Heart Failure

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. (Circulation. 2012;125:1757-1764.)

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. 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 effects and activation of sirtuins. Several studies have 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.

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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 (LICOR 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 Licor 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. 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 transmittal and tissue Doppler echocardiography. We found prolongation of the isovolumic 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

<table>
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<tr>
<th>Table 1. Body and Organ Weights</th>
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<tr>
<td>Normal Diet</td>
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<tr>
<td>Body weight, g</td>
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<tr>
<td>Tibia length, mm</td>
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<tr>
<td>Heart weight, mg</td>
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<tr>
<td>Heart weight/tibia length, mg/mm</td>
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<tr>
<td>LV weight, mg</td>
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<tr>
<td>LV weight/tibia length, mg/mm</td>
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<tr>
<td>RV weight, mg</td>
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<td>RV weight/tibia length, mg/mm</td>
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</table>

Values are mean±SEM. HFHS indicates high fat/high sugar; LV, left ventricular; and RV, right ventricular. n=3 to 4.

*P<0.05 vs normal diet.
†P<0.05 vs HFHS diet.
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 5).

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...
are alterations in protein function due to changes in protein
nel, all of which were unaffected by HFHS feeding (Figure V
plasmic reticulum calcium ATPase, the ryanodine receptor,
for several key calcium regulatory proteins including sarco-
calcium handling in this model, we measured mRNA levels
findings are indicative of impaired LV relaxation26 and are
reflective of an increase in left atrial pressure. All of these
Mayo Clinic, Jacksonville, Florida, USA
experimental conditions, both S17834 and resveratrol
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 hypertro-
phic signaling in cardiac myocytes. First, because we32 and
others33 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. Further-
more, we found increased HNE adducts of LKB, a signaling
molecule that has been implicated in the regulation of
myocardial growth. In spontaneously hypertensive rats, Do-
linsky et al28 demonstrated increased LKB-HNE adducts
in the myocardium that were associated with decreased activity
of LKB and its downstream substrate AMPK, leading to
deinhibition 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.28 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 ox-
idative stress as reflected by generalized decreases in nitro-
tyrosine 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,34 has
been noted previously in this model.9,29 Our finding that
S17834 decreased plasma insulin is consistent with similar
observations showing that resveratrol decreases plasma insu-
lin in other models of type 2 diabetes mellitus.35 A decrease
in plasma insulin levels might oppose myocardial hypertro-
phy by decreasing the stimulation of the phosphatidylinositol
3-kinase/Akt/mTOR/p70S6 pathway.28 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>
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<th>Normal Diet</th>
<th>HFHS Diet</th>
<th>HFHS Diet = S17834</th>
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<tbody>
<tr>
<td>Fasting plasma glucose, mg/dL</td>
<td>87.2 ± 7</td>
<td>126.1 ± 10*</td>
<td>92.1 ± 12</td>
</tr>
<tr>
<td>Fasting plasma insulin, µU/mL</td>
<td>8.5 ± 0.5</td>
<td>15.8 ± 2.7*</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>33 ± 4</td>
<td>93 ± 22†</td>
<td>44 ± 7</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/dL</td>
<td>86 ± 10</td>
<td>96 ± 10</td>
<td>101 ± 11</td>
</tr>
<tr>
<td>Plasma free fatty acid, mEq/L</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dL</td>
<td>88 ± 4</td>
<td>126 ± 10†</td>
<td>145 ± 12†</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>
</tr>
</tbody>
</table>

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).

*P < 0.05 vs normal diet.
†P < 0.01 vs normal diet.
‡P < 0.05 vs HFHS diet.

the E/A wave measured by transmitral Doppler. Tissue
Doppler further demonstrated a decrease in E_m, indicative of
slowed LV relaxation and an increase in the ratio of E/E_m,
reflective of an increase in left atrial pressure. All of these
findings are indicative of impaired LV relaxation26 and are
typical of patients with metabolic syndrome.5 Diastolic dys-
function 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 Langen-
dorff perfusion.

The HFHS-fed mouse has been used extensively to study
the metabolic consequences of obesity.8–11 Despite the pop-
ularity 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.5

An important mechanism of diastolic dysfunction is im-
paired myocardial relaxation due to abnormal calcium han-
dling.30 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 calcium chan-
nel, 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 de-
velopment 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.21,28 Likewise,
resveratrol improved diastolic function in mice with type 1
diabetes mellitus due to streptozocin17 or in db/db mice with
type 2 diabetes mellitus.16 In contrast, resveratrol did not
alleviate the extent of LV remodeling after myocardial
infarction.31 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.13

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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 P01 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 employed by a provider of Servier.

References


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**CLINICAL PERSPECTIVE**

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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Data Supplement (unedited) at:
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Supplemental Methods

*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 $2^{\Delta \Delta C_T} = \frac{2^{\Delta C_T\text{target gene}}} {2^{\Delta C_T\text{GAPDH}}}$ 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
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 (E_m) and the ratio of peak early mitral inflow velocity to myocardial peak early diastolic velocity (E/E_m) 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