The Polyphenols Resveratrol and S17834 Prevent the Structural and Functional Sequelae of Diet-Induced Metabolic Heart Disease in Mice

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Background—Diet-induced obesity is associated with metabolic heart disease characterized by left ventricular hypertrophy and diastolic dysfunction. Polyphenols such as resveratrol and the synthetic flavonoid derivative S17834 exert beneficial systemic and cardiovascular effects in a variety of settings including diabetes mellitus and chronic hemodynamic overload.

Methods and Results—We characterized the structural and functional features of a mouse model of diet-induced metabolic syndrome and used the model to test the hypothesis that the polyphenols prevent myocardial hypertrophy and diastolic dysfunction. Male C57BL/6J mice were fed a normal diet or a diet high in fat and sugar (HFHS) with or without concomitant treatment with S17834 or resveratrol for up to 8 months. HFHS diet–fed mice developed progressive left ventricular hypertrophy and diastolic dysfunction with preservation of systolic function in association with myocyte hypertrophy and interstitial fibrosis. In HFHS diet–fed mice, there was increased myocardial oxidative stress with evidence of oxidant-mediated protein modification via tyrosine nitration and 4-OH-2-nonenol adduction. HFHS diet–fed mice also exhibited increases in plasma fasting glucose, insulin, and homeostasis model assessment of insulin resistance indicative of insulin resistance. Treatment with S17834 or resveratrol prevented left ventricular hypertrophy and diastolic dysfunction. For S17834, these beneficial effects were associated with decreases in oxidant-mediated protein modifications and hyperinsulinemia and increased plasma adiponectin.

Conclusions—Resveratrol and S17834 administered concurrently with a HFHS diet prevent the development of left ventricular hypertrophy, interstitial fibrosis, and diastolic dysfunction. Multiple mechanisms may contribute to the beneficial effects of the polyphenols, including a reduction in myocardial oxidative stress and related protein modifications, amelioration of insulin resistance, and increased plasma adiponectin. The polyphenols resveratrol and S17834 may be of value in the prevention of diet-induced metabolic heart disease.

Key Words: diastolic dysfunction ■ left ventricular hypertrophy ■ metabolic syndrome ■ 4-OH-2-nonenol ■ oxidative stress

The prevalence of diet-induced obesity, diabetes mellitus, and the metabolic syndrome is increasing at an alarming rate and is now a major contributor to cardiovascular morbidity and mortality,1,2 including heart failure.3 Metabolic syndrome, defined as the constellation of obesity, diabetes mellitus, hypertension, and increased triglycerides,4 is associated with left ventricular (LV) hypertrophy and impaired diastolic function that can lead to heart failure with a preserved ejection fraction.5 The mechanism responsible for myocardial hypertrophy and diastolic dysfunction in metabolic syndrome is incompletely understood.

Clinical Perspective on p 1764

Transgenic mouse models with inherent derangements in glucose and/or lipid handling have provided important insights regarding the pathobiology of diastolic dysfunction in metabolic heart disease.6–7 However, because metabolic syndrome is often diet induced, it is desirable to study the cardiovascu-
lar consequences in a model in which the syndrome is also diet induced. The C57BL/6J mouse fed an “American” diet high in fat and sugar (HFHS) is a commonly used model of diet-induced obesity that is associated with diabetes mellitus, hypertension, and increased serum triglycerides.8–11 Very little is known about the cardiac phenotype of these mice, and there is no information about LV diastolic function. We theorized that HFHS feeding would cause a cardiac phenotype typical of metabolic heart disease with myocardial hypertrophy, diastolic dysfunction, and preservation of systolic function. Accordingly, our first goal was to characterize the myocardial structural and functional features associated with a chronic HFHS diet.

Polyphenols exert pleiotropic actions that may be beneficial in metabolic syndrome, including anti-inflammatory and antioxidant effects12,13 and activation of sirtuins.14 Several studies have demonstrated beneficial effects of resveratrol or the synthetic flavonoid derivative S17834 [6,8-diallyl 5,7-dihydroxy 2-(2-ethylhexyl-3-oxo-4-phenyl)pyran-4-one] on systemic15 and cardiovascular12,13 abnormalities associated with diabetes mellitus,16,17 ischemia/reperfusion,18 pressure overload,19,20 hypertension,21 and myocardial infarction.22 However, the ability of the polyphenols to ameliorate metabolic heart disease associated with diet-induced metabolic syndrome is not known. Accordingly, our second goal was to test the hypothesis that S17834 and resveratrol prevent LV hypertrophy and diastolic dysfunction in mice fed a HFHS diet. The mechanism responsible for the beneficial effect of the polyphenols on cardiac structure and function is not understood at the molecular level and may vary depending on the underlying pathophysiology. HFHS feeding is associated with multiple metabolic abnormalities that may cause myocardial hypertrophy or otherwise adversely affect cardiac structure and function including oxidative stress and hyperinsulinemia.9 Accordingly, our third goal was to identify the potential role of these mechanisms in mediating the cardiac effects of polyphenols in this model of metabolic syndrome.

Methods

Experimental Animals
Male C57BL/6J mice 8 weeks of age were fed a normal chow diet (Teklad Global 18% protein rodent diet, product No. 2018, Harlan Laboratories) or a HFHS diet (Bio-Serv diet, product No. F1850) containing 35.5% fat (primarily lard) and 36.3% carbohydrate (primarily sucrose). Some of the mice in the HFHS group also received S17834 (130 mg/kg per day) or resveratrol (130 mg/kg per day) compounded into the food for 8 months (S17834) or 4 months (resveratrol). S17834 was obtained from the Institut de Recherches Servier, and resveratrol was obtained from Orchid Chemicals and Pharmaceuticals (Nungambakkam, Chennai, India). The protocol was approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine.

Measurement of Plasma Glucose, Insulin, Lipids, and Adiponectin
Blood was collected from the tail vein and centrifuged, and the plasma was separated. Plasma glucose levels were measured by glucose meters (ACCU-CHEK, Roche Applied Science, Indianapolis, IN). Plasma insulin level was measured with the use of enzyme-linked immunosorbent assay kits (Crystal Chem, Downers Grove, IL). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated with the following formula: [fasting plasma glucose (mg/dL) × fasting plasma insulin (µU/mL)]/405. Plasma triglycerides, cholesterol, and free fatty acids were measured enzymatically with the use of infinity reagents (Thermo Fisher, Louisville, CO) according to the manufacturer’s instructions. Plasma adiponectin levels were determined with adiponectin enzyme-linked immunosorbent assay kits (Otsuka Pharmaceutical Co Ltd, Tokyo, Japan).

Two-Dimensional and M-Mode Echocardiography
LV dimensions and systolic function were measured in nonanesthetized mice with an Acuson Sequoia C-256 echocardiograph machine equipped with a 15-MHz linear transducer (model 15L8), as we have described.23 Briefly, the heart was imaged in the 2-dimensional parasternal short-axis view, and an M-mode echocardiogram of the midventricle was recorded at the level of papillary muscles. Anterior wall thickness, posterior wall thickness, and LV end-diastolic and end-systolic dimensions were measured from the M-mode image. LV fractional shortening was calculated as follows: (end-diastolic dimension − end-systolic dimension)/end-diastolic dimension×100.

Doppler Echocardiography
LV diastolic function was assessed by transmitral and tissue Doppler echocardiography with the use of a VisaulSonic Vevo 770 high-resolution imaging system (Toronto, Ontario, Canada) equipped with a 30-MHz RMV-707B transducer.24,25 Briefly, mice were anesthetized with isoflurane by a facemask at a concentration of 2.5% for induction and then 1.5% for maintenance. Pulsed-wave Doppler images were collected in the apical 4-chamber view to record the mitral Doppler flow spectra. Peak early (E) and late (A) mitral inflow velocities, E/A ratio, deceleration time of early filling, and isovolumetric relaxation time were measured. Tissue Doppler images were collected in the parasternal short-axis view. Myocardial peak early diastolic velocity (Eₑ) was measured, and E/Eᵥ was calculated. Doppler spectra were recorded for 12 to 14 cardiac cycles, from which at least 5 consecutive cardiac cycles were selected, and the values were averaged in accordance with the American Society of Echocardiography guidelines.26 Data analysis was performed offline with the use of a customized version of Vevo 770 Analytic software.

Organ Weight and Histology
The mice were euthanized at the end of study. Heart and LV with septum were weighed, and LV samples were fixed in 10% buffered formalin, embedded with paraffin, and sectioned. Myocyte cross-sectional area and fibrosis were measured as described previously.23 Briefly, sections were stained with hematoxylin and eosin and examined under a light microscope (BX 40, Olympus). Five random fields from each of 4 sections per animal were analyzed, and 60 myocytes per animal were measured. The quantification of myocyte diameter was determined with the use of NIH ImageJ software. To assess fibrosis, sections were stained with a Masson trichrome kit (Sigma) and examined under a light microscope (BX 40, Olympus).

Immunohistochemistry for 3-Nitrotyrosine and 4-Hydroxy-2-Nonenal
Immunohistochemistry was performed as described previously.23 Briefly, LV tissue sections (4 µm) were blocked with 10% goat serum in phosphate-buffered saline, incubated with rabbit anti-3-nitrotyrosine polyclonal antibody or mouse anti-4-OH-2-nonenol (HNE) monoclonal antibody, and incubated with goat biotin-conjugated anti-rabbit IgG or goat biotin-conjugated anti-mouse IgG (Vector Laboratories, Burlingame, CA). The sections were incubated with avidin and biotinylated horseradish peroxidase macromolecular complex (Vector Laboratories) and stained with 3-amino-9-ethylcarbazole (Vector Laboratories) and hematoxylin (Vector Laboratories). The samples were examined under a light microscope (BX 40, Olympus).

Immunoprecipitation and Immunoblots
For immunoprecipitation of LKB1, frozen LV was homogenized in 1× RIPA buffer (Cell Signaling) with 1 mmol/L phenylmethylsulfonyl fluoride and 1% protease inhibitor set I (Calbiochem). Total...
protein (250 µg) was incubated with mouse anti-LKB1 (Santa Cruz) overnight at 4°C. Protein A/G agarose beads were added and incubated for 1 hour at 4°C. After 3 washes, proteins were eluted in Laemmli buffer, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride membranes. Blots were incubated with rabbit anti-HNE (Calbiochem) and then goat anti-mouse IgG IRDye 800CW and quantified with the use of the Odyssey Infrared Imaging System (LI-COR Biosciences). Blots were stripped and reprobed with goat anti-LKB1 (Santa Cruz) and then donkey anti-goat IgG IRDye 680.

Immunoblots for AMPK were performed on frozen LV that was homogenized in tissue lysis buffer (HEPES, pH 7.4, 20 mmol/L, B-glycerol phosphate 50 mmol/L, EGTA 2 mmol/L, dithiothreitol 1 mmol/L, NaF 10 mmol/L, NaVO₄ 1 mmol/L, Triton X-100 1%, glycerol 10%, and 1 protease inhibitor complete mini tablet, EDTA free, 20 mL [Roch]). Total protein (25 µg) was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Blots were incubated with rabbit anti-phosphoThr172-AMPK (Cell Signaling) and detected with the use of the Lior Odyssey fluorescent system.

### Statistical Analysis

Results are presented as mean±SEM. The statistical significance of differences among groups or between 2 means was determined with ANOVA and the Bonferroni adjustment for multiple comparisons. A Bonferroni-adjusted P value <0.05 was considered significant. Pressure-volume curves were analyzed by repeated-measures 2-way ANOVA.

### Results

#### HFHS Diet Induces Time-Dependent LV Hypertrophy

In HFHS-fed mice, wall thickness was increased at 2 months and increased further at 5 and 8 months (Figure 1). The LV end-diastolic dimension likewise was increased at 2 months and increased further at 5 and 8 months. LV wall thickness relative to LV end-diastolic dimension was unchanged at 2 and 5 months but was increased at 8 months, indicating the development of concentric hypertrophy. LV fractional shortening was unchanged at any time, indicating that systolic function was preserved. Mice were euthanized after 8 months of HFHS feeding. In HFHF-fed mice, heart and LV weights were increased 14% and 11%, respectively, relative to tibia length (Table 1), confirming the echocardiographic finding of LV hypertrophy.

#### HFHS Diet Induces Diastolic Dysfunction

LV diastolic function was assessed after 8 months of HFHS feeding with the use of transmitral and tissue Doppler echocardiography. We found prolongation of the isovolumetric relaxation time and deceleration time, associated with a decrease in the E/A ratio (Figure 2). Eₐ was decreased, indicative of slowed LV relaxation, and E/Eₐ was increased, indicative of an increase in left atrial filling pressure. Taken together, these findings are internally consistent and demonstrate that HFHS feeding leads to impaired LV relaxation and filling.²⁶

To further characterize LV function, hearts were subjected to Langendorff perfusion with the use of the isovolumic, balloon-in-LV technique to allow assessment of LV function over a range of LV volumes.²⁷ For any given LV volume, end-diastolic pressure was higher in HFHS-fed mice (Figure 3). LV systolic pressure was likewise shifted leftward, whereas LV developed pressure was similar to that in normal diet–fed mice, albeit at a smaller LV volume. These data indicate impaired LV filling with preserved systolic function, thus confirming the echocardiographic findings.

#### S17834 and Resveratrol Prevent LV Hypertrophy and Diastolic Dysfunction in HFHS-Fed Mice

In HFHS-fed mice, the addition of S17834 to the diet prevented the increases in LV wall thickness (Figure 1) and heart and LV weights (Table 1). These effects were associated with improvement in diastolic function as assessed by
Doppler echocardiography. The E/A ratio, deceleration time, and isovolumetric relaxation time measured by transmitral Doppler were normalized, as was Em measured by tissue Doppler and the ratio of E/Em (Figure 2). In HFHS-fed mice, resveratrol also prevented LV hypertrophy (Figure I in the online-only Data Supplement) and improved diastolic function (Figure II in the online-only Data Supplement). These effects were qualitatively and quantitatively similar to those observed with S17834.

**S17834 Prevents Myocyte Hypertrophy and Interstitial Fibrosis in HFHS-Fed Mice**

Myocyte diameter was increased in HFHS-fed mice compared with mice fed a normal diet (Figure 4A and 4B). In HFHS-fed mice, there was increased interstitial and perivascular fibrosis visualized by Masson trichrome staining (Figure 4C and 4D). Myocardial concentrations of glycogen and triglyceride were not increased in HFHS-fed mice (Figure III in the online-only Data Supplement). Both myocyte hypertrophy and interstitial fibrosis were prevented by treatment with S17834 (Figure 4).

**S17834 Prevents Oxidant-Mediated Posttranslational Protein Modifications**

Myocardial oxidative posttranslational protein modifications were assessed histochemically with the use of antibodies to 3-nitrotyrosine and the lipid peroxidation product HNE. HNE and 3-nitrotyrosine were markedly increased diffusely over myocytes in HFHS-fed mice, and the accumulation of both was prevented by treatment with S17834 (Figure 5A through 5D).

In cardiac myocytes, HNE adducts have been shown to inhibit the activity of LKB, an upstream kinase for AMPK, thereby leading to increased downstream hypertrophic signaling via mTOR/p70S6 kinase. To test for HNE adducts of LKB, myocardium was immunoprecipitated with an antibody directed against HNE-lysine adducts and immunoblotted for LKB. LKB-HNE adducts were increased in HFHS-fed mice, and the increase was prevented by treatment with S17834 (Figure 5E and 5F). Although LKB is a regulator of AMPK, AMPK activity was not affected by HFHS feeding or S17834 treatment (Figure IV in the online-only Data Supplement).

**S17834 Improves Insulin Sensitivity and Increases Plasma Adiponectin Level**

Consistent with prior reports, fasting glucose, insulin, and HOMA-IR index were increased in HFHS-fed mice (Table 2). Treatment with S17834 decreased fasting glucose, insulin, and HOMA-IR, suggesting improved insulin sensitivity. Plasma cholesterol was increased in HFHS-fed mice but was not affected by S17834. Free fatty acids were not increased by HFHS and were not affected by S17834. The plasma adiponectin level was not different in HFHS diet– versus normal diet–fed mice but was increased by treatment with S17834.
Discussion
This study provides several new findings with regard to the pathophysiology and treatment of metabolic heart disease. First, we demonstrate in mice that diet-induced obesity is associated with metabolic heart disease characterized by myocardial hypertrophy, diastolic dysfunction, myocyte hypertrophy, interstitial fibrosis, oxidant-mediated protein and lipid products, hyperinsulinemia, and insulin resistance. Second, we show that treatment with S17834 or resveratrol prevents the cardiac structural and functional consequences of metabolic syndrome. Third, we show that treatment with S17834 exerts multiple actions that may account for the beneficial structural and functional effects including (1) decreases in oxidative stress and oxidant-mediated protein modifications, (2) amelioration of hyperinsulinemia/insulin resistance, and (3) an increase in plasma adiponectin.

LV Hypertrophy and Diastolic Dysfunction in HFHS-Fed Mice
HFHS-induced obesity was associated with LV hypertrophy. HFHS feeding caused a progressive increase in heart size with wall thickening and chamber growth leading to concentric hypertrophy. Heart and LV weights confirmed LV hypertrophy, and histological analysis revealed that organ growth was associated with increases in myocyte size and interstitial fibrosis. Myocardial triglycerides and glycogen were not increased in HFHS diet–fed mice, indicating that myocardial hypertrophy in this model is not due to accumulation of triglycerides or glycogen.

LV hypertrophy was associated with impaired diastolic function. Doppler assessment of transmitral flow demonstrated prolongation of deceleration time and isovolumetric relaxation time in association with a decrease in the ratio of

Figure 4. Effects of high-fat/high-sugar (HFHS) diet and S17834 (S) on myocyte diameter and cardiac fibrosis in left ventricular myocardium. A. Photomicrographs of left ventricular tissue sections stained by hematoxylin and eosin. B. Quantification of myocyte diameter measured by National Institutes of Health ImageJ. C. Representative photomicrographs of Masson trichrome staining for cardiac fibrosis showing myocardium in red and fibrosis in blue. D. Quantification of cardiac fibrosis measured by National Institutes of Health ImageJ. Bar=25 μm. Values are mean±SEM; n=3 to 4. *P<0.05 vs normal diet–fed mice; †P<0.05 vs HFHS diet–fed mice.

Figure 5. Effects of high-fat/high-sugar (HFHS) diet and S17834 (S) on myocardial nitrotyrosine and 4-hydroxy-2-nonenal (HNE) staining and HNE adducts of LKB. Shown are representative photomicrographs of myocardial nitrotyrosine staining (A and B) and HNE staining (C and D). E, Representative immunoprecipitation (IP) and immunoblot (IB) data for HNE adducts of LKB. F, Group densitometry analysis. Bar=25 μm. Values are mean±SEM; n=3 to 4. *P<0.05 vs normal diet–fed mice; †P<0.05 vs HFHS diet–fed mice.
Fasting plasma insulin, are alterations in protein function due to changes in protein handling proteins in this model, it remains possible that there exclude a role for transcriptional dysregulation of calcium-handling. Although these data

Fasting plasma glucose, mg/dL 87±7 126±10* 92±12
Fasting plasma insulin, μU/mL 8.5±0.5 15.8±2.7* 10.6±1.0
HOMA-IR 33±4 93±22† 44±7
Plasma triglycerides, mg/dL 86±10 96±10 101±11
Plasma free fatty acid, mEq/L 1.5±0.1 1.4±0.1 1.6±0.1
Plasma cholesterol, mg/dL 88±4 126±10† 145±12†
Plasma adiponectin, μg/mL 14.1±1.3 13.6±1.1 20.7±1.8‡

Values are mean±SEM. Plasma was obtained from mice fed a normal diet, high-fat/high-sugar (HFHS) diet, or HFHS diet plus S17834 for 8 weeks for triglycerides, free fatty acids, cholesterol, and adiponectin (n=9 to 14) or for 5 weeks for fasting glucose, insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) index (n=6).

the E/A wave measured by transmitral Doppler. Tissue Doppler further demonstrated a decrease in Eₘ, indicative of slowed LV relaxation and an increase in the ratio of E/Eₘ, reflective of an increase in left atrial pressure. All of these findings are indicative of impaired LV relaxation and are typical of patients with metabolic syndrome. Diastolic dysfunction was further confirmed by isovolumic Langendorff perfusion, which demonstrated an upward shift in the diastolic pressure-volume relationship. In contrast, systolic function was preserved, as evidenced by normal fractional shortening on echocardiography and a normal developed pressure by Langendorff perfusion.

The HFHS-fed mouse has been used extensively to study the metabolic consequences of obesity. Despite the popularity of this model, the cardiac phenotype has not been characterized, and diastolic function in particular has not been assessed. Our findings indicate that the cardiac phenotype of the HFHS-fed mouse is very similar to that in humans with metabolic heart disease.

An important mechanism of diastolic dysfunction is impaired myocardial relaxation due to abnormal calcium handling. As an initial approach to assessing the role of altered calcium handling in this model, we measured mRNA levels for several key calcium regulatory proteins including sarcoplasmic reticulum calcium ATPase, the ryanodine receptor, the sodium-calcium exchanger, and the L-type channel, all of which were unaffected by HFHS feeding (Figure V in the online-only Data Supplement). Although these data exclude a role for transcriptional dysregulation of calcium-handling proteins in this model, it remains possible that there are alterations in protein function due to changes in protein turnover and/or posttranslational modifications.

**S17834 and Resveratrol Prevent LV Hypertrophy and Diastolic Dysfunction**

Both S17834 and resveratrol effectively prevented the development of LV hypertrophy and diastolic dysfunction. These effects were associated at the cellular level with prevention of cardiac myocyte hypertrophy and interstitial fibrosis. Prior studies with resveratrol have demonstrated beneficial effects on cardiac function in a variety of pathological models. In spontaneously hypertensive rats, resveratrol prevented LV hypertrophy and improved diastolic function. Likewise, resveratrol improved diastolic function in mice with type 1 diabetes mellitus due to streptozocin or in db/db mice with type 2 diabetes mellitus. In contrast, resveratrol did not alleviate the extent of LV remodeling after myocardial infarction. Our report is the first demonstration of the cardiac effects of the synthetic flavonoid derivative S17834 in any condition. Prior studies have shown that S17834 can inhibit atherosclerosis in diabetic low-density lipoprotein receptor-deficient mice.

**Mechanism of Antihypertrophic Effect of S17834**

A prominent effect of S17834 and resveratrol was to prevent cardiac hypertrophy induced by HFHS feeding. Accordingly, we assessed mechanisms that are associated with hypertrophic signaling in cardiac myocytes. First, because we and others have shown that oxidant signaling can stimulate myocyte growth, we examined whether HFHS feeding was associated with increased oxidative stress in the myocardium and, if so, whether the increase was prevented by S17834. Immunohistochemistry showed generalized increases in 3-nitrotyrosine and the lipid peroxidation product HNE, indicative of oxidative stress in the myocardium. Furthermore, we found increased HNE adducts of LKB, a signaling molecule that has been implicated in the regulation of myocardial growth. In spontaneously hypertensive rats, Dolinsky et al demonstrated increased LKB-HNE adducts in the myocardium that were associated with decreased activity of LKB and its downstream substrate AMPK, leading to deactivation of hypertrophic signaling via the mTOR-p70S6 kinase pathway. They further demonstrated that resveratrol prevented the increase in LKB-HNE adducts, restored LKB and AMPK activities, and inhibited hypertrophic signaling via mTOR-p70S6 kinase. In contrast, in HFHS-fed mice, the increase in LKB-HNE adduct was not associated with a decrease in AMPK activity and was not affected by S17834 treatment. Thus, although S17834 decreased myocardial oxidative stress as reflected by generalized decreases in nitrotyrosine and HNE and prevented the oxidant-mediated lipid modification of at least 1 specific protein (LKB) implicated in the regulation of myocyte growth, myocyte hypertrophy in this model cannot be attributed to a decrease in LKB activity leading to a decrease in AMPK activity.

Second, we found that S17834 treatment (1) ameliorated hyperinsulinemia/insulin resistance and (2) increased plasma adiponectin. Hyperinsulinemia, which may contribute to myocardial hypertrophy in type 2 diabetes mellitus, has been noted previously in this model. Our finding that S17834 decreased plasma insulin is consistent with similar observations showing that resveratrol decreases plasma insulin in other models of type 2 diabetes mellitus. A decrease in plasma insulin levels might oppose myocardial hypertrophy by decreasing the stimulation of the phosphatidylinositol 3-kinase/Akt/mTOR/p70S6 pathway. Finally, we found that S17834 treatment is associated with an increase in plasma adiponectin. The increase in plasma adiponectin with S17834

**Table 2. Metabolic Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Diet</th>
<th>HFHS Diet</th>
<th>HFHS Diet S17834</th>
<th>HFHS Diet plus S17834</th>
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<tbody>
<tr>
<td>Fasting plasma glucose, mg/dL</td>
<td>87±7</td>
<td>126±10*</td>
<td>92±12</td>
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<tr>
<td>Fasting plasma insulin, μU/mL</td>
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<td>15.8±2.7*</td>
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<td>HOMA-IR</td>
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<td>93±22†</td>
<td>44±7</td>
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<tr>
<td>Plasma triglycerides, mg/dL</td>
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<td>96±10</td>
<td>101±11</td>
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<td>Plasma free fatty acid, mEq/L</td>
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<td>1.4±0.1</td>
<td>1.6±0.1</td>
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<tr>
<td>Plasma cholesterol, mg/dL</td>
<td>88±4</td>
<td>126±10†</td>
<td>145±12†</td>
<td></td>
</tr>
<tr>
<td>Plasma adiponectin, μg/mL</td>
<td>14.1±1.3</td>
<td>13.6±1.1</td>
<td>20.7±1.8‡</td>
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</tbody>
</table>
Figure 6. Schema summarizes the observed mechanisms by which polyphenols may exert beneficial effects in the high-fat/high-sugar (HFHS)-fed mouse. HFHS feeding is associated with oxidative stress and oxidant-mediated protein modifications as evidenced by generalized immunohistochemical staining for nitrotyrosine and 4-hydroxy-2-nonenal staining and specific 4-hydroxy-2-nonenal adducts of LKB that are associated with decreased enzyme activity and increased hypertrophic signaling. Modifications of other proteins (eg, calcium handling or sarcomeric proteins) may contribute to impaired relaxation. HFHS feeding is also associated with insulin resistance and hyperinsulinemia, which may promote hypertrophic growth. Finally, oxidative stress can lead to interstitial fibrosis. Together, these and possibly other mechanisms may contribute to diastolic dysfunction, which is common in metabolic heart disease. By decreasing oxidative stress and inflammation, treatment with S17834 may decrease (1) the generation of oxidative protein modifications, (2) interstitial fibrosis, and (3) insulin resistance/hyperinsulinemia. S17834 also increases plasma adiponectin, which may inhibit hypertrophic signaling. RSV indicates resveratrol.

Implications

The HFHS-fed mouse provides a valuable model of diet-induced myocardial hypertrophy and diastolic dysfunction that should prove useful in elucidating the pathobiology and treatment of metabolic heart disease. This model is associated with increased myocardial oxidative stress and systemic hyperinsulinemia/insulin resistance, both of which may promote myocardial hypertrophy. The polyphenols exert multiple effects in this model that may contribute to decreased myocardial hypertrophy and improved diastolic function including (1) a decrease in myocardial oxidative stress, (2) a decrease in oxidant-mediated protein modifications, (3) an improvement in hyperinsulinemia/insulin sensitivity, and (4) an increase in plasma adiponectin (Figure 6). The ability of the HFHS-fed mouse to reproduce the cardinal myocardial abnormalities of metabolic heart disease observed in humans and the ability of resveratrol and S17834 to prevent the structural and functional consequences of diet-induced heart disease in this model suggest that these polyphenols could be of value in the treatment of metabolic heart disease in humans.

Sources of Funding

This study was supported by National Institutes of Health grants HL-061639 (Dr Colucci), HL-064750 (Dr Colucci), HL031607 (Dr Cohen), and PO1 HL 068758 (Drs Cohen and Walsh), the National Heart, Lung, and Blood Institute–sponsored Boston University Cardiovascular Proteomics Center (contract N01-HV-28178; Drs Cohen and Colucci), and a Strategic Alliance between Servier and the Vascular Biology Section, Boston University Medical Center (Dr Cohen).

Disclosures

This work was performed as part of a Strategic Alliance between the Vascular Biology Section, Boston University Medical Center (Dr Cohen) and Servier, which provided the S17834. Dr Cohen is a consultant for Servier, and Dr Verbeuren is provided an employee of Servier.

References


The prevalence of diet-induced obesity and the metabolic syndrome is increasing at an alarming rate and is a major contributor to cardiovascular morbidity and mortality, including heart failure with a preserved ejection fraction. Polyphenols such as resveratrol and the synthetic flavonoid derivative S17834 exert beneficial systemic and cardiovascular effects in a variety of settings, including diabetes mellitus and chronic hemodynamic overload. However, the ability of the polyphenols to ameliorate metabolic heart disease associated with diet-induced metabolic syndrome is not known. We fed mice an “American” diet high in fat and sugar with or without concomitant treatment with S17834 or resveratrol for up to 8 months. High-fat/high-sugar diet–fed mice developed left ventricular hypertrophy and diastolic dysfunction. Treatment with the polyphenols prevented the cardiac structural and functional consequences of high-fat/high-sugar feeding. We conclude that the high-fat/high-sugar diet–fed mouse provides a valuable model of diet-induced myocardial hypertrophy and diastolic dysfunction that should prove useful in elucidating the pathobiology and treatment of metabolic heart disease. The polyphenols exerted multiple effects that may have contributed to amelioration of metabolic heart disease, including decreases in myocardial oxidative stress and oxidant-mediated protein modifications, improved insulin sensitivity, and an increase in plasma adiponectin. These findings suggest that the polyphenols could be of value in the treatment of metabolic heart disease in humans.
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Circulation. 2012;125:1757-1764; originally published online March 2, 2012;
doi: 10.1161/CIRCULATIONAHA.111.067801
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/125/14/1757

Data Supplement (unedited) at:
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Quantitative PCR for mRNA expression of myocardial calcium handling. Frozen hearts were ground under liquid nitrogen and total RNA was extracted with the mirVana miRNA Isolation Kit (Applied Biosystems). Total RNA was treated with DNase before cDNA synthesis with the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Quantitative PCR was performed with TaqMan Universal PCR Master Mix and TaqMan primers (Applied Biosystems) specific for mouse SERCA2a (Mm01201431_m1), ryanodine receptor (Mm00465877_m1), sodium/calcium exchanger (Mm01232254_m1), L-type calcium channel alpha 1c subunit (Mm00437917_m1) and GAPDH (4352339E) using the Applied Biosystems Step One Plus Real Time PCR System. Data is normalized to GAPDH using the equation 2^{\text{exp}(\text{C}_{T} \text{ target gene}-\text{C}_{T} \text{GAPDH})} and expressed as arbitrary units.

Myocardial triglyceride and glycogen levels. Triglycerides were measured from myocardial tissue samples lysed in 5% Triton-X using a Triglyceride Quantification Kit (Abcam, Cambridge, MA). Tissue glycogen from freeze-clamped, KOH-digested myocardial specimens was measured using the amyloglucosidase method \(^1\).
Supplemental Figures

Figure S1.

A. Total wall thickness (mm)

B. RWT

C. LV EDD (mm)

D. LV ESD (mm)

E. LV FS (%)
Figure S2.

A. IVRT (ms)

B. DT (ms)

C. E/A ratio

D. Em (cm/s)

D. E/Em
Figure S3.

Figure S4.

Figure S5.
Supplemental Figure Legends

Figure S1. Total wall thickness, relative wall thickness (RWT), LV end-diastolic (EDD) and end-systolic (ESD) dimensions, and LV fractional shortening (FS) in mice fed a normal chow diet, a HFHS diet, or a HFHS diet + resveratrol (R). Values are means ± SEM; n=6. *P<0.05 vs. normal diet-fed mice. †P<0.05 vs. HFHS diet-fed mice.

Figure S2. Isovolumic relaxation time (IVRT), deceleration time (DT), the ratio of early-to-late diastolic mitral inflow velocity (E/A), myocardial peak early diastolic velocity (Eₘ) and the ratio of peak early mitral inflow velocity to myocardial peak early diastolic velocity (E/Eₘ) in mice fed a normal chow diet, a HFHS diet, or a HFHS diet + resveratrol (R). Values are means ± SEM; n=6. *P<0.01 vs. normal diet-fed mice. †P<0.05 vs. HFHS diet-fed mice.

Figure S3. Myocardial triglyceride and glycogen levels in mice fed a normal chow diet (ND) or a HFHS diet. Values are means ± SEM; n=4.

Figure S4. The mRNA expression of sarcoplasmic reticulum calcium ATPase (SERCA), ryanodine receptor (RyR), sodium-calcium exchanger (NCX) or L-type calcium channel (LCC) in myocardium of mice fed a normal chow diet (ND) or a HFHS diet. Data is normalized to GAPDH and expressed as arbitrary units. Values are means ± SEM; n=6.

Figure S5. Effect of HFHS diet and treatment with S17834 (S) on phosphorylated AMPK. Shown is a representative Western blot and mean densitometry analysis expressed as the ratio of
phosphorylated AMPK to GAPDH. Total AMPK expression was unchanged (data not shown).

Values are means ± SEM; n=3-4.

Supplemental References