). Considering that $Q_{\text{max}}$ is load dependent and is inversely related to the magnitude of afterload, $Q_{\text{max}}$ was normalized by multiplying by $E_a$ (59). With this adjustment, the afterload-normalized $Q_{\text{max}}$ ($Q_{\text{max, ad}}$) was used for comparisons between controls and diabetic rats.

**Determination of Blood Glucose and Insulin Concentrations**

After completion of the hemodynamic study, the chronic diabetic rats and age-matched controls were euthanized and blood samples were obtained. Whole blood glucose was measured immediately with the Accu-Check Compact kit (Roche Diagnostics), and the plasma samples were collected and frozen at −80°C until analysis. Plasma insulin levels were subsequently measured in duplicate by using a rat insulin ELISA kit (Mercodia, Uppsala, Sweden).

**Tissue Extracts**

Cytosolic and nuclear extracts were prepared as described by Meldrum et al. (27) and were stored at −80°C until analysis. Protein concentration was determined by use of the bicinchoninic acid protein assay kit (Pierce).

**Oxidative Biochemical Parameters**

Total lipid peroxides in the LV were determined as the malondialdehyde (MDA) content by using a commercially available kit (Bioxtech LPO-586; Oxis Research, Portland, OR). The reduced glutathione-to-oxidized glutathione ratio (GSH/GSSG), which is considered a good parameter of antioxidant status, was measured in the cytosolic fraction by a Bioxtech GSH/GSSG-412 colorimetric assay kit, as described by the manufacturer (OxisResearch, Portland, OR).

**Western Blotting of Transcription Factor**

The expression of heart autonomic nervous system and neural crest derivatives (HAND) and myocyte enhancer factor-2 (MEF-2) were investigated by Western blotting. Both dHAND and eHAND, isoforms of HAND, proteins were detected in cytosolic extracts, whereas MEF-2 was detected in nuclear extracts by Laminil’s method (25). Sixty micrograms of protein were loaded on 10% SDS-PAGE. Blots were blocked in 5% skimmed milk in 5 mM Tris-HCl (pH 7.4) containing 200 mM NaCl and 0.05% (vol/vol) Tween 20 for 1 h at 25°C and then incubated overnight with rabbit polyclonal antibodies against dHAND and eHAND and with MEF-2 (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C. After washing, horseradish peroxidase-linked secondary antibody was added (1:5,000, Chemicon). The membranes were then stripped and reprobed with anti-α-actin and anti-lamin B1 antibodies for loading controls for cytosolic and nuclear proteins, respectively. Chemiluminescence detection was done with a Western blotting ECL system (Millipore) and the blot signals were...
quantified by a luminescent image analyzer (LAS-4000, Fujifilm, Tokyo, Japan).

Measurement of Cardiac MHC Isoform mRNA Expression by Reverse Transcription and Quantitative Polymerase Chain Reaction

Total RNA was extracted from the LV with Trizol reagent (Invitrogen) according to the manufacturer’s recommendations. The first-strand cDNA synthesis was performed from 0.75μg of total RNA and reverse transcribed by using ImProm-II reverse transcriptase (Promega, Madison, WI) and random hexamers at 42°C for 1 h, and 70°C for 15 min. A fluorescence quantitative real-time PCR detection system (LightCycler DNA Master SYBR Green I; Roche Molecular Biochemicals, Indianapolis, IN) was used to determine the change in messenger RNA (mRNA) levels of α-MHC and β-MHC expression. The reactions were performed in a 10-μl volume containing 50 pmol of sense and antisense primers, SYBR Green I dye, Taq DNA polymerase (FastStart; Roche), reaction buffer, dNTP mixture, and 4 mM MgCl₂. The PCR conditions were set at 95°C for 10 min to activate the Taq DNA polymerase, followed by 45 cycles at 95°C for 10 s, 60°C for 10 s, and 72°C for 20 s. The amount of α-MHC and β-MHC mRNA was normalized by that of cyclophilin mRNA and is presented in arbitrary units. The primer sequences used for α-MHC, β-MHC, and cyclophilin were as described previously (4).

MHC Isoform Analysis by Gel Electrophoresis

Samples were prepared for gel electrophoresis according to a method described previously with some modifications (9). The frozen tissues were minced in 50 volumes of ice-cold homogenization buffer containing 50 mM Tris·HCl (pH 6.8), 0.2% Triton X-100, and protease inhibitor cocktail (Roche). The homogenates were then centrifuged at 12,000 g for 10 min. The resulting pellets were placed in 30 volumes of sample buffer, containing 62.5 mM Tris·HCl (pH 6.8), 2% SDS, 10% (vol/vol) glycerol, and 5% mercaptoethanol and then boiled for 3 min and incubated on ice for 5 min to yield the homogenate. The original homogenate samples were diluted 1:300 in sample buffer for sample loading. Ten microliters of diluted sample were loaded in each lane. The preparation of the gel was performed according to Talmadge and Roy (43). The upper electrode buffer consisted of 100 mM Tris, 150 mM glycine, 0.1% SDS, and 10 mM 2-mercaptoethanol, whereas the lower electrode buffer consisted of 100 mM Tris, 150 mM glycine, 0.1% SDS, and 10 mM HCl (pH 6.8), 0.2% Triton X-100, and 0.05% SDS. Gel electrophoresis was performed at 4°C at a constant voltage of 275 V for 24 h, fixed for a minimum of 2 h in 5% glutaraldehyde before being silver stained and scanned with a densitometer to determine the amount of α-MHC and β-MHC.

Statistical Analysis

All data are expressed as mean ± SE. Statistical analysis with one-way ANOVA, followed by least significant difference post hoc test for multiple comparisons, and linear regression analysis were performed using SPSS for Windows version 12.0. A value of P < 0.05 was considered significant.

RESULTS

Baseline Values in Control and Chronic Diabetics Rats With a 12-wk Treatment of Either DMTU or Vehicle

At randomization, BW and blood glucose levels were comparable between DMTU-treated and vehicle-treated controls and between DMTU-treated and vehicle-treated diabetic rats. After a 12-wk treatment, the BW, LV weight (LVW), HR, and blood glucose level remained comparable between DMTU-treated and vehicle-treated controls. In chronic diabetic rats, whether DMTU treated or vehicle treated, the BW and LVW were both significantly lower, whereas blood glucose level and normalized LVW (LVWn) were significantly higher than controls (P < 0.05, Table 1). Regarding plasma insulin, chronic STZ-diabetic rats had significantly lower plasma levels than age-matched controls (P < 0.05, Table 1). However, the levels were comparable in DMTU-treated and vehicle-treated chronic STZ-diabetic rats (Table 1). In addition, DMTU treatment resulted in a nearly complete recovery of depressed HR in chronic diabetic rats (P < 0.05, Table 1). The data are shown in Table 1.

Baseline Values in Control and Early Diabetic Rats With a 6-wk Preventive Treatment

After a 6-wk treatment in early diabetic rats, the trends of change in BW, LVW, LVWn, blood glucose level, and HR were similar to those seen in chronic diabetic rats (Table 2). DMTU treatment significantly attenuated the alternations in LVWn and HR in early diabetic rats without changing blood glucose and plasma insulin levels (0.27 ± 0.06 μg/l vs. 0.24 ± 0.02 μg/l, P > 0.05, n = 6) (Table 2). On the other hand, insulin treatment restored blood glucose levels, HR, and LVWn as well (P < 0.05, Table 2).

Oxidative Stress in Cytosolic Extracts of LV From DMTU-Treated and DMTU-Untreated Control and Chronic Diabetic Rats

The MDA level was significantly higher, whereas the GSH/GSSG ratio was significantly lower, in the hearts of chronic STZ-diabetic rats (P < 0.05, Fig. 1A). DMTU treatment ameliorated the oxidative imbalance by reducing lipid peroxidation and normalizing the GSH/GSSG ratio in chronic diabetic rats (P < 0.05, Fig. 1B). However, DMTU did not have any significant effects on either MDA level or GSH/GSSG ratio in control rats.

Effective Arterial Elastance in DMTU-Treated and DMTU-Untreated Control and Chronic Diabetic Rats

Psys, which approximated mean aortic pressure, did not differ significantly between DMTU-treated and vehicle-treated control and chronic diabetic rats.

Table 1. Baseline values and hemodynamic profiles in control and chronic diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Con(D)</th>
<th>DM</th>
<th>DM(D)</th>
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</thead>
<tbody>
<tr>
<td>At randomization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>353±6</td>
<td>344±10</td>
<td>278±30*</td>
<td>255±23*</td>
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<tr>
<td>Glucose, mM</td>
<td>7.3±0.6</td>
<td>7.1±0.5</td>
<td>26.4±1.5*</td>
<td>24.6±2.0*</td>
</tr>
<tr>
<td>At completion of study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>450±14</td>
<td>464±17</td>
<td>283±14*</td>
<td>299±23*</td>
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<tr>
<td>LVW, g</td>
<td>0.8±0.03</td>
<td>0.83±0.04</td>
<td>0.7±0.03*</td>
<td>0.61±0.03*</td>
</tr>
<tr>
<td>LVWn × 10⁻³</td>
<td>1.7±0.05</td>
<td>1.8±0.07</td>
<td>2.2±0.11*</td>
<td>2.2±0.08*</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>7.2±0.4</td>
<td>7.1±0.2</td>
<td>28.7±1.0*</td>
<td>27.5±2.4*</td>
</tr>
<tr>
<td>Insulin, μg/l</td>
<td>1.8±0.2</td>
<td>1.6±0.2</td>
<td>0.34±0.04*</td>
<td>0.35±0.05*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>337±13</td>
<td>328±7</td>
<td>268±24*</td>
<td>316±12†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>93±2.7</td>
<td>96±2.5</td>
<td>86±4.4</td>
<td>87±2.5</td>
</tr>
<tr>
<td>CO₃, ml·min⁻¹·kg⁻¹</td>
<td>223±20.2</td>
<td>223±13.8</td>
<td>392±47.9*</td>
<td>347±18.0*</td>
</tr>
</tbody>
</table>

Data are means ± SE of 6 rats per group. Con, vehicle-treated controls; Con(D), dimethylthiourea (DMTU)-treated controls (DM); DM, vehicle-treated diabetic rats; DM(D), DMTU-treated diabetic rats; BW, body weight; LVW, wet left ventricular weight; LVWn, normalized LV weight (= wet LV weight/BW); HR, heart rate; MAP, mean aortic pressure; CO₃, normalized cardiac output (= cardiac output/BW). *P < 0.05 vs. controls. †P < 0.05 vs. vehicle-treated diabetic rats.

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controls and chronic diabetic rats (Fig. 2A). Consistent with previous studies (15, 26), we found that chronic diabetic rats were in a high-cardiac-output state with significantly higher SV and lower Ea, which were significantly attenuated after DMTU treatment (both \( P < 0.05 \), Fig. 2, B and C).

Therapeutic Effect of DMTU on LV Systolic Mechanics in Control and Chronic Diabetic Rats

Consistent with our previous study (59), \( E_{\text{max}} \) and \( E_{\text{max}}^{\text{n}} \) were significantly lower in the diabetic groups (both \( P < 0.05 \), Fig. 3, A and B). DMTU treatment exerted an insignificant benefit on \( E_{\text{max}} \) (\( P < 0.07 \), Fig. 3A) in chronic diabetic rats, but this benefit became neutralized after normalization by LV weight (i.e., \( E_{\text{max}}^{\text{n}} \)) (\( P > 0.05 \), Fig. 3B). \( V_{\text{es}} \) was significantly increased (by 35%) in diabetic rats (\( P < 0.01 \), Fig. 3C). After DMTU treatment, there was a trend toward a decrease in \( V_{\text{es}} \) in diabetic rats, although not statistically significant. \( E_{\text{es}} \) remained unchanged in all study groups (\( P > 0.05 \), Fig. 3D).

On the other hand, there was no significant difference in \( Q_{\text{max}} \) between the DMTU-treated or vehicle-treated control and diabetic groups (\( P > 0.05 \), Fig. 4A). Considering that \( Q_{\text{max}} \) is enhanced under the condition of lower afterload, which is frequently seen in diabetic rats, \( Q_{\text{max}} \) was normalized by the magnitude of the afterload (i.e., \( Q_{\text{max}}^{\text{ad}} \)) for making comparisons between diabetic and control groups. Our results showed that \( Q_{\text{max}}^{\text{ad}} \) was significantly lower in the vehicle-treated diabetic group than in the control group and DMTU treatment significantly ameliorated the impairment in \( Q_{\text{max}}^{\text{ad}} \) in diabetic animals (\( P < 0.05 \), Fig. 4B).

Preventive Treatment of DMTU on LV Systolic Mechanics in Control and Early Diabetic Rats

Both \( E_{\text{max}} \) and \( Q_{\text{max}}^{\text{ad}} \) were significantly depressed in diabetic rats 6 wk after induction compared with controls (both \( P < 0.05 \), Fig. 5, A and B) and were significantly improved after insulin or DMTU treatment in early STZ-diabetic rats.

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**Table 2. Baseline values and hemodynamic profiles in control and early diabetic rats**

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>DM</th>
<th>DM(D)</th>
<th>DM(I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>364±12</td>
<td>240±31</td>
<td>290±20</td>
<td>372±20</td>
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<tr>
<td>LVW, g</td>
<td>0.68±0.03</td>
<td>0.54±0.06</td>
<td>0.55±0.02</td>
<td>0.71±0.04†</td>
</tr>
<tr>
<td>LVW(_{\text{m}}) ( \times 10^{-3} )</td>
<td>1.87±0.05</td>
<td>2.29±0.06*</td>
<td>1.89±0.1†</td>
<td>1.9±0.4†</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>8.0±1.0</td>
<td>26.2±1.4*</td>
<td>23.8±1.4*</td>
<td>10.0±1.7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>297±9</td>
<td>244±18*</td>
<td>281±11†</td>
<td>309±5†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>114±2.6</td>
<td>100±4</td>
<td>118±5</td>
<td>116±5</td>
</tr>
<tr>
<td>CO(_{\text{es}}) ml:mmHg(^{-1})kg(^{-1})</td>
<td>166±9.3</td>
<td>327±34*</td>
<td>231±33†</td>
<td>190±22†</td>
</tr>
</tbody>
</table>

Data are means ± SE of 6 rats per group. DM(I), insulin-treated diabetic rats. *\( P < 0.05 \) vs. controls. †\( P < 0.05 \) vs. vehicle-treated diabetic rats.

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**Fig. 1.** Oxidative biochemical markers determined by malondialdehyde (MDA, A) and the ratio of reduced to oxidized glutathione (GSH/GSSG ratio, B) in control and chronic diabetic rat hearts. Data are means ± SE of 6 rats per group. CON, vehicle-treated controls; CON(D), DMTU-treated control rats; DM, vehicle-treated diabetic rats; DM(D), DMTU-treated diabetic rats. *\( P < 0.05 \) vs. controls. †\( P < 0.05 \) vs. vehicle-treated diabetic rats.

**Fig. 2.** Determinants of the magnitude of the afterload. \( P_{\text{es}} \), end-systolic pressure of the left ventricle (LV; A); SV, stroke volume (B); Ea, effective arterial elastance (\( \frac{P_{\text{es}}}{SV} \); C). Data are means ± SE of 6 rats per group. *\( P < 0.05 \) vs. controls. †\( P < 0.05 \) vs. vehicle-treated diabetic rats.

**Fig. 3.** A and B). DMTU treatment exerted an insignificant benefit on \( E_{\text{max}} \) (\( P = 0.07 \), Fig. 3A) in chronic diabetic rats, but this benefit became neutralized after normalization by LV weight (i.e., \( E_{\text{max}}^{\text{n}} \)) (\( P > 0.05 \), Fig. 3B). \( V_{\text{es}} \) was significantly increased (by 35%) in diabetic rats (\( P < 0.01 \), Fig. 3C). After DMTU treatment, there was a trend toward a decrease in \( V_{\text{es}} \) in diabetic rats, although not statistically significant. \( E_{\text{es}} \) remained unchanged in all study groups (\( P > 0.05 \), Fig. 3D).

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H1414 DMTU AMELIORATES SYSTOLIC DYSFUNCTION IN DM

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DMTU AMELIORATES SYSTOLIC DYSFUNCTION IN DM

H1415

Fig. 3. Effects of dimethylthiourea (DMTU) on force-dependent indexes of myocardial contractility in control and chronic diabetic rats. $E_{\text{max}}$, maximal systolic elastance ($=P_{\text{isomax}}/V_{\text{eed}}$; A); $E_{\text{maxn}}$, normalized $E_{\text{max}}$ ($=E_{\text{max}} \times LV$ weight; B); $V_{\text{eed}}$, effective end-diastolic volume (C); $P_{\text{nomax}}$, estimated peak isovolumic pressure of LV (D). Data are means ± SE of 6 rats per group. *$P < 0.05$ vs. controls.

(both $P < 0.05$, Fig. 5, A and B). However, DMTU seemed to exert a lesser benefit on both $Q_{\text{maxad}}$ and $E_{\text{max}}$ than insulin did, although not statistically significant ($P > 0.05$, Fig. 5, A and B). Again, $P_{\text{isomax}}$ remained comparable in both control and early diabetic rats. As seen in chronic diabetic rats, early diabetic rats had significantly higher $V_{\text{eed}}$, higher normalized SV ($SV_n$) and lower HR compared with their age-matched controls (all $P < 0.05$, Fig. 5, C–E). Both insulin and DMTU treatment significantly attenuated the changes in $V_{\text{eed}}$, $SV_n$, as well as HR in early diabetic rats.

Switch of MHC Isoforms and Its Correlation With Cardiac Function in Chronic Diabetic Rats

Alternations in the isomyosin composition of MHC have been linked to cardiac dysfunction (28). We therefore examined the expressed levels of MHC. Real-time PCR analysis of mRNA expression in the LV showed that $\alpha$-MHC was the predominant isoform in controls, whereas $\beta$-MHC became the predominant isoform in chronic diabetic rats, and DMTU significantly reversed this switch in chronic diabetic rats ($P < 0.05$, Fig. 6A). Gel electrophoresis of the MHC isozyme composition confirmed the real-time PCR result (Fig. 6B). By linear regression analysis, our study showed that the proportion of slow myosin ($=100\% \times \beta$-MHC/ total MHC) was inversely proportional to $Q_{\text{maxad}}$ with statistical significance ($P < 0.05$, Fig. 6C).

Myocardial Expression of Oxidative Stress-Sensitive Transcription Factors MEF-2, dHAND, and eHAND

Both MEF-2 and HAND proteins are cardiac-specific transcription factors and are also the upstream regulators of MHC isoform switch, which is a hallmark of cardiac stress response. Figure 7 shows the expression MEF-2 in the nuclear fraction and of dHAND and eHAND in the cytosolic fraction of the LV in the DMTU-treated or vehicle-treated control and diabetic groups. MEF-2 protein expression was significantly higher in the diabetic group ($P < 0.05$, Fig. 7A). On the other hand, eHAND protein expression was significantly depressed ($P < 0.05$, Fig. 7B), whereas no significant changes in dHAND
protein expression occurred in diabetic groups \(P > 0.05\), Fig. 7C). DMTU treatment significantly normalized the expression of MEF-2 and eHAND in diabetic rat hearts \((P < 0.05, \text{Fig. } 7, A \text{ and } B)\).

**DISCUSSION**

The most striking finding in the present study is that DMTU, a potent hydroxyl free radical scavenger, has disparate effects on force-dependent and velocity-dependent indexes of cardiac contractility in diabetic rats. In contrast to depressed \(E_{\text{max}}\) and \(Q_{\text{max}}\) in STZ-diabetic rats 20 wk after induction remained comparable to that of age-matched controls. This result is consistent with our previous report (59) showing that \(Q_{\text{max}}\) increased and became significantly higher than in controls from 8 wk onward, reached its maximum at 16 wk after STZ injection, and then declined to a level comparable to that of age-matched controls at 22 wk after STZ injection. We and other investigators reported that \(Q_{\text{max}}\) is not load independent and is inversely proportional to the magnitude of the afterload \((15, 59)\). The STZ-diabetic rats are in a high-cardiac-output state and are frequently associated with lower afterload \((15, 26, 59)\). The depressed afterload might augment \(Q_{\text{max}}\), and, therefore, SV and CO are preserved in diabetic rats. In addition, there is a modest but significant decrease in fractional shortening of the LV in diabetic rats compared with control rats \((49 \pm 2.8 \text{ vs. } 63.7 \pm 1.9\%, n = 9, P < 0.01)\). This is similar to previous studies \((24, 59)\) and could explain why the changes in \(Q_{\text{max}}\) and SV are not evident in the diabetic group. We introduced an afterload-adjusted \(Q_{\text{max}}\), i.e., \(Q_{\text{maxad}}\), to make comparisons between the control and diabetic groups in this study. \(Q_{\text{maxad}}\) is conceptually analogous to maximal unloaded velocity of shortening in muscle. Consistent with previous studies showing that the maximal unloaded velocity of shortening is attenuated in isolated cardiomyocytes of diabetic rats \((32, 56)\), our study showed that \(Q_{\text{maxad}}\) is markedly depressed in diabetic rats and the impairment in \(Q_{\text{maxad}}\) is ameliorated after DMTU treatment. In contrast to marked improvement in \(Q_{\text{maxad}}\), DMTU treatment exerted an insignificant benefit on
Emaxn in our STZ-diabetic model, suggesting that the isomyosin composition might not be directly associated with Emaxn (39). Two factors may be responsible for this result. First, according to the model proposed by Alpert and colleagues (2), peak isometric twitch tension is proportional to cross-bridge cycling rate, strength, and loading sequence. In association with changes in MHC isoforms, the individual value may change differently depending on the stimulus. Second, other cellular and/or structural remodeling in hearts may also be responsible for changes in Emax (39). Further studies are needed to evaluate the relative contribution of the contractile apparatus, extracellular components, and LV geometry to both Emax and Qmax. To our knowledge, this is the first in vivo study showing that antioxidant treatment can markedly ameliorate the velocity-dependent index, but not the force-dependent index, of cardiac contractility in diabetic rats. Quantifying both force-dependent and velocity-dependent indexes of cardiac contractility could provide us with a better understanding of the pathogenesis of systolic dysfunction and possible therapeutic effects in diabetic rat hearts.

We also explored the upstream regulators of the MHC isoform switch, such as MEF-2 and the HAND family. Several studies have shown that MEF-2 and HAND proteins are important transcription factors in mediating cardiac hypertrophy and remodeling and may reinitiate and/or repress the fetal gene program in response to stress, such as hyperglycemia, pressure overload, or volume overload (5, 6, 8, 18, 29). A hallmark of cardiac stress response is a MHC isoform switch in which the embryonic β-MHC is upregulated with concomitant downregulation of the adult α-MHC isoform (5). The isoform switch has been proposed to contribute to attenuated myofibrillar ATPase activity and impaired cardiac contractility (44). HAND isoforms are myogenic basic helix-loop helix transcription factors that have distinct roles in cardiac development (1). Analysis of eHAND-null mice defined an essential role of eHAND in myocardial differentiation of the LV (1). It is noticeable that reduced eHAND is characteristic of human ischemic or dilated cardiomyopathy (31) and might play a role in reinitiating the fetal gene program during cardiac stress (45). In the present study, DMTU treatment ameliorates contractile dysfunction in diabetic rat hearts, suggesting that oxidative stress-dependent attenuation in the expression of eHAND and the resultant MHC isoform switch might be involved in the pathogenesis of diabetic cardiomyopathy. By contrast, the expression of dHAND remains unchanged in diabetic rat hearts. This result is in accordance with the known cardiac chamber-specific expression pattern; that is, eHAND is known to be restricted to the left-sided systemic ventricle and dHAND to the right-sided pulmonary ventricle. On the other hand, our present study showed that MEF-2 is enhanced in response to oxidative stress in diabetic rat hearts. Cardiac-specific overexpression of MEF-2A or MEF-2C was shown to induce a phenotype of dilated cardiomyopathy in mice and sarcomere degeneration in vitro (58). MEF-2 activity has been shown to be enhanced in rat hearts subjected to glucose and pressure-volume overload (6, 18, 29), and MEF-2 functions as an essential effector of divergent intracellular signaling pathways in mediating cardiac hypertrophy and initiating cardiac fetal...
and subsequent activation of PGC-1α-dependent protein kinase, leading to enhanced MEF-2 activity. 

AMP-activated protein kinase and Ca²⁺ during short-term stress, increased intracellular calcium plays a role in balancing cardiac contractility and energy metabolism. PPARγ coactivator-1 (PGC-1α) in conjunction with peroxisome proliferator-activated receptor (PPAR) gamma coactivator-1 (PGC-1α), plays an essential role in balancing cardiac contractility and energy metabolism, then, leads to a preserved cardiac function. Under conditions of prolonged cardiac stress, MEF-2 activity is enhanced in response to increased intracellular calcium but PGC-1α is downregulated (17, 33). The underlying mechanism regulating the interaction of both factors remains unclear, but the net result is downregulation of fatty acid oxidation enzymes and glycolysis. Uncoupling of MEF-2 and PGC-1α pathways may be a critical feature of pathological cardiac remodeling (17) and might play a role in the pathogenesis of diabetic cardiomyopathy.

A switch in MHC isomyosin composition has been reported to cause reduced contracting velocity and energy expenditure in chronic heart failure and in diabetic hearts (19). In the present study, we show that a marked MHC isoform switch and increased percent of slow myosin occur in diabetic rat hearts. Shroff et al. (39) demonstrated that Q_max was inversely proportional to percent slow myosin in spontaneously hypertensive rats. Herein, we demonstrate that Q_max is inversely proportional to percent slow myosin measured by mRNA expression and DMTU treatment partially normalizes MHC isoform switch as well as Q_max. These results suggest that the MHC isoform switch might be mediated by myocardial oxidative stress and might be one of the determinants of Q_max. Further studies are needed to clarify the causality of the relationship.

Antioxidant therapy has been extensively explored for the prevention of cardiovascular disease (51), but results are still controversial. Some studies have even suggested that they can be potentially harmful and that treatment with antioxidants cannot be currently recommended as a therapeutic option. However, the controversies may be attributable to factors such as difficulty in maintaining a consistent circulating antioxidant level, inadequate tissue distribution, and lack of suitable exogenous antioxidants (52, 55). In addition, most human trials used end points that were not directly related to oxidative stress, but rather gross markers of health, such as effect on mortality. In addition, most studies were not specifically designed to assess the effect of antioxidant in diabetic patients, in whom oxidative stress is much higher than in the general population. Indeed, it has been shown that patients with diabetes and end-stage renal disease responded favorably to antioxidant supplement in recent reports (10, 61), suggesting that antioxidants would be more effective in patients with higher levels of oxidative stress. Therefore, the role of antioxidant in high-oxidative-stress subjects, such as diabetic patients, needs further investigations. In our present study, apart from being effective in scavenging hydrogen peroxide and hydroxyl radicals, DMTU has also been shown to readily enter the myocardium, to have a long serum half-life of 43 h, and have no cardiac toxicity in vivo (16, 24). The benefits of DMTU treatment might come either directly from its antioxidant effect to reduce myocardial damage or indirectly from facilitating residual insulin action without changing insulin secretion in chronic diabetic rats. Further studies are needed to define the underlying molecular mechanisms.

In summary, the present study demonstrates that DMTU, by reducing lipid peroxidation and enhancing antioxidant capacity, has disparate effects on modulation of the myocardial response to increased oxidative stress in chronic diabetic rat

![Fig. 7. Analysis of oxidative stress-sensitive transcription factors in hearts. Western blots showed the protein expression of myocyte enhancer factor-2 (MEF-2; A) in the nuclear extracts, eHAND (B), and dHAND (C) in the cytosolic fractions of hearts from 4 study groups. The corresponding bar charts represent the net intensity ratio with lamin-B1 or α-actin; data are means ± SE of 4 rats per group. Statistics were one-way ANOVA followed by least significant difference post hoc test. *P < 0.05 vs. controls. **P < 0.05 vs. vehicle-treated diabetic rats.](http://ajpheart.physiology.org/)

expression of contractile proteins in mediating cardiac stress response. A coupled expression of cardiac contractile proteins and energy metabolism, then, leads to a preserved cardiac function. Under conditions of prolonged cardiac stress, MEF-2 activity is enhanced in response to increased intracellular calcium but PGC-1α is downregulated (17, 33). The underlying mechanism regulating the interaction of both factors remains unclear, but the net result is downregulation of fatty acid oxidation enzymes and glycolysis. Uncoupling of MEF-2 and PGC-1α pathways may be a critical feature of pathological cardiac remodeling (17) and might play a role in the pathogenesis of diabetic cardiomyopathy.

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hearts, with a dramatic restoration of velocity-dependent index of cardiac contractility but an insignificant benefit on force-dependent index. The advantages of DMTU treatment might involve normalization of cardiac-specific transcription factors, such as MEF-2 and eHAND, as well as the reversal of MHC isoform switch in chronic diabetic rats. These observations suggest a way toward an additional therapeutic approach to systolic dysfunction in diabetes.

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