Female Rats Fed a High-Fat Diet Were Associated with Vascular Dysfunction and Cardiac Fibrosis in the Absence of Overt Obesity and Hyperlipidemia: Therapeutic Potential of Resveratrol

Marie-Claude Aubin, Claude Lajoie, Robert Clément, Hugues Gosselin, Angelino Calderone, and Louis P. Perrault

Department of Pharmacology, Université de Montréal, Montreal, Quebec, Canada (M.-C.A., A.C., L.P.P.); Department of Science of Physical Activity, Université du Québec à Trois-Rivières, Trois-Rivières, Quebec, Canada (C.L.); Research Center of the Montreal Heart Institute, Montreal, Quebec, Canada (M.-C.A., R.C., H.G., A.C., L.P.P.); Department of Physiology, Université de Montréal, Montreal, Quebec, Canada (A.C.); and Department of Surgery, Université de Montréal, Montreal, Quebec, Canada (L.P.P.)

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ABSTRACT

It remains presently unknown whether vascular reactivity is impaired and whether maladaptive cardiac remodeling occurs before the onset of overt obesity and in the absence of hyperlipidemia. Normal female rats were fed a high-fat diet for 8 weeks and were associated with a modest nonsignificant increase of body weight (standard diet, 300 ± 10 g; vs high-fat diet, 329 ± 14 g) and a normal plasma lipid profile. In rats fed a high-fat diet, systolic (171 ± 7 mm Hg) and diastolic blood pressures (109 ± 3) were increased compared to a standard diet (systolic blood pressure, 134 ± 8; diastolic blood pressure, 96 ± 5 mm Hg), and acetylcholine-dependent relaxation of isolated aortic rings (high-fat diet, 22 ± 5%, versus standard diet, 53 ± 8%) was significantly reduced. Furthermore, perivascular fibrosis was detected in the heart of rats fed a high-fat diet. The exogenous addition of resveratrol (trans-3,5,4′-trihydroxystilbene) (0.1 μM) to aortic rings isolated from rats fed a high-fat diet restored acetylcholine-mediated relaxation (47 ± 9%). The administration of resveratrol (20 mg/kg/day for 8 weeks) to rats fed a high-fat diet prevented the increase in blood pressure and preserved acetylcholine-dependent relaxation of isolated aortic rings. However, resveratrol therapy failed to attenuate the perivascular fibrotic response. These data have demonstrated that a high-fat diet fed to normal female rats can elicit a hypertensive response and induce perivascular fibrosis before the development of overt obesity and in the absence of hyperlipidemia. Resveratrol therapy can prevent the hypertensive response in female rats fed a high-fat diet but is without effect on the progression of perivascular fibrosis.

Obesity represents a growing health problem in industrialized countries contributing to the onset and/or development of various metabolic-associated disease states, including dyslipidemia and type 2 diabetes. Furthermore, in patients diagnosed with metabolic disorders, hypertension and coronary artery disease are prevalent and the subsequent risk of myocardial infarction is increased (Grundy et al., 2006; Kenchaiah et al., 2002; Klein et al., 2004). In the experimental setting, animals provided with a high-fat diet and associated with overt obesity were likewise documented with elevated blood pressure (Dobrian et al., 2000, 2001; Erdei et al., 2006). The study by Dobrian et al. (2000) demonstrated that the increase in systolic blood pressure in hypercholesterolemia obese rats that were fed a high-fat diet may be related to the 2-fold increase of plasma renin. Furthermore, increased oxidative stress was also highlighted in obese patients and in experimental animal models of obesity and delineated as a seminal event contributing to the development of hypertension (Dobrian et al., 2000; Roberts et al., 2001; Erdei et al., 2006). This work was supported by the Heart and Stroke Foundation of Canada and Quebec, Canadian Institutes of Health Research, and “Fonds de la Recherche de l’Institut de Cardiologie de Montréal” (FRICM). M.-C.A. is a Ph.D. student funded by the Heart and Stroke Foundation of Canada. L.P.P. is a Chercheur-Boursier Senior of the “Fonds de la Recherche en Santé du Québec” (FRSQ). A.C. is a Chercheur-Boursier National of the FRSQ. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. doi:10.1124/jpet.107.135061.

ABBREVIATIONS: LV, left ventricle; ANP, atrial natriuretic peptide; TGF, transforming growth factor; PCR, polymerase chain reaction; SERCA2, sarcoplasmic reticulum Ca²⁺-ATPase 2; ANOVA, analysis of variance.
2006; Vachharajani and Vital, 2006; Van Gaal et al., 2006; Yamato et al., 2007). The underlying mechanism attributed to the hypertensive action of oxidative stresske is secondary to a reduction of biologically active NO via the interaction with superoxide anions (Dobrian et al., 2001; Erdei et al., 2006).

Despite the unequivocal relationship between obesity and cardiovascular disease, it remains presently unknown whether vascular reactivity is impaired and whether mal-adaptive remodeling of the heart occurs before an overt gain in body weight or a change in the plasma lipid profile. In this regard, the present study tested the hypothesis that an impairment of endothelial-mediated relaxation and a reactive fibrotic response in the heart are prevalent in normal adult female rats fed a high-fat diet before the manifestation of overt obesity and in the absence of hyperlipidemia. Furthermore, as previously discussed, elevated oxidative stress represents a putative feature of obesity and is implicated in both vascular dysfunction and cardiac fibrosis (Dobrian et al., 2001; Erdei et al., 2006; Van Gaal et al., 2006; Lu et al., 2008). Thus, a second series of experiments tested the hypothesis that the coadministration of the natural antioxidant resveratrol (Baur et al., 2006; Das and Maulik, 2006) would counteract the deleterious effect of a high-fat diet on endothelial-mediated relaxation and reactive fibrosis.

**Materials and Methods**

**Animal Care.** Female Sprague-Dawley strain 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). All experiments were performed in compliance with recommendations of the Guidelines on the Care and Use of Laboratory Animals issued by the Canadian Council on Animal Research and the Guidelines of the Animal Care and were approved by the Animal Care Committee of the Montreal Heart Institute.

**Diet and Treatment Protocol.** One week after their arrival, rats were randomly assigned to either a standard or high-fat diet for a period of 8 weeks. The high-fat diet consisted of 42% lipids, 36% carbohydrates, and 22% proteins (kcal) and was provided in small pellets (MP Biomedicals, Irvine, CA). The standard rat diet consisted of 12.5% lipids, 63.2% carbohydrates, and 24.3% proteins (kcal) (Agribrands Purina Canada, Woodstock, ON). To assess the therapeutic benefit of resveratrol, 20 mg/kg/day resveratrol was added to achieve a contraction that was 100% of the maximal contraction to phenylephrine (Baur et al., 2006; Das and Maulik, 2006) would counteract the deleterious effect of a high-fat diet on endothelial-mediated relaxation and reactive fibrosis.

**Vascular Reactivity.** After sacrifice, the aorta was harvested and placed in a modified Krebs bicarbonate solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 0.026 mM EDTA, and 11.1 mM dextrose). The aorta was subsequently dissected free of adherent fat and connective tissue and divided into rings (~4 mm in length). The aortic rings were suspended between two metal stirrups connected to an isometric force transducer in a chamber containing Krebs bicarbonate solution maintained at 37°C and oxygenated with a mixture of 95% O2/5% CO2. After 30 min of stabilization, tension was progressively increased to the optimal tension of its active length-tension curve (approximately 3.5 g), as determined by the contractile response to potassium chloride (KCl; 30 mM) at increasing levels of stretch. Maximal contraction was determined with KCl (60 mM), and aortic rings were excluded if the contractile response to potassium chloride was not observed (exclusion rate of less than 5%). After extensive washing, aortic rings were incubated in the presence of indomethacin (10−5 M) and propranolol (10−6 M). After a 45-min period of stabilization, phenylephrine (2 × 10−9 to 10−6 M) was added to achieve a contraction that was a 100% of the maximal contraction observed with KCl (60 mM). Subsequent endothelial-dependent relaxation of isolated aortic rings was assessed with the addition of acetylcholine (10−10 to 10−5 M), and the vascular response was expressed as a percentage of the maximal contraction to phenylephrine. In a separate series of experiments, resveratrol (0.1 μM) was added to aortic rings isolated from both rats fed a high-fat diet for 10 min before the addition of acetylcholine. Lastly, endothelial-independent relaxation was assessed via the addition of sodium nitroprusside, and the vascular response was expressed as a percentage of the maximal contraction to phenylephrine.

**Isolation of Total RNA, Reverse Transcription-PCR, and Real-Time PCR.** Total myocardial RNA was isolated by a modification of the guanidine thiocyanate-phenol-chloroform extraction method, as described previously (Calderone et al., 1995). The reverse transcriptase reaction contained 5 ng/μl total RNA (each sample), Moloney murine leukemia virus reverse transcriptase (800 U), RNaseOUT (40 U), random-hexamer primers (0.04 U), and dNTPs (0.5 mM) and supplied optimal buffers. The reaction protocol con-
gene were obtained from distinct exons that spanned an intron by using the Ensembl Genome Browser program (http://www.ensembl.org). The sequence specificity of each primer was verified with the Blast program derived from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The primers used are as follows: rat atrial natriuretic peptide (ANP), forward, 5'-AGAGCG-GACTAGGCTGCAACA-3', and reverse, 5'-ATTTGGCTGTTATCTTCCGTA-3'; rat sereplasmic reticulum calcium ATPase (SERCA2), forward, 5'-TGATAGCAGGACAGAAGAAG-3', and reverse, 5'-TGATGGCAGGAGACACATTCACTG-3'; rat transforming growth factor-β1 (TGF-β1), forward, 5'-GCTGCTAATTGGTGACCCGCAACA-3', and reverse, 5'-AGCTTGCACCGGAGACCAAT-3'; rat transforming growth factor-β3 (TGF-β3), forward, 5'-AGAAGATCCATAAATTGACAT-3', and reverse, 5'-ACAGATGAAACGGAAAACCT-3'; rat connective tissue growth factor, forward, 5'-AGGCCCTGTGAGC-TGACCTAGA-3', and reverse, 5'-TTTGTAGCCGGTCGATGCACT-3'; and rat β-actin, forward, 5'-CCCTAAGCCACCCGCTGAA-3', and reverse, 5'-GAGGCTACAGGGACACACAG-3'. Appropriate negative controls were used for each experiment.

**Drugs.** All reagents were prepared daily in Ultrapure distilled water with the exception of indomethacin, and resveratrol was prepared in ethanol and dimethyl sulfoxide, respectively. Acetylcholine, bradykinin, indomethacin, and sodium nitroprusside were obtained from Sigma Chemical Co. (Mississauga, ON, Canada). Phenylephrine was obtained from Cayman Chemical Co. (Ann Arbor, MI), dobutamine came from Sandoz Canada Inc. (Boucherville, QC, Canada), and propranolol was obtained from Biomol Research Laboratories Inc. (Plymouth Meeting, PA). For real-time PCR, Moloney murine leukemia virus reverse transcriptase and RNaseOUT were obtained from Invitrogen (Burlington, ON, Canada), random-hexamer primers came from Amersham Biosciences (Baie-d'Urfe, QC, Canada), dNTPs came from MBI Fermentas (Burlington, ON, Canada), and SYBR Green PCR master mix was from Applied Biosystems (Foster, CA). Resveratrol was obtained from Royalmount Pharma (Montreal, QC, Canada).

**Statistical Analysis.** All values are expressed as the mean ± S.E.M. The half-maximal effective concentration (EC_{50}) of acetylcholine-mediated relaxation of isolated aortic rings was measured from the individual dose-response curve using a logistic curve-fitting program (Allfit for Windows 2.12; Dr. De Léan, Université de Montréal, QC, Canada). The pD_{2} value, the negative log of the EC_{50}, was likewise obtained with the program Allfit. A one-way analysis of variance was performed to assess differences in collagen content, vessel lumen area, and wall thickness, and a significant difference was determined by the Bonferroni’s post hoc test and a p value less than 0.05 was considered statistically significant (StatView; SAS Institute Inc., Cary, NC). The effects of a high-fat diet and resveratrol treatment on body weight, cardiac morphology, left ventricular contractility, TGF-β mRNA, and acetylcholine-mediated relaxation (maximal) of aortic rings were analyzed by a two-way ANOVA (Statistica; StatSoft, Tulsa, OK), applied to determine whether there was an interaction between the two main effects. In the case of a significant interaction, a Tukey’s honest significant difference for equal or unequal n) post hoc test was used to compare diet (high-fat or standard) and treatment (with or without resveratrol), and a p value less than 0.05 was considered statistically significant.

**Results**

**Morphometric Data and the Plasma Lipid Profile.** A modest nonsignificant increase in body weight was observed in female rats fed a high-fat diet for a period of 8 weeks compared with female rats fed a standard diet (Table 1). Despite a modest change in body weight, absolute heart weight and left ventricular weight were similar in rats fed a high-fat diet compared with rats fed a standard diet (Table
In rats fed a high-fat diet, plasma cholesterol, high-density lipoprotein, and low-density lipoprotein concentrations were similar to rats fed a standard diet (Table 2). Likewise, plasma glucose levels were comparable between both groups (standard diet, 7.94 ± 1.07 mM; high-fat diet, 7.31 ± 0.79 mM). Lastly, in the high-fat fed rats, plasma triglyceride levels were modestly elevated compared with rats fed a standard diet but did not reach statistical significance (Table 2).

**Mean Arterial Pressure and Left Ventricular Function.** In rats fed a high-fat diet, systolic and diastolic blood pressure, mean arterial pressure, and left ventricular systolic pressure were significantly (p < 0.05) increased compared with rats fed a standard diet (Table 3). Furthermore, left ventricular rate of contraction (+dP/dt) and relaxation (−dP/dt) were likewise increased but did not reach statistical significance (Table 3). Lastly, to assess myocardial reserve, the contractile response to a dobutamine challenge was examined. In rats fed either a standard or high-fat diet, the increase in contractile response to dobutamine was identical (Table 4).

**Cardiac Remodeling.** Despite an increase of mean arterial pressure, the steady-state mRNA levels of ANP and SERCA2 in the LV were comparable in rats fed a high-fat diet and standard diet (Table 5). Employing the Sirius red technique, collagen α1 protein content in the LV of rats fed a high-fat diet was not significantly increased compared with rats fed a standard diet (Table 6). By contrast, extensive and significant perivascular fibrosis was detected in rats fed a high-fat diet (Table 6; Fig. 1). Vessel lumen area (standard diet, 0.0122 ± 0.0024 mm²; versus high-fat diet, 0.0201 ± 0.003 mm²) and wall thickness (standard diet, 0.0128 ± 0.0014 mm; high-fat diet, 0.0156 ± 0.002 mm) were not significantly different between rats fed a standard or high-fat diet. The reactive fibrotic response in rats fed a high-fat diet was not associated with a change in the expression of putative profibrotic peptides as the steady-state mRNA levels of connective tissue growth factor and transforming growth factor-β1 in the LV were identical to that observed in rats fed a standard diet (Table 5). Moreover, TGF-β3 mRNA levels were significantly decreased in the LV of rats fed a high-fat diet compared with rats fed a standard diet (Table 5).

**Vascular Reactivity and the in Vitro Effect of Resveratrol.** Endothelial-mediated relaxation may be compromised in hypertensive rats fed a high-fat diet. Indeed, the maximal relaxation of isolated aortic rings to acetylcholine was significantly decreased (p < 0.05) in rats fed a high-fat diet (22 ± 5%) compared with rats fed a standard diet (53 ± 8%) (Fig. 2). By contrast, vascular sensitivity (pD2) to acetylcholine was similar in rats fed a standard (7 ± 0.1) and high-fat diet (6.9 ± 0.2). The impaired response to acetylcholine in rats fed a high-fat diet was not related to an alteration in soluble guanylate cyclase responsiveness as the administration of the NO donor sodium nitroprusside promoted an identical dose-dependent relaxation of aortic rings from both groups (Fig. 3).

The impaired acetylcholine-mediated relaxation of aortic rings isolated from rats fed a high-fat diet may be related to either a decrease of NO synthesis and/or a reduction of NO bioavailability secondary to an increase of vascular oxidative stress. In this regard, the antioxidant resveratrol (0.1 μM) reported to also stimulate NO synthase activity was added to aortic rings isolated from rats fed a high-fat diet, and acetylcholine-mediated relaxation was normalized (47 ± 9%) (Fig. 2). By contrast, the exogenous administration of resveratrol to aortic rings isolated from rats fed a standard diet had no effect on acetylcholine-mediated relaxation (53 ± 5%) (Fig. 2). Lastly, vascular sensitivity (pD2) to acetylcholine of resveratrol-treated aortic rings isolated from rats fed either a standard (7 ± 0.1) or high-fat diet (7 ± 0.1) was similar. Thus, the beneficial effect in vitro of resveratrol on acetylcholine-mediated relaxation of aortic rings isolated from high-fat fed rats provided the impetus to examine whether the in vivo administration of the antioxidant could exert a therapeutic effect.

**Morphometric Data and Plasma Lipid Profile: The in Vivo Effect of Resveratrol.** The administration of resvera-
MAP and left ventricular contractility in female rats fed a standard or high-fat diet and the effect of resveratrol (RES).

Data are presented as mean ± S.E.M. (n = number of rats per group).

<table>
<thead>
<tr>
<th></th>
<th>Standard (n = 8)</th>
<th>High Fat (n = 10)</th>
<th>Standard + RES (n = 8)</th>
<th>High Fat + RES (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beat/min)</td>
<td>254 ± 5</td>
<td>242 ± 6</td>
<td>244 ± 12</td>
<td>244 ± 9</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>134 ± 8</td>
<td>171 ± 7*</td>
<td>115.0 ± 7</td>
<td>107 ± 6**</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>96 ± 5</td>
<td>109 ± 3*</td>
<td>89 ± 5</td>
<td>81 ± 5**</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>188 ± 6</td>
<td>130 ± 4*</td>
<td>97 ± 6</td>
<td>90 ± 5**</td>
</tr>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>122 ± 6</td>
<td>148 ± 9*</td>
<td>106 ± 5</td>
<td>106 ± 4**</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>9.1 ± 1.0</td>
<td>10.9 ± 2</td>
<td>6.3 ± 0.7</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>LV + dP/dt</td>
<td>6066 ± 289</td>
<td>6973 ± 282</td>
<td>5297 ± 520</td>
<td>5564 ± 249**</td>
</tr>
<tr>
<td>LV - dP/dt</td>
<td>−5189 ± 312</td>
<td>−6012 ± 333</td>
<td>−4257 ± 503</td>
<td>−4336 ± 263**</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure.
*p < 0.05 versus standard diet.
**p < 0.05 versus high fat diet as determined by a two-way ANOVA.

Vascular Reactivity: The in Vivo Effect of Resveratrol.

The administration of resveratrol to rats receiving a standard diet enhanced acetylcholine-mediated relaxation of aortic rings compared to untreated rats (Fig. 4). Moreover, resveratrol treatment of rats fed a high-fat diet significantly improved acetylcholine-mediated relaxation of isolated aortic rings, and the magnitude of relaxation was comparable to that observed in rats fed a standard diet (Fig. 4). The vascular sensitivity (pD2) to acetylcholine was unchanged in both resveratrol-treated rats groups (resveratrol + standard diet, 7 ± 0.02; resveratrol + high-fat diet, 7 ± 0.12) compared to their respective untreated groups.

Discussion

The relationship between obesity and cardiovascular disease has been unequivocally established clinically and in experimental models. Despite these observations, it remains presently unknown whether vascular reactivity is impaired and maladaptive cardiac remodeling occurs before an overt gain in body weight and in the absence of hyperlipidemia.

The present study has demonstrated that normal female rats provided with a high-fat diet for a period of 8 weeks were associated with a significant elevation of blood pressure and extensive perivascular fibrosis in the myocardium despite a nonsignificant increase in body weight and a normal plasma lipid profile and glucose concentration. Furthermore, the blood pressure increase in rats fed a high-fat diet was prevented by the coadministration of the antioxidant resveratrol. However, the therapeutic effect was limited to the vasculature as resveratrol treatment failed to attenuate the perivascular fibrotic response in the heart of rats fed a high-fat diet. An important paradigm established in the present conditions...

Table 4

Cardiac response to dobutamine infusion (5 μg/kg/min) in female rats fed a standard or high-fat diet and the effect of resveratrol (RES)

Data are presented as mean ± S.E.M. (n = number of rats per group).

<table>
<thead>
<tr>
<th></th>
<th>Standard (n = 7)</th>
<th>High Fat (n = 6)</th>
<th>Standard + RES (n = 6)</th>
<th>High Fat + RES (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beat/min)</td>
<td>24 ± 4</td>
<td>35 ± 3</td>
<td>31 ± 6</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>32 ± 5</td>
<td>29 ± 8</td>
<td>31 ± 6</td>
<td>34 ± 6</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/s)</td>
<td>43 ± 2</td>
<td>47 ± 4</td>
<td>47 ± 3</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>−dP/dt (mm Hg/s)</td>
<td>42 ± 6</td>
<td>44 ± 6</td>
<td>45 ± 8</td>
<td>49 ± 78</td>
</tr>
</tbody>
</table>
study was that feeding normal female rats a high-fat diet for a period of 8 weeks caused a modest nonsignificant increase in body weight and plasma triglyceride levels without a change in the plasma lipid profile or glucose concentration. 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 (Dobrian et al., 2000; Roberts et al., 2001; Erdei et al., 2006). Likewise, a similar sex-dependent relationship with regard to body weight gain was documented in various strains of mice fed a high-fat diet (Nishikawa et al., 2007). Thus, these data further support the premise that the effect of a high-fat diet on body weight gain is temporally delayed in female rats compared to male rats.

Elevated blood pressure was reported in normal male rats fed a high-fat diet characterized by a marked increase in body weight gain and hyperlipidemia (Dobrian et al., 2000; Erdei et al., 2006). In the present study, normal female rats fed a high-fat diet were likewise associated with an increase in blood pressure and left ventricular systolic pressure. However, these changes occurred before a significant increase of body weight and in the absence of hyperlipidemia and hyperglycemia. Previous studies have demonstrated that elevated mean arterial pressure in obese rats was related to an impairment of endothelium-mediated relaxation of vascular tissue (Dobrian et al., 2001; Erdei et al., 2006; Galili et al., 2007). In the present study, acetylcholine-mediated relaxation of aortic rings isolated from rats fed a high-fat diet was significantly compromised, whereas sodium nitroprusside-mediated relaxation was intact. Collectively, these data suggest that NO production was diminished and/or bioavailability was reduced secondary to the increased production of superoxide anions (Dobrian et al., 2001; Erdei et al., 2006; Roberts et al., 2006; Galili et al., 2007). In addition to its well-established antioxidant property, resveratrol was also reported to increase NO synthesis (Wallerath et al., 2002; Wang et al., 2007). In the present study, the exogenous administration of resveratrol to aortic rings isolated from rats fed a high-fat diet normalized acetylcholine-mediated relaxation. Thus, these data strongly support the premise that impaired vascular relaxation in female rats fed a high-fat diet was related to an increased oxidative stress and/or decreased synthesis of NO and occurred in the absence of overt obesity, hyperlipidemia, or hyperglycemia.

It has been well established that elevated systemic arterial hypertension promotes a concentric pattern of cardiac hypertrophy characterized by the increased expression of ANP mRNA and concomitant down-regulation of SERCA2 mRNA (Grossman et al., 1975; Calderone et al., 1995). In the present study, despite an increase in blood pressure in rats fed a high-fat diet, neither ANP nor SERCA2 mRNA levels were altered in the LV. These data were consistent with the lack of change of either absolute LV weight or LV/body weight ratio in rats fed a high-fat diet compared with rats fed a standard diet. Thus, at least in female rats fed a high-fat diet for a period of 8 weeks, elevated blood pressure was not associated with a concomitant hypertrophic response. It is possible that the increase in blood pressure was insufficient to promote

**Table 5**

<table>
<thead>
<tr>
<th>mRNA expression in the left ventricle of female rats fed a standard or high-fat diet</th>
<th>Standard</th>
<th>High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP (n = 8)</td>
<td>10.60 ± 1.97</td>
<td>11.65 ± 2.10</td>
</tr>
<tr>
<td>SERCA2α (n = 8)</td>
<td>1.67 ± 0.21</td>
<td>1.43 ± 0.12</td>
</tr>
<tr>
<td>TGF-β1 (n = 4)</td>
<td>0.36 ± 0.07</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>TGF-β3 (n = 5)</td>
<td>3.38 ± 0.50</td>
<td>1.49 ± 0.36*</td>
</tr>
<tr>
<td>CTGF (n = 4)</td>
<td>7.57 ± 0.98</td>
<td>6.32 ± 1.05</td>
</tr>
</tbody>
</table>

CTGF, connective tissue growth factor.

* p < 0.05 versus standard diet.

**Table 6**

<table>
<thead>
<tr>
<th>Left ventricular and perivascular collagen α1 type 1 content in rats fed a standard or high-fat diet and the effect of resveratrol (RES)</th>
<th>Standard</th>
<th>High Fat</th>
<th>High Fat + RES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>0.0057 ± 0.002</td>
<td>0.0092 ± 0.002</td>
<td>0.0129 ± 0.001</td>
</tr>
<tr>
<td>Perivascular</td>
<td>0.1141 ± 0.03</td>
<td>0.267 ± 0.02*</td>
<td>0.324 ± 0.02*</td>
</tr>
</tbody>
</table>

* p < 0.05 versus standard diet as determined by a one-way ANOVA.
fed a high-fat diet provided the impetus to assess whether the in vivo administration would likewise attenuate the rise in blood pressure. Indeed, resveratrol administration to rats fed a high-fat diet prevented the hypertensive response. Furthermore, the beneficial in vivo hemodynamic effect of resveratrol was associated with the preservation of acetylcholine-mediated relaxation of aortic rings isolated from rats fed a high-fat diet. Thus, these data support the premise that resveratrol represents an appropriate pharmacological approach to prevent vascular dysfunction in female rats fed a high-fat diet. The scavenging of superoxide anions, increasing NO synthesis, and the phytoestrogenic property of resveratrol represent underlying mechanisms that may have synergistically prevented the rise of mean arterial pressure in rats fed a high-fat diet (Gehm et al., 1997; Wallerath et al., 2002; Baur and Sinclair, 2006; Wang et al., 2007).

The relationship between hypertension and cardiac fibrosis has been well established (Berk et al., 2007). Moreover, increased oxidative stress represents a putative feature of obesity and was further shown to participate in the progression of cardiac fibrosis either directly or indirectly via the suppression of NO synthesis (Dobrian et al., 2000; Ruetten et al., 2005; Erdel et al., 2006; Lu et al., 2008). In this regard, an attenuation of the perivascular fibrotic response would be expected in the heart of resveratrol-treated rats fed a high-fat diet based on the beneficial effect on blood pressure and its established antioxidant property. Furthermore, a direct antifibrotic action was identified in vitro as resveratrol suppressed cardiac fibroblast proliferation (Olson et al., 2005; Wang et al., 2007). Despite these observations, the in vivo administration of resveratrol failed to prevent the progression of perivascular fibrosis in the heart of rats fed a high-fat diet. Nonetheless, cardiac remodeling was sensitive to resveratrol therapy as the decreased expression of transforming growth factor-β3 mRNA in the heart of rats fed a high-fat diet was prevented. The latter beneficial effect may be related in part to the phytoestrogenic property of resveratrol as 17β-estradiol treatment of either cardiac fibroblasts or osteoclasts increased transforming growth factor-β3 mRNA expression (Yang et al., 1996; Mercier et al., 2002a). Thus, in female rats fed a high-fat diet, the perivascular fibrotic response was at least independent of a rise in blood pressure. Furthermore, the normalization of acetylcholine-mediated relaxation of aortic rings isolated from resveratrol-treated female rats fed a high-fat diet indirectly suggests that the increased perivascular fibrotic response in these hearts may not be secondary to a decreased bioavailability or synthesis of NO.

In conclusion, the present study has demonstrated that, prior to the development of overt obesity and in the absence of hyperlipidemia and hyperglycemia, female rats fed a high-fat diet were associated with vascular dysfunction and perivascular fibrosis. Resveratrol administration preserved vascular function, whereas the perivascular fibrotic response in the heart of female rats fed a high-fat diet persisted. Thus, these data demonstrate that disparate events are linked to the development of hypertension and perivascular fibrosis in female rats fed a high-fat diet.

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References


Dr. Louis P. Perrault, Montreal Heart Institute, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada. E-mail: louis.perrault@icm-mhi.org


Address correspondence to: Dr. Louis P. Perrault, Montreal Heart Institute, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada. E-mail: louis.perrault@icm-mhi.org

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