A high-fat diet increases risk of ventricular arrhythmia in female rats: enhanced arrhythmic risk in the absence of obesity or hyperlipidemia

Marie-Claude Aubin,2,6 Sophie Cardin,5,6 Philippe Comtois,1,6 Robert Clément,6 Hugues Gosselin,6 Marc-Antoine Gillis,6 Khaï Le Quang,2,6 Stanley Nattel,3,5,6 Louis P. Perrault,2,4,6 and Angelino Calderone1,2,6

Departments of 1Physiology, 2Pharmacology, 3Medicine, and 4Surgery, Université de Montréal, Montreal; 5Department of Pharmacology, McGill University, Montreal; and the 6Research Center, Montreal Heart Institute, Montreal, Quebec, Canada

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A high-fat diet increases risk of ventricular arrhythmia in female rats; enhanced arrhythmic risk in the absence of obesity or hyperlipidemia. J Appl Physiol 108: 933–940, 2010. First published February 4, 2010; doi:10.1152/japplphysiol.02812.2009.—Obesity increases the incidence of cardiac arrhythmias and impairs wound healing. However, it is presently unknown whether a high-fat diet affects arrhythmic risk or wound healing before the onset of overt obesity or hyperlipidemia. After 8 wk of feeding a high-fat diet to adult female rats, a nonsignificant increase in body weight was observed and associated with a normal plasma lipid profile. Following ischemia/reperfusion injury, scar length (standard diet 0.29 ± 0.09 vs. high-fat 0.32 ± 0.13 cm), thickness (standard diet 0.047 ± 0.02 vs. high-fat 0.059 ± 0.01 cm), and collagen α1 type 1 content (standard diet 0.21 ± 0.04 vs. high-fat 0.20 ± 0.04 arbitrary units/mm2) of infarcted hearts were not altered by the high-fat diet. However, the mortality rate was greatly increased 24 h postinfarction (from 5% to 46%, P < 0.01 for ischemia/reperfusion rats; from 20% to 89%, P < 0.0001, in complete-occlusion rats) in high-fat fed rats, in association with a higher prevalence of ventricular arrhythmias. Ventricular arrhythmia inducibility was also significantly increased in noninfarcted rats fed a high-fat diet. In the hearts of rats fed a high-fat diet, connexin-40 expression was absent, connexin-43 was hypophosphorylated and lateralized, and neurofilament-M immunoreactive fiber density (standard diet 2,020 ± 260 vs. high-fat diet 2,830 ± 250 µm2/mm2) and tyrosine hydroxylase protein expression were increased (P < 0.05). Thus, in the absence of overt obesity and hyperlipidemia, sympathetic hyperinnervation and an aberrant pattern of gap junctional protein expression and regulation in the heart of female rats fed a high-fat diet may have contributed in part to the higher incidence of inducible cardiac arrhythmias.

Address for reprint requests and other correspondence: A. Calderone, Montreal Heart Institute, 5000 Belanger St., Montreal, Quebec H1T 1C8, Canada (e-mail: angelo.calderone@umontreal.ca).

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Methods

High-Fat Diet

Female Sprague-Dawley rats (180–200 g; Charles River, St-Constant, QC, Canada), were housed on arrival and had access to food and tap water ad libitum. The environment was controlled in terms of light (12:12-h light-dark cycle starting at 6:00 AM), humidity, and room temperature (20–23°C). One week after their arrival, rats were randomly assigned to a standard or high-fat diet for a period of 8 wk. The 18% high-fat diet (no sucrose) consisted of 42.9% lipids (corn oil, 34 g/kg; lard, 146 g/kg), 38.6% carbohydrates, and 18.5% proteins (kcal) (Agribrands Purina Canada, Woodstock, ON, Canada). Aortic reactivity to acetylcholine was determined in a subpopulation of rats in each group. As previously demonstrated (4), the vasorelaxant action of acetylcholine on aortic rings isolated from rats fed a high-fat diet was significantly reduced (data not shown). Additional female rats fed a standard (295 ± 10 g; n = 7) or high-fat (331 ± 17 g; n = 7) diet were used exclusively for immunofluorescence experiments, and left ventricular function and mean arterial pressure are depicted in Supplemental Table 1 (available with the online version of this article). All experiments were performed in compliance with recommendations and guidelines on the care and use of laboratory animals issued by the Canadian Council on Animal Research, and the contractile function (29). Potential arrhythmogenic mechanisms linked to obesity include a prolonged QTc interval and increased sympathetic activity (3, 29, 33).

Despite the unequivocal relationship between obesity and cardiovascular disease, it remains presently unknown whether inadequate scar healing following a cardiac ischemic insult and/or increased arrhythmic risks are prevalent before the establishment of overt obesity and hyperlipidemia. Previous work from our lab has demonstrated that feeding a high-fat diet to normal female rats for a period of 8 wk increased mean arterial pressure in the absence of obesity, hyperlipidemia, and hyperglycemia (4). By contrast, the administration of a comparable high-fat diet in a similar time frame to normal male rats led to a significant increase in body weight, plasma glucose, and cholesterol levels (13, 16). Thus the advantage of our model is that it provides the unique opportunity to examine the influence of a high-fat diet on scar healing and arrhythmogenesis in the absence of obesity and associated complications. Therefore, the present study tested the hypothesis that hearts of female rats fed a high-fat diet for 8 wk are associated with an arrhythmogenic phenotype and/or an impaired reparative fibrotic response following an ischemic insult.
Sirius red staining and scar remodeling by planimetry. rats because the heart was used to assess scar collagen content by injury. Ventricular weights were not obtained from these additional standard or high-fat diet group subjected to ischemia/reperfusion both body and ventricular weights were obtained. Table 2 includes the planimetry. Table 1 depicts the number of rats in each group for which described (4). The plasma lipid profile was determined in blood model SPR-407; Millar Instrument, Houston, TX) as previously high-fat-fed rats subjected to the sham operation. One week later, extrasystoles. Arrhythmic events were not detected in standard or high-fat diet, a subpopulation of rats from each group was subjected to ischemia/reperfusion injury were anesthetized under complete coronary artery ligation. Following 8 wk of feeding a standard or high-fat diet, a subpopulation of rats from each group was arbitrarily selected and subjected to either a sham operation or complete coronary artery ligation. Briefly, rats were sedated with a mixture of ketamine (50 mg/kg; Rogarsetic, Toronto, ON, Canada) and xylazine (10 mg/kg; Rompun, Cambridge, ON, Canada), and the left anterior descending coronary artery was permanently occluded. In the sham-operated group, rats were subjected to an identical surgical procedure, but the artery was not ligated. Last, neither heart weight nor contractile function was determined in the permanent coronary artery occlusion study.

Ischemia/reperfusion injury. Rats fed either a standard or high-fat diet were arbitrarily selected, anesthetized, and subjected to either a sham operation or complete coronary artery ligation. Following 45 min of occlusion, the ligature was removed. Sham-operated rats underwent an identical surgical procedure, but the coronary artery was not ligated. During the occlusion period of rats subjected to either a sham procedure or ischemia/reperfusion injury, surface ECG (conventional DII position) measurements were recorded. An arrhythmic event was defined by the appearance of >5 successive ventricular extrasystoles. Arrhythmic events were not detected in standard or high-fat-fed rats subjected to the sham operation. One week later, hemodynamic measurements were made with a Millar catheter (2 F; model SPR-407; Millar Instrument, Houston, TX) as previously described (4). The plasma lipid profile was determined in blood samples taken from the carotid artery before the animal was euthanized, as previously described (4). Thereafter, the heart was removed and used for protein and mRNA expression, immunofluorescence, or planimetry. Table 1 depicts the number of rats in each group for which both body and ventricular weights were obtained. Table 2 includes the hemodynamic status of rats from Table 1 and additional rats from the standard or high-fat diet group subjected to ischemia/reperfusion injury. Ventricular weights were not obtained from these additional rats because the heart was used to assess scar collagen content by Sirius red staining and scar remodelling by planimetry.

In Vivo Electrophysiological Measurements

High-fat diet (n = 11) and standard diet (n = 9) rats not submitted to ischemia/reperfusion injury were anesthetized under 3% isoflurane, intubated, and ventilated. An octopolar catheter (1.9 F, 0.5-mm electrode spacing, Scisense) was inserted into the left ventricle for programmed stimulation (2× threshold voltage, 2-ms square pulse duration timed with a homemade stimulator triggering a Grass Telefactor model SD9K). Surface ECG (lead 1), intracardiac electrogram, and stimulation impulse trace were band pass-filtered and recorded with IOX software (EMKA). Ventricular effective refractory period (VERP) were evaluated (10-stimuli drive train S1 followed by a premature stimulus S2). Ventricular tachycardia was induced by rapid pacing (20 stimuli per cycle length) using a decremental pacing program (from 100 ms to 30 ms by 5-ms steps) repeated twice per cycle. Rapid rhythm (>500 beats/min) consisting of >10 extrasystoles with varying electrocardiogram morphology and activation times were considered as ventricular tachycardia.

Planimetry and Collagen Content

The heart was excised, immersed in 10% formalin, and cut halfway between the base and apex. The apex and base sections of the heart were fixed, dehydrated, and embedded in paraffin. Serial cryostat sections (6 µm) of ventricular tissue were prepared. Sirius red staining was used to measure collagen α₁ type 1 content (arbitrary units normalized to surface area; mm²) in the left ventricle and infarct region, and hematoxylin-phloxine-safran staining was employed to assess scar remodelling by planimetry, as previously described (4, 18).

Real-Time PCR

Real-time PCR was performed by standard methodology on total RNA isolated from the left ventricle as previously described (15). In all experiments, the left ventricle was divided in two and a sample used for real-time PCR and Western blot analysis. Real-time PCR was performed according to the manufacturer’s instructions employing the molecule SYBR Green (Applied Biosystems). Primers for each gene were obtained from distinct exons that span an intron employing the program Ensembl Genome Browser (www.ensembl.org). The sequence specificity of each primer was verified with the program Blast derived from the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov). The primers used are as follows: rat atrial natriuretic peptide (ANP), forward 5’-AGACGGGACTAAGGCG-CAACA-3’ and reverse 5’-ATTTGCTGTATCTCCGGTA-3’; rat nerve growth factor-β (NGF-β), forward 5’-CAGCTTCTACCTG-GGCCACTC-3’ and reverse 5’-GAGTCTCCCTCTGGACATT-3’; and rat β-actin, forward 5’-CCCTAAGGGCAACCGTGTA-3’.

In Vivo Electrophysiological Measurements

High-fat diet (n = 11) and standard diet (n = 9) rats not submitted to ischemia/reperfusion injury were anesthetized under 3% isoflurane, intubated, and ventilated. An octopolar catheter (1.9 F, 0.5-mm electrode spacing, Scisense) was inserted into the left ventricle for programmed stimulation (2× threshold voltage, 2-ms square pulse duration timed with a homemade stimulator triggering a Grass Telefactor model SD9K). Surface ECG (lead 1), intracardiac electrogram, and stimulation impulse trace were band pass-filtered and recorded with IOX software (EMKA). Ventricular effective refractory period (VERP) were evaluated (10-stimuli drive train S1 followed by a premature stimulus S2). Ventricular tachycardia was induced by rapid pacing (20 stimuli per cycle length) using a decremental pacing program (from 100 ms to 30 ms by 5-ms steps) repeated twice per cycle. Rapid rhythm (>500 beats/min) consisting of >10 extrasystoles with varying electrocardiogram morphology and activation times were considered as ventricular tachycardia.

Table 1. Body and heart weights of female rats fed a standard or high-fat diet and subjected to I/R injury

<table>
<thead>
<tr>
<th></th>
<th>Standard Diet (n = 6)</th>
<th>High-Fat Diet (n = 6)</th>
<th>Standard + I/R (n = 11)</th>
<th>High-Fat Diet + I/R (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>303 ± 9</td>
<td>339 ± 20</td>
<td>334 ± 11</td>
<td>338 ± 10</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>0.40 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.46 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>RV weight, g</td>
<td>0.14 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>LV/body weight ratio, × 10³</td>
<td>1.34 ± 0.04</td>
<td>1.36 ± 0.06</td>
<td>1.37 ± 0.04</td>
<td>1.46 ± 0.03</td>
</tr>
<tr>
<td>RV/body weight ratio, × 10³</td>
<td>0.45 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.46 ± 0.02</td>
<td>0.54 ± 0.03</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = no. of rats per group. I/R, ischemia/reperfusion; LV, left ventricle; RV, right ventricle. Data are presented as means ± SE; n = no. of rats per group. Two-way ANOVA was used to assess statistical difference.

Body and heart weights of female rats fed a standard or high-fat diet and subjected to I/R injury

<table>
<thead>
<tr>
<th></th>
<th>Standard Diet (n = 6)</th>
<th>High-Fat Diet (n = 6)</th>
<th>Standard + I/R (n = 19)</th>
<th>High-Fat Diet + I/R (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>136 ± 8</td>
<td>180 ± 5*</td>
<td>111 ± 5*</td>
<td>115 ± 5*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>97 ± 4</td>
<td>112 ± 2*</td>
<td>80 ± 3*</td>
<td>84 ± 2*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>110 ± 6</td>
<td>135 ± 3*</td>
<td>90 ± 4*</td>
<td>94 ± 3*</td>
</tr>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>121 ± 5</td>
<td>157 ± 8*</td>
<td>110 ± 5</td>
<td>110 ± 5*</td>
</tr>
<tr>
<td>LV +dP/dt, mmHg/s</td>
<td>6.042 ± 107</td>
<td>7.224 ± 310*</td>
<td>5.361 ± 165</td>
<td>5.496 ± 136†</td>
</tr>
<tr>
<td>LV −dP/dt, mmHg/s</td>
<td>−5.078 ± 305</td>
<td>−6.302 ± 337*</td>
<td>−4.204 ± 177*</td>
<td>−4.367 ± 130†</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = no. of rats per group. Statistical difference was determined by 2-way ANOVA. LV +dP/dt, rate of contraction; LV −dP/dt, rate of relaxation. *P < 0.05 vs. standard diet. †P < 0.05 vs. high-fat diet.
and reverse 5′-GAGGCATACGGGACACACAG-3′. Appropriate negative controls were used for each experiment. Target mRNAs were normalized to β-actin mRNA.

**Immunofluorescence**

The heart was excised, immersed directly in 2-methyl butane (−80°C), and stored at −80°C. Immunofluorescence on cardiac tissue (cryostat sections of 14-μm thickness) was performed as previously described (15). Antibodies used include the rabbit polyclonal anti-connexin 43 (1:100; Chemicon); mouse monoclonal anti-connexin-43 (1:500; Chemicon); and the rabbit polyclonal anti-neurofilament-M (1:500; Chemicon). Secondary antibodies were used as a goat anti-mouse IgG conjugated to Alexa-488 (1:500; Invitrogen; emission wavelength, 560 nm) and a goat anti-rabbit IgG conjugated to Alexa-568 (1:500; Invitrogen; emission wavelength, 560 nm). Nonspecific staining was determined following the addition of an isotype control antibody or the conjugated secondary antibody alone. The nucleus was identified with To-Pro 3 (Invitrogen; 1.5 μM; emission wavelength, 661 nm). Immunofluorescence was visualized with a 10×- or 63×-oil 1.4 NA DIC plan apochromat objective mounted on a Zeiss Axiosvert 100 M confocal microscope. To determine sympathetic fiber density, a transverse section from the heart of each rat was used, and the density (μm²) of neurofilament-M-immunoreactive fibers in the left ventricle was determined in 8–10 fields (μm²; each field represents an area of 0.7–0.84 μm²) using the program LSM 5 Image Browser (Zeiss).

**Western Blot**

The left ventricle was lysed in 10 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 1% Triton X-100, 0.5% nonidet P-40, and 1 μg/ml of leupeptin and aprotinin. The homogenate was centrifuged for 10 min, and the supernatant was frozen and stored at −80°C. Protein content was determined with the Bio-Rad assay. The heart was excised, immersed directly in 2-methyl butane (−80°C), and stored at −80°C. Immunofluorescence on cardiac tissue (cryostat sections of 14-μm thickness) was performed as previously described (15). Antibodies used include the rabbit polyclonal anti-connexin 43 (1:100; Chemicon); mouse monoclonal anti-connexin-43 (1:500; Chemicon); and the rabbit polyclonal anti-neurofilament-M (1:500; Chemicon). Secondary antibodies were used as a goat anti-mouse IgG conjugated to Alexa-488 (1:500; Invitrogen; emission wavelength, 560 nm) and a goat anti-rabbit IgG conjugated to Alexa-568 (1:500; Invitrogen; emission wavelength, 560 nm). Nonspecific staining was determined following the addition of an isotype control antibody or the conjugated secondary antibody alone. The nucleus was identified with To-Pro 3 (Invitrogen; 1.5 μM; emission wavelength, 661 nm). Immunofluorescence was visualized with a 10×- or 63×-oil 1.4 NA DIC plan apochromat objective mounted on a Zeiss Axiosvert 100 M confocal microscope. To determine sympathetic fiber density, a transverse section from the heart of each rat was used, and the density (μm²) of neurofilament-M-immunoreactive fibers in the left ventricle was determined in 8–10 fields (μm²; each field represents an area of 0.7–0.84 μm²) using the program LSM 5 Image Browser (Zeiss).

**Statistical Analysis**

All values are expressed as means ± SE. Changes in body weight, cardiac morphology, hemodynamics, gene expression, and collagen content in rats fed a standard or high-fat diet subjected to ischemia/reperfusion injury were assessed by a two-way ANOVA, and significant difference (P value < 0.05) was determined by the Neuman-Keuls post hoc test (STATISTICA; StatSoft, Tulsa, OK). A Student’s unpaired t-test was performed to assess scar remodeling, protein expression, the density of neurofilament-M-immunoreactive fibers, and the number of inducible ventricular extrasystoles, and a P value < 0.05 was considered statistically significant. A chi-square test (SAS Version 9.1; SAS Institute, Cary, NC) was performed to compare the number of arrhythmic events and death of rats fed a high-fat or standard diet subjected to an ischemic insult, and a P value < 0.05 was considered statistically significant.

**RESULTS**

**Body and Heart Weights, Hemodynamics, and Plasma Lipid Profile of Female Rats Fed a Standard or High-Fat Diet**

A modest nonsignificant increase in body weight was observed in female rats fed a high-fat diet for a period of 8 wk compared with rats fed a standard diet (Table 1). Left and right ventricle-to-body weight ratios were similar in rats fed either a standard or high-fat diet (Table 1). Systolic and diastolic blood pressures, mean arterial pressure (MAP), left ventricular systolic pressure, and dP/dt indexes were significantly (P < 0.05) increased in female rats fed a high-fat diet (Table 2). Last, the feeding of a high-fat diet to normal female rats for 8 wk did not increase plasma cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), or triglyceride levels (Table 3).

### Table 3. Lipid profile of female rats fed a standard or high-fat diet and subjected to I/R injury

<table>
<thead>
<tr>
<th></th>
<th>Standard Diet (n = 4)</th>
<th>High-Fat Diet (n = 5)</th>
<th>Standard Diet + I/R (n = 4)</th>
<th>High-Fat Diet + I/R (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/l</td>
<td>1.59 ± 0.14</td>
<td>1.47 ± 0.09</td>
<td>1.53 ± 0.09</td>
<td>1.64 ± 0.14</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>0.50 ± 0.05</td>
<td>0.41 ± 0.04</td>
<td>0.52 ± 0.05</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>0.54 ± 0.09</td>
<td>0.50 ± 0.08</td>
<td>0.55 ± 0.09</td>
<td>0.78 ± 0.18</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.20 ± 0.23</td>
<td>1.03 ± 0.25</td>
<td>1.04 ± 0.22</td>
<td>0.99 ± 0.18</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = no. of rats per group. Statistical difference was assessed by 2-way ANOVA.

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fusion injury was associated with a significantly higher mortality rate \( (P < 0.01 \text{ vs. standard diet}) \) 24 h postinjury (high-fat, 13 of 28 rats died; 46% mortality rate) compared with rats fed a standard diet (1 of 21 rats died; 5% mortality rate). Furthermore, within the high-fat fed group, ventricular fibrillation occurred in 12 rats during the coronary artery occlusion and 6 of these rats died immediately afterward. Ventricular fibrillation was also observed in 3 rats fed a standard diet during coronary artery occlusion and all survived thereafter.

**Ventricular Arrhythmia Inducibility in Rats Fed a High-fat Diet**

Effective refractory periods were similar in right ventricles of rats fed a standard and high-fat diet (see Supplemental Table 2, available with the online version of this article). The number of ventricular tachycardias (e.g., appearance of a minimum of 10 successive extrasystoles) induced in rats fed a high-fat diet were significantly greater than rats fed a standard diet (standard diet 0.6 ± 0.3 vs. high-fat diet 3.4 ± 1.2; \( P < 0.05 \); \( n = 9–11 \) rats per group). In the atria of rats fed a standard or high-fat diet, the effective refractory periods were equivalent, and the number of atrial extrasystoles following extrastimulation was similar (data not shown).

**Cardiac and Scar Remodeling Following Ischemia/Reperfusion Injury**

Although a higher incidence of cardiac arrhythmias may have contributed to the low survival rate in rats fed a high-fat diet subjected to ischemia/reperfusion injury, it is possible that greater infarct expansion and subsequent pump failure may represent an additional underlying event. Following ischemia/reperfusion injury to the heart of rats fed a standard diet, infarct size was predominantly small and associated with a modest significant decrease of systolic/diastolic blood pressures and left ventricular relaxation (Tables 2 and 3). Left ventricle-to-body weight ratio in the ischemically damaged heart of rats fed a standard diet was similar to the noninfarcted heart, suggesting the absence of significant cardiac remodeling (Table 1). Indeed, the steady-state mRNA levels of ANP (sham operated 1.2 ± 0.1 vs. ischemia/reperfusion 1.9 ± 0.4; \( n = 3–5 \) for each group) and ventricular collagen \( \alpha_1 \) type 1 protein content (sham operated 0.015 ± 0.005 vs. ischemia/reperfusion 0.0310 ± 0.01; \( n = 5–6 \) for each group) were not significantly different in sham-operated and ischemia/reperfusion rats fed a standard diet. Following ischemia/reperfusion injury, MAP and left ventricular contractility were significantly decreased in rats fed a high-fat diet (Table 2), albeit infarct size, thickness, and scar collagen were similar to those in the infarcted heart of rats fed a standard diet (Table 4). In the noninfarcted left ventricle of rats fed a high-fat diet, the steady-state mRNA levels of ANP (sham operated 1.0 ± 0.1 vs. ischemia/reperfusion 2.1 ± 0.5; \( n = 3–5 \) for each group) and ventricular collagen \( \alpha_1 \) type 1 protein content (sham operated 0.011 ± 0.001 vs. ischemia/reperfusion, 0.020 ± 0.011; \( n = 5 \) for each group) were not significantly increased following ischemia/reperfusion injury. Last, ischemia/reperfusion injury did not alter the plasma lipid profile of rats fed a standard diet or high-fat diet (Table 3).

**Sympathetic Fiber Hyperinnervation and Gap Junctional Protein Expression/Distribution in the Heart of Rats Fed a High-fat Diet**

In the left ventricle of rats fed a high-fat diet for 8 wk, sympathetic hyperinnervation was apparent, reflected by the elevated protein expression of tyrosine hydroxylase and an increased density of neurofilament-M immunoreactive fibers (standard diet 2,020 ± 260 vs. high-fat diet 2,830 ± 250 \( \mu \)m\(^2\)/mm\(^2\); \( n = 6–7 \) for each group; \( P < 0.05 \text{ vs. standard} \)) (Figs. 1 and 2). Despite sympathetic hyperinnervation, the steady state mRNA levels of NGF-\( \beta \) in the left ventricle of rats fed a high-fat diet (0.73 ± 0.06; \( n = 6 \)) was similar to rats fed a standard diet (0.84 ± 0.05; \( n = 6 \)).

Altered expression and/or distribution of gap junctional proteins may also be involved in the genesis of cardiac arrhythmias. Connexin-40 protein expression in the left ventricle was undetectable by a Western blot approach. However, immunofluorescence revealed modest connexin-40 immunoreactivity at intercalated discs of ventricular myocytes whereas the signal was absent in the heart of rats fed a high-fat diet (\( n = 4 \)) (Fig. 2). A Western blot approach revealed that connexin-43 protein content was significantly increased in the left ventricle of rats fed a high-fat diet (Fig. 1). However, phosphorylation of the Ser\(^{168} \) residue of connexin-43 was markedly reduced in the hearts of rats fed a high-fat diet (Fig. 1). Consistent with dephosphorylation, connexin-43 immunoreactivity was detected laterally along the plasma membrane of ventricular myocytes in rats fed a high-fat diet (Fig. 2).

**DISCUSSION**

Work from our lab has demonstrated that female rats fed a high-fat diet for a period of 8 wk have a modest increase in mean arterial pressure in the absence of overt obesity, hyperlipidemia, and hyperglycemia (4). The present study has further revealed that the hearts of rats fed a high-fat diet show an arrhythmic phenotype, characterized by an increased incidence of inducible ventricular tachyarrhythmias and spontaneous ventricular tachyarrhythmias with cardiac ischemia. Possible underlying mechanisms include sympathetic hyperinnervation and an aberrant pattern of gap junctional protein expression and distribution. By contrast, the hearts of rats fed a high-fat diet were not predisposed to excessive scar expansion and/or inadequate healing following ischemia/reperfusion injury. Nonetheless, these hearts were sensitized to arrhythmic effects of an ischemic insult and showed a higher infarct-related mortality rate.

Previous studies have demonstrated that ischemia/reperfusion injury to the heart of animals with overt obesity leads to

### Table 4. Scar planimetry and collagen content of female rats fed a standard or high-fat diet subjected to I/R injury

<table>
<thead>
<tr>
<th></th>
<th>Scar Thickness, cm</th>
<th>Scar Length, cm</th>
<th>Collagen, arbitrary units/mm(^2)</th>
<th>Infarct Size, % of total area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet + I/R</td>
<td>0.047 ± 0.017</td>
<td>0.288 ± 0.094</td>
<td>0.21 ± 0.04</td>
<td>9.00 ± 4.49</td>
</tr>
<tr>
<td>High-fat diet + I/R</td>
<td>0.059 ± 0.008</td>
<td>0.320 ± 0.134</td>
<td>0.20 ± 0.04</td>
<td>11.8 ± 4.23</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; \( n \) = no. of rats per group. Statistical difference was assessed by Student’s unpaired \( t \)-test.
scar expansion and impaired healing (14, 34). Furthermore, inadequate scar healing contributes to left ventricular dilatation and subsequent adverse cardiac remodeling (11, 34). An ischemic insult was superimposed on the hearts of female rats fed a high-fat diet to determine whether the reparative fibrotic response was compromised. Permanent coronary artery ligation of the heart of rats fed a high-fat diet led to an 89% mortality rate within 24 h. Previous studies have reported that cardiac arrhythmias lead to sudden cardiac death postischemic injury (2, 9, 32). Unfortunately, cardiac rhythm was not monitored in rats fed a high-fat diet subjected to permanent coronary artery occlusion. Therefore, a second series of experiments was performed with ischemia/reperfusion injury to better correlate the possible relationship between cardiac arrhythmias and death. During the coronary artery occlusion phase, a greater number of rats fed a high-fat diet exhibited arrhythmic events, the onset of spontaneous ventricular tachyarrhythmias was earlier, and these rats showed a higher mortality rate 24 h post-ischemia/reperfusion injury. Furthermore, ventricular fibrillation was evident just before death in high-fat fed rats that died during the coronary artery occlusion phase. Ventricular tachyarrhythmias could also be induced in the nonischemic heart of rats fed a high fat diet and the frequency was significantly increased compared with rats fed a standard diet. Collectively, these data confirm the observations first reported by McLennan’s group that the feeding of a saturated animal fatty acid diet for 9 –18 mo to male rats increased the incidence of ventricular extrasystoles post-ischemia/reperfusion injury and associated with a higher mortality rate secondary to ventricular fibrillation (25, 27). The novelty of the present study is that an arrhythmogenic phenotype can be imposed on the heart of female rats after only 8 wk of feeding a high-fat diet in the absence of obesity, hyperlipidemia, and hyperglycemia. Heart failure secondary to an inadequate reparative fibrotic response could potentially contribute to the increased incidence of death following ischemia/reperfusion injury to rats fed a high-fat diet (11, 34). One week post-ischemia/reperfusion injury, scar length, thickness, and collagen type 1 content were similar in rats fed a standard or high-fat diet. These data unequivocally demonstrate that in the

Fig. 1. Tyrosine hydroxylase and connexin-43 protein expression and phosphorylation. A: tyrosine hydroxylase (TH) and connexin-43 (CX43) protein content were elevated in the heart of female rats fed a high-fat diet. Ser368 phosphorylation of connexin-43 was reduced in the heart of rats fed a high-fat diet. GAPDH protein levels were similar in the heart of rats fed a standard or high-fat diet. Semi-quantitative assessment of tyrosine hydroxylase (B), connexin-43 (C), and phosphorylation of the serine368 residue of connexin-43 (D) in the left ventricle of rats fed a standard or high-fat diet. Data were normalized to GAPDH or connexin-43. *P < 0.05, **P < 0.01 vs. rats fed a standard diet.
absence of overt obesity, hyperlipidemia, and hyperglycemia, a high-fat diet does not predispose the heart to excessive scar expansion and/or inadequate scar healing. In addition, infarct size was relatively small in both groups, consistent with previous studies demonstrating that the female rat heart is more resistant to ischemic damage (6, 21). Last, the underlying presence of a hypertensive state in rats fed a high-fat diet did not lead to greater infarct expansion or inadequate scar healing (20, 22, 26).

Myocardial fibrosis is an important potential contributor to cardiac arrhythmias (5, 8). Collagen α1 type 1 protein content in the left ventricle of rats fed a high-fat diet was not signifi-

Fig. 2. Sympathetic innervation and gap junctional protein expression and distribution. A: sympathetic innervation of the left ventricle of a normal female rat heart was visualized by the presence of neurofilament-M immunoreactive fibers. B: in the heart of rats fed a high-fat diet, neurofilament-M fiber density was significantly increased (see RESULTS). These data were corroborated by the increased protein expression of tyrosine hydroxylase (see Fig. 1). Modest connexin-40 immunoreactivity was detected at the intercalated discs of ventricular cardiac myocytes in the normal female rat heart (C), whereas staining was absent in the heart of rats fed a high-fat diet (D). E: robust connexin-43 immunoreactivity was detected at the intercalated discs of normal rat ventricular myocytes. F: in the heart of female rats fed a high-fat diet, connexin-43 staining was observed at the intercalated discs and lateralized along the plasma membrane of numerous ventricular myocytes (indicated by arrow). To-Pro3 staining (blue fluorescence) was used to label the nucleus.
cantly increased compared with rats fed a standard diet. Thus the increased incidence of spontaneous and inducible arrhythmic events in the ischemic and nonischemic heart of rats fed a high-fat diet was not secondary to a prevailing reactive fibrotic response. Several lines of evidence in both patients and animal models support the premise that obesity-induced hypertension leads to increased sympathetic nerve activity (3, 23, 33). In the ischemically damaged heart, sympathetic fiber sprouting was reported and identified as a seminal trigger of cardiac arrhythmias and sudden death (9, 10, 15, 35, 37, 38). Collectively, these observations provided the impetus to test the hypothesis that the greater incidence of inducible cardiac arrhythmias in female rats fed a high-fat diet was associated at least in part with sympathetic hyperinnervation. In the left ventricle of rats fed a high-fat diet for 8 wk, neurofilament-M immunoreactive fiber density and tyrosine hydroxylase protein content were significantly increased. In the ischemically damaged heart, synthesis of the neurotrophin NGF-β and its subsequent uptake and retrograde transport by the left stellate ganglion was identified as a seminal event implicated in sympathetic fiber sprouting (19, 38). In the present study, left ventricular steady state mRNA levels of NGF-β were comparable in female rats fed a high-fat and standard diet. Nonetheless, adipose tissue is an important source of numerous peptides acting on nerve tissue, including NGF-β, and levels of the neurotrophin are increased in obese patients (7, 17). Plasma levels of NGF-β were not measured in the present study, but a high-fat diet may have induced the synthesis of the neurotrophin from an extracardiac source and contributed to the sympathetic hyperinnervation of the heart. Hypercholesterolemia alone promotes cardiac sympathetic hyperinnervation and increased vulnerability to ventricular fibrillation (24). However, sympathetic hyperinnervation of the heart of rats fed a high-fat diet for 8 wk cannot be attributed to hypercholesterolemia, as plasma cholesterol levels were normal.

Gap junctional proteins located at the intercalated disc of cardiac myocytes play a seminal role in action potential propagation and a decreased expression, hypophosphorylation and/or redistribution contributes to the generation of an arrhythmogenic substrate (2, 30, 32). Previous studies have demonstrated that connexin-43 represents the major gap junctional protein isoform expressed in the ventricles, whereas connexin-40 expression is either modest or undetectable (32). Consist with this premise, a Western blot approach was unable to detect an appreciable level of connexin-40 protein in the left ventricle whereas connexin-43 expression was robust. However, a modest connexin-40 immunoreactive signal was detected exclusively at the intercalated discs of ventricular myocytes in the normal rat heart. In the heart of rats fed a high-fat diet, the connexin-40 immunoreactive signal was absent. Although the latter finding demonstrates that ventricular connexin-40 expression was sensitive to a high-fat diet, the biological impact of this modest population on cardiac arrhythmogenicity in the rat heart is questionable (32). By contrast, in the heart of rats fed a high-fat diet, connexin-43 protein expression was increased whereas Ser368 phosphorylation of the gap junctional protein was significantly reduced. The increased protein expression of connexin-43 in the heart of rats fed a high-fat diet may be directly attributed to sympathetic hyperinnervation as both in vitro and in vivo studies have reported a stimulatory effect of α- and β-adrenergic receptor agonists (31, 36). The basis for the hypophosphorylated state of connexin-43 in the heart of rats fed a high-fat diet is unknown but may likewise be related in part to sympathetic hyperinnervation. The serine phosphatase PP2A reported to dephosphorylate connexin-43 is often associated with protein kinase A/A anchoring proteins and recruited via a cAMP-dependent pathway. (1, 12) It has been suggested that the concomitant recruitment and activation of PP2A plays a role in limiting the biological action of protein kinase A (12). In addition, an aberrant pattern of connexin-43 distribution on ventricular myocytes was also observed in the heart of rats fed a high-fat diet with lateralization of the gap junction protein. Collectively, these findings were consistent with previous studies demonstrating that the higher risk of cardiac arrhythmias was associated with a hypophosphorylated state and lateralization of connexin-43 (1, 2). Thus sympathetic hyperinnervation of the heart and the concomitant altered expression, reduced phosphorylation state, and aberrant distribution of gap junction proteins represent potential trigger and substrate contributors, respectively, for the generation of cardiac arrhythmias in female rats fed a high-fat diet.

In the absence of overt obesity, hyperlipidemia, and hyperglycemia, the hearts of female rats fed a high-fat diet were associated with an arrhythmogenic phenotype. Sympathetic hyperinnervation of the heart and an aberrant pattern of expression, reduced phosphorylation, and altered distribution of gap junctional proteins may represent underlying mechanisms. By contrast, a high-fat diet did not compromise the reparative fibrotic response of the ischemically damaged female rat heart. However, their arrhythmic phenotype predisposed these rats to a higher mortality rate following an ischemic insult. Thus, based on the findings of the present study, it is tempting to speculate that a high-fat diet (e.g., Western diet) may predispose individuals to a greater risk of cardiac arrhythmias in the absence of overt obesity.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

HIGH-FAT DIET AND CARDIAC ARRHYTHMIA


