Reversibility of Diabetic Cardiomyopathy with Insulin in Rats

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SUMMARY Diabetes appears to cause a cardiomyopathy independent of atherosclerotic coronary artery disease and hypertension. Left ventricular papillary muscle function studies in rats made severely diabetic with streptozotocin have shown a slowing of relaxation and a depression of shortening velocity. However, the effects of insulin therapy on the myocardial mechanics of diabetic rats have not been studied. Therefore, rats diabetic for 6-10 weeks were treated with PZI insulin for 2, 6, 10, or 28 days and the mechanical performance of their left ventricular papillary muscles was compared to that of untreated diabetics and age-matched controls; cardiac contractile protein enzymatic activity was also measured. Neither 2 nor 6 days of therapy had any effects on the depressed cardiac muscle performance of diabetic animals, although plasma glucose concentration was restored to normal. By 10 days of therapy, recovery of mechanical performance was nearly complete, and by 28 days of therapy, complete reversal of the altered myocardial mechanics was observed. Crystalline insulin added to the bath (9 mU/ml) had no effect on myocardial mechanics in either diabetics or controls. A gradual recovery of actomyosin and myosin ATPase activity in the hearts of insulin-treated diabetic animals was also found, complementing the mechanical studies. In addition to demonstrating a gradual but complete reversibility of the abnormalities in papillary muscle function in diabetic rats (although control of hyperglycemia was less than ideal), this study confirms that this model of a cardiomyopathy is not a result of streptozotocin-induced cardiac toxicity. Additional data are provided indicating that depressed thyroid hormone levels in diabetic rats are not responsible for the mechanical changes observed. Circ Res 49: 1251-1291, 1981

EPIDEMIOLOGICAL data strongly suggest that diabetes mellitus is associated with congestive heart failure even in the absence of associated coronary artery disease or hypertension (Kannel et al., 1974). This conclusion is supported by clinical (Regan et al., 1977) and pathological data (Hamby et al., 1974). Studies of experimental diabetes have suggested alterations in left ventricular diastolic compliance in mildly diabetic dogs (Regan et al., 1974), and a slowing of relaxation as well as a depression of shortening velocity in left ventricular papillary muscles from severely diabetic rats has been shown (Fein et al., 1980). A biochemical basis for the findings of Fein et al. (1980) has been described (Penpargkul et al., 1981; Malhotra et al., in press); both depressed sarcoplasmic reticulum function and contractile protein enzymatic activity were found.

Very little is known about the reversibility, if any, of altered myocardial function with treatment of diabetes. Prior studies have merely looked at apparent compliance changes in the intact heart, and then with variable results (Wu et al., 1977; Pogatsa et al., 1979). The clearly defined changes in papillary muscle function of diabetic cardiac tissue (Fein et al., 1980) suggests that this preparation would also be useful in evaluating the influence of hypoglycemic therapy.

The purpose of this study is to examine the effects of insulin therapy on papillary muscle function and contractile protein biochemistry of severely diabetic rats, evaluating the influence of duration of treatment on the completeness of reversibility of the mechanical alterations. The results of this study have been reported, in part, in abstract (Fein et al., 1981).

Methods

Diabetic Rat Model

Female Wistar rats (Camm and Charles River), 170-200 g in weight, were made diabetic at 9 weeks of age with a single intravenous injection of 60 mg/kg of streptozotocin (Upjohn Company), as previously described (Fein et al., 1980). The diabetic state was assessed by measurement of nonfasting plasma glucose concentration (in tail vein blood obtained during ether anesthesia) with the glucose oxidase method 2-3 weeks after streptozotocin administration. Only animals with plasma glucose concentrations that exceeded 300 mg/100 ml were considered diabetic. Total T4 (Ratcliffe et al., 1974) and T3 resin uptake (Mitchell et al., 1958) was assayed in serum samples taken from diabetic,
treated diabetic, and control rats at the time of sacrifice. Free T4 index was calculated as the product of total T4 and T3 resin uptake, divided by the normal T3 resin uptake (30.7 in humans at the Albert Einstein College of Medicine).

Basic Study Design

Two- and 10-Day Studies

Six to 9 weeks after streptozotocin injection, a subgroup of diabetic animals (Camnn) was begun on insulin therapy. In all studies, the treatment group was chosen to closely match the untreated diabetics with respect to body weight and plasma glucose concentration. PZI insulin (U40, Eli Lilly) was injected subcutaneously each evening (usually between 4 and 6 p.m.). For the first 3–4 days of therapy, either 5 or 6 units of insulin were administered daily. Blood was obtained during the morning after the third or fourth dose of insulin, the plasma glucose concentration was determined, and the insulin dose was adjusted to bring this concentration to approximately 100 mg/100 ml. Because of each animal’s variable response to insulin, the following criteria for inclusion of a treated animal in this study were developed and applied to all the studies described in this paper: (1) treated animals had glucose concentrations below 300 mg/100 ml on all occasions during therapy and (2) mean glucose concentration during therapy for each animal was below 200 mg/100 ml. Insulin therapy was continued for a total of 10.2 ± 0.7 (SEM) days; the average daily dose was 4.7 ± 0.3 units. The animals then were killed and mechanical studies performed. Body weights plus blood for glucose concentration and thyroid function studies were also obtained at sacrifice from the treated diabetics (D+I). In addition, untreated diabetics (D) and age-matched controls (C) were studied.

Several additional diabetic animals were given 5 units of PZI insulin subcutaneously daily for 2 days (beginning 9–10 weeks after streptozotocin injection), and mechanical studies were performed. They were compared with the same groups of untreated diabetics and controls used in the 10-day insulin study described above.

Six- and 28-Day Studies

Seven weeks after streptozotocin injection, a subgroup of diabetic animals (Charles River) was begun on PZI insulin. The dosage chosen was 4 units/day for 2 days, which in most cases was then decreased to 3 units daily. Blood for plasma glucose concentration studies was obtained during the morning after the fourth dose of insulin, and the dosage was adjusted to maintain the glucose concentration at about 100 mg/100 ml. Animals were killed after a total of 6.3 ± 0.2 days of insulin therapy (average dose, 3.5 ± 0.1 units), and mechanical studies were performed along with those of untreated diabetics and age-matched controls.

Five to 8 weeks after streptozotocin injection, a subgroup of diabetic animals (Charles River) was also begun on PZI insulin. For the first 2 days of therapy, 3–5 units were given daily, followed for the remainder of the first week by 2–4 units daily. Blood for plasma glucose concentration measurements then was obtained, and again at weekly intervals thereafter (for a total of four or five determinations for each treated animal). Insulin dosages were adjusted to attempt to regulate glucose levels at 100–200 mg/100 ml. A higher concentration of glucose was chosen for this study because of concern that finer regulation of hyperglycemia over a 4-week period would lead to substantial mortality from hypoglycemia. Mortality (from hypoglycemia) in insulin-treated diabetics was about 40% compared to 20% in untreated diabetics; there was no mortality among controls.

Animals were killed after 28.0 ± 0.3 days of therapy (average daily dose 2.7 ± 0.2 units) and compared mechanically with untreated diabetics and age-matched controls.

Instrumentation and Experimental Design

Mechanical Studies

At the time of study, the rat was anesthetized with ether and the heart quickly removed. The papillary or trabecular muscle was rapidly dissected from the left ventricle and suspended vertically in a myograph as previously described (Fein et al., 1980). The bath was continuously gassed with a mixture of 95% O2/5% CO2 and maintained at a temperature of 30 ± 1°C. The muscle was stimulated with rectangular pulses 5–15 msec in duration and 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 great vessels and atria were trimmed from the heart, which was blotted dry and weighed.

After an equilibration period of 1 hour, during which the muscle contracted isometrically at a resting force of 1 g, bath [Ca2+] was raised from 0.6 to 2.4 mm and the muscle stretched to Lmax, the length associated with maximum developed force. At Lmax, isometric and a series of afterloaded isotonic contractions were obtained and analyzed as previously described (Fein et al., 1980). Approximately 25% of animals killed, both diabetics and controls, yielded muscles unsuitable for study because of a branched structure and/or a maximal developed tension less than 3 g/mm2 at a bath [Ca2+] of 2.4 mm.

The effects of insulin were also analyzed by adding crystalline insulin to the bath at a concentration of 9 μU/ml. Isometric and preloaded isotonic contractions were recorded at least 15–20 minutes later.

Studies of Contractile Proteins

The methods for preparing and analyzing cardiac actomyosin from individual hearts have been de-
scribed previously (Bhan and Scheuer, 1972). Actomyosin was used to survey cardiac contractile proteins in individual animals to avoid the need to pool hearts, which is required for purification of myosin. Actomyosin ATPase has been shown generally to correlate with the calcium ATPase of myosin- and actin-activated magnesium ATPase activity of myosin (Mahotra et al., in press). When findings with actomyosin were positive, studies were repeated with pure myosin (see below). The ventricles were minced and homogenized in 0.05 M KCl, 0.01 M KPO₄⁻ (pH 7.0) and centrifuged. The pellets were further treated with 0.05 M KCl, 0.01 M KPO₄⁻, and 2 mM EGTA (pH 7.0), followed by washing with buffer containing Triton X-100. Actomyosin was extracted and isolated from the myofibrils with 10 volumes of 0.6 M KCl, 10 mM imidazole, 1 mM dithiothreitol (DTT) (pH 7.0) for 20 hours.

After extraction, the brei was centrifuged at 10,000 g for 30 minutes. The supernatant was diluted 10-fold with cold de-ionized water containing 10 mM imidazole and 1 mM DTT (pH 7.0). The precipitated actomyosin was redissolved in 1 M KCl-20 mM imidazole (pH 7.0), and the volume adjusted to bring the KCl concentration to 0.6 M. The dilution-precipitation cycle was repeated one more time. The final precipitate was dissolved in 0.6 M KCl, 10 mM imidazole, 1 mM DTT (pH 7.0) and used as actomyosin. All the homogenization, centrifugation, and extraction procedures were carried out in the cold (4°C).

Myosin was purified as described earlier (Bhan and Malhotra, 1976). Four to six hearts were pooled in each group. Myofibrils washed with Triton X-100 were extracted with 10 volumes of buffer (0.47 M KCl, 0.02 M Na-pyrophosphate, 0.01 M KH₂PO₄, pH 6.8) for 25 minutes. This was followed by fractionation with a solution of saturated (NH₄)₂SO₄, pH 7.0, containing 10 mM EDTA. The fraction precipitating between 35 to 45% (NH₄)₂SO₄ was collected, dissolved in 0.6 M KCl, and dialyzed against 0.4 M KCl, 1 mM EDTA, pH 7.0, to get rid of (NH₄)₂SO₄. All steps, except the final dialysis, were carried out in the presence of 1 mM dithiothreitol (DTT). Myosin obtained in this manner has been shown by SDS gel electrophoresis to be free of actin, troponin, and tropomyosin and to be without evidence of proteolytic degradation of myosin. It has a A_{280}/260 ratio of 1.7-1.75, indicating absence of nucleotide material.

ATPase activity measurements were performed in a final volume of 2 ml at pH 7.6 and 30°C. For the Ca²⁺-dependent ATPase of actomyosin and myosin, the reaction mixture consisted of 0.3 M KCl, 0.05 M Tris-Cl, pH 7.6, 0.01 M CaCl₂, 0.005 M Na₃-ATP, and 75-100 µg of contractile protein. The reaction was initiated by the addition of the substrate and terminated after 10 minutes by the addition of 1.0 ml of cold 10% trichloroacetic acid. Inorganic phosphate (Pi) was determined by the method of Fiske and Subbarow (1925). Protein concentration was determined by the biuret technique using bovine serum albumin as a standard. Results are expressed as micromoles of Pi liberated per minute per mg protein at 30°C.

Variability of Plasma Glucose Concentration Control during Insulin Therapy

Five treated diabetics and five controls had blood samples taken on two consecutive days. Blood was taken at noon on the first day and at 9 a.m., 11 a.m., 1 p.m., and 3 p.m. on the second day. The within- and day-to-day variations of plasma glucose levels in the treated diabetics were compared to that in controls.

Data Analysis

Parameters dependent upon load were computed at intervals of 10% relative load (total isotonic load/total isometric load × 100) by linear interpolation of experimental data. Statistical comparison between two groups was performed using the unpaired Student's t-test. When three groups were compared, a one-way analysis of variance was used, followed when appropriate by Scheffé's test. P < 0.05 was considered statistically significant.

Results

General Features of Animals

In Table 1, plasma glucose concentration and body weight are compared prior to beginning insulin treatment.

Table 1: Comparison of Diabetic and Insulin-Treated Diabetic Animals Prior to Therapy

<table>
<thead>
<tr>
<th></th>
<th>2 Days insulin</th>
<th>6 Days insulin</th>
<th>10 Days insulin</th>
<th>28 Days insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D (12)</td>
<td>D + I (3)</td>
<td>D (7)</td>
<td>D + I (7)</td>
</tr>
<tr>
<td></td>
<td>D (12)</td>
<td>D + I (3)</td>
<td>D (12)</td>
<td>D + I (13)</td>
</tr>
<tr>
<td></td>
<td>D (8)</td>
<td>D + I (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose concentration (mg/100 ml)</td>
<td>535 ± 47</td>
<td>709 ± 31</td>
<td>620 ± 32</td>
<td>550 ± 32</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>535 ± 47</td>
<td>581 ± 49</td>
</tr>
<tr>
<td></td>
<td>569 ± 35</td>
<td>518 ± 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>168 ± 3</td>
<td>165 ± 8</td>
<td>196 ± 5</td>
<td>212 ± 4</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>168 ± 3</td>
<td>167 ± 3</td>
</tr>
<tr>
<td>BW (g)</td>
<td>204 ± 3</td>
<td>209 ± 5</td>
<td></td>
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</tr>
</tbody>
</table>

D = streptozotocin-induced diabetic; D + I = diabetic to be insulin-treated; BW = body weight. Values are expressed as the mean ± SEM. Numbers of animals are given in parentheses. NS = nonsignificant. The same diabetic animals were used for the 2- and 10-day studies. Measurements were made before beginning insulin therapy (2 weeks after streptozotocin injection in the 2- and 10-day studies, and 4 weeks after streptozotocin injection in the 6- and 28-day studies).
therapy in untreated and to-be-treated diabetics. The only differences were in body weight in the 6-day study. Otherwise, the untreated and to-be-treated groups were very closely matched. The general features of diabetic, control, and insulin-treated diabetic animals at the time of study are summarized in Table 2.

### Two Days of Insulin Therapy

Untreated diabetics were markedly hyperglycemic (plasma glucose concentration 643 ± 22 mg/100 ml) and had lower body and heart weights than controls. The T3 resin uptake and free T4 index were depressed. After 2 days of insulin therapy, the plasma glucose level was normalized (plasma glucose concentration, 108 ± 12 mg/100 ml), but body and heart weights were similar to that of untreated diabetics. Thyroid function tests were not different from that of controls in this small group of treated diabetics.

### Six Days of Insulin Therapy

After 6 days of insulin therapy, plasma glucose concentration was normal. In contrast to the 2-day study, body weight in the treated group was in the normal range, whereas heart weight was above the control level. Thyroid function tests were similar in treated diabetics and controls.

### Twenty-eight Days of Insulin Therapy

After 28 days of insulin therapy, plasma glucose concentration was normalized. Body and heart weights in treated animals were no different from controls. Unlike the 2-, 6-, and 10-day studies, free T4 in treated diabetics exceeded control values.

### Muscle Mechanics

Data have been derived from the study of two separate litters of animals from different breeders. As a result, different values for various parameters, when comparing the four studies, may not only be

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### Table 2 General Features of Diabetic, Insulin-Treated Diabetic, and Control Animals

<table>
<thead>
<tr>
<th></th>
<th>2 Days insulin</th>
<th>6 Days insulin</th>
<th>10 Days insulin</th>
<th>28 Days insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D+I</td>
<td>C(13)</td>
<td>D+I(13)</td>
<td>D+I(1)</td>
</tr>
<tr>
<td></td>
<td>D+I</td>
<td>C(13)</td>
<td>D+I(13)</td>
<td>D+I(12)</td>
</tr>
<tr>
<td>Plasma glucose concentration (mg/100 ml)</td>
<td>D(12)</td>
<td>643 ± 22</td>
<td>621 ± 12</td>
<td>643 ± 22</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05*</td>
<td>NS°</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BW (g)</td>
<td>197 ± 10</td>
<td>202 ± 5</td>
<td>197 ± 5</td>
<td>222 ± 7</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>HW (g)</td>
<td>0.57 ± 0.63</td>
<td>0.55 ± 0.9</td>
<td>0.57 ± 0.7</td>
<td>0.58 ± 0.6</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T3 (μg/100 ml)</td>
<td>2.6(6)</td>
<td>2.0(3)</td>
<td>2.0(3)</td>
<td>2.0(3)</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T3R (%)</td>
<td>41.9(11)</td>
<td>46.3(1)</td>
<td>31.0(1)</td>
<td>52.9(1)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>FT4 (μg/100 ml)</td>
<td>5.3(11)</td>
<td>2.0(8)</td>
<td>5.3(11)</td>
<td>5.3(11)</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

D = streptozotocin-induced diabetic; D + I = insulin-treated diabetic; C = age-matched control; BW = body weight; HW = heart weight; T3 = total serum thyroxin; T3R = serum triiodothyronine resin uptake; FT4 = free T4 index (T3 × T4R/30.7). Values are expressed as the mean ± SEM. Numbers of animals are given in parentheses. NS = nonsignificant. The same diabetic (and control) animals were used for the 2- and 10-day studies. P values under each group of data refer to the following: a = D vs. D+I, b = D+I vs. C, c = D vs. C.
a result of the influence of age, duration of diabetes, or duration of therapy [e.g., the higher control values for shortening and relaxation velocity as well as the extent of shortening in the 6-day study (Table 5)]. In considering the effect of duration of insulin therapy on muscle mechanics, the emphasis will therefore be placed on the differences between treated and untreated diabetics and between treated diabetics and controls, comparing the 2-, 6-, 10-, and 28-day studies.

Muscle Characteristics

Data summarizing muscle length, weight, and cross-sectional area are shown in Table 3. Muscles from treated diabetics were shorter than those of untreated diabetics in the 28-day study. There were no other significant differences between groups in any of the studies.

Isometric Studies

Representative isometric contractions from a diabetic and age-matched control rat are shown in Figure 1a. Resting (RT) and peak developed tensions (DT) are similar. Time-to-peak tension (TPT), however, is moderately prolonged and there are marked relaxation abnormalities, including a prolonged time for tension to fall from peak DT to 50% DT (T1/2RI), and an increased time from peak DT to the peak rate of tension fall (TPN). Isometric measurements were made at a muscle length of Lmax and at a bath [Ca2+] of 2.4 mM, and the results are summarized in Table 4.

Two Days of Insulin Therapy. Papillary muscles from three diabetic rats treated for only 2 days with insulin were essentially identical to those of untreated diabetics with respect to the isometric contraction.

Six Days of Insulin Therapy. In this study, muscles from untreated diabetics had a greater resting tension than did muscles from controls. This difference was not observed in other studies here or in those previously reported (Fein et al., 1980). With regard to all parameters of the isometric contraction, treated and untreated diabetics were very similar.

Ten Days of Insulin Therapy. In sharp contrast to the 2- and 6-day studies, after 10 days of insulin the isometric contraction in muscles from treated diabetics differed greatly from that of untreated diabetics. TPT in treated animals was intermediate between that of untreated diabetics and controls. Furthermore, the treated diabetics did not differ from controls with respect to either T1/2R or TPN.

Twenty-eight Days of Insulin Therapy. After 4 weeks of insulin, treated diabetics were essentially identical to controls with respect to every parameter of the isometric contraction.

Isotonic Studies

Typical records of preloaded isotonic contractions from a diabetic and age-matched control rat are shown in Figure 1b. The degree of muscle shortening (PS) was similar. However, time-to-peak shortening (TPS) was prolonged and peak velocity of shortening (Vs) was lower in the diabetic contraction. Time-to-peak relaxation velocity (TVr) was increased in the diabetic trace.

The results of the isotonic studies are summarized in Table 5. Force velocity curves are shown in Figure 2. Mechanical measurements were made at an initial muscle length of Lmax. The isotonic studies described in Table 5 were performed on the same muscles from which the data of Tables 3 and 4 were obtained.

Two Days of Insulin Therapy. Muscles from three treated diabetic animals were very similar to those from untreated diabetics with respect to all features of the isotonic contraction (Table 3). Figure

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**Table 3** General Features of Diabetic, Insulin-Treated Diabetic, and Control Muscles

<table>
<thead>
<tr>
<th></th>
<th>2 Days insulin</th>
<th>6 Days insulin</th>
<th>10 Days insulin</th>
<th>28 Days insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML (mm)</td>
<td>6.0 ±0.2</td>
<td>5.0 ±0.1</td>
<td>5.6 ±0.2</td>
<td>5.2 ±0.3</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MW (mg)</td>
<td>4.1 ±0.3</td>
<td>2.7 ±0.4</td>
<td>4.2 ±0.3</td>
<td>3.3 ±0.3</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>XS (mm²)</td>
<td>0.68 ±0.04</td>
<td>0.53 ±0.04</td>
<td>0.72 ±0.05</td>
<td>0.63 ±0.06</td>
</tr>
<tr>
<td></td>
<td>NS</td>
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</tbody>
</table>

D = streptozotocin-induced diabetic; D + I = insulin-treated diabetic; C = age-matched controls; ML = muscle length at Lmax, MW = muscle weight, XS = muscle cross-sectional area. Values are expressed as the mean ± SEM. Numbers of animals are given in parentheses. NS = nonsignificant. The same diabetic (and control) animals were used for the 2- and 10-day studies.
TPT - time to peak tension; +T - peak rate of tension rise; TWR - time to study, muscles from treated diabetics did not differ lower in untreated diabetics than controls.

Figure 2a shows the near identity of the force-velocity reported experiments (Fein et al., 1980), Vr was relation in muscles from treated and untreated diabetics observed after 10 days of insulin therapy were identical to controls with respect to TPS, Vs, Vr, and TVr. Figure 2c shows the identity of the force-velocity curves in these two groups.

Insulin Added to the Bath

Crystalline insulin was added to the muscle bath to achieve a concentration of 9 mU/ml. Five untreated diabetics and five controls from the 10-day study were studied. In neither group were there any substantial effects of insulin on the isometric or isotonic contraction. The differences in papillary muscle function between these groups were essentially unaffected by insulin. For example, T1/2R < 0.05 before insulin was 156 ± 15 and 104 ± 7 msec (P < 0.05) in diabetics and controls, respectively; after insulin, the values were 147 ±11 and 98 ± 7 msec, respectively (P < 0.05). The Ca2+-ATPase activity of purified actomyosin was markedly depressed in preparations from hearts of untreated diabetic rats (Fig. 3a). There was no change after 2 days of insulin therapy, modest improvement after 5 days, substantial recovery at 10 days, and complete reversal after 28 days of insulin therapy.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>2 Days insulin</th>
<th>6 Days insulin</th>
<th>10 Days insulin</th>
<th>28 Days insulin</th>
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<tr>
<td></td>
<td>D+1 (12)</td>
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<td>D+1 (13)</td>
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<td></td>
<td>(3)</td>
<td></td>
<td>(13)</td>
<td></td>
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<tr>
<td></td>
<td>D (7)</td>
<td></td>
<td>D (7)</td>
<td></td>
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<td></td>
<td>C (12)</td>
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<td></td>
<td>D (8)</td>
<td></td>
<td>D (8)</td>
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<tr>
<td></td>
<td>C (8)</td>
<td></td>
<td>C (8)</td>
<td></td>
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<tr>
<td>RT (g/mm²)</td>
<td>2.35 ± 0.14</td>
<td>2.36 ± 0.18</td>
<td>2.83 ± 0.25</td>
<td>2.19 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>±0.39 ±0.18</td>
<td>±1.58 ±0.23</td>
<td>±0.14 ±0.16</td>
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</tr>
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<td></td>
<td>±0.22 ±0.16</td>
<td>±0.18 ±0.30</td>
<td>±0.18 ±0.16</td>
<td>±0.16 ±0.30</td>
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<tr>
<td>P</td>
<td>NS</td>
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<td>NS</td>
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<td>&lt;0.05</td>
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<tr>
<td>DT (g/mm²)</td>
<td>5.81 ±0.55</td>
<td>6.80 ±1.81</td>
<td>7.50 ±1.37</td>
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<td>±0.95 ±0.64</td>
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<td>TPN (msec)</td>
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<td>131 ±7</td>
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D = streptozotocin-induced diabetic; D + 1 = insulin-treated diabetic; C = age-matched control; RT = resting tension; DT = developed tension; TPT = time to peak tension; +T = peak rate of tension rise; TWR = time to 1/2 relaxation (50% DT); -T = peak rate of tension fall; TPN = time from peak tension to peak rate of tension fall. Values are expressed as the mean ± SEM. Numbers of animals are given in parentheses. Values are obtained at a muscle length of Lmax. NS = nonsignificant. The same diabetic (and control) animals were used for the 2- and 10-day studies.
myosin gave similar results (Fig. 3b); no prepara-
tions were obtained from the 2-day study. Again,
gradual but complete reversal of the depressed
Ca$^{2+}$-ATPase activity of myosin was observed after
4 weeks of insulin therapy.

Glucose Concentration Control during Insulin
Therapy

Plasma glucose concentration values were mea-
sured in five treated diabetic and five control ani-
mals; these particular animals were not used in the
mechanical studies. The variation of glucose con-
centration during the day is much greater in treated
diabetics than in controls. Highest - lowest glucose
concentration on day 2 averaged 184 ± 41 and 63
± 8 mg/100 ml in treated diabetics and controls,
respectively (P < 0.05). Also, the day-to-day vari-
ation in glucose levels in diabetics substantially
exceeded that of controls. Highest - lowest glucose
concentration, comparing values on noon, day 1 and
day 2, averaged 155 ± 29 and 25 ± 7 mg/100 ml in
treated diabetics and controls, respectively (P <
0.05).

Discussion

Diabetes mellitus has been shown to be associ-
ated with the development of a cardiomyopathy,
performance was discussed previously (Fein et al., 1980). The duration of diabetes did not substantially alter the mechanical differences between diabetic and control muscles in studies carried out after 5, 10, and 30 weeks of diabetes. Correlated with these mechanical alterations were the observations in diabetic hearts of decreased calcium binding and uptake by isolated sarcoplasmic reticulum and depressed actomyosin and myosin ATPase activities (Penpargkul et al., 1981; Malhotra et al., in press). Further biochemical as well as histological studies of this model revealed no change in collagen synthesis and no structural abnormalities.

Whereas the myocardial changes in diabetes have been clarified to some extent, there is much less information about reversibility of these changes with insulin or oral hypoglycemic agents. Insulin administration was evaluated experimentally in a study of the isolated perfused heart of severely diabetic rats (Miller, 1979) and in vivo hemodynamic studies of diabetic dogs (Wu et al., 1977; Pogatsa et al., 1979). Miller (1979) observed a diminished response of cardiac output and systolic pressure to a rise in atrial pressure in hearts from rats made diabetic 3 days earlier. When insulin (10^{-8} M) was included in the perfusate, these changes were reversed. This study showed that certain features of cardiac performance in short term diabetes in rats were acutely reversible with insulin; these cardiac alterations were attributed to insufficient glucose transport. In contrast, Penpargkul et al. (1980), using similar techniques in chronically diabetic rats, found that insulin (10 mU/ml) in the perfusate did not reverse the abnormalities in ventricular function.

Wu et al. (1977) and Pogatsa et al. (1979) studied the effects of long-term hypoglycemic therapy or diabetic dogs. Wu et al. (1977) studied dogs that manifested glucose intolerance but not fasting hyperglycemia. Treatment with tolbutamide was instituted after the development of glucose intolerance and actually resulted in greater ventricular stiffness. In contrast, Pogatsa et al. (1979) studied more severely diabetic dogs manifesting fasting hyperglycemia, glycosuria, and diminished body weight. Untreated animals had hemodynamic alterations similar to those described by Wu et al. (1977). However, hypoglycemic therapy resulted in significant but incomplete improvement of left ventricular diastolic stiffness.

In the present study, the effects of treatment of diabetes mellitus with insulin on papillary muscle function have been delineated for the first time. In addition, the time course of insulin's effect on con-

### Table 5 Isotonic Contraction

<table>
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<tr>
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<th>2 Days insulin</th>
<th>6 Days insulin</th>
<th>10 Days insulin</th>
<th>28 Days insulin</th>
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</thead>
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<tr>
<td>PS (%ML)</td>
<td>±0.07 ±0.07 ±0.06</td>
<td>±0.03 ±0.06 ±0.03</td>
<td>±0.03 ±0.06 ±0.03</td>
<td>±0.03 ±0.06 ±0.03</td>
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<tr>
<td>Vₚ (ML/sec)</td>
<td>±0.02 ±0.02 ±0.02</td>
<td>±0.07 ±0.07 ±0.07</td>
<td>±0.07 ±0.07 ±0.07</td>
<td>±0.07 ±0.07 ±0.07</td>
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<tr>
<td>Vₛ (ML/sec)</td>
<td>±0.02 ±0.02 ±0.02</td>
<td>±0.07 ±0.07 ±0.07</td>
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</tr>
<tr>
<td>Tₚ (msec)</td>
<td>±0.01 ±0.01 ±0.01</td>
<td>±0.06 ±0.06 ±0.06</td>
<td>±0.06 ±0.06 ±0.06</td>
<td>±0.06 ±0.06 ±0.06</td>
</tr>
<tr>
<td>Tₛ (msec)</td>
<td>±0.01 ±0.01 ±0.01</td>
<td>±0.06 ±0.06 ±0.06</td>
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</table>

D = streptozotocin-induced diabetes, D + I = insulin-treated diabetes; C = age-matched controls. PS = peak shortening, TPS = time to peak shortening, Vₚ = peak velocity of shortening divided by Lₚ, Vₛ = peak velocity of relaxation divided by Lₛ, TVₚ = time to peak velocity of relaxation, MI = muscle lengths. Values are expressed as the mean ± SEM. Numbers of animals are given in parentheses. NS = nonsignificant. Values are obtained at an initial muscle length = Lₛ, and at a relative load (total isometric load/total isometric load x 100) of 50%. The same diabetic and control animals were used for the 2- and 10-day studies.
The major finding of this study is that 4 weeks of insulin therapy resulted in complete reversal of all mechanical and one of the major biochemical features of cardiac tissue from chronically diabetic rats. After only 2 and 6 days of therapy, no reversal of contractile abnormalities was observed. These observations are compatible with the concept that altered cardiac function in chronic diabetes is not simply a short-term result of decreased glucose transport. Further support for this idea is the failure of insulin added to the muscle bath or high concentration of bath glucose in this and the previous study (Fein et al., 1980), respectively, to correct the changes in papillary muscle function from chronically diabetic rats; the work of Penpargkul et al. (1980) also supports this concept (see above).

This study confirms our previous report of depressed contractile protein enzymatic activity in hearts of diabetic rats (Malhotra et al., in press). In the present study, the time course of recovery of mechanical function of diabetic heart muscle correlated with the gradual but complete recovery of contractile protein enzymatic activity. Dillman (1980) also reported reversal of the depressed myosin ATPase after 4 weeks of insulin therapy in diabetic rats. These findings are not surprising considering the known relation between shortening velocity and actomyosin or myosin ATPase in both species studied, and both the severity and duration of diabetes, might be of substantial importance in influencing the results. It has already been noted that mildly or moderately diabetic dogs do not exhibit complete reversal of their hemodynamic alterations with therapy. The potential for reversibility may relate to the presence or absence of interstitial changes in the myocardium. Whereas histochemical studies of chronically diabetic dogs have shown increased interstitial glycoprotein (Regan et al., 1974), recent studies of the severely diabetic rat indicate no changes in interstitial collagen formation (Modrak, 1980; Factor et al., 1981). It is noteworthy that complete mechanical reversibility was demonstrated in this study despite lack of ideal 24-hour control of hyperglycemia in the insulin-treated animals.

The observation that shortening and relaxation velocities in the 28-day study were greater in muscles from treated diabetics than in those of control animals was unexpected. While this may be an indication that insulin therapy may result in more than a simple reversal of the effects of diabetes, this is speculative and will need to be studied further.

**Figure 2** Force-velocity relations. Values were obtained from a series of afterloaded isotonic contractions at an initial muscle length of Lmax. Force is expressed as the % relative load (total isotonic load/total isometric load × 100). Velocity is expressed as the number of muscle lengths per second, calculated as the peak velocity (in mm/sec) divided by Lmax. Values are plotted as the mean ± SEM. *P < 0.05. a: Two-day study. Velocities in controls exceed those of untreated and treated diabetics at all loads except at 50%, where controls and treated diabetics are not significantly different. b: Six-day study. Control velocities exceed those of untreated and treated diabetics at all loads. c: Ten-day study. Velocities in treated diabetics and controls exceed those of untreated diabetics at all loads. d: Twenty-eight-day study. Velocities in treated diabetics and controls exceed those of untreated diabetics at all loads. Also, velocities in treated diabetics exceed those of controls at all relative loads except 90% and 90%.
In the previous study (Fein et al., 1980), the possibility that the myocardial alterations in diabetic rats was due to direct cardiotoxicity from streptozotocin was considered. Rats were given 3-O-methyl glucose (a nonmetabolizable glucose analogue) before streptozotocin, which prevented the development of diabetes as well as any of the mechanical changes found in papillary muscle from diabetic animals. Similar findings were found when sarcoplasmic reticulum and contractile proteins were studied (Penparkkul et al., 1981; Malhotra et al., in press). It was recognized, however, that 3-O-methyl glucose might also inhibit streptozotocin-induced cardiac damage. This study, demonstrating reversibility with insulin of the diabetes-associated changes, makes it extremely unlikely that they were due to drug toxicity.

Another feature of diabetic animals previously described is the depression of free T4 index. Although this index was depressed in diabetic animals from the 6- and 10-day studies, this was not the case in the 28-day study, yet diabetics still differed mechanically from controls. Furthermore, the free T4 index in insulin-treated animals in the 28-day study.
study did not differ from that of untreated diabetics, whereas the mechanical differences were profound. Thus, a dissociation between papillary muscle mechanics and free T4 was observed, supporting the concept that hypothyroidism, even if present in diabetic rats, does not mediate the altered myocardial function in diabetes.

This study has therefore demonstrated the gradual but complete reversal of altered papillary muscle function and contractile protein enzymatic activity in insulin-treated chronically diabetic rats. The results must be extended further to examine the influence of duration and severity of diabetes and the degree of control of hyperglycemia with insulin. It will also be of pathophysiological and possibly therapeutic importance to establish whether reversibility is contingent upon recovery of serum insulin levels or restoration of normal plasma glucose; experiments designed to separate the influence of these factors will be required. The findings presented here provide the background for such future work. This study also provides a basis for the speculation that the cardiomyopathy observed in some human diabetics may be at least partially reversible with insulin.

Acknowledgments

We thank John R. Katt for his excellent technical assistance; Dr. Lakman Chervu for performing thyroid function studies; and Toni Maio, Laura Salerno, and Marilyn Sasso for their efforts in preparing the manuscript. Streptozotocin was given to us by the Upjohn Company, Kalamazoo, Michigan.

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Reversibility of diabetic cardiomyopathy with insulin in rats.
F S Fein, J E Strobeck, A Malhotra, J Scheuer and E H Sonnenblick

doi: 10.1161/01.RES.49.6.1251

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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