Myocardial Mechanics and Titin in Experimental Insulin-resistant Rats

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SUMMARY

We investigated the intrinsic cardiac mechanics of myocardium and changes in titin in insulin-resistant rats. Microsonometry and micromanometry were used to evaluate the maximal elastance (Emax) and myocardial stiffness constant (Km) of the left ventricle, in addition to the traditional systolic and diastolic cardiac function, with an isolated working heart preparation. Thirty 150 g Wistar rats were divided into three groups of 10. Group A was fed rat chow, while groups B and C were fed a 66% fructose diet for 7–8 months. Group C also received clonidine. Group B rats developed insulin resistance, as well as elevated plasma glucose and blood pressure. Group C rats also had insulin resistance and elevated plasma glucose, but not higher blood pressure. Group B rats had decreased Emax, decreased peak-dp/dt, prolonged Tau and increased Km compared to normal control rats. Group C rats, which mimicked the clinical condition of diabetic cardiomyopathy, maintained normal global left ventricular function as revealed by cardiac output, peak + dp/dt, peak - dp/dt and Tau of relaxation. However, they had a lower Emax slope (355 ± 51 vs 535 ± 56 mmHg·mm than group A rats, p < 0.05) and increased Km (81.6 ± 9.9 vs 25.5 ± 4.8 in group A, p < 0.001), even though the extent of elevation of plasma glucose was only mild (71.3 ± 2.0 to 108.9 ± 4.4 mg/dl, p < 0.001). Their left ventricular mass, myocyte size, interstitial fibrosis and vascular picture did not change. However, the content of myocardial titin decreased significantly (intensity ratio of titin/actin was 0.23 ± 0.01 and 0.29 ± 0.02 in group C and group A rats respectively, p < 0.05). These findings suggest that changes in titin play a role in the change in myocardial functional characteristics and may be one of the causes of diabetic cardiomyopathy. (Jpn Heart J 1997; 38: 717–728)


**Key words:** Insulin resistance, Titin, Isolated heart preparation, Maximal elastance, Myocardial stiffness constant

**Diabetic** cardiomyopathy independent of coronary atherosclerosis has been extensively studied with various methods in terms of pathology,\(^1\)\(^-\)\(^3\) hemodynamics\(^4\)\(^-\)\(^9\) and biochemistry.\(^10\)\(^-\)\(^14\) The hemodynamics of this disease have been investigated using cardiac catheterization,\(^9\) echocardiography\(^5\)\(^-\)\(^7\) and radionuclide techniques.\(^8\)\(^-\)\(^9\) Studies on hemodynamics have focused on cardiac performance, which is influenced by neuroendocrinal, circulatory and peripheral metabolic factors.

The intrinsic cardiac mechanical properties should be evaluated with an isolated working heart preparation. Traditionally, the cardiac function of an isolated heart preparation is evaluated in terms of the rate of pressure rise (peak + dp/dt), rate of pressure decline (peak – dp/dt) and developed left ventricular pressure.\(^15\)\(^-\)\(^17\) These parameters are affected by ventricular size, shape and architecture, and reflect functional changes in the cardiac chamber rather than in the myocardium per se.\(^18\)\(^-\)\(^19\) Systolic function with the relation between intraventricular force and length of the midwall circumference in end-systole (maximal elastance: Emax)\(^19\)\(^-\)\(^21\) and diastolic function with the end-diastolic stress-strain relation (myocardial stiffness constant: Km)\(^22\)\(^-\)\(^23\) may reflect intrinsic myocardial function. There has been no report regarding intrinsic myocardial function in experimental diabetic cardiomyopathy.

In animal studies hyperglycemia is usually induced by destruction of the pancreas with alloxan or streptozotocin.\(^10\)\(^-\)\(^14\) In this kind of animal model, the islet cells of the pancreas are destroyed and the hyperglycemic state is due to hyposecretion of insulin. This model does not fit the hyperinsulinemic condition of type II diabetes, which is more often encountered in humans.\(^24\)

Information regarding type II diabetes is important. Zavaroni *et al.*\(^25\) and Tobey *et al.*\(^26\) established an animal model of insulin resistance in rats using a fructose diet. Rats fed fructose develop a condition of hyperglycemia, hyperinsulinemia and hypertension which mimicks non-insulin dependent diabetes mellitus (NIDDM). Adding clonidine to the drinking water can prevent hypertension in insulin-resistant rats, but the metabolic changes still occur.\(^27\) Using this model, we have attempted to evaluate experimentally the cardiac mechanics in insulin-resistant rats mimicking type II diabetic patients.

Biochemical analysis may explain the alteration of cardiac function in diabetic cardiomyopathy. A decrease in myosin ATPase with a shift in the isoenzyme form of myosin from the faster V\(_1\) to the slower V\(_3\) has been reported.\(^28\) Titin is an elastic protein, and is the third most abundant protein in the myocardium next to actin and myosin. In the myocardium of failing hearts with dilated
cardiomyopathy, the amount of titin is decreased and the organization of titin in the sarcomere disrupted. Nevertheless, no such information has been reported in diabetic hearts. Thus, we attempted to assess any change in titin content, and its contribution to the change in cardiac mechanics in the diabetic heart.

**MATERIALS AND METHODS**

Thirty male Wistar rats weighing approximately 150 g were divided into three groups of 10 rats. Group A (normal control rats) was fed usual rat chow (Fwu-Sow, Taipei, Taiwan, ROC), group B a high fructose diet containing 66% fructose, 22% protein, 12% fat, 4.9 g/kg sodium and 4.9 g/kg potassium (TD 89247; Teklad Labs, Madison, WI, USA), and Group C 0.75 µg/ml clonidine in the drinking water in addition to the high fructose diet.

Body weight and blood pressure were determined using the tail cuff method (model 65~12, IITc Woodland Hills, CA, USA). Fasting plasma glucose concentration was determined using the glucose oxidase method (Glucose Analyzer 2, Beckman, CA, USA) and fasting plasma insulin concentration was measured using a Linco Rat Insulin RIA Kit (Linco Research, Inc., St. Louis, MO, USA). All values were determined once a week for 2 months and then once a month until the rats were sacrificed.

On the morning of sacrifice, the overnight fasted rats were anesthetized with 5 mg/100 g body weight of pentobarbital, intraperitoneally. Laparotomy was done at the midline. The inferior vena cava and abdominal aorta were cannulated. The intraaortic pressure was recorded, a glucose tolerance test performed by injecting 5 g/100 g body weight of 50% glucose water into the inferior vena cava, and a 1 ml blood sample obtained from the aorta at 0, 1, 2, 5 and 10 minutes to measure glucose and insulin. The heart was then isolated and immediately immersed in ice-cold saline for cannulation of the ascending aorta and left atrium through the pulmonary vein.

A Langendorff preparation was prepared using the cannulated heart. The perfusate was 37°C S.T. Thomas’ solution (118.5 mM NaCl, 25 mM NaHCO$_3$, 4.75 mM KCl, 1.19 mM MgSO$_4$, 1.18 mM KH$_2$PO$_4$, 1.36 mM CaCl$_2$ and 11.1 mM glucose) mixed with 95% O$_2$ and 5% CO$_2$ at a height of 100 cm. With the heart being perfused without working, two piezoelectric crystal transducers of a microsonometer (Triton Tech Inc. San Diego, CA, USA) were sutured onto the epicardium, one 2 mm lateral to the mid-left anterior descending artery, and the other 2 mm lateral to the mid-posterior descending artery. The distance between these two epicardial transducers was equal to the outer minor diameter of the left ventricle. A third transducer was inserted obliquely into the endocar-
dium near the first transducer. By adjusting the location of the endocardial transducer until a minimum distance between this pair of transducers was obtained, we could determine the thickness of the myocardium from the signals displayed by the microsonometer.

After the isolated heart had been perfused for 30 minutes, it was shifted to the working mode; the flow from the perfusion bottle was clamped and the flow to the left atrium opened. The opening of the drain tube from the aorta was set 100 cm above the heart. Consequently, the working heart contracted against an afterload of about 100 cm H$_2$O, mimicking the condition in vivo. A 5 F Millar micromanometer catheter was then inserted into the left ventricular cavity from the apex to record the intraventricular pressure by varying the height of the perfusate bottle connected to the left atrium between 15 cm and 25 cm. Intraventricular pressure, dp/dt, cardiac output, myocardial thickness and the outer diameter of the left ventricle were recorded at different levels of preload (Figure).

At the end of these experiments, the atria and vessels were excised from the

**Figure.** Representative recordings of intraventricular pressure, dp/dt and outer diameter and myocardial thickness of left ventricle in isolated working heart preparation. LVP = left ventricular pressure; ED = end-diastole; ES = end-systole.
heart. The heart was then divided into the septum, free wall of the left ventricle, and free wall of the right ventricle and each portion weighed. A part of the left ventricular free wall was processed for microscopic examination with HE stain and Orcein-Carmine-Picroidigocarmine stain. The rest of the left ventricle was treated with Rigor buffer containing 75 mM KCl, 10 mM Tris-base, 2 mM MgCl2 and 2mM EGTA to extract the myofibril proteins. The extract was then applied to 10% SDS gel (sodium dodecyl sulfate-polyacrylamide gel) for electrophoresis. The intensity ratios of titin/actin and myosin/actin were determined by densitometry.32)

**Calculations**

*Insulin resistance index:* The insulin resistance index was calculated from fasting plasma levels of insulin and glucose by adopting the homeostasis assessing model developed by Matthews et al.33) Insulin resistance index equals insulin/22.5 e:

\[ \text{ln}(\text{glucose/18}). \]

*Insulinogenic index:* The insulinogenic index was defined as the ratio of the post-stimulation insulin increment area divided by the corresponding glucose increment area during the intravenous glucose tolerance test.

*Tau (Time constant of isovolumic pressure decline)*34: The time course of myocardial tension decline in the isovolumic period approximated an exponential process. A linear relation was obtained by plotting the pressure of the left ventricle versus time in a semilog scale. Tau is defined as the negative inverse slope of this line, representing isovolumic relaxation, and is unaffected by peak + dp/dt or peak - dp/dt. From the plot mentioned above, the following equations can be deduced.

\[ P = e^{At+B} \]
\[ \frac{dp}{dt} = A(e^{At+B}) \]

when \( t = 0, \frac{dp}{dt} = \text{peak} - \frac{dp}{dt} = Ae^B = AP_0 \)

\[ P_0 \] is the left ventricular pressure at peak - dp/dt then,

\[ \text{Tau} = 1/-A = P_0/\text{peak} - \frac{dp}{dt} \]

where \( P = \) pressure; \( A = \) slope of exponential pressure fall; \( t = \) time after peak - dp/dt; and \( B = \) intercept.

*Emax (maximal elastance)*20-21: At end-systole, dp/dt begins to move toward negativity from baseline, the diameter of the left ventricle is almost minimal and the myocardial thickness (h) is maximal. From the recorded tracings, end-systolic P (left ventricular pressure), Ro (outer radius of the left ventricle) and Ri (inner radius of the left ventricle equals Ro minus h) can be obtained. The intraventricular force \( F = P \times \pi R_i^2 \) versus length of the midwall circumference \( [l = \pi (R_o + R_i)] \) was then plotted to obtain an approximate linear relation; that is Emax (myocardial elastance). The slope of Emax represents the contractility of
the myocardium.

$K_m$ (myocardial stiffness constant)$^{22-23}$: At end-diastole, $dp/dt$ begins to move toward positivity from the baseline, the diameter of the left ventricle is almost maximal and the myocardial thickness minimal. End-diastolic stress ($σ = P × πR^2/2h^2$) versus strain $[ε = (l - l_p)/l_p$, where $l_p$ is the length of the midwall circumference at minimal intraventricular pressure] was plotted. The stress-strain relation is curvilinear and the slope of any tangent to this line represents myocardial elastic stiffness $(dσ/dε)$. Furthermore, myocardial elastic stiffness versus stress was plotted to gain a linear relation. $K_m$, the slope of this line, represents myocardial stiffness.

**Statistical analysis**

All values are expressed as mean ± SEM. Each rat served as its own control. The paired t-test was used to assess the changes in blood pressure, plasma glucose and insulin resistance index between day 1 and the last day. The results obtained in the 3 groups of rats were compared by one-way analysis of variance. When a statistically significant difference was found, a comparison between the 2 groups was performed using an unpaired t-test. A $p$ value < 0.05 was considered to be statistically significant.

**RESULTS**

**Basic data of experimental rats before sacrifice (Table I):** Rats were fed their respective diets for 7 to 8 months. The body weights at sacrifice were about 500 g, without any significant difference between the 3 groups. Systolic BP at day 1 was 105.3 ± 1.3, 107 ± 4.9 and 105.3 ± 1.9 mmHg in groups A, B and C, respectively ($p > 0.05$). BP remained stable in group A and group C until the day

<table>
<thead>
<tr>
<th>Table I. Basic Data of Experimental Rats before Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong> A ($n = 10$)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Blood pressure (mmHg)</strong></td>
</tr>
<tr>
<td>day 1</td>
</tr>
<tr>
<td>final day</td>
</tr>
<tr>
<td><strong>Plasma glucose (mg/dl)</strong></td>
</tr>
<tr>
<td>day 1</td>
</tr>
<tr>
<td>final day</td>
</tr>
<tr>
<td><strong>Insulin resistance index</strong></td>
</tr>
<tr>
<td>day 1</td>
</tr>
<tr>
<td>final day</td>
</tr>
<tr>
<td><strong>Insulinogenic index</strong></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Group A = normal control rats; group B = rats with high fructose diet; Group C = rats with high fructose diet and clonidine; final day = the day of sacrifice, 7–8 months after day 1. Compared with group A; *$p < 0.05$, ***$p < 0.005$, ****$p < 0.001$, **Group C vs Group B, $p < 0.005$. 
Table II. Systolic Function of Experimental Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 10)</th>
<th>Group B (n = 10)</th>
<th>Group C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (ml/min)</td>
<td>28.6 ± 1.6</td>
<td>29.4 ± 1.8</td>
<td>26.6 ± 2.3</td>
</tr>
<tr>
<td>Peak + dp/dt (mmHg/sec)</td>
<td>2290 ± 245</td>
<td>1730 ± 132</td>
<td>1965 ± 192</td>
</tr>
<tr>
<td>Slope of Emax (mmHg·mm)</td>
<td>535 ± 56</td>
<td>234 ± 32**</td>
<td>355 ± 51*</td>
</tr>
</tbody>
</table>

Group A = normal control rats; Group B = rats with high fructose diet; Group C = rats with high fructose diet and clonidine. Emax = maximal elastance. Compared with group A, *p < 0.05, **p < 0.001.

Table III. Diastolic Function of Experimental Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 10)</th>
<th>Group B (n = 10)</th>
<th>Group C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak-dp/dt (mmHg/sec)</td>
<td>1959 ± 192</td>
<td>1442 ± 115*</td>
<td>1589 ± 141</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>13.6 ± 2.9</td>
<td>27.8 ± 4.9*</td>
<td>13.8 ± 0.9*</td>
</tr>
<tr>
<td>Km</td>
<td>25.5 ± 4.8</td>
<td>185.1 ± 15.9**</td>
<td>81.6 ± 99**</td>
</tr>
</tbody>
</table>

Group A = normal control rats; Group B = rats with high fructose diet; Group C = rats with high fructose diet and clonidine; Tau = time constant of relaxation; Km = myocardial stiffness constant. Compared with group A; *p < 0.05, **p < 0.001; *Group C vs Group B, p < 0.05; **Group C vs Group B, **p < 0.001.

Table IV. Histochemical Pictures of Experimental Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 10)</th>
<th>Group B (n = 10)</th>
<th>Group C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>0.74 ± 0.05</td>
<td>0.74 ± 0.03</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>Titin/actin</td>
<td>0.29 ± 0.02</td>
<td>0.22 ± 0.01*</td>
<td>0.23 ± 0.01*</td>
</tr>
<tr>
<td>Myosin/actin</td>
<td>1.80 ± 0.11</td>
<td>1.49 ± 0.05</td>
<td>1.74 ± 0.12</td>
</tr>
</tbody>
</table>

Group A = normal control rats; Group B = rats with high fructose diet; Group C = rats with high fructose diet and clonidine; Compared with group A; *p < 0.05.

of sacrifice, being 103.1 ± 2.3 and 106.1 ± 1.8 mmHg, respectively. In group B, BP increased gradually in the first 3 weeks, then remained stationary thereafter and was 123.6 ± 4.5 mmHg at the day of sacrifice. This was higher than that at day 1 (p < 0.05), and also higher than those on the day of sacrifice in group A (p < 0.001) and group C (p < 0.005).

The blood glucose at day 1 was 72.2 ± 2.8 mg/dl in group A, 74.4 ± 1.8 mg/dl in group B and 71.3 ± 2.0 mg/dl in group C (p > 0.05). In group B and group C, blood glucose increased significantly by the day of sacrifice to 116.6 ± 8.3 mg/dl (p < 0.01) and 108.9 ± 4.4 mg/dl (p < 0.001), respectively. Both of these values were higher than the 74.0 ± 1.8 mg/dl in group A (group B vs group A, p < 0.001; group C vs group A, p < 0.001). The blood glucose levels in groups B and C were similar at the day of sacrifice.

The insulin resistance index at day 1 was 30.4 ± 3.1 in group A, 32.2 ± 1.9 in group B and 25.3 ± 3.4 in group C, (p < 0.05 between groups). At the day of sacrifice it had increased to 53.4 ± 1.7 (p < 0.001), 100.5 ± 6.3 (p < 0.001) and 90.3 ± 7.3 (p < 0.001) in groups A, B and C, respectively, being significantly
higher in group B and group C than in group A (group B vs group A, \( p < 0.001 \); group C vs group A, \( p < 0.001 \)). In contrast to the insulin resistance index, the insulinogenic index calculated from intravenous glucose tolerance immediately before sacrifice was \( 40.2 \pm 3.2 \) in group B and \( 40.9 \pm 6.4 \) in group C, both values being significantly lower than that of \( 62.6 \pm 5.4 \) in group A (group B vs group A, \( p < 0.005 \); group C vs group A, \( p < 0.05 \)).

**Systolic function of experimental rats (Table II):** Cardiac output was \( 28.6 \pm 1.6 \text{ ml/min} \) in group A, \( 29.4 \pm 1.8 \text{ ml/min} \) in group B and \( 26.6 \pm 2.3 \text{ ml/min} \) in group C (\( p > 0.05 \) between groups). Peak + dp/dt was \( 2290 \pm 245 \), \( 1730 \pm 132 \) and \( 1965 \pm 192 \) mmHg/sec in the 3 groups, respectively (NS). The slope of Emax was \( 535 \pm 56 \text{ mmHg•mm} \) for group A, \( 234 \pm 32 \text{ mmHg•mm} \) for group B and \( 355 \pm 51 \text{ mmHg•mm} \) for group C. There were significant differences between groups A and B (\( p < 0.001 \)) and groups A and C (\( p < 0.05 \)).

**Diastolic function of experimental rats (Table III):** Peak - dp/dt was \( 1959 \pm 192 \text{ mmHg/sec} \) in group A, \( 1442 \pm 115 \text{ mmHg/sec} \) in group B and \( 1589 \pm 141 \text{ mmHg/sec} \) in group C, being significantly higher in group A than in group B (\( p < 0.05 \)). Tau of relaxation was \( 13.6 \pm 2.9 \text{ ms} \), \( 27.8 \pm 4.9 \text{ ms} \) and \( 13.8 \pm 0.9 \text{ ms} \) for groups A, B and C, respectively, being significantly higher in group B than in group A (\( p < 0.05 \)) and group C (\( p < 0.05 \)). Km was \( 25.5 \pm 4.8 \) in group A, \( 185.1 \pm 15.9 \) in group B and \( 81.6 \pm 9.9 \) in group C, being the highest in group B and the lowest in group A. There were significant differences among the 3 groups (group A vs group B, \( p < 0.001 \); group B vs group C, \( p < 0.001 \); group A vs group C, \( p < 0.001 \)).

**Histochemical pictures of experimental rats (Table IV):** Left ventricular mass was \( 0.74 \pm 0.05 \text{ g} \) in group A, \( 0.74 \pm 0.03 \text{ g} \) in group B and \( 0.73 \pm 0.03 \text{ g} \) in group C (NS). The mean diameter of cardiomyocytes was \( 16.1 \pm 1.2 \text{ µm} \) in group A, \( 16.5 \pm 0.1 \text{ µm} \) in group B and \( 16.7 \pm 0.5 \text{ µm} \) in group C (no statistically significant difference among the groups). Microscopically, no remarkable fibrosis was seen in the perivascular area or interstitium in any group. There was no vascular change in terms of endothelial proliferation or capillary microaneurysm.

The intensity ratios of titin/actin were \( 0.22 \pm 0.01 \) in group B and \( 0.23 \pm 0.01 \) in group C, both being significantly less than the \( 0.29 \pm 0.02 \) in group A (group B vs group A, \( p < 0.05 \); group C vs group A, \( p < 0.05 \)). The intensity ratios of myosin/actin were similar among the 3 groups (\( 1.80 \pm 0.11 \) in group A, \( 1.49 \pm 0.05 \) in group B and \( 1.74 \pm 0.12 \) in group C).

**DISCUSSION**

Rats fed a high fructose (66%) diet have been reported to develop hyperinsulinemia, hyperglycemia (up to 150 mg/dl) and hypertension (up to 150
mmHg) within 1 to 2 weeks. In the present study, rats were fed a high fructose diet for 7 to 8 months, much longer than the 1 to 2 months reported in other studies. Their blood glucose and pressure increased to around 110 mg/dl and 120 mmHg, respectively, beginning at the 2nd week and remained stationary thereafter. Although not reaching levels of definite hyperglycemia and hypertension, their blood glucose and pressure were significantly higher than those of the normal control rats. Clonidine in the drinking water prevented the elevation of blood pressure induced by the high fructose diet, but not the relative hyperglycemia.

Even with such a long duration of elevated blood glucose and BP, the rats failed to develop ventricular hypertrophy, shown by the left ventricular mass and the diameter of cardiomyocytes. Perivascular and interstitial fibrosis were not detected either. The usual pathological changes in patients with type II diabetes, such as endothelial hyperplasia, capillary microaneurysm and deposition of mucopolysaccharides were not found in our experimental rats. This may be because the degree of elevation of blood glucose and BP was only mild in our study, or because of other limitations of the experimental model. Actually, the insulin resistance in type II diabetic patients is much more complex, and cannot be directly extrapolated from the present animal model.

There was no significant change in systolic function in terms of cardiac output and peak $+\frac{dp}{dt}$ even after prolonged elevation of blood glucose and BP. Nevertheless, peak $+\frac{dp}{dt}$ tended to become worse with insulin resistance alone (group C), and decreased even more when BP was elevated together with insulin resistance (group B). However, the 3 groups could be differentiated clearly by the slope of the maximum elastance, which represents the maximal potential of myocardial contractility, independent of preload, afterload, and left ventricular size and shape.

On the other hand, diastolic function was the worst in rats with elevated blood glucose and BP, as revealed by decreased peak $-\frac{dp}{dt}$, prolonged Tau and an increased myocardial stiffness constant. The rats that only had elevated blood glucose also had increased myocardial stiffness. It seems that diastolic function is impaired more markedly than systolic function in this rat model. This is compatible with the clinical condition of type II diabetic patients, in which diastolic dysfunction usually appears earlier than systolic dysfunction.

The diastolic properties of the myocardium consist of relaxation and compliance. The former is an active process requiring an energy supply. When relaxation is prolonged in diabetes, it is attributed to inadequate ATP production due to altered myocardial substrates and decreased Ca$^{2+}$-reuptake by the sarcoplasmic reticulum. The latter is a passive process and usually decreases in diabetes due to interstitial fibrosis and myocardial hypertrophy. Peak $-\frac{dp}{dt}$
and Tau are used to describe relaxation, while the myocardial stiffness constant is used to measure compliance. The rats with elevated blood sugar without elevation of BP in our study exhibited impairment of compliance only. As there was no remarkable myocardial hypertrophy or fibrosis histologically, the reasons for the deterioration of ventricular compliance still need to be ascertained. Is it simply because high blood glucose is hazardous to myocardial mechanics?

Titin is an elastic protein which extends throughout the whole sarcomere. It stabilizes the structure of sarcomeres and provides passive tension when the myocardium is stretched in diastole. Within a range, the more an isolated titin is stretched, the stronger the tension becomes. Although there were no remarkable pathological changes in the myocardium of our experimental rats, the contents of titin in rats with elevated blood glucose and BP and rats with elevated blood glucose only were significantly less marked than those of the normal control rats.

From the above findings, it is possible that elevated blood glucose interferes with the genesis or organization of titin in the sarcomere, and that an elevated glucose concentration causes an increase in polyol pathway activity. In addition, the high fructose diet increases the fructose concentration which might prevent sorbitol being metabolized further, so that the metabolites in the polyol pathway accumulate. Cardiac muscle contractile properties have been reported to be changed by the polyol pathway-mediated mechanisms in galactose-fed rats. Is the genesis or organization of titin affected by the accumulation of metabolites in the polyol pathway? This remains to be studied. The myocardium loses some elasticity and becomes less stretchable so that the potential of the maximal contractile force decreases according to the Frank-Starling law. Furthermore, comparing the rats with elevated blood glucose and BP and the rats with elevated blood glucose only, the former possessed less maximal elastance, longer relaxation time and greater myocardial stiffness. This is similar to previous reports in which elevated BP indeed aggravates the myocardial dysfunction caused by elevated blood glucose.

Group C rats with elevated blood glucose but without elevation of BP had lower maximum elastance and more myocardial stiffness than normal control rats, mimicking the clinical condition of diabetic cardiomyopathy. The influence of neurohumoral factors can be avoided using an isolated heart preparation. Furthermore, the maximum elastance and myocardial stiffness are independent of preload and afterload, and have been used to evaluate the properties of the myocardium itself, instead of the chamber as a whole. Actually, in this study, rats with elevated blood glucose for such a long period still maintained normal global left ventricular function, as indicated by cardiac output, peak + dp/dt, peak - dp/dt and Tau of relaxation. However, the specific myocardial functional char-
acteristics became worse. Alteration of titin plays a role in these changes and may have been a cause of diabetic cardiomyopathy.

REFERENCES