Alterations in cardiac contractile performance and sarcoplasmic reticulum function in sucrose-fed rats is associated with insulin resistance

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Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by abnormally high levels of glucose in the blood (28a). Type 2 DM is the most prevalent form of this disease and has been recognized as a serious health threat that is growing significantly worldwide (61). Resistance to the biological actions of insulin in glucose and lipid metabolism is the core defect in Type 2 DM and develops years before disease diagnosis.

One of the major complications of DM is the development of a specific heart muscle disease independent of coronary artery, hypertensive, or valvular heart disease (50). This diabetic cardiomyopathy is characterized by structural and functional abnormalities in the heart that result from the metabolic derangements present in DM (47). Furthermore, prediabetic metabolic derangements may produce abnormalities in cardiac structure and function before the development of overt DM (20). The diabetes-related cardiac contractile dysfunction has been described as early diastolic dysfunction preceding systolic damage (10).

The sarcoplasmic reticulum (SR) is an intracellular membranous network in cardiac cells that plays a critical role in regulating the intracellular concentration of Ca$^{2+}$, thereby controlling cardiac contraction and relaxation (18). The sarcoplasmic reticulum Ca$^{2+}$-ATPase (SERCA2a) is responsible for a species-specific amount of intracellular Ca$^{2+}$ reuptake into the SR (70–92%), facilitating cardiac relaxation, and thereby makes Ca$^{2+}$ available for the next wave of contraction (4). SERCA2a, therefore, is considered to be a key determinant of cardiac contractility. The function of SERCA2a is modulated through its physical interaction with the phosphoprotein phospholamban (PLB), which inhibits SERCA2a activity in its unphosphorylated state (18, 31). Phosphorylation of PLB by the SR-associated AMP-dependent protein kinase (PKA) or Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMK II) relieves this inhibition, thereby increasing SR Ca$^{2+}$ uptake (18, 31). Conversely, dephosphorylation of PLB by the SR-associated protein phosphatases (PP), specifically PP1 and PP2A, which are the major PP in the heart (30), depresses SR Ca$^{2+}$ uptake (27). Although it has been well established that Type 1 DM results in a cardiomyopathy associated with alterations in SR function and its regulation (5, 7, 19, 26, 29, 35, 39, 43, 44, 55, 56, 59), very little is known about the status of the SR in Type 2 DM.

Diet in sucrose has been shown to induce whole body insulin resistance in rats (1, 11, 16, 22–24, 38, 40, 41, 57), a condition intimately associated with the metabolic syndrome, obesity, and the early stages of Type 2 DM in humans. Moreover, previous studies have shown that high-sucrose feeding in rats leads to the development of cardiac contractile dysfunction (11, 16, 22–24, 40, 57). However, no study has determined the impact of insulin resistance on the function and regulation of cardiac SR vesicles. Accordingly, this study examined cardiac contractile performance, SR Ca$^{2+}$ cycling, and its regulatory mechanisms in sucrose-induced insulin-resistant (i.e., prediabetic nonobese) rats.

MATERIALS AND METHODS

The following experimental protocol was approved by the Animal Care Committee of the University of Manitoba and conforms to the guidelines of the Canadian Council on Animal Care Concerning the Care and Use of Experimental Animals (vol. 1, 2nd ed., 1993).

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Animal model. Four-week-old male Sprague-Dawley rats (Central Animal Care, University of Manitoba, Manitoba, Canada) weighing 75–100 g at the time of arrival were housed two to three per cage under controlled temperature (20 ± 2°C), humidity (30–70%), and lighting (12:12-h light-dark cycle). On arrival, all rats were given standard chow and water ad libitum. After 1 wk, rats were randomly assigned to one of two groups. The control group continued to receive water and rat chow, whereas the experimental group received 935 mM (32%) sucrose (Sigma, St. Louis, MO) in their drinking water and rat chow.

Experimental protocol. Non-fasted control and sucrose-fed rats underwent echocardiographic and metabolic assessments at 1, 2.5, 5, and 10 wk postdiet initiation. At the 2.5-, 5-, and 10-wk time points, a random subset of control and sucrose-fed rats were euthanized. Before death, rats were weighed and anesthetized using a cocktail of ketamine (90 mg/kg) and xylazine (10 mg/kg). Hearts were excised under controlled temperature (20 ± 0.5°C), humidity (30–70%), and lighting (12:12-h light-dark cycle). On arrival, all rats were given standard chow and water ad libitum. After 1 wk, rats were randomly assigned to one of two groups. The control group continued to receive water and rat chow, whereas the experimental group received 935 mM (32%) sucrose (Sigma, St. Louis, MO) in their drinking water and rat chow.

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Echocardiographic assessment of cardiac structure and function. Rats were weighed and anesthetized with 5% isoflurane and maintained under anesthesia with 2% isoflurane (Ohio Medical Products, Madison, WI) carried by oxygen at a flow rate of 2 l/min. Two-dimensional transthoracic echocardiography was performed using a SONOS 5500 ultrasound system (Aliquant Technologies, Andover, MA). A 12-MHz probe coated with ultrasonic transmission gel (Parker Laboratories, Fairfield, NJ) was placed in the parasternal short-axis orientation to obtain M-mode and pulsed-wave Doppler images. Left ventricle (LV) M-mode tracings were taken at the level of the papillary muscles at a sweep speed of 150 mm/s and a depth setting of 3 cm. M-mode images were used to measure the following parameters according to the leading-edge method of the American Society of Echocardiography (46): interventricular septal dimensions, left ventricular posterior wall dimensions, and left ventricular internal dimensions at end diastole (IVSd, LVPWd, and LVIDd, respectively) and end systole (IVSs, LVPWs, and LVIDs, respectively). The M-mode echocardiograms in Fig. 1 illustrate these dimensions. Moreover, these dimensions were used to calculate the systolic parameters: LV fractional shortening (FS), ejection fraction (EF), and cardiac output as follows: FS = [(LVIDd − LVIDs)/LVIDd] × 100; EF = [(end diastolic volume − end systolic volume)/end diastolic volume]; cardiac output = [(end diastolic volume − end systolic volume)/1,000] × heart rate (2). End diastolic volume and end systolic volume were calculated by the cubic formulas LVIDd3 and LVIDs3, respectively (2).

Doppler waveforms of mitral inflow and aortic outflow were obtained from the apical four- and five-chamber views, respectively. Diastolic variables determined from the apical four-chamber view included the peak early diastolic filling velocity (E wave), peak late diastolic filling velocity (A wave), ratio of the peak early to late diastolic filling velocity (E/A), and E wave deceleration time (E decel time), which is the time interval of peak E wave velocity to zero. Isovolumic relaxation time, the time from the end of systolic ventricular outflow to mitral valve opening, and aortic ejection time were obtained from the apical five-chamber view. All measurements were based on the average of three selected cardiac cycles.

Metabolic measurements. Immediately following echocardiographic assessment, 10% of total circulating blood volume was collected through puncture of the jugular vein of the anesthetized rats. Blood samples were centrifuged at 1,500 rpm for 10 min, and the plasma was stored at −20°C for later analysis. Plasma glucose, triglyceride, and cholesterol levels were measured using the respective diagnostic kits (Roche Diagnostics, Indianapolis, IN). Plasma insulin levels were determined with an enzyme-linked assay kit (Alpco Diagnostics, Windham, NH).

Isolation of SR vesicles. SR vesicles were obtained using a method described previously (33–35, 53, 56). Heart tissue was pulverized and homogenized using a Polytron homogenizer (Brinkman, Westbury, NY) for a period of 20 and 25 s, separated by a 1-min interval, at a speed of 12,000 rpm. The homogenization buffer contained (in mM) 10 NaHCO3, 5 Na2S, 15 Tris-HCl (pH 6.8), and the protease inhibitors (in μM) 1 leupeptin, 1 pepstatin, and 100 phenylmethyl-sulphonyl fluoride. Samples of homogenate were aliquoted, quick frozen in liquid nitrogen, and subsequently stored at −85°C until experimentation. The remaining homogenate was then centrifuged for 20 min at 10,919 g, and the supernatant obtained was further centrifuged for 45 min at 43,666 g (JA 20.0 rotor; Beckman Instruments, Palo Alto, CA). The resultant pellet was suspended in a buffer containing 0.6 M KCl and 20 mM Tris-HCl (pH 6.8) and centrifuged at the same speed and duration as in the previous step. The final pellet containing the SR fraction was suspended in a buffer containing 250 mM sucrose and 10 mM histidine (pH 7.0), from which aliquots were quick frozen in liquid nitrogen and then stored at −85°C until experimentation. All steps were performed in the cold room (4°C).

Measurement of SR Ca2+ uptake. Ca2+ uptake by the SR vesicles was measured using a procedure described previously (33–35, 53, 56). The reaction mixture contained 50 mM Tris-maleate (pH 6.8), 5 mM NaN3, 5 mM ATP, 5 mM MgCl2, 120 mM KCl, 5 mM K-oxalate, 0.1 mM EGTA, 0.1 mM45CaCl2, and 25 μM ruthenium red. The reaction was initiated with the addition of SR vesicles (20 μg) to the reaction mixture at 37°C and was terminated after 1 min by filtering 200 μl of the reaction mixture through 0.45-μm membrane filters (Fisher Scientific, Whitby, ON, Canada). The filters were then washed, dried at 60°C for 1 h, and counted in a β-lucid-scintillation counter.

Measurement of SR-associated PKA and CaMK II activities. Determination of PKA and CaMK II activities of the SR vesicles was based on a technique previously established (33–35, 56). PKA and CaMK II activities of the SR vesicles was measured using assay kits from Upstate Biotechnology (Lake Placid, NY). The assay kit for PKA activity is based on the phosphorylation of a specific substrate, kemptide, by the transfer of the γ-phosphate of [γ-32P]ATP by PKA. The assay kit for CaMK II activity is based on the phosphorylation of a specific substrate, autocomtide, by the transfer of the γ-phosphate of [γ-32P]ATP by CaMK II.
spotting the reaction mixture on phosphocellulose filter papers. The respective assay dilution buffers, substrates, and inhibitor cocktails exogenous substrate.

(200 wells in the absence of phosphopeptide, presence of phosphopeptide phosphatase assay kit (Upstate Biotechnology), which is based on the

SR vesicles were measured using a method described previously

-32P]ATP by CaMK II. The reactions for PKA and CaMK II were

initiated by adding [γ-

Measurement of SR-associated PP activities. PP activities of the SR vesicles were measured using a method described previously (33–35, 56). PP activities were determined using the serine/threonine phosphatase assay kit (Upstate Biotechnology), which is based on the dephosphorylation of the phosphopeptide KRpTIRR. The reaction was initiated with the addition of SR vesicles (25 μg) to microtiter wells in the absence of phosphopeptide, presence of phosphopeptide (200 μM), or presence of phosphopeptide and 2 nM okadaic acid (Upstate Biotechnology) and incubated for 30 min. The reaction was terminated by adding malachite green, and the absorbance was read after 15 min at a wavelength of 650 nm to determine the amount of inorganic phosphate released. The total PP activity was calculated as the difference between the amounts of inorganic phosphate released in the presence and absence of the exogenous substrate. PP1 activity was defined as the total PP activity in the presence of 2 nM okadaic acid, and PP2A activity was calculated as the difference between the total PP and PP1 activities. Okadaic acid at a concentration of 2 to 10 nM specifically inhibits PP2A and has therefore been used in previous studies (8, 36) to differentiate between the activities of PP1 and PP2A.

Western blot analysis. The protein content of the SR proteins SERCA2a, PLB, and its phosphorylated forms, serine-16 PLB and threonine-17 PLB, were determined by Western blot analysis according to a procedure described previously (34, 35, 53, 56). SR samples (20 μg) were separated on 10% (for SERCA2a) or 20% (for PLB, serine-16 PLB, and threonine-17 PLB) gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were probed with monoclonal SERCA2a (Affinity Bioreagents, Golden, CO), monoclonal PLB (Upstate Biotechnology), polyclonal serine-16 PLB (Badrilla, Leeds, UK), or polyclonal threonine-17 PLB (Badrilla). Appropriate secondary antibodies were used, and the antibody-antigen complexes of all probed membranes were detected using an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK). An imaging densitometer (model GS-800; Bio-Rad Laboratories) was used to scan the protein bands, and the data were quantified using Quality One 4.5.0 software (Bio-Rad Laboratories). Western blot analysis was also performed to determine the protein content of SERCA2a in homogenous samples (20 μg).

Statistical analysis. Unpaired t-test was used to examine the effect of diet (control vs. sucrose) on an experimental parameter measured at one time point. In contrast, two-way analysis of variance followed by Bonferroni post hoc test was used to examine the effect of diet (control vs. sucrose) and weeks on diet (1, 2.5, and 5 vs. 10 wk) on an experimental parameter. A difference between the means of groups was considered significant when P < 0.05. Data are expressed as means ± SE in the figures and tables.

RESULTS

The general characteristics of the control and sucrose-fed rats are provided in Table 1. The body weights of the control and sucrose-fed rats were similar until 10 wk, when significant increases were observed in the sucrose-fed rats. The heart weights were also comparable between the two groups throughout the time course. Plasma insulin, glucose, and triglyceride levels were significantly elevated at all time points, and plasma cholesterol levels were significantly increased at the final two time points in the sucrose-fed rats compared with control rats.

To determine whether changes in cardiac structure and function occurred in the sucrose-fed rats, in vivo echocardiography was performed and compared with control rats. Parameters of cardiac structure and function obtained by M-mode and Doppler echocardiography in the control and sucrose-fed rats are provided in Tables 2 and 3, respectively. With regard to cardiac structure, the dimensions of the IVS did not differ between the control and sucrose-fed rats until the 10-wk time point when significant thinning of the IVS was observed at diastole and systole in the sucrose-fed rats. Similarly, the internal dimensions of the LV did not change until 10 wk when increases in the LVID were evident at diastole and systole in the sucrose-fed rats. The dimensions of the LVPW at diastole and systole were similar between the two groups at all time points. The M-mode echocardiograms of representative left ventricles from the control (Fig. 1A) and sucrose-fed (Fig. 1B) rats at 10 wk illustrate the aforementioned alterations in cardiac structure in the sucrose-fed rats.

LV systolic function was significantly impaired as FS and EF were decreased in the sucrose-fed rats at the final time point. The HR of the control and sucrose-fed rats were comparable at the 1-wk time point, after which significant increases in HR were observed in the sucrose-fed rats. The cardiac output of the control and sucrose-fed rats were similar until 10 wk, when significant increases were evident in the sucrose-fed rats.

E wave, A wave, and E/A ratio were measured as parameters of LV diastolic function. The control and sucrose-fed rats exhibited similar E waves throughout the time course. The two groups showed equivalent A waves at 1 wk, after which the sucrose-fed rats exhibited significant increases in the A wave.

Table 1. General characteristics of the control and sucrose-fed rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 8–17)</th>
<th>Sucrose (n = 10–19)</th>
<th>Control (n = 9–15)</th>
<th>Sucrose (n = 9–17)</th>
<th>Control (n = 5–16)</th>
<th>Sucrose (n = 5–16)</th>
<th>Control (n = 8–38)</th>
<th>Sucrose (n = 6–40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>199.4 ± 5.0</td>
<td>202.6 ± 4.5</td>
<td>277.7 ± 7.7</td>
<td>302.2 ± 6.5</td>
<td>421.4 ± 5.6</td>
<td>438.3 ± 6.6</td>
<td>544.9 ± 6.6</td>
<td>617.2 ± 12.1*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>9.4 ± 0.2</td>
<td>10.0 ± 0.2</td>
<td>12.0 ± 0.7</td>
<td>12.0 ± 0.7</td>
<td>1.19 ± 0.2</td>
<td>1.25 ± 0.3</td>
<td>1.41 ± 0.2</td>
<td>1.45 ± 0.2</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>10.7 ± 0.27</td>
<td>12.5 ± 0.31*</td>
<td>8.56 ± 0.50</td>
<td>12.0 ± 0.7*</td>
<td>11.8 ± 0.60</td>
<td>14.9 ± 0.96*</td>
<td>10.3 ± 0.25</td>
<td>11.6 ± 0.52*</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>15.7 ± 3.1</td>
<td>78.6 ± 21.0*</td>
<td>34.1 ± 8.5</td>
<td>81.6 ± 12.5*</td>
<td>66.1 ± 16.7</td>
<td>147 ± 15.7*</td>
<td>60.4 ± 7.5</td>
<td>200 ± 18.3*</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>1.17 ± 0.09</td>
<td>1.99 ± 0.16*</td>
<td>1.81 ± 0.12</td>
<td>2.63 ± 0.18*</td>
<td>1.80 ± 0.16</td>
<td>2.63 ± 0.28*</td>
<td>2.42 ± 0.12</td>
<td>3.74 ± 0.22*</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>2.05 ± 0.04</td>
<td>2.26 ± 0.09</td>
<td>1.89 ± 0.05</td>
<td>2.06 ± 0.06</td>
<td>1.70 ± 0.03</td>
<td>2.04 ± 0.12*</td>
<td>1.78 ± 0.04</td>
<td>2.18 ± 0.06*</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 vs. control rats.
As a result, the sucrose-fed rats had reduced E/A ratios after 2.5 wk comparative to the control rats. The parameters concerning time, E decel time, isovolumic relaxation time, and aortic ejection time, did not differ between the control and sucrose-fed rats, with the exception of a more rapid ejection time at 5 wk in the sucrose-fed rats.

Given the critical role of the SR in regulating cardiac contraction and relaxation, its function was examined commencing at the 2.5-wk time point when a difference in LV contraction and relaxation, its function was examined comparatively to the control rats. The parameters concerning time, E decel time, isovolumic relaxation time, and aortic ejection time, did not differ between the control and sucrose-fed rats, with the exception of a more rapid ejection time at 5 wk in the sucrose-fed rats.

To determine whether the significant reduction in SR Ca\(^{2+}\) uptake in the hearts of the 10-wk sucrose-fed rats was directly attributed to abnormalities in the phosphorylation of PLB, the reduction in SR Ca\(^{2+}\) uptake in the hearts of the 10-wk sucrose-fed rats may also be attributed to abnormalities in the phosphorylation of PLB. Examination of the phosphorylation status of PLB in the 10-wk sucrose hearts showed a significant 71% decrease in the PKA-mediated phosphorylation of PLB at serine-16 as well as a significant 68% reduction in the CaMK II-mediated phosphorylation of PLB at threonine-17 compared with control hearts (Fig. 5). To find the underlying mechanisms for the significant reduction in PLB phosphorylation at the serine and threonine residues, the SR-associated PAK and CaMK II activities were assessed. There were significant increases in the activities of both SR-associated PAK (67%) and CaMK II (41%) in the 10-wk sucrose hearts compared with control hearts.

**Table 2. Parameters of cardiac function and structure obtained by M-mode echocardiography in control and sucrose-fed rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 16–17)</th>
<th>Sucrose (n = 17–18)</th>
<th>Control (n = 69–72)</th>
<th>Sucrose (n = 70–74)</th>
<th>Control (n = 63–69)</th>
<th>Sucrose (n = 65–69)</th>
<th>Control (n = 35–39)</th>
<th>Sucrose (n = 37–39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS, %</td>
<td>40.8 ± 1.2</td>
<td>41.1 ± 1.1</td>
<td>44.4 ± 0.67</td>
<td>43.2 ± 0.66</td>
<td>41.9 ± 0.67</td>
<td>42.5 ± 0.65</td>
<td>43.2 ± 0.90</td>
<td>39.3 ± 1.4*</td>
</tr>
<tr>
<td>EF</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>0.79 ± 0.01</td>
<td>0.78 ± 0.01</td>
<td>0.78 ± 0.01</td>
<td>0.79 ± 0.01</td>
<td>0.73 ± 0.02*</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>266 ± 9.10</td>
<td>281.6 ± 14.4</td>
<td>272.1 ± 8.7</td>
<td>265.5 ± 7.8</td>
<td>324.6 ± 10.5</td>
<td>343.5 ± 9.7</td>
<td>392.5 ± 11.1</td>
<td>427.1 ± 15.3*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>391 ± 4.5</td>
<td>398 ± 5.5</td>
<td>382 ± 2.7</td>
<td>399 ± 2.7*</td>
<td>358 ± 2.9</td>
<td>376 ± 2.9*</td>
<td>337 ± 4.1</td>
<td>354 ± 3.6*</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>1.49 ± 0.06</td>
<td>1.39 ± 0.06</td>
<td>1.69 ± 0.03</td>
<td>1.74 ± 0.04</td>
<td>1.80 ± 0.03</td>
<td>1.84 ± 0.03</td>
<td>1.90 ± 0.06</td>
<td>1.74 ± 0.05*</td>
</tr>
<tr>
<td>IVs, mm</td>
<td>2.50 ± 0.07</td>
<td>2.41 ± 0.09</td>
<td>2.88 ± 0.04</td>
<td>2.88 ± 0.05</td>
<td>3.06 ± 0.04</td>
<td>3.10 ± 0.05</td>
<td>3.17 ± 0.05</td>
<td>2.91 ± 0.07*</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>7.32 ± 0.10</td>
<td>7.41 ± 0.12</td>
<td>7.29 ± 0.08</td>
<td>7.17 ± 0.09</td>
<td>8.08 ± 0.08</td>
<td>8.10 ± 0.08</td>
<td>8.81 ± 0.09</td>
<td>9.26 ± 0.18*</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>4.34 ± 0.11</td>
<td>4.37 ± 0.12</td>
<td>4.05 ± 0.07</td>
<td>4.08 ± 0.08</td>
<td>4.68 ± 0.06</td>
<td>4.66 ± 0.08</td>
<td>5.02 ± 0.10</td>
<td>5.68 ± 0.25</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>1.22 ± 0.03</td>
<td>1.30 ± 0.04</td>
<td>1.72 ± 0.05</td>
<td>1.77 ± 0.06</td>
<td>1.87 ± 0.04</td>
<td>1.79 ± 0.04</td>
<td>1.80 ± 0.04</td>
<td>1.77 ± 0.06</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>2.15 ± 0.07</td>
<td>2.21 ± 0.07</td>
<td>2.70 ± 0.05</td>
<td>2.76 ± 0.05</td>
<td>2.89 ± 0.04</td>
<td>2.85 ± 0.04</td>
<td>3.03 ± 0.05</td>
<td>2.98 ± 0.08</td>
</tr>
</tbody>
</table>

Data are means ± SE. FS, fractional shortening; EF, ejection fraction; CO, cardiac output; HR, heart rate; IVS, interventricular septal dimensions; LVID, left-ventricular internal dimensions; LVPW, left-ventricular posterior wall dimensions; s, systole; d, diastole. *P < 0.05 vs. control rats.

**Table 3. Parameters of cardiac function obtained by Doppler echocardiography in control and sucrose-fed rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 6–18)</th>
<th>Sucrose (n = 6–19)</th>
<th>Control (n = 28–37)</th>
<th>Sucrose (n = 27–38)</th>
<th>Control (n = 26–36)</th>
<th>Sucrose (n = 31–36)</th>
<th>Control (n = 8–17)</th>
<th>Sucrose (n = 8–15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E wave, cm/s</td>
<td>116 ± 4.2</td>
<td>120 ± 4.9</td>
<td>110 ± 3.2</td>
<td>110 ± 2.5</td>
<td>116 ± 2.3</td>
<td>119 ± 2.1</td>
<td>109 ± 3.5</td>
<td>119 ± 5.9</td>
</tr>
<tr>
<td>A wave, cm/s</td>
<td>77.4 ± 6.7</td>
<td>75.9 ± 5.3</td>
<td>72.9 ± 2.6</td>
<td>79.2 ± 1.2*</td>
<td>76.1 ± 2.4</td>
<td>87.8 ± 2.2*</td>
<td>69.8 ± 4.0</td>
<td>81.6 ± 3.0*</td>
</tr>
<tr>
<td>E/A</td>
<td>1.55 ± 0.10</td>
<td>1.60 ± 0.06</td>
<td>1.55 ± 0.06</td>
<td>1.40 ± 0.03*</td>
<td>1.56 ± 0.05</td>
<td>1.36 ± 0.02*</td>
<td>1.61 ± 0.13</td>
<td>1.47 ± 0.09</td>
</tr>
<tr>
<td>E decel, ms</td>
<td>40.7 ± 1.6</td>
<td>41.5 ± 4.9</td>
<td>42.1 ± 1.8</td>
<td>40.1 ± 1.6</td>
<td>47.5 ± 1.6</td>
<td>46.2 ± 1.5</td>
<td>50.9 ± 1.6</td>
<td>52.4 ± 3.8</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>19.3 ± 0.74</td>
<td>18.8 ± 0.56</td>
<td>19.2 ± 0.48</td>
<td>19.0 ± 0.58</td>
<td>20.3 ± 0.42</td>
<td>19.7 ± 0.39</td>
<td>21.9 ± 0.72</td>
<td>20.1 ± 0.43</td>
</tr>
<tr>
<td>ET, ms</td>
<td>68.2 ± 0.85</td>
<td>66.8 ± 0.86</td>
<td>69.2 ± 0.80</td>
<td>67.8 ± 0.61</td>
<td>69.5 ± 0.73</td>
<td>67.5 ± 0.85*</td>
<td>70.6 ± 0.92</td>
<td>70.0 ± 0.99</td>
</tr>
</tbody>
</table>

Data are means ± SE. E wave, peak early diastolic filling velocity; A wave, peak late diastolic filling velocity; E/A, ratio of the peak early to late diastolic filling velocity; E decel, E wave deceleration time; IVRT, isovolumic relaxation time; ET, aortic ejection time. *P < 0.05 vs. control rats.
varied due to the different strains of rat used, as well as the mode, amount, and duration of sucrose feeding. In this study, the sucrose rats exhibited higher plasma insulin levels than control rats within 1 wk of feeding, suggesting that these rats had developed whole body insulin resistance. Concurrently, the sucrose-fed rats exhibited hyperglycemia and hypertriglyceridemia, followed by moderate increases in plasma cholesterol levels and body weight. The addition of sucrose to either drinking water or rat chow has been shown to produce similar changes in the levels of circulating insulin (1, 17, 40, 42, 54), triglycerides (17, 40, 42, 54), cholesterol (1, 42), and glucose (40, 42, 54), as well as in body weight (1, 24, 40). The sucrose-fed rats in our study exhibited metabolic derangements and elevated body weight consistent with the metabolic syndrome (21) and the insulin-resistant stage of Type 2 DM in humans (20).

Previous studies have shown that the development of insulin resistance in rats caused by high-sucrose feeding results in cardiac contractile dysfunction, specifically depressed myocyte contractility (11, 16, 22–24, 57) and reduced myofibrillar protein ATPase activities (40). However, no study to date has examined cardiac structure and function by in vivo echocardiography in a sucrose-induced model of insulin resistance. Hence, we performed serialized echocardiographic assessments that revealed abnormalities in diastolic and systolic performance as well as alterations in myocardial structure in the sucrose-fed rats.

With regard to diastolic function, the sucrose-fed rats exhibited early abnormalities in LV filling as demonstrated by higher A waves and reduced E/A ratios from the 2.5-wk time point onward. These changes are consistent with echocardiographic findings in other murine models of Type 2 DM (12, 25, 51). Moreover, our results correspond with human studies that have shown early diastolic dysfunction marked by a significant reduction in the E/A ratio in patients with impaired glucose tolerance or overt Type 2 DM (6, 13). The early abnormality in diastolic function was followed by reductions in FS and EF between the 5- and 10-wk time points, indicating decreased cardiac contractility in the sucrose-fed rats. The late development of systolic dysfunction has been previously observed in 12-wk-old diabetic (db/db) mice (51) and in 20-wk-old Zucker diabetic fatty rats (60). Alterations in myocardial structure also occurred between 5 and 10 wk, as the sucrose-fed rats exhibited a thinner interventricular septal wall and concomitant enlarged left ventricular internal cavity, suggesting minor left ventricular dilatation.

Given the critical role of the SR in regulating cardiac contraction and relaxation, defective SR function may explain the alterations in cardiac contractile function observed in our sucrose-induced insulin-resistant rats. Currently, there is scanty
and conflicting information available on the status of the SR in insulin-resistant (i.e., prediabetic) and Type 2 diabetic murine models (3, 32, 49, 57). Schaffer et al. (49) reported a decrease in SR Ca\(^{2+}\) uptake in 12-mo-old diabetic rats that had been injected with streptozotocin as neonates to produce a Type 2 diabetic-like condition. However, the experimental model used in their study (49) had certain limitations that included no elevation in basal insulin levels and normal body weight, which differ from the clinical state seen in the majority of Type 2 diabetic patients. In contrast, Misra et al. (32) observed an increase in SR Ca\(^{2+}\) uptake in 3-mo-old JCR:LA corpulent (cp/cp) rats. Despite this observation, Misra et al. (32) found no changes in active cell shortening and intracellular Ca\(^{2+}\) concentration under basal conditions in cardiomyocytes from the cp/cp rats. Belke et al. (3) reported a decreased rate of relaxation in isolated working hearts and a reduced rate of decay of the Ca\(^{2+}\) transient in isolated myocytes from 12-wk-old db/db mice. They attributed these changes to diminished SR function as the sarcolemmal sodium-calcium exchanger activity was unchanged. Similarly, Wold et al. (57) reported that slowed cytosolic Ca\(^{2+}\) removal and myocyte relaxation (with normal sodium-calcium exchanger function) involved impaired SERCA activity in sucrose-induced insulin-resistant rats. However, the limitation with these studies (3, 57) was that functional parameters were not assessed in isolated SR vesicles, which restricts the conclusions of these studies.

In this study, we examined Ca\(^{2+}\) uptake in isolated SR vesicles commencing at the 2.5-wk time point when abnormalities in LV diastolic function first became apparent. We observed no difference in SR Ca\(^{2+}\) uptake in the hearts of the 2.5- and 5-wk control and sucrose-fed rats. These results suggest that other cellular mechanisms, such as altered Ca\(^{2+}\) affinity of the contractile proteins or incomplete Ca\(^{2+}\) removal across the sarcolemmal membrane, may have contributed to the diastolic dysfunction. In support of this, Schaffer et al. (48) reported the activity of the sodium-calcium exchanger to be depressed in a murine model of Type 2 DM. However, we did observe a
significant decrease in SR Ca\(^{2+}\) uptake in the hearts of the 10-wk sucrose-fed rats. Since a decline in SR Ca\(^{2+}\) uptake would decrease SR Ca\(^{2+}\) loading, thereby making less Ca\(^{2+}\) available for the next wave of contraction, it is not surprising that we observed significant reductions in FS and EF in the 10-wk sucrose-fed rats.

The significant reduction in SR Ca\(^{2+}\) uptake in the hearts of the 10-wk sucrose-fed rats may be attributed to changes in the SR Ca\(^{2+}\) cycling proteins. We observed no changes in SERCA2a and its inhibitory protein PLB and therefore no changes in the ratio of PLB/SERCA2a in the 10-wk sucrose hearts compared with control hearts. Conversely, Belke et al. (3) reported a significant elevation in the protein content of PLB and therefore an increase in the ratio of PLB/SERCA2a in 12-wk-old db/db mice. Furthermore, it is possible that changes in SERCA2a function precede changes in protein expression, which has been observed in Type 1 diabetic rat hearts (59).

The decline in SR Ca\(^{2+}\) uptake in the hearts of the 10-wk sucrose-fed rats may also be attributed to decreased PLB phosphorylation by the SR-associated kinases. We observed a significant decrease in the PKA-mediated phosphorylation of PLB at serine-16 as well as a significant decrease in the CaMK II-mediated phosphorylation of PLB at threonine-17. A similar observation has been made by Belke et al. (3) in 12-wk-old db/db mice in which the phosphorylation status of serine-16 was reduced. Since PKA and CaMK II are endogenous to the SR (28), the observed decrease in PLB phosphorylation at the serine and threonine residues may be attributed to reductions in SR-associated PKA and CaMK II activities, respectively. However, our results show an augmentation in the activities of both SR-associated PKA and CaMK II in the hearts of the 10-wk sucrose-fed rats. One other study (15) assessed the activity of PKA in vitro using normal rat ventricular myocytes cultured in a high-glucose medium. Although their study found the total PKA activity to be depressed in the high-glucose myocytes, the phosphorylation status of serine-16 was unaffected. A great limitation of their study was that global PKA activity was measured, which may or may not have affected sarcoplasmic reticular PLB. This global measurement contrasts our study, which measured PKA activity localized to the SR.

Increased SR-associated PP activity may have also contributed to the decreased PLB phosphorylation observed in the hearts of the 10-wk sucrose-fed rats. PP1 has been reported to be endogenous to the SR (52) and dephosphorylates PLB at both the PKA and CaMK II phosphorylation sites (30), thereby inhibiting SR Ca\(^{2+}\) uptake. PP2A has also been shown to dephosphorylate PLB (30). In this study, however, we observed no changes in the total PP, PP1, and PP2A activities in the 10-wk sucrose hearts compared with control hearts.

Collectively, our protein kinase and protein phosphatase results would suggest augmented PLB phosphorylation in the hearts of the 10-wk sucrose-fed rats; however, this was not observed. This indicates that additional factors may affect the regulation of SR function such as anchoring proteins. Anchoring proteins are thought to target protein kinases and protein phosphatases to intracellular locations positioning them close to their substrates, thereby facilitating the phosphorylation or dephosphorylation of specific targets (9). In this respect, A-kinase anchoring proteins (AKAPS) may modulate the compartmentalization of PKA to the cardiac SR (45, 58), while \(\alpha\)-kinase anchoring proteins may modulate the compartmentalization of CaMK II to the cardiac SR (37). AKAPS specific to PP1 have also been identified (9). Therefore, it is plausible that anchoring defects may have contributed to the impaired phosphorylation of PLB despite augmented activities of the SR-associated PKA and CaMK II in the 10-wk sucrose hearts.

Our study is novel in that it is the first to examine serial alterations in cardiac structure and function by echocardiography while determining the status of cardiac SR Ca\(^{2+}\) cycling and its regulatory mechanisms in sucrose-induced insulin-resistant rats. The early abnormalities in LV diastolic filling and late impairments in systolic function concomitant with alterations in myocardial structure in the sucrose-fed rats suggest the development of a diabetic-like cardiomyopathy at an insulin-resistant state before the manifestation of overt Type 2 DM. Furthermore, the cardiac contractile dysfunction in the sucrose-induced insulin-resistant heart is associated with impairments in SR function and its regulation.

In conclusion, our study provides a better understanding of the phenotypic changes that occur in the myocardium during the insulin-resistant state and specifically identifies potential targets for improving SR function. This information is of paramount importance as it will enable the prevention or therapeutic treatment of cardiac contractile dysfunction in human disorders involving insulin resistance such as the metabolic syndrome, Type 2 DM, obesity, and hypertension.

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CARDIAC PERFORMANCE AND SR FUNCTION IN SUCROSE-FED RATS


