Impairment of cardiac insulin signaling and myocardial contractile performance in high-cholesterol/fructose-fed rats

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Deng J-Y, Huang J-P, Lu L-S, Hung L-M. Impairment of cardiac insulin signaling and myocardial contractile performance in high cholesterol-fructose-fed rats. Am J Physiol Heart Circ Physiol 293: H978–H987, 2007. First published March 30, 2007; doi:10.1152/ajpheart.01002.2006.—Although insulin resistance is recognized as a potent and prevalent risk factor for coronary heart disease, less is known as to whether insulin resistance causes an altered cardiac phenotype independent of coronary atherosclerosis. In this study, we investigated the relationship between insulin resistance and cardiac contractile dysfunctions by generating a new insulin resistant animal model with rats on a high cholesterol-fructose diet. Male Sprague-Dawley rats were fed high cholesterol-fructose (HCF) diet for 15 wk; the rats developed insulin resistance and cardiac contractile dysfunctions characterized by increased blood pressure, hyperlipidemia, hyperinsulinemia, impaired glucose tolerance, and insulin resistance. The results show that HCF induced insulin resistance not only in metabolic response tissues (i.e., liver and muscle) but also in the heart as well. Insulin-stimulated cardiac glucose uptake was significantly reduced after 15 wk of HCF feeding, and cardiac insulin resistance was associated with blunted Akt-mediated insulin signaling along with decreased FATP1 levels. The cardiac performance of the HCF rats exhibited a marked reduction in cardiac output, ejection fraction, stroke volume, and end-diastolic volume. Akt; glucose transporter; cardiac dysfunction; high cholesterol-fructose diet; insulin resistance

HEART DISEASE IS A LEADING CAUSE of death in diabetic patients (43), with coronary artery disease (CAD) and atherosclerosis being the primary reasons for increased incidence of cardiovascular dysfunction (43, 38). However, a predisposition to heart failure might also reflect the effects of underlying abnormalities in cardiac diastolic function that can be detected in asymptomatic patients with diabetes (13, 4). Several etiological factors have been put forward to explain why hyperglycemia and/or diabetes tends to lead to diabetic cardiomyopathy. The accumulation of connective tissues, insoluble collagens (1), and abnormalities of various proteins that regulate ion flux (specifically, intracellular calcium) (18), has been proposed as an explanation for left ventricular wall stiffness and contractile dysfunctions. Recently, it was speculated that diabetic cardiomyopathy could also occur as a consequence of metabolic alterations (7, 8, 23). It is well known that under normal conditions, the adult heart utilizes predominantly long-chain fatty acids for most of its energy requirements (60–90%), with glucose and lactate providing the rest (25). Since Randle et al. (33, 34) proposed the existence of a glucose-fatty acid cycle in 1963, the link between glucose and fatty acid metabolism has been widely accepted. Disruption of the balance between glucose and fatty acid metabolism is often a primary defect observed in cardiac pathologies such as hypertrophy, heart failure, diabetes, dilated cardiomyopathy, and myocardial infarction (6, 11). Cardiac muscle is also a target of insulin (16); impairment of insulin-stimulated cardiac glucose uptake has been described in animal models of diabetes (12), obesity (15), and hypertension (27). Binding of insulin to its receptor activates the tyrosine kinase activity of the receptor’s β-subunit (22). This leads to autophosphorylation as well as tyrosine phosphorylation of several insulin receptor substrates. These substrates, in turn, interact with phosphatidylinositol 3-kinase and stimulate Akt, a downstream serine/threonine kinase that induces glucose uptake via translocation of glucose transporter GLUT4 to the plasma membrane (10). Abnormalities in insulin signaling account for insulin resistance. Insulin resistance is an important risk factor for the development of hypertension, atherosclerotic heart disease, left ventricular hypertrophy and dysfunction, and heart failure (17, 19, 32). It reflects a disturbance of glucose metabolism and can potentially worsen metabolic efficiency of both skeletal and cardiac muscles. The exact mechanisms of cardiac insulin resistance on progression of left ventricular contractile dysfunctions are not fully elucidated. In addition, there have been no studies of cardiac dysfunction in type II diabetic rodent models other than genetically obese or diabetic animals. The rodent model has the advantage of having atherosclerosis not present to confuse the interpretation of the mechanism of diabetic cardiomyopathy. Therefore, in this experiment, we chose the high cholesterol-fructose diet to induce insulin resistance in rats and investigated whether insulin resistance has an effect on cardiac insulin signaling and left ventricular contractile dysfunctions.

METHODS

Animals and diets. This investigation abides by the rules written in the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health [DHEW Publication No. (NIH) 85-23, revised 1996]. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Four-week-old Sprague-Dawley (SD) rats (body weights 150–170 g) were maintained in the Animal Center of Chang Gung University under an ambient temperature of 25 ± 1°C and a light-dark period of 12 h. The animals were maintained on either the chow diet (LabDiet 5010), containing 5.1% fat (linoleic acid, C18:2, unsaturated fatty acid), 23.5% protein, and 50.3% carbohydrate with water, or a high-cholesterol diet (Harlan Teklad TD03468, Indianapolis, IN) with 10% fructose solution for 15 wk. The high-cholesterol diet contained 10.1% fat (5% coconut oil and 5.1% linoleic acid), 17% protein, 51.6% carbohydrate, and 4% cholesterol. Both diets contained a standard mineral and vitamin mixture. Body weight, water, and food intake were recorded weekly.

**Biochemical analysis.** Blood was collected from the femoral vein after pentobarbital (65 mg/kg ip) anesthesia for biochemical measurements. Plasma was used for the measurements of total cholesterol, high-density lipoprotein, and triglyceride (Randox, Antrim, UK). Insulin was measured using a sandwich enzyme-linked immunosorbent assay (ELISA; Mercodia, Uppsala, Sweden). Insulin and glucose tolerance tests were performed on animals that had been fasted overnight. Animals were either intraperitoneally injected with 1 U/kg body wt of human regular insulin (Lilly) or intravenously injected with fructose for 15 wk.

![Fig. 1. General characteristics of rats fed with chow (control) and high cholesterol-fructose diet (HCF) for 15 wk. Body weight (A), water intake (B), food intake (C), and fasting cholesterol (D), triglyceride (E), and insulin levels (F) were examined in control and HCF rats. Data are means ± SE (n = 25–30). *P < 0.05; **P < 0.01; ***P < 0.001 vs. control.](#)
with 0.5 gm/kg body wt of glucose. Blood glucose samples (0.2 ml for each time point) were collected from the femoral vein at 0, 5, 10, 20, 30, 60, 90, and 120 min after glucose administration and were determined using the glucose oxidase method (Chemistry Analyzer; Quik-Lab, Ames).

**Hemodynamic measurements.** The animals were anesthetized with pentobarbital sodium (65 mg/kg ip) and placed on controlled heating pads (TC-1000 temperature controller; CWE) with the core temperature measured via a rectal probe maintained at 37°C. A microtipped pressure-volume catheter (SPR-838; Millar Instruments, Houston, TX) was inserted into the right common carotid artery and advanced into the left ventricle (LV) under pressure control as described previously (3, 30, 31). Polyethylene cannulas (PE-50) were inserted into the right femoral artery for the measurement of mean arterial pressure (MAP). After stabilizing for 20 min, the signals were continuously recorded at a sampling rate of 1,000/s by using an ARIA pressure-volume (P-V) conductance system (Millar Instruments) coupled to a Powerlab/4SP analog-to-digital converter (AD Instruments, Mountain View, CA). Data were displayed and recorded on a computer. All P-V loop data were analyzed using a cardiac P-V analysis program (PVAN3.2; Millar Instruments), and the heart rate, end-systolic volume, end-diastolic volume (EDV), end-systolic pressure (ESP), end-diastolic pressure (EDP), stroke volume (SV), ejection fraction (EF), cardiac output (CO), stroke work (SW), arterial elastance (end-systolic pressure/SV), MAP, and maximal slopes of systolic pressure increment (dP/dt max) and diastolic decrement (dP/dt min) were computed. The relaxation time constant (tau), an index of diastolic function, was calculated using two different methods [Weiss method (tau w): regression of log (pressure) vs. time; Glantz method (tau g): regression of dP/dt vs. pressure] using PVAN3.2. Total peripheral resistance (TPR) was calculated using the following equation: TPR = MAP/CO. The hemodynamic parameters were also determined under conditions of changing preload, elicited by transiently compressing the inferior vena cava using a cotton swab inserted through a small, transverse, upper abdominal incision. This technique yields reproducible occlusions in animals without opening the chest cavity. Because dP/dt max may be preload dependent, the P-V loops recorded at different preloads were used to derive other useful systolic function indexes that may be less influenced by loading conditions and cardiac mass. These measurements include the dP/dt EDV relation (dP/dt-EDV), end-systolic P-V relation (ESPVR), maximum chamber elasticity, and the preload-recruitable stroke work, which represents the slope between SW and EDV and is independent of chamber size and mass. The slope of the end-diastolic PV relationship (EDPVR), an index of LV stiffness, was also calculated from P-V relations using PVAN3.2.

**Immunoblotting.** Tissue lysates (membranes and cytosolic fraction) were isolated from soleus muscle, epididymal adipose, and cardiac tissues according to a previously published procedure with slight modifications (24). In brief, tissues were first homogenized in a lysis buffer (M-PER; Pierce) with 1 mM phenylmethylsulfonyl fluoride as a protease inhibitor. The tissue lysates were then ultracentrifuged at 50,000 rpm for 1 h at 4°C. The resulting supernatant was labeled as a cytosolic fraction. The resulting pellet, which contained the crude membrane, was resuspended in M-PER (~300–500 μl) with 0.5% Triton X-100, incubated at 4°C overnight, and centrifuged again at 15,700 g for 20 min. Finally, the supernatant was collected and labeled as a membrane fraction. Protein samples of cytosolic and

### Table 1. General characteristics of rats fed with chow (control) and high cholesterol-fructose diet for 15 wk

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HCF</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>566.67±6.67</td>
<td>466.36±7.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Food intake, g/day/hat</td>
<td>30±0.73</td>
<td>18±1.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water intake, ml/day/hat</td>
<td>34.17±2.12</td>
<td>91.82±14.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>86.33±3.58</td>
<td>89.36±2.54</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, μg/l</td>
<td>0.35±0.012</td>
<td>3.15±0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>66.56±4.25</td>
<td>324.24±35.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>93.81±6.41</td>
<td>126.97±9.25</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>42.74±1.96</td>
<td>39.20±1.18</td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>106.9±3.97</td>
<td>127.78±1.51</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE; (n = 20–26 rats per group) HCF, high cholesterol-fructose diet. HDL, high-density lipoprotein; MAP, mean arterial pressure.

Fig. 2. Intravenous glucose tolerance tests (IVGTT; A and B) and intraperitoneal insulin tolerance tests (IPITTs; C) in rats fed with chow (control) and HCF diet for 15 wk. During IVGTTs, plasma glucose (A) and insulin levels (B) increased significantly in HC rats compared with control rats. C: HCF impaired insulin sensitivity during IPITTs. Data are means ± SE (n = 8). *P < 0.05; **P < 0.01; ***P < 0.001 vs. control.
membrane lysates were subjected to 10% SDS-PAGE and electro-
phoretically transferred to polyvinylidene difluoride protein sequenc-
ing membrane for 2 h. The membrane was blocked in 5% nonfat milk
in Tris-buffered saline with 0.1% Tween-20. It was then washed and
blotted with GLUT1 (Chemicon), GLUT4 (Chemicon), fatty acid
transporter FATP1 (Santa Cruz Biotechnology), and/or fatty acid
translocase CD36/FAT (Santa Cruz Biotechnology) antibodies. Phos-
phorylation of Akt was detected with anti-phospho-Akt (Ser473) and
anti-phospho-Akt (Thr308) (Santa Cruz Biotechnology); Akt was
determined with anti-Akt antibody (Santa Cruz Biotechnology). The
membrane was then incubated with horseradish peroxidase-conju-
gated secondary antibody before chemiluminescence detection
(Pierce).

Histology. Tissues were fixed overnight with 4% paraformalde-
hyde in PBS, dehydrated, embedded in paraffin, sectioned (6–8 μm), and
stained with hematoxylin and eosin.

Statistical analysis. Data are means ± SE. The differences in body
weight, water intake, food intake, cholesterol level, triglyceride level,
insulin level, glucose tolerance test, and insulin tolerance test were
analyzed using two-way repeated-measures ANOVA; others were
analyzed using Student’s t-test, and the significant difference was set
at \( P < 0.05 \).

RESULTS

General characteristics in control and HCF rats. Male SD
rats were fed with a high-cholesterol diet with 10% fructose
solution (HCF) for 15 wk to induce insulin resistance. HCF rats
developed insulin resistance syndrome, which was character-
ized by elevated blood pressure, an impaired glucose tolerance
during glucose challenge, and increased fasting plasma chole-
sterol, triglyceride, and insulin levels. As shown in Fig. 1A and
Table 1, HCF rats gained slightly less weight than the control
rats over the study period. HCF rats also increased water intake
and reduced food intake compared with the control rats (Fig. 1, B and C). After 15 wk of
feeding, there was no difference in fasting glucose levels and
plasma high-density lipoprotein (HDL) among the groups (see
Table 1); however, HCF rats showed higher levels of total

Fig. 3. Glucose transporter (GLUT) protein levels of the skeletal muscles and epididymal adipose
tissues in rats fed with chow (control) and HCF diet for 15 wk. The cytosolic and membranous GLUT1
and GLUT4 protein levels were examined for observation of GLUT1 and GLUT4 trafficking in soleus
muscles (A–D) and epididymal fat pad (E–H). Equal amounts of proteins were resolved on 10%
SDS-PAGE and blotted with respective GLUT1 and GLUT4 antibodies. All blots were stripped and
reprobed with an antibody to GAPDH or Na⁺-K⁺-ATPase. C, D, G, and H show densitometric
measurements of protein bands in A, B, E, and F, respectively. All experiments were performed in
quintuplicate from 5 animals.
plasma cholesterol, triglyceride, and insulin than control rats (Fig. 1, D–F). In addition, HCF rats had an increase in MAP (106.9 ± 1.48 vs. 127.8 ± 1.51 mmHg, P < 0.001), systolic blood pressure (122.1 ± 1.96 vs. 145.3 ± 2.03 mmHg, P < 0.001), and diastolic blood pressure (105.3 ± 1.55 vs. 119.0 ± 1.58 mmHg, P < 0.01).

Intravenous glucose tolerance test (IVGTT) was performed on rats that had fasted overnight and had intravenously received bolus injections of glucose (500 mg/kg) through the femoral vein. After glucose loading, the plasma glucose concentration was elevated from 77.3 ± 3.81 to 216.2 ± 5.86 mg/dl (5 min after administration, P < 0.001) and then dropped to 80.2 ± 2.13 mg/dl (2 h after administration, P < 0.001; Fig. 2A) in the control rats. HCF impaired glucose tolerance (Fig. 2A) and the efficiency of insulin responses in IVGTTs (Fig. 2B). In addition, HCF also impaired insulin sensitivities in intraperitoneal insulin tolerance tests (IPTTs) compared with the control rats (Fig. 2C).

Impaired insulin signaling in HCF rats. Expression of glucose transporter (GLUT1 and GLUT4) proteins were examined using immunoblotting methods in experimental rat soleus muscle and epididymal fat pad after 15 wk of HCF diet. HCF rats had a dramatic reduction of membranous GLUT4 protein levels in soleus muscle compared with the control rats (Fig. 3A). In contrast, there was no significant difference in the GLUT1 protein levels between the two groups (Fig. 3).

To confirm the effects of HCF on insulin-stimulated recruitment of glucose transporters and fatty acid transport protein to the cell surface of the heart, we subjected protein extracts from the heart to Western blot analysis (Fig. 4). After insulin stimulation, the GLUT4 protein levels in the membrane were increased 1.63- and 1.30-fold in control and HCF rats, respectively (Fig. 4, A and C, P < 0.05). The basal membranous fatty acid transporter 1 (FATP1) was significantly increased in the heart of HCF rats (Fig. 4, E and F, P < 0.05). However, the membranous FATP1 and CD36 protein levels were not affected by insulin stimulation in both groups (Fig. 4, E and F).

Insulin-mediated phosphorylation of Akt was measured in the hearts of HCF rats to determine whether decreased cardiac insulin signaling (insulin resistance) was responsible for impaired GLUT4 membrane translocation. Intravenous injections of insulin significantly increased cardiac Akt phosphorylation (residue Ser473 and Thr308 of Akt) in control rats (Fig. 5). Insulin increased cardiac Akt-Ser473 phosphorylation in rats fed with chow and HCF (residue Ser473 and Thr308 of Akt) in control rats (Fig. 5). Although insulin induced Akt-Ser473 phosphorylation in both diet groups, the insulin-mediated induction in cardiac Akt-Thr308 phosphorylation was almost completely blocked in HCF rats (insulin increased Akt-Thr308 phosphorylation 1.43- and 0.98-fold in control and HCF rats, respectively, P < 0.05, Fig. 5).

Attenuated cardiac contractile functions in HCF rats. Impaired insulin signaling may have divergent or distinct effects on the progression of cardiomyopathy in rats fed with HCF diet for 15 wk. We sought to directly measure the cardiac performance by using Millar pressure-volume instruments. The hemodynamic parameters [CO, SW, maximal power, EF, SV, maximum volume, EDV, maximal [(dV/dt)max] and minimal rates of volume change [(dV/dt)min]] were reduced significantly in HCF rats compared with the control rats (Fig. 6 and Table 2). Animals fed with HCF diet for 15 wk also prolonged τ (Fig. 6J, P < 0.05) and increased the effective arterial elasticity (P < 0.01, Fig. 6K). Figure 7 illustrates typical P-V loops obtained after inferior vena cava occlusions in both groups.

![Fig. 4](http://ajpheart.physiology.org/)

**Fig. 4.** Cardiac GLUT1 and GLUT4, fatty acid transport protein 1 (FATP1), and CD36 protein levels in rats fed with chow (control) and HCF diet for 15 wk. Heart tissues were harvested 5 min after intravenous injection of 0.9% NaCl or insulin (10 units of human regular insulin; Lilly). The cytosolic and membranous GLUT1 and GLUT4 (A–D) and membranous FATP1 and CD36 protein levels (E and F) were examined in the heart. Equal amounts of proteins were resolved on 10% SDS-PAGE and blotted with respective GLUT1, GLUT4, FATP1, and CD36 antibodies. All blots were stripped and reprobed with an antibody to GAPDH or Na+–K+–ATPase. C, D, and F show densitometric measurements of protein bands in A, B, and E, respectively. All experiments were performed in quadruplicate from 4 animals.
The slopes of systolic P-V relations (ESPVR, Fig. 7A) were dramatically decreased in HCF rats compared with the control rats (Fig. 7B, P < 0.001).

Altered heart morphology in HCF rats. After 15 wk of HCF feeding, the appearances of HCF rat hearts were larger than those of control rats (Fig. 8A, left). The hearts weighed 1.41 ± 0.15 and 1.17 ± 0.1 g in control and HCF rats, respectively (P < 0.05, n = 8). However, both groups had identical weights when normalized to the body weight: 2.55 ± 0.19 and 2.58 ± 0.10 mg/g for control and HCF rats, respectively (Fig. 8A, right). Transverse sections of HCF hearts showed dilatation of the ventricle chamber and a decrease in ventricular wall thickness (Fig. 8B). Hematoxylin- and eosin-stained sections of HCF rat hearts presented an increase in distance between myocytes and thinner cardiomyocytes compared with the control group (Fig. 8C).

**DISCUSSION**

Both genetic and environmental factors contribute to the development of metabolic abnormalities. Several experimental studies have demonstrated that the macronutrient composition of a diet is an important environmental determinant of the quality of insulin action (2, 5). High fat and high fructose intakes were shown to contribute to conditions such as hyperlipidemia, glucose intolerance, hypertension, and atherosclerosis (26, 39). In addition, brief feeding of excess atherogenic diet (chow with 45% kcal from fat and 2% cholesterol) produces striking features of metabolic syndrome and coronary artery disease (14). High sugar intake is linked to an increased risk of heart diseases. Simple sugars are the primary source of high triglycerides (a type of blood fat) and very low-density lipoproteins (LDL), which are independent risk factors for atherosclerosis. Sugar lowers HDL cholesterol and raises LDL cholesterol along with blood pressure levels. In addition, it has been suggested that fructose-induced hyperuricemia results in endothelial dysfunction and insulin resistance and might be a causal mechanism of the metabolic syndrome (28). In the present study, HCF rats also showed hyperuricemia (0.62 ± 0.06 vs. 1.36 ± 0.15 mg/dl, P < 0.001). Sugar-sweetened beverages in the market today contain 12–15% sucrose; this factor should not be ignored with regard to the development of metabolic syndrome.

**Table 2. Hemodynamic parameters of rats fed with chow (control) and HCF diet for 15 wk**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>HCF (n = 8)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>358.2 ± 8.27</td>
<td>372.3 ± 15.67</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum volume, μl</td>
<td>422.5 ± 28.1</td>
<td>318.1 ± 23.01</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Minimum volume, μl</td>
<td>162.0 ± 30.4</td>
<td>173.6 ± 17.42</td>
<td>NS</td>
</tr>
<tr>
<td>End-systolic volume, μl</td>
<td>179.3 ± 33.4</td>
<td>195.5 ± 21.98</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic volume, μl</td>
<td>406.3 ± 28.0</td>
<td>311.0 ± 15.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Maximum pressure, mmHg</td>
<td>128.8 ± 6.51</td>
<td>131.9 ± 7.23</td>
<td>NS</td>
</tr>
<tr>
<td>Minimum pressure, mmHg</td>
<td>4.07 ± 0.47</td>
<td>3.16 ± 0.59</td>
<td>NS</td>
</tr>
<tr>
<td>End-systolic pressure, mmHg</td>
<td>117.0 ± 7.88</td>
<td>122.8 ± 8.06</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic pressure, mmHg</td>
<td>7.63 ± 0.56</td>
<td>8.56 ± 1.74</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>260.0 ± 14.8</td>
<td>160.3 ± 12.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>62.8 ± 4.57</td>
<td>47.3 ± 2.23</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cardiac output, μl/min</td>
<td>101.009 ± 7.441</td>
<td>62.689 ± 4.185</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stroke work, mmHg/μl</td>
<td>24.919 ± 1.312</td>
<td>15.185 ± 1.467</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Arterial elastance, mmHg/ml</td>
<td>0.47 ± 0.06</td>
<td>0.83 ± 0.10</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;, mmHg/ml</td>
<td>36.97 ± 7.29</td>
<td>312.4 ± 19.8</td>
<td>NS</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;min&lt;/sub&gt;, mmHg/ml</td>
<td>8.923 ± 767</td>
<td>8.071 ± 436</td>
<td>NS</td>
</tr>
<tr>
<td>dV/dt&lt;sub&gt;max&lt;/sub&gt;, μl/s</td>
<td>9.249 ± 520</td>
<td>8.735 ± 853</td>
<td>NS</td>
</tr>
<tr>
<td>dV/dt&lt;sub&gt;min&lt;/sub&gt;, μl/s</td>
<td>1.0124 ± 692</td>
<td>6.796 ± 801</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Pressure at dV/d&lt;sub&gt;max&lt;/sub&gt;, mmHg</td>
<td>8.8833 ± 729</td>
<td>5.182 ± 545</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pressure at dP/d&lt;sub&gt;min&lt;/sub&gt;, mmHg</td>
<td>5.74 ± 0.66</td>
<td>4.91 ± 1.08</td>
<td>NS</td>
</tr>
<tr>
<td>Volume at dP/d&lt;sub&gt;max&lt;/sub&gt;, μl</td>
<td>87.0 ± 3.91</td>
<td>88.2 ± 2.38</td>
<td>NS</td>
</tr>
<tr>
<td>Volume at dP/d&lt;sub&gt;min&lt;/sub&gt;, μl</td>
<td>367.9 ± 27.9</td>
<td>312.4 ± 19.8</td>
<td>NS</td>
</tr>
<tr>
<td>Tau&lt;sub&gt;W&lt;/sub&gt;, ms</td>
<td>166.1 ± 32.6</td>
<td>185.1 ± 22.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Tau&lt;sub&gt;g&lt;/sub&gt;, ms</td>
<td>9.90 ± 0.54</td>
<td>12.0 ± 0.81</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Maximal power, mW</td>
<td>143.6 ± 8.30</td>
<td>96.2 ± 7.85</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Preload-adjusted maximal power, mW/μl</td>
<td>9.77 ± 1.19</td>
<td>9.82 ± 1.06</td>
<td>NS</td>
</tr>
<tr>
<td>End-systolic elastance, mmHg/ml</td>
<td>1.02 ± 0.099</td>
<td>0.50 ± 0.079</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

dP/dt<sub>max</sub> and dP/dt<sub>min</sub>, maximal slopes of systolic pressure increment and diastolic pressure decrement, respectively; dV/dt<sub>max</sub> and dV/dt<sub>min</sub>, maximum and minimum rate of volume change; tau<sub>W</sub> and tau<sub>g</sub>, relaxation time constant of left ventricular pressure calculated using the Weiss or Glantz method, respectively. NS, no significant difference.
insulin resistance and CAD in the population. Therefore, in this study we chose a high-cholesterol diet combination with 10% fructose in drinking water to investigate whether diet-induced insulin resistance causes cardiac contractile dysfunctions. This diet is relevant to human nutrition because it mimics a common Western diet with high consumption of sugary drinks. The result shows that feeding with a HCF diet to SD rats resulted in a phenotype of insulin resistance syndrome characterized by an increase in blood pressure, hyperlipidemia, hyperinsulinemia, and insulin resistance. Body weight gain observed in rats fed a HCF diet was slightly lower than in control rats over the study period (Fig. 1 and Table 1). This was due to HCF rats having an increase in water (10% fructose solution) intake along with a reduction in food (high-cholesterol diet) intake during the experimental period. As observed through the calculation of the energy expenditure in the 15th week, the energy intakes did not differ significantly between the two groups (121.8 and 120.43 kcal/rat/day in the control and HCF rats, respectively); thus the animals fed the HCF diet did not develop obesity. Although ~70% of individuals in insulin resistance were overweight/obese, 30% of those were underweight/lean (35). Obesity promotes states of both chronic low-grade inflammation and insulin resistance. However, even in the absence of obesity, infusion of animals with inflammatory cytokines or lipids can cause insulin resistance (42). Elevation of plasma triglycerides and reduction of HDL are frequently observed in patients with insulin resistance and/or diabetes (21). Feeding an HCF diet to SD rats resulted in dramatic increases in plasma cholesterol and triglyceride with slight decreases in HDL (Fig. 1 and Table 1). Since elevation of plasma triglycerides in humans has been associated with consumption of high-carbohydrate diets (20), intake of 10% fructose could have been responsible for increases in plasma triglyceride levels in HCF rats.

Insulin is a potent anabolic hormone and is essential for tissue development, growth, and maintenance of whole body glucose homeostasis. Failure of the target cells to respond to insulin stimulation (such as insulin resistance) is commonly observed under acute stress conditions and in individuals with obesity, metabolic syndrome, or diabetes (41). In the present

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**Fig. 6.** Hemodynamic parameters were measured using a Millar pressure-volume conductance catheter system in rats fed with chow (control) and HCF diet for 15 wk. Cardiac output (A), stroke work (B), maximal power (C), ejection fraction (D), stroke volume (E), maximal volume (F), end-diastolic volume (G), and maximal (dV/dt_max; I) and minimal rate of volume change (dV/dt_min; J) were significantly reduced in the HCF group. On the other hand, the HCF rats showed a significantly increased relaxation time constant of left ventricular pressure (tau_L; J) and arterial elastance (K). Data are means ± SE of 8–9 independent experiments.
HCF-induced insulin resistance in skeletal muscles was associated with a significant decrease in membrane GLUT4 levels (Fig. 3). According to our findings, GLUT1 and GLUT4 levels of adipose tissues were not altered with HCF feeding; this might be due to HCF-induced insulin resistance without development of obesity (Fig. 1 and Table 1). Interestingly, the HCF rats also developed defects in cardiac insulin action associated with blunted Akt-Thr308 phosphorylation in the heart (Fig. 5). The results suggest that HCF was shown to develop insulin resistance not only in metabolic-response tissues (i.e., liver and muscle) but also in the heart. Furthermore, HCF decreased GLUT4 and increased FATP1 levels, which indicated that cardiac glucose uptake was reduced whereas fatty acid uptake might have been elevated. Transgenic over-expression of FATP1 in the heart caused lipotoxic cardiomyopathy, suggesting that increases in fatty acid supply to the heart adversely affect cardiac contractile functions (9).

Recent findings have indicated that the perturbations in cardiac energy metabolism and insulin resistance are among the earliest diabetes-induced events in the myocardium, preceding both functional and pathological changes (36, 40). Furthermore, studies have found myocardial insulin resistance in advance dilated cardiomyopathy limits both glucose uptake and oxidation and impairs the heart’s ability to generate much needed adenosine triphosphate (37). To evaluate cardiac functions, the Millar pressure-volume instrument was used to determine left ventricular contractile functions. The data show that feeding rats a HCF diet resulted in left ventricular con-

Fig. 7. A: representative pressure-volume relations following inferior vena cava occlusions in rats fed with chow (control) and HCF diet for 15 wk. Note that the slopes of end-systolic and end-diastolic pressure-volume relations (ESPVR and EDPVR, respectively) indicate left ventricular contractility and stiffness, respectively. B: the end-systolic elastance was reduced significantly in the HCF groups. Data are means ± SE of 8–9 independent experiments.
tractile dysfunctions (Figs. 6 and 7 and Table 2). Under conditions of changing preload, the ESPVR were significantly decreased in the HCF rats; this caused a dramatic reduction in the SV because EDV was decreased. These findings indicate the important role of cardiac insulin resistance in the pathogenesis of heart contractile dysfunctions in diabetes and/or metabolic syndrome individuals.

Cardiac insulin signal not only regulates metabolic energy homeostasis but also generates signals for cardiac growth, programmed cell death, and programmed cell survival. During insulin resistance or diabetes, the heart rapidly modifies its energy metabolism, resulting in augmented fatty acid and decreased glucose consumption. Accumulating evidence suggests that this alteration of cardiac metabolism plays an important role in the development of cardiomyopathy (29). Our results have demonstrated that cardiomyocytes were dramatically shrunken in HCF hearts. The transverse sections also show ventricular dilation and a decrease in ventricular wall thickness. These results suggest that cardiac insulin resistance may lead to the development of dilated cardiomyopathy. Overall, the results indicate that high-cholesterol food and sugar-sweetened beverages that lead to maladaptive metabolic processes may interfere with the action of insulin and increase susceptibility for the development of cardiomyopathy.

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REFERENCES
Components of metabolic syndrome and coronary artery disease in patients with well-controlled type 2 diabetes mellitus.

Dyson MC, Alloosh M, Vuchetich JP, Mokelke EA, Sturek M.

Initial steps of insulin signaling and glucose transport are defective in the type 2 diabetic rat heart.


