Absence of meal-induced insulin sensitization (AMIS) in aging rats is associated with cardiac dysfunction that is protected by antioxidants

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Ming Z, Legare DJ, Lautt WW. Absence of meal-induced insulin sensitization (AMIS) in aging rats is associated with cardiac dysfunction that is protected by antioxidants. J Appl Physiol 111: 704–714, 2011. First published May 26, 2011; doi:10.1152/japplphysiol.00057.2011.—We have previously demonstrated that progressive development of absence of meal-induced insulin sensitization (AMIS) leads to postprandial hyperglycemia, compensatory hyperinsulinemia, resultant hyperlipidemia, increased oxidative stress, and obesity, progressing to syndrome X in aging rats. The present study tested the hypothesis that progressive development of AMIS in aging rats further resulted in deterioration in cardiac performance. Anesthetized male Sprague-Dawley rats were tested at 9, 26, and 52 wk to determine their dynamic response to insulin and cardiac function. Dynamic insulin sensitivity was determined before and after atropine to quantitate hepatic insulin sensitizing substance (HISS)-dependent and -independent insulin action. Cardiac performance was evaluated using a Millar pressure-volume conductance catheter system. AMIS developed with age, as demonstrated by significant decrease in HISS-dependent insulin action, and this syndrome was increased by sucrose supplementation and inhibited by the antioxidant treatment. Associated with progressive development of AMIS, aging rats showed impaired cardiac performance, including the reduction in cardiac index, heart rate, dP/dtmax, dP/dtmin, ejection fraction and decreased slope of left ventricular end-systolic pressure-volume relationship, and increased relaxation time constant of left ventricular pressure as well as increased left ventricular end-diastolic pressure. Total peripheral vascular resistance also increased with age. Sucrose supplementation and antioxidant treatment, respectively, potentiated and attenuated cardiac dysfunction associated with age. In addition, poor cardiac performance correlated closely with the development of AMIS. These results indicate that AMIS is the first metabolic defect that leads to homeostatic disturbances and dysfunctions, including cardiovascular diseases. Age; HISS-dependent insulin sensitivity; cardiac dysfunction; metabolic syndrome

AGE IS THE MAJOR RISK FACTOR for cardiovascular disease (6, 17, 39). Cardiac function deteriorates with aging and is exacerbated with high carbohydrate consumption (4, 6, 15, 17). Development of insulin resistance, specifically in people with long-term intake of a high-sugar diet, is a key step toward development of cardiovascular disease (12, 14, 39). Insulin resistance triggers sustained greater insulin release, resulting in compensatory hyperinsulinemia (12, 39). A cluster of metabolic dysfunctions arise, including hyperglycemia, dyslipidemia, obesity, and hypertension. All these metabolic dysfunctions are risk factors strongly associated with the development of cardiovascular disease related to aging. Due to the increase in intake of sugar over the past decades, and the increase in the average lifespan, the morbidity and mortality associated with cardiovascular disease have increased substantially in the elderly (12, 14, 39).

The mechanism(s) for the development of insulin resistance associated with aging is not yet fully understood. Among the suggested theories, one unique hypothesis is that aging is associated with absence of meal-induced insulin sensitization (AMIS), resulting from gradual inability of the liver to produce hepatic insulin sensitizing substance (HISS) (for reviews see 21, 22, 25). According to this theory, in the immediate postprandial state, a pulse of insulin will result in the release of a pulse of the putative hormone, HISS, from the liver. HISS action is selective for skeletal muscle and leads to uptake and storage of glucose as glycogen in the large skeletal muscle mass. HISS release in response to insulin occurs only in the presence of two permissive feeding signals after a meal, the first being a hepatic parasympathetic-mediated signal and the second being a 30–50% elevation in hepatic glutathione levels (20, 37, 46). The glucose disposal effect of insulin in the fed state is decreased by ~55% by blocking HISS release (20).

Although HISS has not been chemically identified, its dynamic action and hormonal nature are readily quantified (20, 21, 35). For example, the dynamic response to insulin, determined using a rapidly sampled euglycemic clamp, is doubled in response to refeeding fasted rats. This meal-induced insulin sensitization is reversed back to fasting levels by the use of atropine (39) or surgical denervation of the liver (20). The hormonal nature of HISS is shown by reduction of peripheral (but not hepatic or gut) insulin action following hepatic denervation, and restoration of HISS release by intraportal, but not intravenous, continuous infusion of acetylcholine to mimic the permissive signal (47). The parasympathetic nerves act via acetylcholine and activation of nitric oxide synthase. Denervation-induced blockade of HISS release in fed (but not 24 h fasted) rats can be restored to fed levels by intraportal, but not intravenous, administration of a nitric oxide donor (40). HISS, released from the liver, acts on skeletal muscle to produce both metabolic and vascular effects (27).

The insulin resistance due to HISS insufficiency is referred to as HISS-dependent insulin resistance (HDIR). HDIR occurring after a meal is suggested to account for postprandial hyperglycemia, hyperinsulinemia, hyperlipidemia, and increased oxidative stress (24–26, 28). Absence of HISS release/action following a meal results in the postprandial response to insulin being reduced by ~50% resulting in postprandial hyperglycemia and compensatory increased insulin secretion. Insulin is a lipogenic hormone acting primarily on adipose tissue and liver and results in a shift in nutrient partitioning toward lipids and increased production of triglyceride by the liver, thus account-
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Materials and Methods

Animals were treated according to the guidelines of the Canadian Council on Animal Care. The Protocol Management and Review Committee at the University of Manitoba approved all protocols.

Animals and Groups

Male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) 7 wk old (body wt 200–225 g) were pair housed and maintained under controlled conditions (22 ± 1°C, 12:12-h light/dark cycle). They were fed a standard rat chow diet (caloric intake 60% carbohydrates in corn starch, 25% protein, and 14% lipids) with free access to water for 2 wk to adapt to the housing environment. Then the animals were divided into four groups: group 1 (aging control) fed with normal chow; group 2 (antioxidant treatment) fed with normal chow supplemented with antioxidant cocktail [5-adenosylmethionine (SAME) (0.5 g/kg diet), vitamin C (12.5 g/kg diet), and vitamin E (1.5 g/kg diet)]; group 3 (sucrose) fed with normal chow with drinking water containing 5% sucrose (50 ml/day per rat plus access to regular tap water); and group 4 (sucrose + antioxidant treatment) fed with normal chow supplemented with antioxidant plus drinking 5% sucrose water (50 ml/day per rat plus access to regular tap water). Given the average daily food consumption of 20 g, the approximate daily intake for vitamin C is 250 mg/kg body wt, for vitamin E 30 mg/kg body wt, and for SAME 19 mg/kg body wt. For convenience, the antioxidant cocktail is referred to as SAMEC.

Rats included in these four groups were tested at the ages of 6 and 12 mo (n = 13/group). Young adult rats (n = 14) at the age of 9 wk, fed with standard rat chow, served as the young control group for 6- and 12-mo-old rats.

Body weight gain was monitored once every 2 wk. Food and water intake were monitored for 1-wk periods at prescheduled times throughout the treatment. The animal identification was ensured by microchip implantation.

Surgical Preparation

To establish a consistent postprandial state, all rats underwent an 8-h fast and a refeeding period of 2 h immediately before the start of surgical preparation. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (54.7 mg/kg; CEVA Sante Animal, Libourne, France). Anesthesia was maintained by a continuous infusion of pentobarbital sodium (0.5 mg/ml saline given at 50 μl/min) through a cannula in the jugular vein, supplemented with a 0.54 mg (0.01 ml) bolus injection when required. The rats were placed on a temperature-controlled surgical table (Harvard Apparatus, Kent, UK), and rectal temperature was monitored and held at 37.0–37.5°C. Spontaneous respiration was allowed through a tracheal catheter.

An arterial-venous shunt was established, as previously described (21), for monitoring mean arterial blood pressure (MAP), for derivation of arterial blood samples, and for intravenous drug delivery. Briefly, two catheters (polyethylene tubing PE-60), one inserted into the right femoral artery and the other into the right femoral vein, were connected with silicon tubing. A side branch of the circuit was connected to a pressure transducer for the recording of the shunt pressure, which, when the silicon tubing toward the venous side of the circuit was clamped, measured the systemic arterial blood pressure. Blood samples were taken from the arterial side of the shunt for glucose measurement. Flowing blood within the shunt ensures the real-time measurement of the arterial blood glucose concentration, which is essential for the dynamic euglycemic clamp test as mentioned below. An infusion line was inserted into the venous side of the shunt for intravenous drug delivery. Another infusion line connected to the jugular vein was established for glucose infusion. Animals were heparinized (100 IU/kg) to prevent clotting in the vascular shunt.

Glossary

AMIS Absence of meal-induced insulin sensitization
HISS Hepatic insulin sensitizing substance
HDIA HISS-dependent insulin resistance
RIST Rapid insulin sensitivity test
Ped Left ventricular end-diastolic pressure
Pes Left ventricular end-systolic pressure
EDV End-diastolic volume
EF Ejection fraction
SV Stroke volume
SW Stroke work
SWI Stroke work index
CI Cardiac index
CO Cardiac output
dP/dt max Left ventricular pressure upstroke
dP/dt min Left ventricular pressure fall
TPRI Total peripheral resistance index
tau Time constant of left ventricular pressure decay
PRSW Preload recruitable stroke work
ESPVR End-systolic pressure-volume relationship
EDPVR End-diastolic pressure-volume relationship
Emax Maximum elastance
Hemodynamic and Left Ventricle Pressure-Volume Measurement

For the assessment of hemodynamics and left ventricle (LV) pressure-volume (P-V) relationship, a micropipette conductance pressure-volume (P-V) catheter (size 1.9 F, Scisense, London, ON, Canada) was introduced into the right carotid artery and further advanced into the LV. The position of the catheter was carefully adjusted until stable P-V loops were obtained. The catheter was connected to an EMKA signal processor, and all data were acquired digitally and analyzed at a sample rate of 1,000 Hz using IOX data acquisition/analysis system (EMKA Technologies, Falls Church, VA). The abdomen was opened, and the inferior vena cava between liver and diaphragm was identified for the purpose of calibrating the cardiac output measured by the conductance catheter.

The following parameters were recorded and analyzed: heart rate (HR), MAP, maximal left ventricular systolic pressure (Pes), left ventricular end-diastolic pressure (Pd), the maximal rates of LV pressure upstroke and fall (dP/dtmax and dP/dtmin, respectively), time constant of left ventricular pressure decay (tau), ejection fraction (EF), stroke volume (SV), cardiac output (CO), and stroke work (SW). Cardiac output was normalized to body weight (cardiac index; CI). Stroke work was also normalized to body weight (SWI). Total peripheral resistance index (TPRI) was calculated by the equation: TPRI = MAP/CI.

In addition, left ventricular P-V relations were evaluated from P-V loops recorded during transient occlusion of the inferior vena cava by external compression of the vessel. Preload recruitable stroke work (PRSW), end-systolic P-V relationship (ESPVR), end-diastolic P-V relationship (EDPVR), dP/dtmax-end-diastolic volume relationship (dP/dtmax-EDV), and maximum elastance (Emax) were calculated using IOX software.

Calibration of the conductance catheter. The volume signal of the conductance catheter was calibrated for parallel conductance and cardiac output, according to IOX recommendation. Unlike traditional indexes such as ejection fraction (EF) and dP/dtmax, these additional parameters, derived from the P-V relationship, are more specific and more direct indicators of ventricular performance, independent of cardiac loading conditions and HR (16, 31). Briefly, 20 μl of 10% prewarmed saline was injected intravenously, and, from the shift of P-V relations, parallel conductance volume was calculated by the IOX software and used for correction of the cardiac mass volume. At the end of experiments, an ultrasonic perivascular V type flow probe (size 3 mm) was placed around the arch of the thoracic aorta to measure cardiac output (T206, Transonic Systems, NY, USA), for the purpose of calibrating the cardiac output measured by the conductance catheter.

The heart was harvested. Left and right ventricle were separated and weighed.

Chemicals

Human insulin was purchased from Novo Nordisk (Bagsvaerd, Denmark). Atropine, vitamin C (l-ascorbic acid), and vitamin E [±-α-tocopherol] were all purchased from Sigma. S-adenosylmethionine was purchased from New Foods (Bloomingdale, IL). Insulin and atropine were dissolved in saline. The antioxidants were incorporated into regular rat chow by Research Diets (New Brunswick, NJ). Plasma insulin concentration was assayed by ELISA (ALPCO, Windham, NH).

Statistical Analysis

Values are presented as means ± SE. The data were analyzed by paired or unpaired t-test where appropriate. A one-way ANOVA followed by Tukey’s test was employed when the multiple means from different groups were compared. Statistical significance was taken at P < 0.05. Linear regression was used.

RESULTS

Changes in Body Weight, Heart Weight, and General Circulation

Body weight, heart weight, and the parameters for general circulation are summarized in Table 1. Body weight increased, as expected, in aging rats, and this tendency was potentiated by the sucrose diet. Antioxidant treatment prevented the sucrose effect.

Compared with young rats, both right and left ventricle weights increased in the 26-wk-old rats but did not further increase in the 52-wk-old rats. However, the percentage weight gain of the left ventricle was less than that of the body weight; thus left ventricle-to-body weight ratio was reduced in aging rats. The gain in heart weight was also enhanced by the sucrose diet, and this enhancement was prevented by antioxidant treatment.

The arterial pressure increased slightly and the CI decreased with aging, resulting in a significant increase in the total peripheral resistance index (Table 2). The sucrose diet showed a tendency to potentiate, and antioxidant treatment effectively prevented the increment in the vascular resistance (as summarized later in Fig. 4).

Aging and Insulin Sensitivity: Effects of the Sucrose Diet and Antioxidant Treatment

Similar to our previous studies (24, 28), aging was associated with the progression of insulin resistance. Compared with the young rats, the RIST index in the control fed state decreased by ~40% in 6-mo-old rats and further by ~54% in 12-mo-old rats, respectively (Fig. 1, top panel). In rats with the
Table 1. Body and heart weight in 9-, 26-, and 52-wk old rats: effects of taking sucrose and antioxidants

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>n</th>
<th>BW, g</th>
<th>LVW, mg</th>
<th>RVV, mg</th>
<th>LVW/BW, mg/g</th>
<th>RVV/BW, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Control</td>
<td>14</td>
<td>358 ± 9*</td>
<td>721 ± 22*</td>
<td>206 ± 7*</td>
<td>2.02 ± 0.04*</td>
<td>0.58 ± 0.01*</td>
</tr>
<tr>
<td>26 wk-old-rats (C)</td>
<td>13</td>
<td>649 ± 16</td>
<td>1,068 ± 25</td>
<td>303 ± 13</td>
<td>1.65 ± 0.02</td>
<td>0.46 ± 0.01</td>
</tr>
<tr>
<td>26 wk-old-rats (A)</td>
<td>13</td>
<td>664 ± 18</td>
<td>1,044 ± 23</td>
<td>300 ± 9</td>
<td>1.55 ± 0.02</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>26 wk-old-rats (S)</td>
<td>13</td>
<td>674 ± 15</td>
<td>1,035 ± 31</td>
<td>309 ± 9</td>
<td>1.59 ± 0.02</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>26 wk-old-rats (T)</td>
<td>13</td>
<td>638 ± 23</td>
<td>999 ± 33</td>
<td>289 ± 9</td>
<td>1.57 ± 0.02</td>
<td>0.46 ± 0.01</td>
</tr>
<tr>
<td>52 wk-old-rats (C)</td>
<td>13</td>
<td>782 ± 20†</td>
<td>1,123 ± 20</td>
<td>293 ± 11</td>
<td>1.44 ± 0.03†</td>
<td>0.38 ± 0.01†</td>
</tr>
<tr>
<td>52 wk-old-rats (A)</td>
<td>13</td>
<td>767 ± 23†</td>
<td>1,146 ± 32</td>
<td>294 ± 8</td>
<td>1.50 ± 0.02</td>
<td>0.39 ± 0.01†</td>
</tr>
<tr>
<td>52 wk-old-rats (S)</td>
<td>13</td>
<td>864 ± 25†</td>
<td>1,250 ± 37†</td>
<td>311 ± 10</td>
<td>1.45 ± 0.04†</td>
<td>0.37 ± 0.01†</td>
</tr>
<tr>
<td>52 wk-old-rats (T)</td>
<td>13</td>
<td>800 ± 17†</td>
<td>1,198 ± 29†</td>
<td>314 ± 7</td>
<td>1.50 ± 0.03</td>
<td>0.39 ± 0.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE. C, control diet; A, diet with antioxidants; S, control diet + 5% sucrose water; T, 5% sucrose water + diet with antioxidants; BW, body weight; LVW or RVW, left or right ventricular weight, respectively. *P < 0.05 vs. all other groups; †P < 0.05 vs. 26-wk-old rats with same treatment; ‡P < 0.05 vs. same-age control.

Table 2. Hemodynamic indexes in 9-, 26-, and 52-wk-old rats on normal diet

<table>
<thead>
<tr>
<th></th>
<th>9 wk</th>
<th>26 wk</th>
<th>52 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>109.8 ± 3.1</td>
<td>114.1 ± 3.5</td>
<td>120.5 ± 3.5*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>405 ± 9</td>
<td>369 ± 11</td>
<td>341 ± 7*</td>
</tr>
<tr>
<td>Pes, mmHg</td>
<td>122 ± 3.1</td>
<td>126 ± 3.9</td>
<td>132 ± 4.9</td>
</tr>
<tr>
<td>Ped, mmHg</td>
<td>0.5 ± 1.0</td>
<td>5.9 ± 0.6*</td>
<td>8.0 ± 1.5*</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>9,903 ± 219</td>
<td>9,422 ± 267</td>
<td>7,778 ± 198†</td>
</tr>
<tr>
<td>dP/dtmin, mmHg/s</td>
<td>−8,640 ± 342</td>
<td>−8,125 ± 213</td>
<td>−6,970 ± 159†</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>7.5 ± 0.2</td>
<td>8.4 ± 0.3*</td>
<td>9.5 ± 0.2†</td>
</tr>
<tr>
<td>EDV, μl</td>
<td>225 ± 14</td>
<td>379 ± 16*</td>
<td>397 ± 19*</td>
</tr>
<tr>
<td>SV, μl</td>
<td>143 ± 8</td>
<td>215 ± 14*</td>
<td>204 ± 11*</td>
</tr>
<tr>
<td>EF, %</td>
<td>62.0 ± 1.6</td>
<td>57.3 ± 1.6*</td>
<td>52.8 ± 1.3*</td>
</tr>
<tr>
<td>SWI, mmHg·μl·100 g⁻¹</td>
<td>4,873 ± 342</td>
<td>4,201 ± 267</td>
<td>3,153 ± 168*</td>
</tr>
<tr>
<td>CI, ml·min⁻¹·100 g⁻¹</td>
<td>16.2 ± 1.0</td>
<td>11.2 ± 0.7*</td>
<td>9.1 ± 0.4*</td>
</tr>
<tr>
<td>TPR, mmHg·ml⁻¹·min⁻¹·100 g</td>
<td>7.1 ± 0.4</td>
<td>10.3 ± 0.7*</td>
<td>13.5 ± 0.9†</td>
</tr>
<tr>
<td>ESPVR, mmHg/μl</td>
<td>0.85 ± 0.05</td>
<td>0.71 ± 0.04</td>
<td>0.54 ± 0.03*</td>
</tr>
<tr>
<td>EDPRVR, mmHg/μl</td>
<td>0.03 ± 0.001</td>
<td>0.027 ± 0.004</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>dP/dtmax-EDV, mmHg·μl⁻¹</td>
<td>36.9 ± 3.0</td>
<td>29.5 ± 4.6</td>
<td>19.4 ± 2.1</td>
</tr>
<tr>
<td>PRSW, mmHg</td>
<td>87.4 ± 7.2</td>
<td>80.6 ± 6.1</td>
<td>67.9 ± 4.4</td>
</tr>
<tr>
<td>Emax, mmHg/μl</td>
<td>2.64 ± 0.23</td>
<td>2.16 ± 0.17</td>
<td>1.44 ± 0.12†</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; Pes and Ped, end-systolic and end-diastolic pressure, respectively; dP/dtmax and dP/dtmin, maximal slope of the systolic pressure increment and diastolic pressure decrement; tau, time constant of left ventricular (LV) pressure decay; EDV, end-diastolic volume; SV, stroke volume; EF, ejection fraction; SWI, stroke work index; CI, cardiac output index; TPR, total peripheral resistance index; ESPVR and EDPRVR, end-systolic and end-diastolic pressure-volume relationship, respectively; dP/dtmax-EDV, +dP/dt and EDV relationship; PRSW, preload recruitable stroke work and EDV relationship; Emax, maximum chamber elasticity. *P < 0.05 vs. 9-wk-old rats; †P < 0.05 vs. 26-wk-old rats.

Aging with Amis Results in Cardiac Dysfunction

Table 2 presents the data obtained from the P-V conductance catheter showing the baseline cardiac function in animal groups at various ages. Compared with the 9-wk-old rats, both cardiac systolic and diastolic function showed a tendency to be decreased in 26-wk-old rats. At the age of 52 wk, decreased cardiac function reached the level of statistical significance. Among the indexes reflecting the cardiac systolic function, the dP/dtmax decreased by 22% and the EF decreased by 15%. For the indexes reflecting cardiac diastolic function, dP/dtmin decreased by 19%, tau increased by 26%, with a significant increase in Ped. The CI decreased significantly at 26 wk and demonstrated a further significant decrease at 52 wk. HR also decreased continuously. Aged rats also showed a slight tendency of increased end systolic pressure.

Ventricular P-V relations. Typical trace recordings of left ventricular P-V relationships recorded during transient inferior vena cava occlusion from young and 52-wk-old rats are presented in Fig. 2. Compared with young control rats, the slope of ESPVR was less steep (Fig. 2) and overall value of ESPVR (Fig. 3) was lower in 52-wk-old rats, suggesting the development of deterioration in cardiac systolic performance. Other load-independent indexes reflecting cardiac systolic performance, such as dP/dtmax-EDV and PRSW, were also decreased in 52-wk-old rats (Fig. 3). In contrast, EDPRVR, an index reflecting ventricle end-diastolic stiffness, did not show significant difference between young and 52-wk-old rats, although a significant increase in Ped was observed in aging rats (Table 2).

Effect of antioxidants. There was a trend observed from 26-wk-old rats showing beneficial effects of antioxidants in improving cardiac performance (data not shown), and the beneficial effects became statistically significant in 52-wk-old rats, as summarized in Figs. 3 and 4. In rats treated with antioxidants, the beneficial effects in improving cardiac performance included higher dP/dtmax, dP/dtmin, EF%, dP/dtmax-EDV, and major but significant decrease in the RIST index was also observed in aging rats with or without the sucrose diet. Treatment with antioxidants partially prevented this tendency. The data suggested that the decreases in insulin sensitivity associated with aging and the sucrose diet are mainly due to the decreases in HISS action (Fig. 1, bottom panel).

Changes associated with aging: load-dependent indexes. Among the indexes reflecting the cardiac systolic function, the dP/dtmax decreased by 22% and the EF decreased by 15%. For the indexes reflecting cardiac diastolic function, dP/dtmin decreased by 19%, tau increased by 26%, with a significant increase in Ped. The CI decreased significantly at 26 wk and demonstrated a further significant decrease at 52 wk. HR also decreased continuously. Aged rats also showed a slight tendency of increased end systolic pressure.
Insulin action.

Insulin sensitivity are mainly dependent on HISS action and not direct post-atropine consists of only the HISS-independent, or direct insulin action. Control rapid insulin sensitivity test (RIST) consists of hepatic insulin sensitizing substance (HISS)-dependent and HISS-independent insulin action. RIST post-atropine consists of only the HISS-independent, or direct insulin action. The results showed HISS-dependent insulin action was decreased with age, further decreased by sucrose feeding and was protected by antioxidants; n = 13–14 for each group. Young: 9-wk-old rats; 26 or 52C, A, S and T represent 26-wk-old or 52-wk-old rats with control diet (C), antioxidant diet (A), sucrose diet (S), and sucrose + antioxidant diet (T). 

\( \text{PRSW} \) compared with their same-age partners (Figs. 3 and 4). Treatment with antioxidants showed a strong trend in increasing ESPVR and \( E_{\text{max}} \), and in decreasing tau, although statistical significance was not reached for these parameters. The percentage protection provided by the antioxidants for impaired cardiac performance associated with aging is presented in Fig. 5A. The antioxidants provided higher protection for indexes \( \text{EF\%}, \text{PRSW} \), and \( \text{dP/dt}_{\text{max}} \) or \( \text{dP/dt}_{\text{min}} \), and less protection for the changes in CI and SWI associated with aging.

Sucrose Diet on Cardiac Function in Aging Rats and Effects of Antioxidant Treatment

Figure 2 shows the typical trace recordings of left ventricle P-V loops obtained from 52-wk-old rats fed with/without sucrose. The 26-wk-old rats fed with sucrose showed a strong tendency for having a lower cardiac performance (data not shown). At the age of 52 wk, compared with their normal-diet partners, rats fed with sucrose showed more deteriorated cardiac function, as demonstrated by lower \( \text{dP/dt}_{\text{max}} \) \( (P = 0.054) \), \( \text{dP/dt}_{\text{min}} \) \( (P < 0.05) \), and \( \text{EF\%} \) \( (P < 0.05) \), and a tendency of higher tau \( (P = 0.10) \) (Figs. 3 and 4). Evaluation of left ventricle P-V relationships also revealed that rats fed with sucrose had a more flat ESPVR (Fig. 2) and lower values of \( \text{dP/dt}_{\text{max}}-\text{EDV} \) and PRSW, as well as a strong trend of lower ESPVR \( (P = 0.08) \) and \( E_{\text{max}} \) \( (P = 0.09) \) (Figs. 3 and 4). Sucrose feeding also tended to cause further increase in \( \text{Ped} \) but had no significant influence on EDPVR, as shown in Fig. 4.

Effect of antioxidants. Treatment with antioxidants effectively improved cardiac performance in rats fed sucrose, as indicated by increased \( \text{dP/dt}_{\text{max}} \), \( \text{dP/dt}_{\text{min}} \), \( \text{EF\%} \), and SWI and by decreased \( \text{Ped} \) and tau (Figs. 3 and 4). Moreover, the depressed ESPVR, \( E_{\text{max}} \), \( \text{dP/dt}_{\text{max}}-\text{EDV} \), and PRSW associated with sucrose feeding were prevented by the treatment of antioxidants (Fig. 3). Interestingly, the improvement of cardiac performance in sucrose rats receiving antioxidants reached levels similar to their same-age partners not given sucrose. The percentage protection provided by antioxidants for impaired cardiac performance associated with aging is presented in Fig. 5B.

Correlations of Insulin Sensitivity, Blood Glucose, Plasma Insulin, and Cardiac Function

As shown in Table 3, section 1, which pooled the data from all rats fed with normal diet at different ages, decreased cardiac function and increased TPRI were significantly correlated with decreased HISS-dependent insulin action (HDIA) that developed with aging. This tendency was potentiated by sucrose feeding (Table 3, section 1). Provision of antioxidants reduced the regression coefficients (Table 3, sections 2 and 4).

Aging associated deterioration in cardiac function and peripheral circulation (increased peripheral resistance) were also correlated significantly with increased fasting blood glucose and fasting plasma insulin concentrations (Table 3). Sucrose feeding increased and antioxidant treatment decreased the magnitude of the relationships. The concentration of postprandial insulin only showed a weak relationship and postprandial glucose showed no relationship with the cardiac function. The metabolic parameters for these series (adiposity, regional fat masses, HISS-dependent and -independent insulin action, fed and fasted glucose, and lipid profile) were previously reported (28).

DISCUSSION

We have previously demonstrated that in aging Sprague-Dawley rats, with the development of AMIS resulting from lack of postprandial HISS action, postprandial glucose disposal
shifts from storage in skeletal muscle as glycogen to fat in fatty tissue (24, 28). This metabolic shift leads to postprandial hyperglycemia, compensatory hyperinsulinemia, resultant hyperlipidemia, increased oxidative stress, and increasing obesity. In the present study, we have tested the hypothesis that the metabolic dysfunction following AMIS will negatively and progressively affect cardiac performance. The major observations from the present study include: 1) with the development of HDIR in aging rats, the arterial pressure is increased and CI is decreased, leading to significant elevation in total peripheral vascular resistance, suggesting impairment in peripheral vasculature; 2) baseline systolic and diastolic cardiac performance progressively declined with aging; 3) the P-V relationship, during transient reduction in cardiac end systolic/diastolic volume, reveals a significant decline in cardiac systolic performance while diastolic performance remained relatively normal; 4) the deteriorated cardiac performance is closely associated with the development of HDIR, and the acceleration (sucrose diet) or attenuation (antioxidant diet) of HDIR in aging rats potentiates or attenuates the deterioration in cardiac performance.

Aging-Associated Deterioration in Cardiac Performance

Aging-associated functional deterioration in cardiac performance has been mainly studied recently using P-V conductance and Doppler echocardiography techniques in rats and other murine species (1, 2, 5, 7, 8, 30, 32, 36, 44, 45). Previous studies using the P-V conductance catheter have revealed parallel deteriorations in both cardiac systolic and diastolic function with age. The major manifestations include the progressive declines in load-dependent parameters, ±dP/dt, EF%, HR, Pes, SV, and CO, associated with the elevation in Ped, tau, and total peripheral vascular resistance. Analysis of left ventricular P-V relationships further reveals the decline in the more sensitive load-independent parameters, ESPVR, Emax, dP/dt-EDV, and PRSW, representing impairment in intrinsic cardiac contractile function. In the later stages of aging, rats also showed a significant increase in EDPVR, suggesting myocardial fibrosis and structural stiffness at the end-diastolic period. Studies using echocardiography, on the other hand, detect more early and prominent decline in diastolic function in the murine heart with age, whereas systolic function only declines slightly (7). It has been suggested that the pathophysiological characteristics in the murine aging heart are similar to what occur in the elderly human (7).

The present longitudinal study compared cardiac performance in rats during the lifespan of 9, 26, and 52 wk. In agreement with previous studies, our data showed that both systolic and diastolic cardiac performance declined gradually with aging and became statistically significant at the age of 52 wk. Evaluated from load-dependent indexes, the decreases in EF%, dP/dt, and HR were approximately 14, 22, 19, and 16%, respectively. Tau increased by 27%. In addition, load-independent indexes reflecting cardiac intrinsic contractile function, including ESPVR, Emax, dP/dt-EDV, and PRSW, also decreased by approximately 36, 45, 47, and 22%, respectively. However, although we observed a significant elevation in end-diastolic pressure which suggested an end-diastolic myocardial stiffness, there was no significant change in EDPVR in 52-wk-old rats compared with the young control.
group. Bal et al. (1) observed a similar phenomenon in a group of 80-wk-old rats. This suggests that the change in Ped may be a more sensitive index in reflecting left ventricular end-diastolic stiffness in rats.

Effects of Sucrose Supplementation

In a previous study, we fed rats a chronic sucrose supplement to potentiate, and an antioxidant cocktail, SAMEC (combination of vitamin C, vitamin E, and S-adenosylmethionine), to attenuate the impairment of meal-induced insulin sensitization to determine if the degree of HISS action correlated with the metabolic dysfunction in aging rats (28). Our data showed that postprandial hyperglycemia, hyperinsulinemia, hyperlipidemia, and adiposity associated with aging were enhanced by the sucrose supplement and were attenuated by antioxidants. Absence of HISS action correlated with the metabolic dysfunctions. The same approach has been used in the present study to evaluate the correlation of impaired cardiac performance with the degree of AMIS development. Our results showed, for the first time, that a sucrose supplement and an antioxidant cocktail, SAMEC, respectively, enhanced and attenuated aging-associated deterioration in cardiac performance.

Increase in the intake of sugar has been well demonstrated to positively associate with metabolic and cardiovascular diseases (8, 10, 11, 42, 43). Sharma et al. (42, 43) reported that hypertensive rats fed with high-sugar diets (61% fructose, 9% starch, 10% fat) for 8 and 13 wk decreased cardiac performance, associated with increased cardiomyocyte apoptosis and mortality. In a rat model fed a 15-wk high-sugar/-cholesterol diet, Deng et al. (8) observed that the rats developed cardiac insulin resistance, associated with marked cardiac dysfunction, including the reduction in cardiac output, EF%, ESPVR, and increased left ventricular relaxation time constant. In agreement with these studies, our study showed that aging rats fed with a low-dose sucrose supplement (5% sucrose, 50 ml allowed per day per rat) developed more severe decrease in cardiac performance. Compared with the same-age rats, rats fed with sucrose showed significantly lower dP/dt_{min}, EF%, and dP/dr-EDV (all P < 0.05) and a strong tendency to lower dP/dr_{max} (P = 0.054), tau (P = 0.09), ESPVR (P = 0.08), Emax (P = 0.09), and PRSW (P = 0.055). The CI and total peripheral resistance also showed a strong tendency to decrease and increase, respectively.

The enhancement of sucrose on aging-associated cardiac dysfunction may relate to early and severe development of HDIR. Ribeiro et al. (37) reported that a 9 wk sucrose diet (35% in unlimited water) resulted in severe HDIR and accumulation of fat. In the present study, aging rats fed with sucrose developed more severe HDIR and correlation was shown...
between the RIST index and cardiac dysfunction (Table 3). This suggests that aging and a high sucrose diet may cause poor cardiac performance through a common mechanism, the impairment of meal-induced insulin sensitization.

**Effect of SAMEC**

Aging and a high sucrose diet increase oxidative stress. We concocted a unique synergistic antioxidant cocktail, abbreviated as SAMEC, consisting of S-adenosylmethionine plus vitamin E and vitamin C (24, 26, 28), to simultaneously protect the aqueous and lipid components of the cell and the mitochondrial function and glutathione levels. The cocktail was developed as a tool to protect against the severe acute free radical hepatotoxicity generated by thioacetamide. The cocktail turned out to show dramatic synergism, working only if all three components were used. However, the specific molecular mechanisms by which age, sucrose, and SAMEC affect HISS release is unknown, and the components of SAMEC each play
numerous roles unrelated to their antioxidant capacity. In the present study, SAMEC provided a protective effect on HISS action which not only prevented the sucrose-induced HDIR but allowed it to remain near levels seen in the young control rats. SAMEC led to significantly higher levels of HISS action which not only prevented the sucrose-induced HDIR but also provided a protective effect on HISS action, blood glucose, plasma insulin, and heart function in rats.

Relevance to Humans

Although these studies were carried out in rats, meal-induced insulin sensitization and the issue of high-sugar diets and the possibility of antioxidant prophylaxis during the aging process has relevance to humans. Meal-induced insulin sensitization has been demonstrated in young lean male human subjects (34, 35) and the parasympathetic control of meal-induced insulin sensitization has also been demonstrated (34, 35). The dynamic curves of insulin and HISS action are qualitatively comparable in humans, cats, rats, and mice.

The known effects of age, sucrose supplement, and SAMEC on HDIR afforded means of manipulating HISS action pos-
tively and negatively over a one-year period and demonstrated that the predicted dysfunctions are dependent on the degree of HDIR (impaired HISS action). The dysfunctions include the full array of clusters of complications associated with obesity, cardiovascular dysfunction, syndrome X, and type 2 diabetes. These dysfunctions represent a mechanistic-based progression of predictable consequences of AMIS and thus might be considered an AMIS syndrome.

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