Altered Myocardial Mechanics in Diabetic Rats

FREDERICK S. FEIN, LAURA B. KORNSTEIN, JOHN E. STROBECK, JOSEPH M. CAPASSO, AND EDMUND H. SONNENBLICK

SUMMARY Diabetes mellitus is associated frequently with congestive heart failure in humans, even in the absence of associated coronary disease or hypertension. Nevertheless, the effects of the diabetic state on myocardial mechanics have not been studied. Accordingly, diabetes was induced in female Wistar rats by injection of streptozotocin (60 mg/kg). Left ventricular papillary muscles were studied 5, 10, and 30 weeks later and compared with controls. Relaxation was delayed significantly and velocity of shortening was depressed at all loads. However, the passive and active force-length curves, as well as the series elastic properties, were not altered. The changes in cardiac performance were found over a range of muscle lengths, stimulus frequencies, and bath concentrations of calcium, glucose, and norepinephrine. The duration of diabetes had no major effect on the mechanical changes observed. The possible influences of drug-induced cardiac toxicity, malnutrition, and altered thyroid hormone levels have been considered; the latter two factors could not be excluded completely from having some influence on the mechanical properties of diabetic cardiac muscle. Evidence is cited showing abnormalities in calcium uptake by sarcoplasmic reticulum and depressed actomyosin ATPase activity. Thus, a cardiomyopathic state has been produced in the rat consequent to the induction of experimental diabetes mellitus. Various mechanisms for this entity have been suggested. Circ Res 47: 922-933, 1980

DIABETES MELLITUS, a disease with wide prevalence in humans, has major cardiovascular effects, including a strikingly increased incidence of congestive heart failure (Kannel et al., 1974). Epidemiological (Kannel et al., 1974), clinical (Regan et al., 1977), and pathological data (Hamby et al., 1974) suggest the existence of a diabetic cardiomyopathy independent of atherosclerotic coronary artery disease, hypertension, or valvular disease. A long-standing question has been whether the complications of diabetes are due to the diabetic state per se or to associated abnormalities that are genetically transmitted. Genetic factors may be avoided in experimental studies in animals in which diabetes is induced by drugs such as alloxan or streptozotocin; such drugs selectively destroy the β cells of the pancreas (Rerup, 1970).

Cardiac function in diabetes has been evaluated in humans and animals by several techniques. In humans, noninvasive and invasive studies have suggested reduced cardiac reserves (Ahmed et al., 1975; Regan et al., 1977; Rubler et al., 1978). In animals, studies of the intact diabetic dog (Regan et al., 1974) have suggested abnormalities of left ventricular diastolic compliance. Unfortunately, such studies do not permit easy quantification of loading conditions or geometry and so do not provide accurate assessment of muscle function per se. Use of the isolated ventricular papillary muscle does permit direct study of cardiac muscle performance (Sonnenblick, 1965).

The purpose of this study is to examine the effects of drug-induced diabetes on mechanics of isolated cardiac muscle to explore whether a diabetic cardiomyopathy exists and, if so, what the mechanisms of its production may be. The results of this study have been reported, in part, in abstract form (Fein et al., 1978, 1979).

Methods

Diabetic Rat Model

Female Wistar rats (Charles River), 170-200 g in weight, were made diabetic at 9 weeks of age with a single intravenous injection of streptozotocin, 60 mg/kg (Upjohn Company). The streptozotocin was dissolved in 0.9% saline containing 0.02 M sodium citrate (pH adjusted to 4.5), and less than 1 ml per rat was injected via the tail vein within 30 minutes of its preparation (Ganda et al., 1976). The diabetic state was assessed by measurement of nonfasting plasma glucose with the glucose oxidase method 2-3 weeks after streptozotocin administration. The diabetic state was assessed by measurement of nonfasting plasma glucose with the glucose oxidase method 2-3 weeks after streptozotocin administration. We used standard techniques to measure concentrations of plasma albumin (Miyada et al., 1972), serum β-hydroxybutyrate (Antonis et al., 1966), and total T₄ (Ratcliffe et al., 1974) and T₃ resin uptake (Mitchell et al., 1958) in blood samples taken from representative diabetic and control rats, at the time they were killed with other anesthesia; hematocrits also were determined. Free T₄ index was calculated.
as the product of total $T_4$ and $T_3$ resin uptake, divided by the normal $T_3$ resin uptake (31.4 in humans at the Albert Einstein College of Medicine). Groups of 10–15 animals with plasma glucose in excess of 300 mg/100 ml (at least 75% of the injected animals) were studied along with age-matched controls at various times following the induction of diabetes. Unless otherwise stated, all rats were fed Purina rat chow and given water ad libitum.

Instrumentation and Experimental Design

At the time of study, the heart was removed quickly after administration of ether anesthesia. The papillary or trabecular muscle was dissected rapidly from the left ventricle and suspended vertically in a myograph mounted on a Palmer stand. The tendinous end was tied with suture (Ethicon silk 5-0) to a lightweight steel rod, which inserted into a magnesium lever (equivalent mass of 125 mg) held in place by an upper micrometer stop. The bathing solution was a modified Krebs bicarbonate buffer (pH 7.4) containing, in millimoles per liter: Na\(^+\), 140.2; K\(^+\), 3.5; Mg\(^2+\), 1.1; Cl\(^-\), 119.0; HCO\(_3\)-, 24.8; H\(_2\)PO\(_4\)-, 1.1; SO\(_4\)-, 1.1; Ca\(^2+\), 0.6; (increased to 2.4 during the experiment), and glucose, 5.5. The bath was gassed continuously with a 95% O\(_2\)/5% CO\(_2\) mixture and maintained at a temperature of 30 ± 1°C. The muscle was stimulated with rectangular pulses 5–15 msec in duration with a voltage 10–15% above threshold at a frequency of 0.1 Hz, provided through platinum electrodes arranged on either side of and parallel to the muscle preparation.

The parameters of muscle force and its first derivative, muscle length, and velocity were displayed as a function of time on a multichannel storage oscilloscope (Tektronix 5103N). Force was measured by a strain gauge transducer (OSC-3, Kistler-Morse Corp.) attached via a spring-loaded clip to the nontendinous end of the muscle. The rate of force change was derived from electronic differentiation of the force signal. Changes in muscle length, i.e., lever position, were detected by a photodiode system. A voltage proportional to lever displacement was passed through an analog differentiator to determine velocity (time constant = 0.05 msec).

After an equilibration period of 1 hour, during which the muscle contracted isometrically at a resting force of 1 gram, the length-tension relationship was obtained at a calcium concentration of 0.6 mM. The length-tension curve was generated by reducing muscle length from the length associated with a maximum developed force ($L_{\text{max}}$) to about 91% $L_{\text{max}}$ in 0.1-mm steps, and the resting and developed force (and its differential) were recorded. The relationship between force and velocity for a series of afterloaded contractions was established at an initial muscle length of $L_{\text{max}}$ and at 0.2 mm below $L_{\text{max}}$.

The load on the muscle was increased progressively from preload to isometric force by varying the current passed through a coil suspended by bearings in a magnetic field, and the muscle length and velocity during shortening and relaxation were recorded. These determinations subsequently were repeated at a bath [Ca\(^2+\)] of 0.6 mM. Series elasticity was measured using a single quick release (duration less than 5 msec) from peak-developed tension to preload tension, analyzing the instantaneous force-length plot. The extent of muscle shortening during the release was expressed as a percent of $L_{\text{max}}$.

Basic Study Design

The mechanical function of cardiac muscle after 5 weeks of diabetes was compared to that of age-matched controls (C\(_1\)). A second group of age-matched controls (C\(_2\)) was created whose daily food intake was limited over the same period. Individually caged C\(_2\) controls and diabetics (D) were weighed three times a week. The number of pellets of Purina rat chow given to C\(_2\) controls was adjusted so that their mean body weight equaled the mean weight of the diabetics fed ad libitum with a similar distribution of weights. Mechanical studies also were carried out in rats that had been diabetic for 10* and 30 weeks. These studies were carried out on animals derived from separate litters.

Interventions

The effects of several interventions on contraction and relaxation processes were evaluated: (1) a glucose dose-response was performed in the bath at glucose concentrations of 100 (initial level), 400, 700, and 1000 mg/100 ml, with increments made every 10 minutes; (2) the frequency of stimulation was increased from 0.1 to 0.8 Hz by successive doublings of rate every 2 minutes; and (3) norepinephrine (Levophed, Winthrop) was added to the bath in 10-fold increments, increasing concentration from $10^{-8}$ to $10^{-7}$ M at 10-minute intervals. No more than one such intervention was performed on each papillary muscle.

\* Two 10-week groups were studied. From the first (10A), the basic isometric and isotonic data were measured. From the second (10B), the force-frequency relation and norepinephrine dose-response were obtained. In addition, serum β-hydroxybutyrate was measured in group 10B.
TABLE 1  General Features of Diabetic and Control Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C1</th>
<th>D</th>
<th>C2</th>
<th>5 Weeks</th>
<th>10 Weeks</th>
<th>30 Weeks</th>
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<tbody>
<tr>
<td>BW (g)</td>
<td>260±5 (9)</td>
<td>205±5 (9)</td>
<td>205±7 (9)</td>
<td>205±8 (10)</td>
<td>221±8 (13)</td>
<td>304±8 (8)</td>
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<tr>
<td>P</td>
<td>≤0.05</td>
<td>NS</td>
<td>≤0.05</td>
<td>NS</td>
<td>≤0.05</td>
<td>NS</td>
</tr>
<tr>
<td>HW (g)</td>
<td>0.63±0.02 (9)</td>
<td>0.53±0.01 (9)</td>
<td>0.52±0.02 (9)</td>
<td>0.52±0.01 (10)</td>
<td>0.64±0.02 (13)</td>
<td>0.79±0.02 (8)</td>
</tr>
<tr>
<td>[glu] (mg/100 ml)</td>
<td>≤0.05</td>
<td>NS</td>
<td>≤0.05</td>
<td>NS</td>
<td>≤0.05</td>
<td>NS</td>
</tr>
<tr>
<td>[BHBA] (mM)</td>
<td>0.34±0.05 (5)</td>
<td>0.14±0.28 (5)</td>
<td>0.72±0.15 (7)</td>
<td>0.39±0.03 (5)</td>
<td>0.56±0.05 (4)</td>
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</tr>
<tr>
<td>[alb] (g/100 ml)</td>
<td>4.0±0.1 (5)</td>
<td>3.9±0.2 (5)</td>
<td>4.2±0.4 (5)</td>
<td>4.2±0.4 (5)</td>
<td>4.2±0.4 (5)</td>
<td>4.2±0.4 (5)</td>
</tr>
<tr>
<td>Hct (vol/100 ml)</td>
<td>47±1 (5)</td>
<td>44±2 (3)</td>
<td>42±1 (3)</td>
<td>42±1 (3)</td>
<td>42±1 (3)</td>
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<tr>
<td>[T₄] (μg/100 ml)</td>
<td>3.3±0.4 (5)</td>
<td>0.9±0.3 (5)</td>
<td>2.1±0.4 (5)</td>
<td>3.8±0.2 (10)</td>
<td>2.2±0.2 (13)</td>
<td>1.6±0.2 (8)</td>
</tr>
<tr>
<td>T₃R (%)</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
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<tr>
<td>FT₄</td>
<td>57±2±0.8 (5)</td>
<td>51±4±2.5 (5)</td>
<td>54±6±1.2 (5)</td>
<td>53±1±0 (10)</td>
<td>50±2±1.2 (13)</td>
<td>48±1±6 (8)</td>
</tr>
<tr>
<td>FT₃R Index (%)</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
</tr>
<tr>
<td>T₄, and T₃R Index</td>
<td>6.2±0.6 (5)</td>
<td>1.5±0.5 (5)</td>
<td>3.6±0.6 (5)</td>
<td>6.5±0.4 (13)</td>
<td>3.5±0.4 (13)</td>
<td>2.3±0.3 (8)</td>
</tr>
<tr>
<td>β²</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
<td>≤0.05</td>
</tr>
</tbody>
</table>

C₁ = age-matched controls (5-week) fed ad libitum; D = streptozotocin-induced diabetic; C₂ = age-matched underfed controls; C = age-matched controls (10- or 30-week) fed ad libitum; BW = body weight; HW = heart weight; glu = plasma glucose; BHBA = serum β-hydroxybutyrate; alb = plasma albumin; Hct = hematocrit; T₄ = total serum thyroxine; T₃R = serum triiodothyronine resin uptake; FT₄ = free T₄ index (T₄ X T₃R/31.4); NS = not significant (P > 0.05). P values in 5-week study refer to comparisons between groups C₁ and D, and between groups D and C₂.

Values are expressed as the mean ± SEM. Numbers of rats are given in parentheses. Ten-week study data are from group 10A except where indicated by an asterisk.

* Values obtained from group 10B.

3-O-Methyl Glucose Study

A group of rats (170–200 g in weight) was given a 1-ml injection per animal of a 1.65 mmol of 3-O-methyl glucose (Sigma) by tail vein immediately before streptozotocin injection (60 mg/kg), to prevent the latter's diabetogenic effect (Ganda et al., 1976). This nonmetabolizable glucose analog was dissolved in 1 N saline and kept in the cold for 24 hours prior to injection. Plasma glucose concentrations were monitored in rats given 3-O-methyl glucose and age-matched controls, and papillary muscle studies were performed 5 weeks after injection.

Data Analysis

Parameters dependent on muscle length and load were computed at intervals of 1% Lₘax, and 5% relative load (total isotonic load/total isometric load X 100), respectively, by linear interpolation of experimental data. Statistical comparison between groups was performed using the unpaired Student's t-test. When three groups were compared, one-way analysis of variance was used and was followed, when appropriate, by the Newman-Keuls test. P ≤ 0.05 was considered statistically significant.

Results

The Diabetic Model

General features of drug-induced diabetes after 5, 10, and 30 weeks of diabetes are shown in Table 1 along with values for controls at the time of study. Body weights were significantly less in the diabetic rats compared to controls. Except for the 10-week group, heart weights also were depressed. After 5 weeks of controlled restriction of food, body and heart weights of underfed controls (C₂) and diabetics were identical. Plasma glucose was several times higher than in controls at all intervals of diabetes studied; the seemingly high values in control groups may reflect the sympathetic response to ether anesthesia (Price et al., 1970). Beta-hydroxybutyrate levels were highest in diabetics; however, the differences from control values were quantitatively small and unlikely to cause systemic acidosis. Plasma albumin and hematocrit were identical in both controls and diabetics. Serum thyroxine (T₄), T₃ resin uptake, and free T₄ index, however, were depressed in the 5- and 10-week studies in the diabetics. Another group of rats, studied after 30 weeks of diabetes, showed no differences in T₄ between diabetics and controls, but control values were low. These data are discussed further below.

Muscle Mechanics

Isometric Studies

Representative isometric contractions in a diabetic (D) and age-matched control (C) are shown in Figure 1a. Resting and developed tensions are similar. Time to peak tension (TPT) is prolonged and the peak rate of tension rise (+T') is slightly less in the diabetic contraction. The most striking differences, however, are noted during relaxation. The time for peak-developed tension (DT) to fall to 50% DT (T½ R) is prolonged, peak rate of tension fall (-T') is depressed, and time from DT to -T' (TPN) is increased in the diabetic trace.
Figure 1 Ten-week study. Papillary muscle contractions at Lmax. Bath [Ca2+] = 2.4 mM. a: Representative isometric contractions from diabetic and age-matched control fed ad libitum. Tension (upper panel) and the rate of tension change (lower panel) are plotted against time. Mechanical parameters are indicated by arrows pointing to the control trace. RT = resting tension; DT = developed tension; TPT = time to peak tension; TVR = time to peak velocity of relaxation; —T' = peak rate of tension fall; TPN = time from peak tension to peak rate of tension fall. b: Representative isotonic contractions from diabetic and age-matched control fed ad libitum. Relative loads (total isotonic load/total isometric load × 100) were similar.

Isometric measurements were made at Lmax and a bath [Ca2+] of 2.4 mM, and these are summarized in Table 2. Data have been derived from the study of three separate litters of diabetic and control animals after 5, 10, and 30 weeks of drug-induced diabetes mellitus. Thus, since these rats were not from the same original litter, intergroup comparisons reflecting the effect of the duration of diabetes cannot be made conclusively. However, intragroup comparisons are valid and will be discussed in detail.

Following 5 weeks of diabetes, papillary and trabecular muscles from age-matched controls (Ci), diabetics (D), and age- and weight-matched controls (C2) did not differ with respect to average muscle length, weight, or cross-sectional area data. Mechanical properties were not different in papillary as compared to trabecular muscles. Resting and developed tension, as well as the peak rate of rise of isometric tension (+T'), also did not differ among groups. However, time to peak tension (TPT'), and several indexes of relaxation, including the half-time of relaxation (T½ R), the peak rate of tension decline (−T'), and the time to reach the peak rate of tension decline (TPN) were all abnormal in diabetics, compared to Ci controls. Food deprivation in C2 control animals produced a slowing of relaxation compared to C1 controls when TPT, T½ R, and TPN were analyzed. The values for these variables were still lower than in diabetics, but the differences did not achieve statistical significance. However, −T' in C2 controls was still significantly different from that of the diabetics, and almost identical to that of C1 controls.

The relations between muscle length and resting and developed tensions in the three groups studied after 5 weeks of diabetes are shown in Figure 2. No statistical differences were detected in the passive length-tension curves of diabetics and controls. Similarly, no differences were seen in active length-tension properties of muscles from the three groups. Figure 3 illustrates the relation between muscle length and the peak rate of tension decline after 5 weeks of diabetes. Significant depressions were observed in the diabetic group over a large portion of the range of muscle lengths studied.

Differences in the time course of relaxation comparing diabetics with either control group also were present at a low (0.6 mM) level of [Ca2+] in the bathing medium; T½ R at Lmax was 87 ± 3 in Ci controls, 111 ± 3 in diabetics, and 101 ± 2 msec in C2 controls (values are mean ± SEM). The differences between diabetics and either control group were significant (P < 0.05).

In a separate group of rats studied 10 weeks after the development of diabetes (group 10A), analysis of isometric contraction revealed similar alterations in relaxation as were seen in the 5-week study (see Table 2). In another group of rats studied 30 weeks after the development of diabetes, the alterations in relaxation parameters persisted.

Isotonic Studies

Typical records of preloaded isotonic contractions of diabetic (D) and age-matched control (C) rats are shown in Figure 1b, at a bath [Ca2+] of 2.4 mM. The extent of muscle shortening did not differ between diabetic and control contractions. Time to peak shortening was prolonged in the diabetic muscle and peak velocities of shortening and relaxation (lengthening) were lower in the diabetic contraction; time to peak relaxation velocity also was increased in the diabetic trace.

In Figure 4a, force-velocity curves are plotted for the 5-week study. Measurements were made at an initial length of Lmax and a bath [Ca2+] of 2.4 mM. Diabetics exhibited depressed shortening velocities over a wide range of loads when compared to either group of controls. Similar results were obtained in studies done at an initial length 0.2 mm below Lmax

† Points on the force-velocity curve were plotted only if all animals had values of shortening velocities at the particular relative load. For individual rats, force-velocity curves could be constructed with relative loads as low as 20%.
question of direct streptozotocin-induced cardiac toxicity. Body and heart weights and plasma glucose concentrations were comparable to those of age-matched controls. No significant differences between the two groups were found (Table 4).

Thyroid Function Studies
Depressions of total T₄ and free T₄ index (a measure of the free T₄ concentration) were observed after 5 and 10, but not 30 weeks of diabetes (Table 1). To assess the possible relationship between the mechanical abnormalities in diabetic cardiac muscle and the lowered hormone levels, T₄/R was plotted vs. free T₄ index for individual rats. No relation was seen for either group (Fig. 8). Four diabetic and four control rats were selected from the larger groups because of closely matched values for free T₄ index (5.1 ± 0.3 in diabetics and 5.4 ± 0.4 in controls); there was still a significant difference in T₄/R (194 ± 15 in diabetics and 115 ± 7 msec in controls; Fig. 8). In these subgroups, as in the entire groups from which they were selected, body weights were lower in diabetics but heart weights in diabetics were identical to that of controls.

Discussion
Although it has long been known that diabetes mellitus is a risk factor for congestive heart failure in humans, only recently has evidence accumulated favoring the existence of a diabetic cardiomyopathy, that is, heart failure independent of large vessel coronary artery disease, hypertension, or valvular disease. The Framingham study (Kannel et al., 1974) showed that the incidence of congestive heart failure was higher in diabetics (about five times higher in females) than nondiabetics when factors such as hypertension, coronary, and rheumatic heart disease were taken into account. The study suggested that diabetes was a discrete cause of congestive heart failure due to either small vessel disease or metabolic disorder. Hamby et al. (1974) came to similar conclusions, noting that the incidence of diabetes was quite high in patients with an unexplained cardiomyopathy.

Studies of the pathology of diabetic hearts usually have involved small numbers of patients with a clinical cardiomyopathy (Hamby et al., 1974; Regan et al., 1977). Ventricular hypertrophy, interstitial fibrosis, and intimal thickening of small coronary arteries have been described. The significance of the small vessel changes was uncertain. Noninvasive clinical studies have provided indirect evidence of the existence of a diabetic cardiomyopathy (Ahmed et al., 1975; Rubier et al., 1976). Of particular interest in relation to the current study is the echocardiographic finding of a prolonged isovolumic relaxation time in diabetics (Rubier et al., 1978). Invasive studies of left ventricular function in diabetics without hypertension or coronary artery disease have been limited. Of 12 such patients studied by Regan et al. (1977), eight had no clinical signs of
heart failure but had changes interpreted as an increased left ventricular wall stiffness and possibly a preclinical cardiomyopathy. The other four patients had clinical evidence of heart failure and ventriculography showed diffuse hypokinesis in three of four.

Experimental models of diabetes have facilitated measurements of cardiac performance. Catheterization studies of dogs made mildly diabetic for 11 months with alloxan suggested an increase in left ventricular wall stiffness (Regan et al., 1974). This conclusion is consonant with their clinical studies (see above). These changes were attributed to the increase in interstitial glycoprotein observed on histochemical study. Studies in the isolated perfused hearts of diabetic rats have shown: (1) decreased response of peak systolic pressure to a rise in left ventricular filling pressure (Miller, 1979); (2) more rapid development of heart failure on exposure to severe global ischemia (Feuvray et al., 1979); and (3) myocardial failure during late recovery from ischemia (Hearse et al., 1975). In isolated, spontaneously beating atria from diabetic rats, a reduced heart rate, increased contractile force, and lessened sensitivity to the inotropic effects of norepinephrine and calcium were noted (Foy and Lucas, 1978).

However, in all of the above studies, ambiguities relating to ventricular cavitary geometry, wall thickness, and fiber orientation in the various experimental preparations prevent a precise description of myocardial function. Analysis of the performance of isolated ventricular papillary muscle avoids these uncertainties.

In the present study, the effects of diabetes mellitus on papillary muscle function have been delineated for the first time. The most prominent abnormalities observed involved the process of relaxation. These included: (1) a delayed onset of relaxation as measured by the time to peak isometric tension and time to peak isotonic shortening; (2) a slowed rate of relaxation characterized by a prolonged time for isometric relaxation and depressed rates of isometric and isotonic relaxation; and (3) a delay in reaching peak isometric and isotonic relaxation rates. The changes in myocardial relaxation that we have noted in diabetic muscles were observed under a wide range of conditions, including altered muscle lengths, stimulus frequencies, and concentrations of calcium, glucose, and norepinephrine. The duration of diabetes did not affect substantially the quantitative differences in relaxation. Series elasticity was unchanged in diabetic muscles, indicating that other factors (see below) must account for the relaxation defects.

Figure 5 Five-week study. Influence of bath [glucose] on developed tension (a) and the time to ½ relaxation (b) at Lmax. Bath \([\text{Ca}^{2+}] = 2.4 \text{ mM}\). Values are plotted as the mean ± SEM. The timing abnormality in the diabetics is present at all but the highest [glucose]. * \(P \leq 0.05\). □ = C; (n = 5); ○ = D (n = 6); △ = C2 (n = 5).

Figure 6 Ten-week study (10B). Influence of the frequency of stimulation on developed tension (a) and the time to ½ relaxation (b) at Lmax. Bath \([\text{Ca}^{2+}] = 2.4 \text{ mM}\). Values are plotted as the mean ± SEM. The timing abnormality in the diabetics is present at all frequencies. * \(P \leq 0.05\). □ = C (n = 4); ○ = D (n = 4).
A major concern in interpreting these results is whether the observed changes in papillary muscle function were due to diabetes itself. The possible influences of malnutrition, direct drug-induced cardiac toxicity, and altered thyroid hormone levels have been considered.

Streptozotocin as used in this study produced severe diabetes which resulted in lower body and heart weights in diabetic animals compared to age-matched controls (group C). Therefore, in the 5-week study, another group of controls (C2) was given a limited daily food intake (see Methods). At the time of study, diabetics and C2 controls had very similar body and heart weights but the differences in many mechanical properties between the groups still were observed. The differences in cardiac performance between underfed rats and rats fed ad libitum resembled those previously described (Cohen et al., 1976). Of particular importance in that study, no depression in shortening velocity was observed in muscles from severely malnourished rats. The 10-week study revealed no differences in heart weight between diabetics and controls, yet the changes in mechanical properties in diabetics were no less striking than in the 5- or 30-week studies. Therefore, malnutrition is probably not the primary factor responsible for the changes observed in diabetic rat papillary muscle function.

The 3-O-methyl glucose experiments provided evidence that streptozotocin itself probably is not cardiotoxic. Rats given 3-O-methyl glucose before streptozotocin did not develop diabetes or any of the mechanical abnormalities described above. However, 3-O-methyl glucose might also inhibit streptozotocin-induced cardiac damage. The absence of early histological changes by both light and electron microscopy in this model of diabetes (Bhan et al., 1978; Factor et al., 1978), as well as the early onset of a stable change in papillary muscle function, suggest that the defect probably is not drug-induced. Final proof would be the finding that changes in relaxation could be prevented with early insulin therapy or pancreatic transplantation after injection with streptozotocin.

A significant depression in total and free T4 was observed in the 5- and 10-week studies among diabetic rats. Hypothyroidism is known to slow relaxation and to depress the force-velocity relation in papillary muscle studies (Buccino et al., 1967). The possibility that the effect of diabetes on rat cardiac tissue is a result of associated hypothyroidism thus was considered and thought unlikely for the following reasons. As shown in Figure 8, no relation was found between the time to ½ relaxation and the free T4 index over a wide range of values for free T4 index. Further, when matched for free T4 index, diabetics and controls show the same substantial differences in relaxation as when the entire groups are compared. The 30-week study did not show differences in free T4 index between diabetics and controls, yet the changes in mechanical properties in diabetics were as marked as in the 5- and 10-week studies.‡ Thus, hypothyroidism, even if pres-
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Table 4  3-O-Methyl Glucose Study

<table>
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<tr>
<th>General features</th>
<th>C (10)</th>
<th>3OM (10)</th>
</tr>
</thead>
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<tr>
<td>BW (g)</td>
<td>246 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>[glu] (mg/100 ml)</td>
<td>NS</td>
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<td>Isometric data</td>
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<tr>
<td>DT (g/mm²)</td>
<td>5.37 ± 0.19</td>
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<tr>
<td>TPT (msec)</td>
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<td>+T' (g/sec per mm²)</td>
<td>NS</td>
<td>72.10 ± 9.56</td>
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<tr>
<td>T½R (msec)</td>
<td>99 ± 2</td>
<td>NS</td>
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<td>-T' (g/sec per mm²)</td>
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</tr>
<tr>
<td>V₀ (ML/sec)</td>
<td>1.14 ± 0.02</td>
<td>1.09 ± 0.08</td>
</tr>
<tr>
<td>Vᵢ (ML/sec)</td>
<td>1.84 ± 0.08</td>
<td>1.76 ± 0.24</td>
</tr>
</tbody>
</table>

C = age-matched controls; 3OM = rats given 1.65 mmol of 3-O-methyl glucose before streptozotocin injection; BW = body weight; HW = heart weight; glu = plasma glucose; DT = developed tension; TPT = time to peak tension; +T' = peak rate of tension rise; T½R = time to ½ relaxation (50% DT); -T' = peak rate of tension fall; PS = peak shortening; TPS = time to peak shortening; V₀ = peak velocity of shortening, divided by Lmax; Vᵢ = peak velocity of relaxation, divided by Lmax; ML = muscle lengths; NS = not significant (P > 0.05).

Values are expressed as the mean ± SEM. Numbers of rats are given in parentheses. Values are obtained at an initial muscle length = Lmax, and at a relative load (total isotonic load/total isometric load X 100) of 35%. Bath [Ca²⁺] = 2.4 mM. Muscles were studied 5 weeks after 3-O-methyl glucose injection.

ent in the diabetic rat, probably does not mediate the observed changes in papillary muscle function.

These findings of abnormal myocardial function in experimental diabetes do not necessarily imply that ventricular failure exists in the intact diabetic animal. In fact, despite clearly demonstrated alterations in relaxation and shortening velocity, two important indexes of muscle performance, i.e., peak developed tension and peak shortening, were not affected by the diabetic state. It is possible, however, that in the intact diabetic animal at physiological heart rates, the relaxation defect might impair diastolic filling of the ventricles. It is also possible that systolic performance of the hearts of such animals might be normal under resting conditions, but abnormal on exposure to the stress of exercise, ischemia, or hypertension.

It is unlikely the changes in diabetic papillary muscle function are simply a result of inadequate substrate and, hence, energy stores, because high bath glucose concentrations, likely to increase glucose transport into the myocytes, did not correct the defects in contraction and relaxation in diabetic tissue. Moreover, in preliminary studies, insulin added to the bath (at concentrations of up to 10⁻² U/ml) did not restore papillary muscle function of these muscles to normal. In histological studies at the light and ultrastructural level, no significant changes in the vascular supply of the heart or structural alterations in the myocytes or interstitium were noted, which would explain the altered cardiac muscle performance in diabetics (Bhan et al., 1978; Factor et al., 1978).

The alterations in relaxation in diabetic myocardium most probably result from a disorder of sarcoplasmic reticum. The sarcoplasmic reticulum, particularly in rat cardiac muscle, has been shown to be the most active subcellular organelle involved with the sequestration of activator calcium (Solaro et al., 1974). Calcium binding and/or uptake by cardiac sarcoplasmic reticum have been shown to be altered in a variety of physiological and pathological states. Increased calcium uptake has been observed in hyperthyroidism (Suko, 1971) and the hypertrophy due to exercise (Penpargkul et al., 1977). Depressed calcium uptake has been observed with aging (Froehlich et al., 1978), hypothyroidism (Suko, 1971), the high output states of anemia and
The decrease in velocity of shortening at all loads strongly implies a decrease in shortening at zero load, i.e., V<sub>max</sub>. We have not extrapolated the force-velocity curves to this theoretical point to avoid potential ambiguity. A decrease in V<sub>max</sub> is of interest in view of the known relation between this parameter and actomyosin or myosin ATPase in both skeletal (Barany, 1967) and cardiac muscle (Delcayre at al., 1975) from different species. Furthermore, in cardiac muscle, V<sub>max</sub> and either myofibrillar or actomyosin or myosin ATPase have been shown to change concomitantly in various physiological conditions such as aging (Alpert et al., 1967) and pathological states such as hyper- and hypothyroidism (Buccino et al., 1967; Yazaki, et al., 1975) and pulmonary artery stenosis (Chandler et al., 1967). Thus, it is of interest that calcium-activated actomyosin ATPase, and both actin-activated and calcium-activated myosin ATPase all were found to be significantly depressed in preparations from rats made diabetic according to the current protocol (Penpargkul et al., 1979).

The precise mechanisms whereby diabetes mellitus results in the alterations in function of the sarcoplasmic reticulum and the biochemistry of contractile proteins remain obscure at present and are the basis for future investigations. The specific derangements in myocardial function described in this paper will provide direction to these studies.

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References

Barnay M (1967) ATPase activity of myosin correlated with speed of muscle shortening. J Gen Physiol 50: 197-218
Fabio A, Fabio F (1973) Activation of skinned cardiac cells: Subcellular effects of cardioactive drugs. Eur J Cardiol 1: 143-155


Hearse DJ, Stewart DA, Chain EB (1975) Diabetes and the survival and recovery of the anoxic myocardium. J Mol Cell Cardiol 7: 397–415


Suko J (1971) Alterations of Ca ++ uptake and Ca ++-activated ATPase of cardiac sarcoplasmic reticulum in hyper- and hypothyroidism. Biochim Biophys Acta 252: 324–327

Altered myocardial mechanics in diabetic rats.
F S Fein, L B Kornstein, J E Strobeck, J M Capasso and E H Sonnenblick

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