Resveratrol Treatment of Mice With Pressure-Overload–Induced Heart Failure Improves Diastolic Function and Cardiac Energy Metabolism

Miranda M. Sung, PhD; Subhash K. Das, MSc; Jody Levasseur; Nikole J. Byrne, BSc; David Fung; Ty T. Kim, BSc; Grant Masson, BSc; Jamie Boisvenue; Carrie-Lynn Solty, MSc; Gavin Y. Oudit, MD, PhD, FRCPC; Jason R.B. Dyck, PhD

Background—Although resveratrol has multiple beneficial cardiovascular effects, whether resveratrol can be used for the treatment and management of heart failure (HF) remains unclear. In the current study, we determined whether resveratrol treatment of mice with established HF could lessen the detrimental phenotype associated with pressure-overload–induced HF and identified physiological and molecular mechanisms contributing to this.

Methods and Results—C57Bl/6 mice were subjected to either sham or transverse aortic constriction surgery to induce HF. Three weeks post surgery, a cohort of mice with established HF (% ejection fraction <45) was administered resveratrol (320 mg/kg per day). Despite a lack of improvement in ejection fraction, resveratrol treatment significantly increased median survival of mice with HF, lessened cardiac fibrosis, reduced gene expression of several disease markers for hypertrophy and extracellular matrix remodeling that were upregulated in HF, promoted beneficial remodeling, and improved diastolic function. Resveratrol treatment of mice with established HF also restored the levels of mitochondrial oxidative phosphorylation complexes, restored cardiac AMP-activated protein kinase activation, and improved myocardial insulin sensitivity to promote glucose metabolism and significantly improved myocardial energetic status. Finally, noncardiac symptoms of HF, such as peripheral insulin sensitivity, vascular function, and physical activity, were improved with resveratrol treatment.

Conclusions—Resveratrol treatment of mice with established HF lessens the severity of the HF phenotype by lessening cardiac fibrosis, improving molecular and structural remodeling of the heart, and enhancing diastolic function, vascular function, and energy metabolism. (Circ Heart Fail. 2015;8:128-137. DOI: 10.1161/CIRCHEARTFAILURE.114.001677.)

Key Words: energy metabolism ■ heart failure ■ remodeling ■ resveratrol

Despite existing therapies for heart failure (HF), the current 1-year mortality rate after diagnosis of symptomatic HF remains at 25% to 40%. As such, new strategies to treat this debilitating syndrome and to improve the quality and length of life of patients with systolic HF must be developed. Although recent work has implicated resveratrol, a naturally occurring polyphenol, as a potential treatment of HF, the vast majority of these studies either use resveratrol to prevent the development of HF or inappropriately label modest cardiac dysfunction as HF. As such, despite many claims to the contrary, it has currently not been clearly established whether resveratrol can be used as a treatment for chronic HF.

Clinical Perspective on p 137

An exception to the aforementioned previous studies is the work by Kanamori et al., which showed that resveratrol treatment of mice with myocardial infarction–induced HF reversed adverse remodeling and improved cardiac function, suggesting that resveratrol may be an effective treatment for HF. However, the beneficial effects of resveratrol in this setting were largely attributed to preventing infarct expansion and cell death instead of treating a stable condition. As a result, it remains to be determined whether resveratrol can be used as a treatment for established HF in a model that precludes confounding effects associated with infarct expansion. This is particularly important because the majority of HF patients with symptomatic HF have an established scar, and beneficial therapies must occur independent of preventing infarct expansion.

On the basis of complications associated with rodent models of myocardial infarction–induced HF, we used a transverse aortic constriction (TAC) mouse model of pressure-overload–induced HF that does not involve an infarct and scar tissue formation. This model also allowed us to test the effectiveness of resveratrol treatment in other clinical conditions that...
are common causes of HF, such as pressure overload resulting from hypertension or aortic valvular stenosis and coronary artery disease. Using this model, we show that acute resveratrol treatment improves diastolic function, reduces HF-induced cardiac remodeling and fibrosis, and restores myocardial glucose oxidation rates in mice with HF. These resveratrol-mediated effects are associated with a dramatic improvement in survival of mice with established HF.

Methods

Experimental Animals

This investigation conforms with the guidelines of the Canadian Council on Animal Care and the University of Alberta Animal Policy and Welfare Committee. Male C57Bl/6 mice (7 weeks were obtained from Charles Rivers Laboratory and maintained on a 12:12-hour light–dark cycle (0600:1800 light) with free ad libitum access to food and water for a 1-week acclimatization period. At 8 weeks, mice were randomly assigned into groups and were subjected to sham (n=13) or transverse aortic constriction (TAC; n=54) surgery to induce pressure-overload–induced HF. Three weeks post surgery, 80% of the TAC mice (n=45) were considered to be in HF with ejection fraction (EF) <45% and were randomly allocated into 2 cohorts of mice where the treatment group was administered resveratrol (4 g resveratrol /kg AIN-93G diet; Dyets Inc, Bethlehem, PA; n=22) in their diet, whereas the control group (n=23) received regular diet without resveratrol for an additional 2 weeks. For mice, the dosage of resveratrol was equivalent to ~320 mg resveratrol/kg body weight per day as described previously. These doses equate to plasma levels of 10 to 20 μmol/L in rodents.

Transverse Aortic Constriction Surgery

TAC surgery was performed as described previously. Sham mice underwent the same open-chest procedure as the TAC mice but without aortic banding.

Statistical Analysis

Results are expressed as mean±SEM. Statistical analyses were performed using GraphPad Prism software. Kaplan–Meier survival curves were generated and log-rank test performed to compare curves. Comparisons between groups were performed by Kruskal–Wallis nonparametric ANOVA and Dunn multiple comparison tests. Detailed Materials and Methods are available in the Data Supplement.

Results

Resveratrol Treatment Increases Survival of Mice With HF in the Absence of Changes in Systolic Function or Cardiac Hypertrophy

Three weeks post surgery, mice with established HF (EF<45%; Table 1) were administered diet with ~320 mg resveratrol/kg body weight per day; calculated plasma levels equivalent to patients receiving 150 mg/d) or without resveratrol. Untreated mice with established HF had a median survival of 35 days, whereas resveratrol treatment increased survival to 78 days (Figure 1A), showing that resveratrol treatment significantly improves survival in mice with established left ventricular (LV) pressure overload-induced HF. On the basis of this finding, we performed subsequent experiments at 5 weeks post TAC when all untreated mice were still alive and could be compared with resveratrol-treated mice (Table 1 in the Data Supplement).

At 5 weeks post TAC, mice had significantly reduced EF (Figure 1B) and increased wet lung weight (Table 2) compared with shams. In addition, compared with these sham controls, mice subjected to TAC displayed significant cardiac hypertrophy, including a 2.2-fold increase in LV mass (Figure 1C) and 1.3-fold increase in diastolic LV posterior and interventricular septal wall thickness (Table 2). Interestingly, resveratrol treatment did not improve EF or indexes of ventricular hypertrophy in mice with HF when compared with untreated mice (Figure 1B and 1C). However, although hearts from mice subjected to TAC were markedly dilated as shown by significant increases in end-diastolic LV internal volume and diameter (Figure 1D and 1E), resveratrol seemed to modestly reduce both LV chamber size and end-diastolic and end-systolic volumes by ~15% (Figure 1D and 1E; Table 2). These effects are particularly important because the severity of LV dilatation and remodeling is a strong predictor of long-term survival in humans.

To determine what effect resveratrol has on gene expression of established molecular markers of pathological cardiac hypertrophy and HF (such as anf, bnp, β-mhc, and skα, which are
generally induced in hypertrophied rodent hearts), we measured these in all groups. Cardiac expression of fetal genes, including anf, bnp, β-mhc, and ska, all returned to near-baseline values of sham mice when mice with established HF were administered resveratrol (Figure 1F). This profound reduction in molecular markers of cardiac hypertrophy by resveratrol indicates that improvements at the transcriptional level may occur acutely in response to resveratrol, and that translation of these molecular changes into structural changes of the heart may require more time to manifest. Alternatively, this may suggest that the regression in markers of pathological hypertrophy is accompanied by a switch to adaptive physiological hypertrophy to maintain cardiac output in response to chronic pressure-overload.

**Resveratrol Treatment Improves Diastolic Function of Mice With HF**

Although resveratrol treatment did not have an effect on systolic function, resveratrol did improve diastolic function in mice with established LV pressure-overload–induced HF. Indeed, left atrial (LA) enlargement induced by pressure overload was reduced by 25% with resveratrol treatment (Figure 2A). Because the LA dilates in response to increasing LV pressure, this is a valuable physiological marker of the duration and severity of diastolic dysfunction. Furthermore, resveratrol treatment was found to reduce mitral E/A ratio (Figure 2B) and E/E' (Figure 2C) when compared with untreated TAC mice further confirming improved diastolic function with resveratrol treatment. Together, these data suggest that resveratrol improves atrial function and improves the diastolic filling properties of the LV. These findings underscore the importance that diastolic function plays in HF and are in agreement with the concept that the severity and prognosis of HF are more closely correlated to diastolic filling abnormalities rather than EF.

**Resveratrol Treatment Restores Mitochondrial Protein Content and Promotes Glucose Oxidation in HF**

Studies from both humans and animals suggest that defects in cardiac mitochondrial oxidative phosphorylation and substrate selection contribute to the development and progression of HF. Importantly, we show that protein levels of oxidative phosphorylation complexes were reduced in hearts...
Table 2. Physical Characteristics and Cardiac Morphology and Function in Mice After 2 Weeks of Vehicle or Resveratrol Treatment

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
<th>TAC+Resv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>26.20±0.60</td>
<td>23.96±1.03</td>
<td>25.88±0.76</td>
</tr>
<tr>
<td>Wet lung weight, g</td>
<td>0.15±0.01</td>
<td>0.26±0.03*</td>
<td>0.27±0.04*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>481±20</td>
<td>473±33</td>
<td>481±22</td>
</tr>
<tr>
<td>24-h food intake, g</td>
<td>4.26±0.19</td>
<td>3.52±0.18</td>
<td>3.50±0.18</td>
</tr>
<tr>
<td>24-h food intake/BW</td>
<td>0.16±0.01</td>
<td>0.15±0.01</td>
<td>0.14±0.01</td>
</tr>
</tbody>
</table>

Wall measurements

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
<th>TAC+Resv</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS-diastole, mm</td>
<td>0.79±0.02</td>
<td>1.07±0.02*</td>
<td>1.02±0.04*</td>
</tr>
<tr>
<td>IVS-systole, mm</td>
<td>1.20±0.06</td>
<td>1.21±0.04</td>
<td>1.17±0.05</td>
</tr>
<tr>
<td>LVPW-diastole, mm</td>
<td>0.81±0.03</td>
<td>1.03±0.03*</td>
<td>0.99±0.04*</td>
</tr>
<tr>
<td>LVPW-systole, mm</td>
<td>1.18±0.05</td>
<td>1.15±0.03</td>
<td>1.18±0.05</td>
</tr>
</tbody>
</table>

Systolic function

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
<th>TAC+Resv</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS, %</td>
<td>31.83±2.03</td>
<td>12.41±1.05*</td>
<td>11.90±1.20*</td>
</tr>
<tr>
<td>LVEDV, μL</td>
<td>65.71±4.79</td>
<td>106.00±3.90*</td>
<td>90.72±4.44*</td>
</tr>
<tr>
<td>LVESV, μL</td>
<td>26.73±3.81</td>
<td>78.65±4.26*</td>
<td>66.35±5.09*</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td>19.43±1.26</td>
<td>13.62±0.81*</td>
<td>12.23±0.86*</td>
</tr>
<tr>
<td>SV, μL</td>
<td>41.23±2.20</td>
<td>29.37±1.12*</td>
<td>25.14±2.28*</td>
</tr>
</tbody>
</table>

Diastolic function

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
<th>TAC+Resv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral E velocity, mm/s</td>
<td>746.75±20.20</td>
<td>622.28±56.95</td>
<td>737.25±51.06</td>
</tr>
<tr>
<td>Mitral A velocity, mm/s</td>
<td>473.97±22.77</td>
<td>324.69±36.05</td>
<td>509.81±48.75</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>17.14±0.51</td>
<td>18.24±0.88</td>
<td>18.25±1.08</td>
</tr>
<tr>
<td>NCT, ms</td>
<td>6.11±0.46</td>
<td>10.71±0.83*</td>
<td>10.52±1.05*</td>
</tr>
<tr>
<td>ET, ms</td>
<td>43.33±1.91</td>
<td>51.98±1.91*</td>
<td>49.70±1.66*</td>
</tr>
</tbody>
</table>

n=11 shams; n=10–14 TAC and TAC+Resv. CO indicates cardiac output; ET, ejection time; FS, fractional shortening; HR, heart rate; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; IVS, interventricular septal wall thickness; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVPW, left ventricular posterior wall thickness; Resv, resveratrol; SV, stroke volume; and TAC, transverse aortic constriction.

(Amyl P; Figure 3C) in hearts from HF mice. Interestingly, activation of Akt was unchanged between groups during fasting when plasma insulin levels are low (Figure 3D). However, the blunted insulin-induced Akt activation normally observed in HF was improved with resveratrol treatment (Figure 3E), suggesting that resveratrol improves myocardial insulin sensitivity in the failing heart. Because restored levels of oxidative phosphorylation complexes, AMPK activation, and improved insulin sensitivity induced by resveratrol could contribute to improved myocardial energetic status via the stimulation of glucose metabolism,9.22 we measured oxidative metabolism in ex vivo perfused working hearts. Although both myocardial glucose oxidation (Figure 3F) and fatty acid oxidation (Figure 3G) rates were reduced in the failing heart, resveratrol treatment increased glucose oxidation levels (Figure 3F). Together, these data suggest that improved myocardial energetic status contributes to the improved diastolic function and increased survival observed in resveratrol-treated HF mice.

Resveratrol Treatment Reduces Cardiac Fibrosis and Markers of Cardiac Stress in Mice With HF

Excessive cardiac fibrosis from chronic pressure-overload affects myocardial compliance resulting in increased myocardial stiffness, which is a major determinant of diastolic dysfunction.23,24 Because of this, we investigated the mechanisms responsible for resveratrol improving diastolic function in mice with established HF by examining cardiac fibrosis and matrix remodeling. Masson trichrome staining and immunoblot analysis showed that collagen was dramatically increased in hearts from mice with HF. Importantly, these parameters were reduced with resveratrol treatment (Figure 4A–4C), demonstrating that resveratrol reduces the degree of cardiac fibrosis in mice with established HF. In addition, the expression of cardiac markers of inflammation, such as tumor necrosis factor-α, interleukin-1β, and transforming growth factor-β, were similar between HF mice treated with or without resveratrol (data not shown). In agreement with a reduction in collagen deposition and beneficial cardiac remodeling, transcription markers of fibrosis and remodeling were also significantly reduced in resveratrol-treated HF mice. In fact, numerous genes involved in extracellular matrix and fibrotic remodeling, such as col1α1, col3α1, timp1, timp2, timp3, and timp4, were almost completely returned to baseline levels observed in hearts from untreated sham control mice (Table II in the Data Supplement; Figure 4D and 4E). Furthermore, gene expression of matrix metalloproteinase2 (mmp2), which is known to degrade type I collagen and play an important role in extracellular matrix remodeling, was significantly increased in hearts from TAC mice (Figure 4E), which is consistent with studies of pressure-overload in humans25 and animal models.26,27 In agreement with matrix metalloproteinase-2 being involved in detrimental remodeling, treatment with resveratrol reduced matrix metalloproteinase-2 levels in hearts from mice with HF (Figure 4E). These findings suggest that the ability of resveratrol to lessen cardiac fibrosis and reduce molecular events contributing to LV remodeling may be involved in improved LV diastolic filling properties and reduced LA size in mice with established HF.
Resveratrol Treatment Does Not Alter Silent Information Regulator 1 Expression but Increases Antioxidant Defense Protein Levels in Hearts From Mice With HF

Resveratrol has been shown to be a potent activator of silent information regulator 1 (SIRT1) and as such we investigated whether SIRT1 is altered by resveratrol treatment in HF. Contrary to previous studies, SIRT1 protein levels were neither changed in TAC hearts nor did resveratrol treatment alter SIRT1 levels (Figure 5A), suggesting that resveratrol may not act through the SIRT1 pathway to mediate the beneficial effects of resveratrol in HF. In addition, oxidative stress in cardiac tissue, as assessed by myocardial 4-hydroxy-2-nonenal levels, was not changed in HF mice in the presence of resveratrol when compared with untreated HF mice (Figure 5B). However, cardiac manganese superoxide dismutase protein expression was significantly reduced in HF compared to control and was restored to control levels by resveratrol (Figure 5C). Together, these data suggest that maintaining antioxidant defense enzymes, such as manganese superoxide dismutase, may be a key factor underlying resveratrol-mediated protection against oxidative stress in HF.

Resveratrol Treatment Increases Physical Activity, Insulin Sensitivity, and Improves Vascular Function in Mice With HF

Although reduced physical activity is a hallmark feature of HF, studies have shown that low LVEF do not correlate with...
exercise intolerance in humans with HF and have thus proven to be poor predictors of exercise capacity.\textsuperscript{29,30} Given that diastolic function may be a better determinant of physical inactivity in HF than systolic function,\textsuperscript{31} we assessed whether the improvement in diastolic function by resveratrol treatment had any effect on physical activity. Using the Oxymax laboratory animal monitoring system with $x$, $y$, and $z$-axis activity monitors, we show that mice with HF displayed a decline in overall physical activity as measured by reductions in total levels of rearing, grooming and ambulatory activity, and that this was at least partly restored with resveratrol treatment (Figure 6A).

Because abnormalities in vascular and endothelial function have been proposed to be important contributors to impaired aerobic capacity and the development and progression of HF,\textsuperscript{32,33} we assessed whether resveratrol may indirectly increase physical activity via its ability to improve vascular function\textsuperscript{9,34–36} and ultimately increase oxygen perfusion to the heart and skeletal muscle. Using flow-mediated dilation of the left femoral artery,\textsuperscript{36} we show that mice with established HF failed to increase femoral artery flow velocity compared with sham animals after 5 minutes of ischemia (Figure 6B). In contrast, resveratrol treatment of mice with HF significantly improved flow-mediated vasodilation (Figure 6B). Together, these data show that in the absence of changes in cardiac output, resveratrol treatment improves vascular function in mice with established HF and this may contribute to the increased physical activity observed in resveratrol-treated mice with HF by allowing greater peripheral oxygen delivery that may ultimately increase exercise capacity.

Finally, several lines of evidence point to HF being an insulin-resistant state, with the degree of insulin resistance correlating with disease severity and reduced exercise capacity in patients with HF.\textsuperscript{37,38} Consistent with this, basal and insulin-induced Akt activation in skeletal muscle were reduced in HF mice (Figure 6C and 6D). However, resveratrol treatment...
Interestingly, studies of patients with HF have shown that the degree of diastolic dysfunction and not EF influenced survival.

Consistent with this, resveratrol-treated mice in our current study may reflect this degree of positivity.36,39 However, it is not known whether resveratrol can be used to treat established pressure-overload–induced HF and prevent or reverse cardiac and vascular remodeling induced by HF. Using the TAC mouse model, we made several key findings starting with resveratrol treatment significantly improving median survival of mice with HF. To investigate the potential physiological and molecular mechanisms that could be responsible for this significant survival benefit, we investigated effects of resveratrol on cardiac structure and function. Interestingly, although resveratrol did not improve systolic function, it did improve diastolic function in HF mice, as well as modestly reduced LV end-diastolic volume, LV end-systolic volume, LV chamber diameter, and LA dimensions. Furthermore, resveratrol treatment reduced cardiac fibrosis and molecular markers of cardiac hypertrophy and remodeling that are commonly observed in the failing heart. These latter findings suggest that resveratrol may prevent LV remodeling in the setting of HF and ultimately reduce LV stiffness and improve LV diastolic filling properties.

Because cardiac remodeling is an independent contributor to HF progression, the prevention or regression of adverse cardiac remodeling is considered an important therapeutic target in the treatment of this syndrome. Indeed, several large HF studies with pharmacological or device therapy have shown that a reduction in LVESV of ≥10% signifies a clinically relevant reversal of LV remodeling, which is a strong predictor of lower long-term mortality and cardiovascular events.12,40 Therefore, the 15% reduction in LVESV in the resveratrol-treated HF mice in our current study may reflect this degree of positive LV remodeling. Consistent with this, resveratrol-treated mice have a lower mortality rate and reduced HF symptoms (ie, physical inactivity) compared with untreated HF mice. Interestingly, studies of patients with HF have shown that the degree of diastolic dysfunction and not EF influenced survival rate, suggesting that diastolic dysfunction is an important contributor to HF independent of EF.41,42 Furthermore, mounting evidence also indicates that LA enlargement is associated with a poorer prognosis and is a powerful predictor of outcomes in patients with HF with reduced EF providing additional prognostic information independent from systolic and diastolic function.15 Together, this supports that resveratrol improves survival rates in mice with established HF, in part, by improving diastolic function and reducing LA enlargement.

To understand the molecular mechanisms responsible for resveratrol’s effects in HF, we investigated AMPK, which has been shown to be activated by resveratrol to mediate several beneficial cardiovascular and metabolic effects.2,21 We observed increased activation of cardiac AMPK in our resveratrol-treated HF mice, as well as restored levels of mitochondrial oxidative phosphorylation proteins. Consistent with previous reports showing that total myocardial oxidative metabolism is diminished in the pressure-overloaded heart,10,43 we also found that glucose and fatty acid oxidation are reduced in the failing heart. Although this likely contributes to myocardial energetic deficiency in HF, it is possible that the failing heart switches to using greater endogenous substrates. However, given the fact that the heart has limited endogenous stores of these substrates,44,45 it is likely that these endogenous stores are not sufficient to meet energetic demand. Related to these other metabolic effects, resveratrol treatment of mice with HF was associated with increased myocardial insulin sensitivity and rates of glucose oxidation. Because myocardial relaxation is a highly energy-consuming process where ATP hydrolysis is necessary for myofilament inactivation and active sequestration of calcium by the sarcoplasmic reticulum,46 increased ATP supplied from glucose oxidation may be partially responsible for improved diastolic function in resveratrol-treated HF mice.

Exercise intolerance is widely recognized as a primary symptom in patients with chronic HF, which limits physical activity and reduces quality of life.28 In addition, a growing body of evidence has shown that exercise training benefits the health of HF patients by improving exercise capacity, peak exercise performance, oxygen extraction in the periphery, and reducing cardiac events.27 Because resveratrol has been suggested to...
be an exercise mimetic, we further hypothesized that resveratrol treatment of mice with HF may also improve physical activity and that this may also contribute to improvement in overall health and subsequent improved survival that we observe in these mice. Consistent with our hypothesis, mice with pressure-overload–induced HF displayed reduced overall levels of physical activity and had a shorter life span than sham mice. Importantly, resveratrol treatment greatly improved both of these parameters. Although the reasons for increased physical activity of HF mice treated with resveratrol are not completely clear, it is well known that alterations in coronary and peripheral vascular function, in particular endothelial dysfunction, may also be one of the mechanisms underlying exercise intolerance and lack of physical activity in HF. Consistent with this, we show that mice with HF also possess impaired vascular function. In agreement with our previous findings that resveratrol has vasodilatory properties and improves vascular function, we show that resveratrol improved flow-mediated dilatation of the femoral artery of mice with pressure-overload–induced HF. Overall, we conclude from these data that the ability of resveratrol to improve vascular function in mice with established HF may partly explain how resveratrol improves total physical activity by increasing blood/oxygen/metabolic substrate delivery to the heart and muscle. However, because we have only measured daily spontaneous activity, it remains unknown whether resveratrol can improve exercise capacity in these mice. That said, because studies in healthy mice suggest that resveratrol acts as an exercise mimetic, resveratrol may also be able to improve exercise capacity in mice with HF. An important point is that the treatment, although effective, was only for 2 weeks and it is unclear why the benefits are not sustained and what other mechanisms are acting at later time points. Although TAC has been widely accepted as an appropriate animal model of HF, the model has limited relationship...
to most forms of HF because of the persistent LV pressure-overload not typically left untreated in most patients with HF.

Conclusions
Although we have likely not identified all of the physiological and molecular mechanisms responsible for the beneficial effects of resveratrol on survival and physical activity in mice with LV pressure-overload–induced HF, we show that resveratrol reduces cardiac fibrosis, improves diastolic cardiac function, increases myocardial glucose utilization, and improves peripheral vascular function. Together, the data presented herein suggest that resveratrol has multiple beneficial physiological effects in the setting of HF, and that these cumulative changes may contribute to lessening the detrimental phenotype associated with pressure-overload–induced HF.

Sources of Funding
This work was supported by grants from the Canadian Institute of Health Research to Dr Dyck and Dr Oudit. Dr Sung was supported by an Alberta HEART trainee award. D. Fung was supported by a graduate studentship from Alberta Innovates-Health Solutions. S.K. Das was supported by a graduate studentship from Alberta Innovates-Health Solutions.

Disclosures
None.

References

function, prevents adverse cardiac remodeling, and improves myocardial insulin sensitivity and glucose metabolism. Further-

significantly increase survival rates in mice with established heart failure. Resveratrol treatment improves diastolic cardiac

syndrome and improve health outcomes are needed. We show that the naturally occurring polyphenol, resveratrol, is able to

treatments/interventions have ultimately also increased the incidence and prevalence of heart failure. Despite existing therapies for heart failure,

Although advancements in therapies have improved cardiac mortality from cardiovascular disease, these treatments/interventions

have also increased the incidence and prevalence of heart failure. Despite existing therapies for heart failure, the current 1-year mortality rate after diagnosis of symptomatic heart failure remains high, and new strategies to treat this syndrome and improve health outcomes are needed. We show that the naturally occurring polyphenol, resveratrol, is able to significantly increase survival rates in mice with established heart failure. Resveratrol treatment improves diastolic cardiac function, prevents adverse cardiac remodeling, and improves myocardial insulin sensitivity and glucose metabolism. Furthermore, several noncardiac aspects of the heart failure syndrome, such as peripheral insulin sensitivity, vascular function, and physical activity, were improved with resveratrol treatment. Overall, our preclinical data suggest that resveratrol supplementation may be a potential therapy to assess in patients with systolic heart failure.
Resveratrol Treatment of Mice With Pressure-Overload–Induced Heart Failure Improves Diastolic Function and Cardiac Energy Metabolism

Miranda M. Sung, Subhash K. Das, Jody Levasseur, Nikole J. Byrne, David Fung, Ty T. Kim, Grant Masson, Jamie Boisvenue, Carrie-Lynn Soltys, Gavin Y. Oudit and Jason R.B. Dyck

Circ Heart Fail. 2015;8:128-137; originally published online November 13, 2014; doi: 10.1161/CIRCHEARTFAILURE.114.001677

Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/8/1/128

Data Supplement (unedited) at:
http://circheartfailure.ahajournals.org/content/suppl/2014/11/13/CIRCHEARTFAILURE.114.001677.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Materials

Primary antibodies were purchased from Cell Signaling Technology, Santa Cruz Biotechnology, EMD Millipore or Mitosciences; human recombinant insulin (Novolin) was from Novo Nordisk Canada Inc. and trans-resveratrol was purchased from Lalilab (Durham, NC).

Transverse aortic constriction (TAC) surgery

In brief, male 8 week-old mice were anesthetized by an intraperitoneal (i.p.) injection of a cocktail of ketamine (100mg/kg) and xylazine (10mg/kg), intubated, and connected to a mouse ventilator (MiniVent, Harvard Apparatus, Holliston, MA). Following midline sternotomy, a double blunted 27-gauge needle was tied to the aorta between the innominate and left common carotid arteries using 6/0 silk suture. The needle was then removed and the chest and skin were sutured closed using 6/0 silk continuous pattern suture. One week post-surgery, trans-stenotic gradients were measured by pulsed-wave Doppler flow studies to confirm similar pressure overload in all groups of mice with pressure gradients ranging from 50-70 mmHg.

Echocardiography and tissue doppler imaging

Mice were mildly anesthetized using 1.5% isoflurane, and transthoracic echocardiography was performed using a Vevo 770 high resolution imaging system equipped with a 30-MHz transducer (RMV-707B; VisualSonics) as described previously1.

Flow mediated dilation after temporary femoral artery ischemia
Mice subjected to either sham or TAC surgery were mildly anesthetized with isoflurane (4% induction and 0.75% maintenance). The femoral artery was visualized with a 40-MHz transducer (RMV-704; VisualSonics, Toronto, Canada) and identified by its characteristic flow pattern. A 0.5 cm vertical incision was made in the left regio femoris, the femoral artery and vein were separated and 3 cm 7/0 nylon (Ethicon) suture was passed around the artery upstream of the site visualized by ultrasound. The site was then closed using 6/0 silk (Ethicon) simple continuous suture and the 7/0 nylon suture tails externalized. Reproducible ischemia and reperfusion was achieved by attaching a bulldog clamp to the exteriorized 7/0 nylon ties and using the weight of the clamp as the constant. Flow arrest was confirmed by abrogation of the Doppler signal. Velocity of flow was monitored at baseline (pre) and after 5 min of ischemia the hindlimb was reperfused by release of the occluder. Reactive hyperemia was monitored by measuring blood flow velocity through the femoral artery at 1 min of reperfusion post-ischemia (post) by Doppler flow measurements.

**Ex vivo heart perfusion**

Hearts were perfused in the working heart mode at 11.5 mm Hg preload and 50 mm Hg afterload with Krebs–Henseleit buffer containing 0.8 mmol/L oleate prebound to 3% delipidated bovine serum albumin (BSA), 5 mmol/L glucose, and 50 μU/mL insulin. At the end of 60 min aerobic perfusion, hearts were snap frozen in liquid N$_2$ with a Wollenberger clamp and stored at -80°C until analysis. Oleate and glucose were labeled using [9,10-3H]oleate and [U-14C]glucose for the metabolic determination of fatty acid and glucose oxidation, respectively.

**Metabolic analysis in vivo**

Total physical activity was measured using the Comprehensive Lab Animal Monitoring System (CLAMS/Oxymax, Columbus Instruments, Columbus, OH) and was calculated by
adding Z counts (rearing or jumping) to total counts associated with ambulatory movement and stereotypical behavior (grooming and scratching) as described previously\textsuperscript{4}.

**Histology**

Masson’s trichrome staining of paraffin-embedded apical heart sections were visualized using a Leica DMLA microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Retiga 1300i FAST 1394 CCD camera (QImaging, Surrey, BC, Canada) as described previously\textsuperscript{5}.

**Gene expression**

Cardiac mRNA expression was determined by real time PCR by using Taqman probes. Total RNA was extracted from flash frozen ventricular tissue by using TRIzol RNA extraction method\textsuperscript{6}. 1µg of RNA was subjected to reverse transcription to synthesize cDNA. Real time PCR was performed by taking 5µl of suitable cDNA dilutions from unknown, standard (brain cDNA) and 8µl Taqman master-mix (includes-primers + Probes) that were then loaded on white 384 Light cycler\textsuperscript{®} 480 multi well plates supplied from Roche with 18s rRNA as internal control. Samples are loaded in triplicate and the data was analyzed by Light cycler\textsuperscript{®} 480 machine from Roche. Gene expression of cardiac disease markers (ANF, BNP, MHC-β, α-SKA), fibrosis (COL-I, COL-III, TGF-β), inflammation (IL-1β, TNF-α) and extracellular matrix (ECM) remodeling (TIMP-1, TIMP-2, TIMP-3, TIMP-4, MMP-2) were analyzed (Supplemental Table 2).

**Insulin signaling studies *in vivo***

A subset of sham, TAC control and Resv-treated TAC mice were fasted for 6 hours and then given i.p. injections with human recombinant insulin (5U/kg). Mice were sacrificed by decapitation 10 min post-injection, and heart and gastrocnemius muscle was rapidly removed, freeze-clamped in liquid nitrogen and stored at −80°C until time of analysis.
Immunoblot analysis

Denatured samples of ventricular or gastrocnemius muscle homogenates were subjected to SDS-PAGE and proteins were transferred onto a nylon membrane. Subsequent immunoblotting to determine expression of target proteins was employed. Immunoblots were developed using the Western Lightning Plus-ECL enhanced chemiluminescence substrate (Perkin Elmer, Waltham, MA). Densitometric analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD). Densitometric data were corrected against tubulin, actin or respective total protein levels as a loading control.
SUPPLEMENTAL TABLES

Supplemental Table 1. Comparison of echocardiographic data from mice pre- and post-Resveratrol treatment

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAC</td>
<td>TAC + Resv</td>
</tr>
<tr>
<td><strong>Wall Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corr. LV mass, mg</td>
<td>122.5 ± 4.8</td>
<td>120.2 ± 7.9</td>
</tr>
<tr>
<td>IVS–diastole, mm</td>
<td>0.93 ± 0.02</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>LVPW–diastole, mm</td>
<td>0.90 ± 0.02</td>
<td>0.93 ± 0.04</td>
</tr>
<tr>
<td>LVID–diastole, mm</td>
<td>4.19 ± 0.04</td>
<td>4.05 ± 0.07</td>
</tr>
<tr>
<td>LA diameter, mm</td>
<td>2.34 ± 0.11</td>
<td>2.37 ± 0.14</td>
</tr>
<tr>
<td><strong>Systolic Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF, %</td>
<td>38.12 ± 1.70</td>
<td>36.24 ± 3.54</td>
</tr>
<tr>
<td>FS, %</td>
<td>18.66 ± 0.97</td>
<td>17.30 ± 1.94</td>
</tr>
<tr>
<td>LVEDV, µl</td>
<td>78.25 ± 1.57</td>
<td>72.35 ± 3.04</td>
</tr>
<tr>
<td>LVESV, µl</td>
<td>47.92 ± 1.65</td>
<td>45.55 ± 3.96</td>
</tr>
<tr>
<td><strong>Diastolic Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral E/A ratio</td>
<td>3.50 ± 0.51</td>
<td>3.48 ± 0.56</td>
</tr>
<tr>
<td>E/E’</td>
<td>43.20 ± 2.91</td>
<td>46.63 ± 4.29</td>
</tr>
</tbody>
</table>

N=10-14 TAC and TAC + Resv. * P<0.05 vs. TAC pre-treatment; † P<0.05 vs TAC + Resv pre-treatment;
‡ P<0.05 vs. TAC post-treatment by Kruskal Wallis and Dunn’s multiple comparison test.

Abbreviations: IVS, interventricular septal wall thickness; LVPW, left ventricular posterior wall thickness; LVID, left ventricular internal diameter; EF, ejection fraction; FS, fractional shortening; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume.
Supplemental Table 2. Primers used in real time PCR

<table>
<thead>
<tr>
<th>gene</th>
<th>Type</th>
<th>Sequence</th>
</tr>
</thead>
</table>
| ANF    | Forward: 5'GGA GGA GAA GAT GCC GGT AGA-3'  
          Reverse: 5'GCT TCC TCA GTC TGC TCA CTC A-3'  
          Probe: 5'FAM-TGA GGT CAT GCC CCC GCA GG-TAMRA-3' |
| BNP    | Forward: 5'CTG CTG GAG CTG ATA AGA GA-3'  
          Reverse: 5'TGC CCA AGG GTC CAG TCT GAG AT-3'  
          Probe: 5'FAM-CTC AAG GCA GCA CCC TCC GGG-TAMRA-3' |
| β-MHC  | Forward: 5'GTG CCA AGG GCC TGA ATG AG-3'  
          Reverse: 5'GCA AAG GCT CCA GGT CTG A-3'  
          Probe: 5'FAM-ATC TTG TGC TAC CCA GCT CTA A-TAMRA-3' |
| A-SKA  | Forward: 5'CAG CCG GCG CCT GTT-3'  
          Reverse: 5'CCA CAG GCC TTT GTT TGA AAA-3'  
          Probe: 5'FAM-TGA CGT GTA CAT AGA TTG ACT TG TTT ACC TCA TTT TG-TAMRA -3' |
| IL-1β  | Forward: 5'AACCTGCTGGTGTGTGACGT-3'  
          Reverse: 5'CAGCAGGGCTTTTTTTGTTG-3'  
          Probe: 5'FAM-TTAGACAGCTGCACTACAGGCTCCGAGATG-TAMRA-3' |
| TNF-α  | Forward: 5'ACAAGGCTGCCCGACTAC-3'  
          Reverse: 5'CTTCTCTGATGAGATAGCAAATC-3'  
          Probe: 5'FAM-TGCTCTCACCACCGTCAGC-TAMRA-3' |
| Pro-Collagen-I | Forward: 5'CTTCACCTACAGCACCCTTGTG-3'  
          Reverse: 5'TGACTGTCTTGGCCCAAAGTTC-3'  
          Probe: 5'FAM-CTGCACGAGTCAACC-TAMRA-3' |
| Pro-Collagen-III | Forward: 5'TGTCTTTTGGATGACATAATCTG-3'  
          Reverse: 5'ATTGGGTACCTGCTGAGGGA-3'  
          Probe: 5'FAM-ATGAGGAGCCTAGACT-TAMRA-3' |
| TGF-β  | Forward: 5'CCTGCAAGACCACATCGACATG-3'  
          Reverse: 5'ACAGGATCTGGCCACGGAT-3'  
          Probe: 5'FAM-CTGGTGAAACGGAAGCGCATCGA-TAMRA-3' |
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP 1</td>
<td>5'-CATGGAAAGCCTCTCTGGATATG-3'</td>
<td>5'-AAGCTGCAGGCACCTGATGTG-3'</td>
<td>5'-FAM-CTCATACGGGCCCCTAAAGGAAC-TAMRA-3'</td>
</tr>
<tr>
<td>TIMP 2</td>
<td>5'-CCAGAAGAGAGCTGATGAACCA-3'</td>
<td>5'-GTCCATCCAGGCACCTCATC-3'</td>
<td>5'-FAM-ACTCGCTGTCCCATGATCTCCTTG-C-TAMRA-3'</td>
</tr>
<tr>
<td>TIMP 3</td>
<td>5'-GGCCTCAATTACCGCTACACCA-3'</td>
<td>5'-CTGATAGCCAGGGTACCCAAA-3'</td>
<td>5'-FAM-TGCTACTACTTGCCCCTTGTGACCTCCA-TAMRA-3'</td>
</tr>
<tr>
<td>TIMP 4</td>
<td>5'-TGCAGAGGGGAGCTGAAAC-3'</td>
<td>5'-GGTACATGGCGACTGACATGCA-3'</td>
<td>5'-FAM-CCACCAGAACTGTAGCTGGCCGAAATC-TAMRA-3'</td>
</tr>
<tr>
<td>MMP-2</td>
<td>5'-AATCGATGAGCGGAGATGTG-3'</td>
<td>5'-TGGCATGGCCGACTCA-3'</td>
<td>5'-TCTGTCTGAACCTTAGATATGCTATTCCCTCG-3'</td>
</tr>
<tr>
<td>18S r RNA</td>
<td>Forward:</td>
<td>Reverse:</td>
<td>Mm03928990_g1*</td>
</tr>
<tr>
<td>Probe</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SUPPLEMENTAL REFERENCES

1. Sung MM, Koonen DP, Soltys CL, Jacobs RL, Febbraio M, Dyck JR. Increased CD36 expression in middle-aged mice contributes to obesity-related cardiac hypertrophy in the absence of cardiac dysfunction. *J Mol Med (Berl).* 2011;89:459-469


