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Resveratrol Prevents the Development of Pathological Cardiac Hypertrophy and Contractile Dysfunction in the SHR Without Lowering Blood Pressure

Sijo J. Thandapilly[1], Peter Wojciechowski[1], John Behbahani[2], Xavier L. Louis[1], Liping Yu[1], Danijel Juric[1], Melanie A. Kopilas[2], Hope D. Anderson[2] and Thomas Netticadan[1]

BACKGROUND
Cardiac hypertrophy is a compensatory enlargement of the heart in response to stress such as hypertension. It is beneficial in reducing stress placed on the heart. However, when the stress is of a chronic nature, it becomes pathological and leads to cardiac dysfunction and heart failure. Current treatments for hypertension and heart failure have proven beneficial but are not highly specific and associated with side effects. Accordingly, there is an important need for alternative strategies to provide safe and effective treatment.

METHODS
Ten-week-old male spontaneously hypertensive rats (SHRs) and Wistar–Kyoto (WKY) rats were treated with resveratrol (2.5 mg/kg/day) for a period of 10 weeks. Systolic blood pressure, and cardiac structure and function were measured in all groups at different time points of resveratrol treatment. Oxidative stress was also determined in all groups after 10 weeks of resveratrol treatment.

RESULTS
SHRs were characterized with high blood pressure and concentric hypertrophy from 15 weeks of age. Cardiac functional abnormalities were also evident in SHR from 15 weeks onwards. Resveratrol treatment significantly prevented the development of concentric hypertrophy, and systolic and diastolic dysfunction in SHR without lowering blood pressure. Resveratrol also significantly reduced the oxidative stress levels of cardiac tissue in SHR.

CONCLUSIONS
Resveratrol treatment was beneficial in preventing the development of concentric hypertrophy and cardiac dysfunction in SHR. The cardioprotective effect of resveratrol in SHR may be partially mediated by a reduction in oxidative stress. Thus, resveratrol may have potential in preventing cardiac impairment in patients with essential hypertension.

Keywords: *blood pressure; cardiac hypertrophy; hypertension; resveratrol; SHR*

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Heart failure is a leading cause of mortality worldwide.[1,2] It is a complicated, multifactorial syndrome resulting from the heart's inability to pump sufficient blood to meet the metabolic demands of tissues. The development of heart failure is secondary to diseases such as hypertension, ischemic heart disease, valvular heart disease, or cardiomyopathy. Heart failure is always preceded by cardiac hypertrophy, which is the enlargement of heart in response to stress (pressure or volume overload). It is an adaptation that is beneficial to the stressed heart in the initial stages as it helps to overcome the stress placed on it. If hypertrophy prolongs, the heart will no longer be able to compensate the stress and will enter into a decompensated stage. This transition from compensated to decompensated stage is often characterized by the presence of cardiac fibrosis, arrhythmia, and apoptosis. This will eventually lead to irreversible functional deterioration and heart failure.[2,3] Current treatments available for patients with heart failure include use of β-adrenergic receptor blockers, angiotensin-converting enzyme inhibitors, and angiotensin-receptor blockers.[4,5] These therapies are not highly specific and often result in a number of side effects.[6] Accordingly, it is imperative to explore alternate strategies to provide safe and effective treatment for heart failure.

Hypertension induces pressure overload resulting in cardiac hypertrophy. Hypertension is a major independent risk factor for the development of heart failure.[7,8] Different plant extracts and phytochemicals have been recently screened for antihypertensive and antihypertrophic properties.[9,10] Cardioprotection by resveratrol (*trans*-3′, 4′, 5-trihydroxystilbene), a polyphenol found predominantly in grapes and berries, or its analogue has been documented in different experimental settings of pressure overload.[11,12] However, no study has examined the effects of res-

[1]Heart Failure Research Laboratory, Canadian Centre for Agri-Food Research in Health and Medicine, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada; [2]Vascular Biology Laboratory, Canadian Centre for Agri-Food Research in Health and Medicine, Department of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada. Correspondence: Thomas Netticadan (tnetticadan@sbrc.ca)

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veratrol in preventing chronic changes in cardiac structure and function due to essential hypertension. Therefore, we examined the effects of resveratrol in spontaneously hypertensive rats (SHRs), an appropriate model of essential hypertension.

METHODS

The experimental protocols used in this article were approved by the University of Manitoba Animal Care Committee and are in agreement with the Canadian Council on Animal Care and Use of Experimental Animals.[13]

Animal model. Ten-week-old male SHR and their controls Wistar–Kyoto (WKY) rats obtained from Charles River, St Constant, Quebec, Canada were used in this study. Animals were acclimatized in temperature and humidity-controlled rooms with a 12-h dark and 12-h light period cycle throughout the study.

Treatment and examinations. Ten-week SHR and WKY were treated daily by oral gavage with resveratrol (2.5 mg/kg/day), a dose previously established by us,[12] for a period of 10 weeks.

Measurement of blood pressure. Systolic blood pressures were measured in SHR and WKY rats starting at 0 weeks, 5 weeks, and 10 weeks of resveratrol treatment using the NonInvasive Blood Pressure Measurement System (CODA) equipped with volume pressure recording (Kent Scientific, Torrington, CT). A total of four groups were examined—WKY, resveratrol-treated WKY, SHR, and resveratrol-treated SHR.

Assessment of cardiac structure and function. Cardiac structure and function were assessed by echocardiography in 10-week SHR and WKY rats starting at 0 weeks, 5 weeks, and 10 weeks of resveratrol treatment.

Examination of cardiac structure in vivo. Two-dimensional-guided (2D) M-mode echocardiography was used to examine cardiac structure as described by us earlier.[14] The following parameters were measured: left ventricular (LV) internal dimensions at diastole and systole, LV posterior wall thickness at diastole and systole, and interventricular septum thickness at diastole and systole.

Measurement of cardiac function in vivo. Two-dimensional-guided M-mode echocardiography and pulse-wave Doppler echocardiography were used to assess cardiac function as described by us earlier.[14] Contractile parameters of systolic function such as LV ejection fraction and cardiac output were assessed by 2D-guided M-mode echocardiography. Diastolic function was assessed by measuring the isovolumic relaxation time (IVRt) using pulse-wave Doppler echocardiography.

Measurement of lipid peroxidation levels. The degree of lipid peroxidation was assessed by measuring malondialdehyde levels in the homogenized LV tissue and blood plasma using the OxiSelect TBARS Assay Kit (Cell Biolabs, San Diego, CA). The thiobarbituric acid reactive substances (TBARS) values in tissue and plasma were expressed as nmol/mg of protein and nmol/ml of plasma, respectively.

Measurement of TAS. The total antioxidant status (TAS) of the blood plasma was determined using the TAS assay kit (Randox Laboratories, Crumlin, UK) according to the manufacturer's protocol. Plasma TAS values were expressed as μmol/ml of plasma.

Statistical analysis. All values are expressed as means ± s.e. One-way analysis of variance was used to analyze variations between the means of the groups. Significant values are defined as $P < 0.05$. When significance was obtained, one-way analysis of variance was followed by Tukey post hoc test.

RESULTS

General observations

There was a significant increase in the heart-to-tibia length ratio in the 20-week-old SHR in comparison to their age-matched WKY controls; this increase was significantly prevented after 10 weeks of resveratrol treatment (**Figure 1a**). Treatment with resveratrol did not affect 20-week WKY.

Blood pressure

Blood pressure measurements were conducted in 10, 15, and 20-week SHR and their age-matched WKY, treated with and without resveratrol. Systolic blood pressure was significantly increased in SHR at all time points, when compared to their age-matched WKY controls. Treatment with resveratrol did not significantly lower systolic blood pressure in SHR (**Figure 1b**).

Cardiac structure

Echocardiographic analysis of cardiac structure was carried out in 10, 15, and 20-week SHR and their age-matched WKY, treated with and without resveratrol. M-mode echocardiography showed no changes in cardiac structure in 10 and 15-week SHR (in comparison to their age-matched WKY controls). However, 20-week SHR exhibited a significant increase in the interventricular septum and LV posterior wall thickness when compared to their age-matched WKY controls; this increase was significantly reduced after 10 weeks of resveratrol treatment (**Figure 1c,d**). Twenty-week SHR did not exhibit any change in LV internal dimension (in comparison with WKY controls) (**Figure 1e**).

Cardiac function

Echocardiographic analysis of cardiac function was carried out in 10, 15, and 20-week SHR and their respective age-matched WKY, treated with and without resveratrol. A significant increase in the diastolic functional parameter, IVRt, was observed in 20-week SHR but not in 10- and 15-week SHR (in comparison with their respective WKY controls); resveratrol treatment prevented this elevation (**Figure 1h**). There was also a significant decrease in the systolic functional parameter ejection fraction in 20-week SHR; resveratrol treatment

significantly prevented this decrease (Figure 1f). Treatment with resveratrol did not affect IVRt and ejection fraction in 20-week WKY. Cardiac output was unchanged in all groups at all time points of the study (Figure 1g).

Oxidative stress

Concentrations of TBARS were significantly elevated in the LV tissues and blood plasma of 20-week SHR when compared to the age-matched WKY controls (Figure 2a,b). Resveratrol

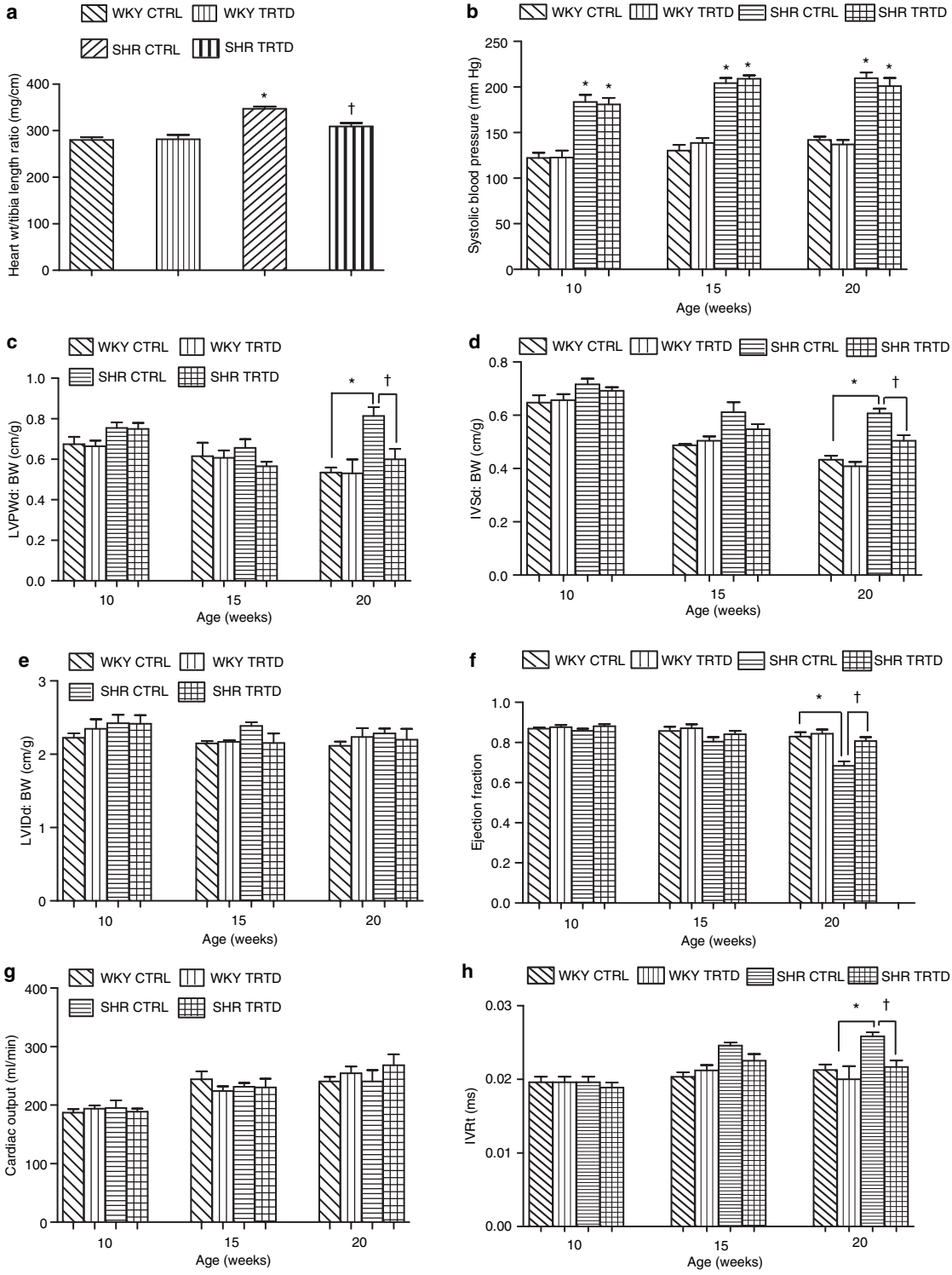

Figure 1 | Effects of resveratrol on cardiovascular parameters in WKY rats and SHR. (**a**) Heart weight/tibia length ratio in 20-week WKY and SHR treated with and without resveratrol. (**b**) Analysis of blood pressure, (**c**) left ventricular posterior wall thickness at diastole (LVPWd), (**d**) interventricular septal wall thickness at diastole (IVSd), (**e**) left ventricular internal dimension at diastole (LVIDd), (**f**) ejection fraction, (**g**) cardiac output, and (**h**) isovolumic relaxation time (IVRt) in 10-, 15-, and 20-week-old WKY and SHR with or without resveratrol treatment. BW, body weight; CTRL, control; SHR, spontaneously hypertensive rat; TRTD, treated; WKY, Wistar–Kyoto. Data are mean ± s.e. $n = 4–8$. *$P < 0.05$ vs. WKY; †$P < 0.05$ vs. SHR CTRL.

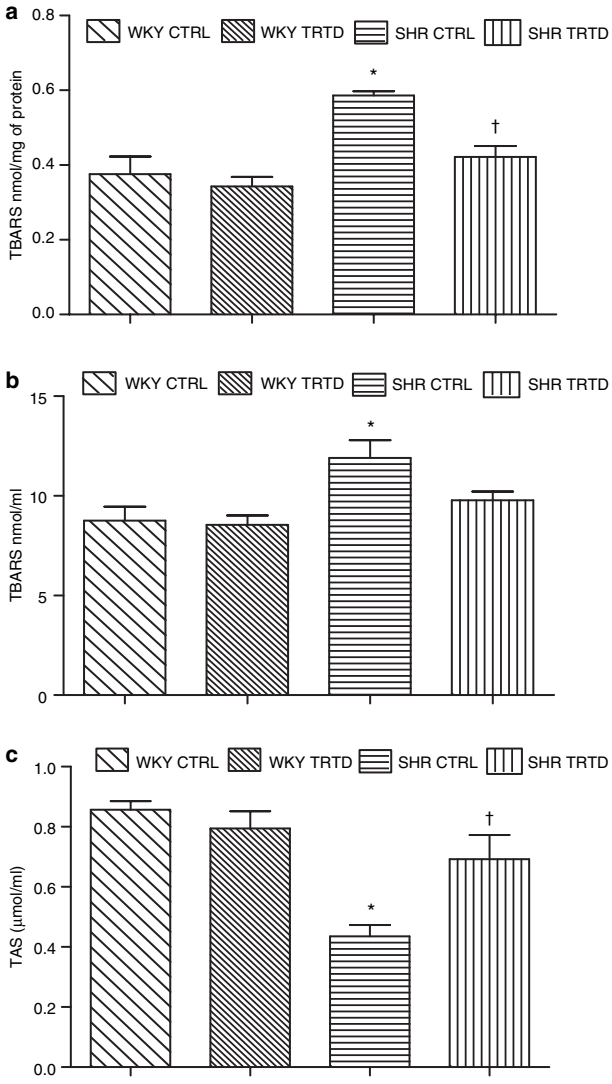

Figure 2 | Effects of resveratrol on oxidative stress in WKY rats and SHR. (**a**) TBARS levels in the heart tissues of 20-week WKY and SHR treated with and without resveratrol. (**b**) TBARS concentration in blood plasma of 20-week WKY and SHR treated with and without resveratrol. (**c**) Total antioxidant status (TAS) in blood plasma of 20-week WKY and SHR treated with and without resveratrol. CTRL, control; SHR, spontaneously hypertensive rat; TBARS, thiobarbituric acid reactive substances; TRTD, treated; WKY, Wistar–Kyoto. Data are mean ± s.e. $n = 3–5$. *$P < 0.05$ vs. WKY; †$P < 0.05$ vs. SHR CTRL.

treatment significantly reduced the elevation in TBARS in the LV tissues of 20-week SHR (**Figure 2a**). There was also a trend toward lowering of the increased TBARS levels by resveratrol treatment in blood plasma of SHR (**Figure 2b**).

On the other hand, a significant decrease was observed in the TAS of blood plasma in 20-week SHR when compared to age-matched WKY controls (**Figure 2c**). The treatment with resveratrol significantly recovered the total antioxidant capacity in 20-week SHR (**Figure 2c**).

DISCUSSION

Regression of pressure overload–induced cardiac hypertrophy and its deleterious consequences on heart function have been reported by us previously in resveratrol-treated abdominal aortic-banded rats.[12] Li *et al.* also reported prevention of cardiac structural and functional alterations by resveratrol treatment in another experimental model of pressure overload created by transverse aortic constriction.[15] Thus, it appears that resveratrol is beneficial in treating pathological pressure overload conditions that include hypertension.

Although the abdominal aortic-banded rat and the transverse aortic constricted rat are experimental models of pressure overload, they do not mimic pressure overload induced by essential hypertension. In this study, we used a suitable model of essential hypertension, the SHR that develop hypertension in very early stages of life as a consequence of genetic, hemodynamic, vascular, renal, and neurohormonal alterations.[16] These rats also develop cardiac hypertrophy gradually in response to the progressive hypertensive disease and not abruptly as a consequence of the surgical procedure. Thus, the occurrence and nature of LV hypertrophy in SHR may resemble development of LV hypertrophy in human secondary to systemic hypertension.[17,18] In the present study, we demonstrate that resveratrol treatment is beneficial in preventing the development of cardiac hypertrophy and cardiac dysfunction due to essential hypertension.

Recently, Dolinsky *et al.*[19] reported that short-term (2-week) administration of resveratrol prevented cardiac structural and functional alterations in 14-week SHR. It is very important to point out that Dolinsky *et al.*[19] presented data on SHR rats (treated with and without resveratrol) only, but not on control Wistar rats (treated with and without resveratrol), making it impossible to determine the development of cardiac structural and functional alterations in SHR, as well as the effects of resveratrol treatment on these parameters in SHR. Furthermore, there was no significant difference observed (in Dolinsky *et al.*'s study) in most of the functional parameters (ejection fraction and IVRt) in SHR treated with resveratrol (when compared to untreated SHR). In view of these serious limitations in Dolinsky *et al.*'s study, our study is the first to report that administration of resveratrol prevented development of pathological cardiac hypertrophy and overt cardiac dysfunction in 20-week-old SHR.

In the present study, systolic blood pressure was significantly elevated in the SHR group starting from 10 weeks of age. Development of concentric hypertrophy was evident from 15 weeks of age (in SHR), as characterized by increased thickness of the ventricular walls (interventricular septum and LV posterior wall) with no change in chamber dimension (LV internal dimension). Systolic and diastolic dysfunctions appeared from 15 weeks of age (in SHR), as characterized by a decline in fractional shortening and an elevation of IVRt, respectively. However, cardiac output was not altered at all time points despite increased blood pressure in SHR; this may be due to the relative increase in arterial peripheral resistance. These results are consistent with the findings of previous studies[20,21] and validate the model used in this study.

In the present study, treatment with resveratrol prevented the development of pressure overload–induced cardiac hypertrophy

and cardiac dysfunction in SHR without removing the actual stress (hypertension) placed on the heart. The ineffectiveness of resveratrol in lowering blood pressure is consistent with previously reported studies in SHRs.[19,22] In other models of pressure overload such as transverse aortic constriction and partially nephrectomized rats, it has been reported that the antihypertrophic effects of resveratrol are partially mediated by a reduction in blood pressure.[11,15] One of the reasons for the observed discrepancy of the effects of resveratrol in lowering blood pressure may be the nature of hypertension in these models—local (transverse aortic constriction and abdominal aortic constriction) vs. systemic (SHR), as well as the strong genetic predisposition in the latter group. On the basis of the results obtained in the present study, we speculate that resveratrol has a direct antihypertrophic effect on the heart. This view is consistent with other reports showing reversal of cardiac hypertrophy in SHR without lowering blood pressure by Matsuoka *et al.*[23] and Tsutsui *et al.*,[24] also suggesting a direct effect of resveratrol on the heart. The underlying mechanisms already reported include a reduction of oxidative stress,[25] stimulation of nitric oxide,[11] or activation of SIRT1 (ref. 26). It is well established that oxidative stress is one of the main causes of development of pathological cardiac hypertrophy in SHR.[27] In this study, we found that resveratrol significantly reduced oxidative stress in SHR; this reduction was consistent with a recovery in the total antioxidant levels in SHR. Thus, the alleviation of the oxidative stress may be one of the mechanisms by which resveratrol prevents the development of pathological hypertrophy and cardiac dysfunction in SHR.

In conclusion, we report for the first time that resveratrol is beneficial in preventing the development of concentric hypertrophy and contractile dysfunction in SHR without affecting blood pressure. Thus, resveratrol may have potential in preventing functional damage to the heart caused by essential hypertension. Accordingly, resveratrol in combination with a blood pressure lowering agent may be useful in treating patients with essential hypertension.

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