Resveratrol Arrests and Regresses the Development of Pressure Overload But Not Volume Overload-Induced Cardiac Hypertrophy in Rats

by

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A Thesis submitted to the Faculty of Graduate Studies of

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ABSTRACT

Heart failure is a multi-factorial syndrome with poor prognosis and is a leading cause of mortality worldwide. Heart failure, however, is preceded by cardiac hypertrophy, a compensatory enlargement of the heart in response to cardiac stress, such as pressure overload (PO) or volume overload (VO). Despite current pharmacological treatments of cardiac hypertrophy and heart failure, such as diuretics, inhibitors of the renin-angiotensin-aldosterone system and β-adrenergic receptor blockers, the rate of morbidity associated with heart failure is still high. Nutraceuticals and functional foods are rapidly evolving as potential treatment for various human pathologies. In this context, resveratrol, a polyphenol with antioxidant properties that is found in grapes and berries, has been demonstrated to regress PO-induced cardiac hypertrophy in rats; however, its effects on VO are unknown. We hypothesize that resveratrol will be able to arrest and regress development of PO and VO-induced cardiac hypertrophies. The objectives of this study were to test the efficacy of resveratrol in arresting the development of cardiac hypertrophy and in regressing developed cardiac hypertrophy resulting from PO and VO.

Sprague Dawley rats were subjected to aortocaval shunt and abdominal aortic banding to create VO and PO, respectively. Sham operated rats served as controls. All rats were maintained for 28 days. Resveratrol was administered by oral gavage at 2.5 mg/kg body weight/day. Treatment was initiated either 2 days post-surgery to test for arrested development of PO and VO cardiac hypertrophy (stage 1), or 14 days post-surgery to test for regression of PO and VO cardiac hypertrophy (stage 2). Cardiac structure and function were assessed by echocardiography at 2, 14 and 28 days post-surgery to evaluate the progression and treatment of PO and VO. PO induced concentric

hypertrophy with increased left ventricular (LV) wall thickness, resulting in diastolic dysfunction. VO resulted in eccentric hypertrophy with LV dilation along with increased cardiac output and improved diastolic function. Resveratrol treatment significantly arrested early changes in cardiac structure and function in PO, but not VO rats. Treatment with resveratrol also significantly regressed changes in cardiac structure and function after it developed in PO but not VO rats. These results demonstrate a potential for resveratrol in the treatment of established cardiac hypertrophy due to PO but not due to VO.

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I. INTRODUCTION

Heart failure is a multi-factorial syndrome that is a leading cause of hospitalization and mortality worldwide [1, 2]. According to statistical analysis by the World Health Organization, fatalities from heart failure will rise to more than 20 million per year by 2020 [3]. Cardiac hypertrophy is the enlargement of the myocardial tissue and is an adaptive mechanism to maintain adequate cardiac function in the presence of chronic pathological stress [4-7]. This hypertrophic growth manifests in two ways: (a) concentric hypertrophy, which is caused by chronic pressure overload (PO) and leads to increased wall thickness, and (b) eccentric hypertrophy, which is caused by chronic volume overload (VO) and results in dilation and thinning of the heart wall [4-7]. Although this myocardial enlargement is initially beneficial, prolonged hypertrophy has deleterious consequences to heart function which can lead to heart failure.

To date, many pharmaceutical agents, such as inhibitors of the renin-angiotensin-aldosterone system (ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; AA, aldosterone antagonists), β-blockers, and diuretics, have been used to treat myocardial hypertrophy and early stages of heart failure. These therapies have proven to be moderately successful but the incidence of heart failure is on the rise [8-10]. In this regard, there is an important need for alternative strategies to prevent and reverse hypertrophy before it develops into heart failure. One such avenue is the use of functional foods or nutraceuticals that can be administered as dietary supplements.

The concept of food and nutrition in the current scenario is changing, where certain foods are being used to promote better health and well-being, which in turn helps to reduce the risk of disease. This link between nutrition and health has given rise to the

concept of nutraceuticals, which is defined as "part of a food that provides medicinal and health benefits" and administered in a concentrated form through a non-food matrix [11]. The ability of nutraceuticals to positively influence cardiovascular risk factors presents an enormous opportunity in the future treatment of cardiovascular disease [12-14]. The medical benefits of foods have been largely credited to components such as carotenoids, fatty acids, isothiocyanates, phenolic acids, plant sterols, vitamins, flavanoids, polyphenols, and sulfides/thiols. In the past few years, resveratrol *(trans-3,5,4,-trihydroxystilbene)*, a phenolic phytoalexin present in grapes and berries and a major constituent of red wine, has been reported to have cardioprotective properties [15]. A recent study from our laboratory demonstrated that resveratrol administration will reverse chronic cardiac hypertrophy, along with its deleterious effects on cardiac function, in rats subjected to PO [16].

To date, no study has evaluated the potential of resveratrol in arresting or regressing cardiac hypertrophy due to volume overload. In the current study, we compared the effects of resveratrol in arresting or regressing cardiac hypertrophy due to both pressure and volume overload. We hypothesize that resveratrol will be able to arrest and regress development of PO and VO-induced cardiac hypertrophies.

II. LITERATURE REVIEW

2.1 Heart Failure

2.1.1 Definition

In its simplest form, heart failure is defined as the impaired pumping ability of the heart. Clinical trials have shown in the last few decades that certain therapies (such as direct-active vasodilators and inotropic drugs) which improve hemodynamic overload, believed to be the underlying cause of heart failure, were not always beneficial and, in several cases, decreased survival; other drugs (such as β-adrenergic receptor antagonists) which initially worsened hemodynamic signs and symptoms of heart failure ultimately improved cardiac function and reduced mortality [17]. These finding have necessitated a more comprehensive description of heart failure beyond simple hemodynamics. A more accurate depiction therefore incorporates a complex interaction of all or some of the following conditions: edematous disorders (renal insufficiencies leading to salt and water retention), hemodynamic disorders (characterized by peripheral vasoconstriction and decreased cardiac output), neurohormonal disorders (hyperactivity of the renin-angiotensin-aldosterone system and adrenergic system), inflammatory syndromes (circulating pro-inflammatory cytokines) and myocardial disease (myocardial injury with subsequent ventricular remodeling) [18] with possible genetic and epigenetic factors [19]. However, because heart failure is a clinical syndrome, there is no agreement on its exact definition among clinicians making its incidence difficult to ascertain [18]. Despite the lack of a universal agreed-upon measure of heart failure, epidemiological data still provides sufficient estimates and insight into this multi-factorial syndrome.

2.1.2 Epidemiology

Despite the significant progress that has been made in the treatment of heart failure, this multi-factorial syndrome continues to remain the leading cause of mortality in industrial nations [18] and is becoming a growing threat in developing nations [20]. Currently, there are over six million cases of heart failure in Europe. Furthermore, in addition to the approximately six million Americans that presently exhibit heart failure, it is estimated that in the United States alone, half a million new cases occur each year [21, 22]. The risk of developing heart failure dramatically increases with every decade of life and is especially high in people over the age of 50 years [23]. The mortality rate in patients due to heart failure is approximately 50% within five years [24]. With a vast aging population, the occurrence of heart failure will continue to rise throughout the world. It is therefore paramount to seek out new alternatives to heart failure therapy to combat this potential epidemic and its risk factors.

2.2 Development of Heart Failure

The development of heart failure in clinical settings is generally a direct result of an insult on the myocardium that produces increased wall stress and subsequent myocardial remodeling [25]. The most common primary diseases for the development of heart failure include coronary artery disease, hypertension, cardiomyopathies and valvular heart disease.

2.2.1 Coronary Artery Disease

Coronary artery disease is considered the most common cause of heart failure in Western society [21]. It is a general classification for any disease which affects the coronary arteries, leading to myocardial dysfunction and subsequent heart failure.

Coronary atherosclerosis is the most common cause of myocardial ischemia, which often leads to a myocardial infarction [26]. Coronary artery vasospasms may also produce myocardial ischemia, although they appear to be dependent on the presence of atherosclerotic lesions [27]. Atherosclerosis is the formation of yellowish, cholesterol-filled plaques which are deposited in the walls of arteries, such as coronary arteries of the heart. Typically, high levels of circulating cholesterol, specifically low density lipoprotein (LDL) cholesterol, infiltrate the arterial wall, especially in the instance of endothelial damage [26]. The entrapped LDL cholesterol may become oxidized by oxygen free radicals produced by neighbouring cells, thus promoting smooth muscle cell growth [28]. Thereafter, an inflammatory response is critical in the progression of atherosclerosis. Circulating monocytes infiltrate through the endothelial layer, where they differentiate to macrophages in the media layer of the coronary artery. Together, the macrophages and proliferating smooth muscle cells endocytotically engulf the LDL and develop into foam cells, creating fatty streaks. These fatty streaks enlarge and produce fibrous plaques which decrease the arterial lumen diameter, leading to symptoms of ischemia such as angina [27]. The fibrous plaques may also rupture leading to localized thrombosis. The blood clot can completely occlude the vessel at the site of the plaque or break off, forming a thromboembolism which would occlude a downstream segment of the coronary artery. In both cases, a myocardial infarction results; if left untreated and the individual survives, the resultant MI would develop into heart failure [26].

2.2.2 Hypertension

Hypertension is the chronic elevation in blood pressure (systolic with or without diastolic) which develops left ventricular hypertrophy and promotes atherosclerosis and

subsequent systolic or diastolic heart failure [29]. The development of heart failure is preceded by hypertension in approximately 75% of cases [30] and is the most common co-morbidity in heart failure patients [18].

Hypertension is typically characterized into two broad categories. Essential or primary hypertension, though an identifiable cause is not yet known, has several risk factors with a strong genetic component. The renin-angiotensin-aldosterone system is critical in the regulation of the cardiovascular system. In addition to direct regulation of vasoconstriction, angiotensin II possesses cytokine-like effects and is a potent stimulator of vascular smooth muscle growth and extracellular matrix production [31]. In this regard, genetic abnormalities in angiotensinogen (the precursor of angiotensin II) remains at the forefront for essential hypertension [32]. Non-essential or secondary hypertension is typically considered to be due to endocrine disorders or environmental factors, such as excess dietary sodium, alcohol, obesity and stress. However, a combination of the two forms typically occurs [31].

2.2.3 Cardiomyopathies

Cardiomyopathies are any diseases that affect the heart muscle and result in decreased myocardial function and insufficient pumping of blood [33]. Several types of cardiomyopathies occur including dilated, hypertrophic, diabetic and restrictive cardiomyopathy.

2.2.3.1 Dilated Cardiomyopathy

Dilated cardiomyopathy is a progressive enlargement of the ventricles with ensuing weakening of the myocardium. These changes impair systolic function and ultimately congestive heart failure develops [34, 35]. As the causes are vast, dilated

cardiomyopathy is the most common type of cardiomyopathy and the most common cause of congestive heart failure. Causes of dilated cardiomyopathy include viruses [36], alcohol and other drugs [37], and parasites (commonly found in Chagas disease) [38], with about a third of patients having a genetic component [35]. Dilated cardiomyopathy may develop ventricular and atrial arrhythmias and may be accompanied by other cardiac disorders (mitral regurgitation); however, dilated cardiomyopathy is considered idiopathic in the absence of any coronary artery, valvular or epicardial disease [35].

2.2.3.2 Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy produces left ventricular hypertrophy in the absence of any hemodynamic overload as a result of genetic alterations in genes that code sarcomeric proteins, specifically thin and thick filaments and Z disk proteins [39]. It is the most common genetic cardiac disease, estimated to occur in 1:500 [40]. Hypertrophic cardiomyopathy can follow a Mendelian pattern of inheritance and often produces asymmetric hypertrophy that normally affects the septal wall more than the left ventricular free wall. Despite being able to maintain normal left ventricular systolic function, hypertrophic cardiomyopathy ultimately develops diastolic dysfunction, cardiac arrhythmias and heart failure [39]. Clinical detection of hypertrophic cardiomyopathy is primarily performed through echocardiography [41].

2.2.3.3 Diabetic Cardiomyopathy

Diabetes mellitus is a metabolic disorder characterized by impaired glucose tolerance and is known to be a major risk factor in the development of heart failure [42]. In this regards, the development of abnormalities in myocardial structure or

function where there is no observed coronary artery disease, significant valvular disease and hypertension can be clinically defined as diabetic cardiomyopathy. Hyperglycemia is believed to drive the development of diabetic cardiomyopathy by causing increased myocardial apoptosis and necrosis, autonomic neuropathy, microvascular disease and endothelial dysfunction, and disturbances in free fatty acid metabolism [43]. Impaired calcium homeostasis, activation of renin-angiotensin-aldosterone system, increased oxidative stress and mitochondrial dysfunction also result from hyperglycemia [42]. These pathways promote the development of cardiac hypertrophy and systolic dysfunction. In addition, collagen deposition and myocardial fibrosis promote impaired ventricular filling leading to diastolic dysfunction [43].

2.2.3.4 Restrictive Cardiomyopathy

Restrictive cardiomyopathy results when the walls of the ventricles become rigid. The stiffened chamber impairs proper filling of one or both ventricles, resulting in decreased diastolic blood volume [44]. It is the least common cardiomyopathy but presents the patient with the poorest outcome [35]. Restrictive cardiomyopathy can also develop with or without cardiac hypertrophy [45]. Several fibrotic disorders lead to restrictive cardiomyopathy, such as endomyocardial fibrosis and amyloidosis. The resultant diastolic dysfunction from impaired filling subsequently leads to heart failure, where right-sided failure tends to predominate [44]. This is common in carcinioid heart disease, where cardiac lesions containing fibrous plaques develop on the tricuspid and pulmonary valves and the right ventricular endocardium [44]. The most severe form of restrictive cardiomyopathy is restrictive obliterative

cardiomyopathy, where ventricular filling is completely impaired and is typically associated with eosinophilia [44].

2.2.4 Valvular Heart Disease

Valvular heart disease represents a group of cardiac pathologies that affect the proper function of either the aortic, pulmonary, tricuspid or mitral valves of the heart. Depending on the valve(s) affected, the resultant increase in hemodynamic load within the heart overloads either the right, left or both ventricles, ultimately leading to cardiac dysfunction and heart failure [46]. Defects may either be a stenotic valve (where a valve does not open fully and the resultant opening is decreased) or an incompetent valve (where a valve does not fully close resulting in regurgitation). Stenotic defects typically decrease the flow of blood across the valve and require greater pressure to normalize blood flow, resulting in pressure overload. Regurgitation through incompetent valves produce either systolic or diastolic leaks which results in volume overload [46]. In most cases, echocardiography provides the best mode of detection for valvular heart disease, especially in asymptomatic patients [47-50].

2.2.4.1 Aortic Valve Defects

Stenotic lesions of the aortic valve are commonly due to congenital defect, rheumatic fever and calcification of the valve leaflets (called calcific bicuspid in early/late adulthood; calcific senile in old age) [51]. The decreased area of the aortic valve creates an increased pressure gradient that the left ventricle must pump across to expel blood to the body during systole. The resultant pressure overload produces concentric hypertrophy of the left ventricle which typically leads to diastolic dysfunction [48, 51].

Incompetent aortic valves that lead to regurgitation of diastolic blood back into the left ventricle are typically the result of congenital defects, rheumatic fever, infective endocarditis, dilatation of the aortic root, or collagen disease, such as systemic lupus erythematosus [47]. The degree of aortic valve regurgitation typically dictates the type of remodeling produced. Mild aortic valve regurgitation will generally produce only left ventricular volume overload, resulting in eccentric hypertrophy. However, severe aortic valve regurgitation causes a massive diastolic reflux of blood with initial volume overload, which is thereafter expelled, in addition to the forward stroke volume, into the aorta. The increased total aortic stroke volume promotes the development of systolic hypertension that produces pressure overload. This combinational overload develops left ventricular systolic dysfunction, decreased left ventricular diastolic compliance and ultimately heart failure [47].

2.2.4.2 Mitral Valve Defects

Mitral valve stenosis is almost always secondary to rheumatic fever and remains a major health concern in developing nations [50]. In this instance, the mitral valve leaflets undergo thickening, calcification and fusion; this increases left atrial pressure which reduces left ventricular filling and cardiac output and ultimately results in left-sided heart failure [46].

Mitral regurgitation, on the other hand, produces a back flow of blood from the left ventricle into the left atria during ventricular systole [52]. Mitral regurgitation is typically caused by rheumatic fever, congenital defect, valve prolapse into the left atria, mitral ring calcification, infective endocarditis, connective tissue disorders and defects of either the chordae tendinae or papillary muscle. The subsequent volume

overload of the left atria during ventricular systole leads to volume overload of the left ventricle during diastole, therefore creating eccentric hypertrophy in both. The result is an increase in left ventricular ejection fraction due to increased left ventricular end diastolic volume, but with reduced forward stroke volume. Although initially compensating for reduced forward stroke volume by a further increase in left ventricular chamber volume, the heart ultimately decompensates and develops ventricular dysfunction; this reduces left ventricular ejection fraction and forward stroke volume, which in turn increases the amount of regurgitation back into the left atria. The ensuing increase in atrial pressure leads to pulmonary hypertension, right ventricular dysfunction and heart failure [52].

2.2.4.3 Tricuspid Valve Defects

The development of tricuspid valve disease is common in the heart failure patient but is typically secondary to left-sided heart valve disease, with mitral valve disease being the most common [49]. The most common cause of tricuspid valve stenosis is rheumatic fever and is usually associated with mitral and aortic valve problems. Typically, tricuspid valve stenosis causes increased right ventricular pressure with ensuing hypertrophy and decreased right ventricular stroke volume [49].

Tricuspid regurgitation is one of the most common congenital cardiac defects and is typically due to dilation of the annulus, valve prolapse into the right atrium during ventricular systole and abnormalities in leaflet tissue due to thickening and shortening, as well as fibrous fusion of the chordae tendinae. Severe tricuspid regurgitation is typically secondary to left-sided heart valve disease and can cause impaired right ventricular function. Dilation of the right ventricle, right atrium and

inferior vena cava, resulting from retrograde blood flow, all contribute to impaired right ventricular function. This cascade of events lead to venous hypertension, decreased cardiac output, sodium retention and fluid retention, leading to edema [49, 53].

2.2.4.4 Pulmonary Valve Defects

Pulmonary valve disease is the least common of the valvular diseases. Pulmonary valve stenosis is usually congenital in nature and causes right ventricular pressure overload. Pulmonary valve regurgitation can also be the result of congenital defects, such as Tetralogy of Fallot. However, pulmonary valve regurgitation usually results from dilatation of the valve ring, a condition secondary to pulmonary hypertension, connective tissue disorders, such as Marfan syndrome, or is idiopathic. Pulmonary valve regurgitation typically creates right ventricular volume overload that, if severe enough, will lead to right ventricular heart failure [54].

2.2.4.5 Congenital Heart Disease

As described above, valvular defects are typically caused by congenital abnormalities. Cardiac defects that occur *in utero* can be classified into three broad categories: stenotic lesions (as described above), left to right shunts and right to left shunts. Left to right shunts are the most common form of congenital heart disease. Included are atrial and ventricular septal wall defects and patent ductus arteriosus, which all create VO and result in both right and left ventricular hypertrophy [54]. The least common forms of congenital heart disease are the right to left shunts, which includes Tetralogy of Fallot, transposition of the great arteries and hypoplastic left heart syndrome which all typically cause cyanosis and infant heart failure [55].

2.3 Cardiac Hypertrophy

In contrast to hyperplasia, which is a proliferation in cell number, hypertrophy is the enlargement of the cell itself [56]. Therefore, cardiac hypertrophy is defined as the enlargement of the heart as a direct result of increased cardiomyocyte size. Alterations in loading conditions of the heart, especially the left ventricle, cause structural remodeling induced by either pressure overload, volume overload or a combination of the two [5].

2.3.1 Pressure Overload

Pressure overload, such as increased afterload (an increase in the systemic blood pressure onto the heart), stimulates an adaptive response of the heart chamber wall, such as the left ventricle, to undergo concentric hypertrophy. In concentric hypertrophy, cardiomyocytes up-regulate protein synthesis of sarcomeres which are added in parallel, resulting in a thicker wall. According to the Law of Laplace, increased wall stress is normalized by increased wall thickness [5]. Several hemodynamic stimuli, including hypertension and valvular stenosis, as well as genetic causes, such as hypertrophic cardiomyopathy, elicit concentric hypertrophy as previously described.

2.3.2 Volume Overload

In contrast, volume overload results when an increased preload (increase in blood volume within the heart), stimulates ventricular remodeling that is eccentric in nature. Eccentric hypertrophy is characterized by increased sarcomere replication in series, resulting in elongated cardiomyocytes and chamber enlargement. Such remodeling is necessary to accommodate the increased blood volume and is necessary to increase forward stroke volume in the presence of such hemodynamic overload. Volume overload occurs in congenital defects and valvular disease with regurgitation, as previously

described. In addition, eccentric hypertrophy can occur in a pregnant woman due to natural volume overload [57] and anemia, where the reduced blood viscosity and arterial dilatation results in decreased vascular resistance and increased venous return [58].

2.3.3 Combination Overload

This form of dual remodeling is most often observed in myocardial infarction. In this instance, the infarcted myocardium undergoes apoptosis and necrosis, leading to wall thinning and weakening of the infarct zone, which expands and leads to volume overload. The infarct expands into non-infarcted regions of the myocardium and the increased volume load induces pressure overload leading to hypertrophy in healthy myocardium [5, 59]. Myocardial infarction is typically the result of coronary artery disease, as described earlier.

2.3.4 Molecular Mechanisms

2.3.4.1 Pro-hypertrophic Pathways

The activation of the renin-angiotensin-aldosterone and β-adrenergic systems occur in response to cardiac insults, activating signaling cascades which promote the development of cardiac hypertrophy. The renin-angiotensin-aldosterone system mediates production of the pro-hypertrophic hormone angiotensin II, which starts with conversion of angiotensinogen to angiotensin I by the enzyme renin, followed by cleavage to angiotensin II by angiotensin converting enzyme. The autocrine and/or paracrine actions of angiotensin II on the cardiomyocyte make it an important regulator of hypertrophy [60]. Angiotensin II binds to angiotensin receptor type 1, which is a G-protein coupled receptor that operates via Gqα. Within the cardiomyocyte, Gqα activation leads to the breakdown of phosphotidylinositol

bisphosphate by phospholipase C into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 increases intracellular calcium levels by activating L-type calcium channels on the sarcolemma and IP_3-gated calcium channels on the sarcoplasmic reticulum. DAG activates protein kinase C, particularly the ε-isoform, which activates downstream mitogen-activated protein kinases (MAPKs) that, in turn, mediate the function of transcription factors that promote cardiac hypertrophy. In addition, increased calcium activates the calcineurin-NFAT (nuclear factor of activated T cells) pathway through Ca^{2+}/calmodulin, where the transcription factor NFAT activates genes involved in pathological cardiac hypertrophy [60, 61]. Angiotensin II also promotes oxidative stress through the generation of reactive oxygen species that promote apoptosis, which is enhanced by the increased intracellular calcium levels [5]. Additional factors which act via the Gqα subunit are endothelin-1, noradrenaline (norepinephrine) and various growth factors [62].

Heart failure patients also have increased levels of circulating catecholamines [63]. In the β-adrenergic system, the catecholamine adrenaline (epinephrine) is produced by the sympathetic nerves and adrenal glands. Hyperadrenergic signaling acts through the β1-adrenergic receptor, a G-protein coupled receptor which utilizes the Gsα to activate adenylyl cyclase (AC). AC catalyzes the breakdown of adenosine triphosphate into pyrophosphate and 3',5'-cyclic adenosine monophosphate (cAMP). The latter molecule, cAMP, acts as a second messenger to activate cAMP dependant protein kinase A (PKA). PKA phosphorylates key intracellular calcium cycling proteins, such as L-type calcium channels on the sarcolemma, ryanodine receptors and phospholamban on the sarcoplasmic reticulum, thus increasing intracellular

calcium levels. The increased calcium levels promote pro-hypertrophic signaling through calcineurin-NFAT pathway and apoptosis as described above [63].

2.3.4.2 Anti-Hypertrophic Pathways

One of the most studied anti-hypertrophic pathways is the nitric oxide-3',5'-cyclic guanosine monophosphate pathway (NO-cGMP). In response to pro-hypertrophic stimuli, natriuretic peptides are synthesized, namely atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), to increase water and electrolyte excretion by the kidneys. These natriuretic peptides additionally oppose the effects of angiotensin II by binding to particulate guanylyl cyclase receptors on the cardiomyocyte sarcolemma, which catalyzes the breakdown of guanosine triphosphate to 3',5'-cyclic guanosine monophosphate (cGMP) and pyrophosphate in the cell. The second messenger cGMP then activates cGMP-dependent protein kinase type 1 (PKG-1). Additionally, nitric oxide (NO) can enter the cardiomyocyte and activate soluble guanylyl cyclase, thereby further enhancing the production of cGMP and the activity of PKG-1. NO is produced by nitric oxide synthase (NOS) enzymes. Three isoforms of NOS exist: NOS1 or neuronal NOS, NOS2 or inducible NOS and NOS3 or endothelial NOS. NOS1 and NOS3 are constitutively expressed and their activity is dependant on intracellular calcium levels [64]. Increased intracellular calcium levels in pathological cardiac hypertrophy therefore increase constitutive NOS activity, resulting in more PKG-1 activity. PKG-1 decreases intracellular calcium levels by inhibiting the sarcolemmal cardiac Na^+/H^+ exchanger 1 and L-type calcium channels, and prevents NFAT translocation into the nucleus by inhibiting calcineurin, therefore inhibiting transcription of pro-hypertrophic genes [63]. PKG-1 can also repress Gqα

signaling through an interaction with the regulator of G-protein-coupled signaling 2 (RGS2) [65].

2.4 *In Vivo* Research on Cardiac Hypertrophy

In vivo animal models are invaluable tools for dissecting the development of cardiac hypertrophy and its progression from a compensatory to decompensatory state. Most cardiac patients are under pharmacological treatment, generally have co-existing diseases and tissues obtained for research are predominantly from end-stage heart failure. Unfortunately, these factors confound the elucidation of cardiac hypertrophy at various stages. In contrast, representative surgical models allow researchers to study pressure overload, volume overload, or combination overload cardiac hypertrophy in their pure form at specific stages. Furthermore, many different animals may be utilized for research, depending on their suitability, and include mice, rats, guinea pigs, rabbits, cats, dogs and pigs.

2.4.1 Models of Pressure Overload

2.4.1.1 Abdominal Aortic Banding

One of the most common models of pressure overload involves surgically tying a band (usually with a silk suture or metal clip of fixed diameter) around the suprarenal aorta in the abdominal region. The resultant decrease in luminal diameter at the level of the band produces an increased afterload for the heart [16, 66, 67].

2.4.1.2 Transverse Aortic Constriction

Unlike abdominal aortic banding, transverse aortic constriction (TAC) requires a less-invasive fine microsurgical technique and requires intubation. Generally, the thoracic cavity is opened at the level of the second left intercostal space and the aortic

arch is constricted (banded with suture) between the brachiocephalic/innominate artery and left common carotid artery [68, 69]. Since the band is more proximal to the heart compared to abdominal aortic banding, the result is a more pronounced hypertrophied heart that progresses to heart failure much earlier. More specifically, TAC is a model of pressure overload dilated cardiomyopathy as indicated by the prominence of left ventricular chamber dilation [70]. TAC is predominantly performed in mice but several studies have been done in rats [71].

2.4.1.3 One-Kidney, One-Clip & Partial Nephrectomy

Although less common, one-kidney, one-clip produces luminal stenosis which mimics systemic hypertension. This procedure can be performed in rabbits, mice, and rats and is obtained by partial constriction of the main left renal artery with a metal (silver) clip of fixed diameter, followed by nephrectomy of the right kidney [72-74]. Similarly, hypertension-induced cardiac hypertrophy can also be induced by the partial nephrectomy model, wherein the right kidney is removed and all arteries except the accessory renal artery in the left kidney are ligated with suture [75].

2.4.1.4 Pulmonary Hypertension

Although the previous models of pressure overload produce left-sided cardiac hypertrophy, surgical techniques on animals exist to induce right sided cardiac hypertrophy. Constriction of the pulmonary artery creates pulmonary hypertension resulting in right ventricular hypertrophy [76]. Also, subcutaneous injection of monocrotaline, a pyrrolizidine alkaloid derived from *Crotalaria spectabilis* [77, 78], chronic exposure to 100% oxygen in rat pups to produce neonatal hyperoxic lung

injury [79], and rats exposed to chronic hypoxia in a hypobaric chamber [80] all induce pulmonary hypertension leading to right ventricular hypertrophy.

2.4.1.5 Neurohormonal Infusion

Injection of pro-hypertrophic agents such as angiotensin II and isoproterenol (a β-adrenergic agonist) also produces concentric hypertrophy *in vivo* [81] by mimicking the neurohormonal activity in clinical settings of pressure overload. Cardiac hypertrophy secondary to hyperthyroidism is also clinically important, where intraperitoneal injections of the hormone L-thyroxine in rats is used to induce concentric hypertrophy [82].

2.4.2 Models of Volume Overload

2.4.2.1 Aortic Valve Regurgitation

Defects in the aortic semi-lunar valve can produce volume overload in the left ventricle [83]. Experimental models of aortic valve regurgitation are created by advancing a catheter, with the aid of echocardiography, through the right carotid artery to the aortic valve leaflets, which are then punctured [84].

2.4.2.2 Mitral Valve Regurgitation

In addition to aortic valve regurgitation, mitral valve regurgitation is another common cause of left ventricular volume overload [85]. Mitral valve regurgitation in animals can be produced without opening the thoracic cavity by using fluoroscopic-guidance to catheterize and surgically rupture the mitral valve chordae tendinae [86]. Similarly, pure mitral valve regurgitation can be created by puncturing a hole in the mitral valve leaflet [87].

2.4.2.3 Tricuspid Valve Regurgitation

Using a similar method to mitral valve regurgitation, right-sided volume overload can be produced by tricuspid valve regurgitation, wherein catheterization and subsequent rupture of the tricuspid chordae tendinae is performed [88].

2.4.2.4 Arteriovenous Shunt/Fistula

Several types of shunt surgeries have been developed to study volume overload in various animal models of cardiac hypertrophy. The most commonly reported model is surgical creation of an aortocaval shunt between the abdominal aorta and inferior vena cava in the area between the renal artery and iliac bifurcation [66, 89]. Additionally, shunts can also be created at the aortopulmonary level [90] and between the left common carotid artery and external jugular vein [91].

2.4.2.5 Anemia

Persistent anemia has been shown to cause left ventricular hypertrophy and heart failure. Animal models of chronic anemia can be induced by feeding a low iron diet combined with regular bleeding, resulting in biventricular hypertrophy [92, 93].

2.4.2.6 Atrio-ventricular Block

Volume overload can also be induced by atrio-ventricular (A-V) block. In this procedure, an electric knife is advanced through the right atrial wall, after which the nodal region is destroyed by electrocoagulation [94].

2.4.3 Models of Pressure and Volume Combination Overload

2.4.3.1 Myocardial Infarction

Models that mimic clinical settings of ischemic heart disease, such as a myocardial infarction, produce left ventricular remodeling that occurs as a mixture of

pressure and volume overload [5]. The most common model employs permanent ligation of a coronary artery in the heart. Specifically, the proximal left anterior descending artery is occluded, resulting in an infarct zone that closely mimics the type seen in clinical settings [95]. Another model is isoproterenol-induced myocardial infarction, which involves injection of high doses of isoproterenol that are believed to create highly cytotoxic free radicals via auto-oxidation, resulting in histological and functional remodeling similar to MI [96, 97].

2.5 Echocardiography

Echocardiography is a non-invasive imaging technique that utilizes ultrasounds to assess the structure and function of the heart, with applications in both basic research and clinical settings [98, 99]. Analysis of the heart may be performed by either the trans-thoracic method (external probe on the thorax) or trans-esophageal method (internal probe in the esophagus) [100]. A more invasive method, epicardial echocardiography, takes advantage of cardiac surgery where the heart is exposed allowing for direct placement of the probe onto the heart itself [101].

In the context of cardiac hypertrophy and ensuing heart failure, echocardiography is vital in the assessment of left ventricular structure and function. M-Mode echocardiography allows assessment of cardiac structure, including interventricular septal wall thickness (IVS), left ventricular posterior wall thickness (LVPW), left ventricular internal dimensions (LVID), and systolic functional parameters including left ventricular ejection fraction (EF), fractional shortening (FS), heart rate (HR) and cardiac output (CO) [66]. In addition, M-Mode allows for the estimation of left ventricular mass. Pulse Wave Doppler allows for additional assessment of left ventricular function. Included is

isovolumetric relaxation time (IVRt), a measure of ventricular diastole function, which is the time from the closing of the aortic valve to the opening of the mitral valve. Mitral valve function, such as early and late diastolic filling velocities, and aortic ejection time can also be measured [66].

Recent advances in echocardiography have implemented the use of real-time three dimensional imaging. The use of real-time-three-dimensional echocardiography has been extensively utilized in the quantitative assessment of left ventricular volumes and function and mechanical dys-synchrony in heart failure patients [102, 103].

2.5.1 Echocardiography in Pressure Overload

Concentric hypertrophy of the left ventricle is observed as an increase in the septal wall (IVS) and posterior wall (LVPW) thickness. As such, echocardiography has been extensively utilized in detection and analysis of pressure overload in clinical settings [104] and in animal models used in research [67]. In addition, echocardiography allows for the analysis of treatment progression for pressure overload, such as post-aortic valvular replacement surgery [105] and experimental treatment in animal models [16]. With respects to function, diastolic dysfunction is a hallmark of pressure overload which has been demonstrated by echocardiography in both animal models [66] and clinical settings [106].

2.5.2 Echocardiography in Volume Overload

Eccentric hypertrophy leads to chamber dilation as previously described. Abnormalities such as mitral valve regurgitation leading to volume overload are commonly diagnosed with echocardiography [85]. This is an important tool used to assess the status of cardiac structure and function in the progression of volume overload

in clinical settings [107]. In addition, echocardiography is typically used to characterize animal models of volume overload with respect to cardiac structure and function [108]. The treatment of volume overload in clinical settings, such as β-blocker therapy in severe mitral valve regurgitation [109] and in animal models of volume overload [110], is extensively used to monitor the treatment progress. Evaluation of patient cardiac function by echocardiography has demonstrated that the majority of patients with volume overload suffer from systolic dysfunction, characterized by decreased left ventricular ejection fraction [85]; however, a substantial proportion of heart failure patients have normal left ventricular ejection fraction, even with severe mitral valve regurgitation [109, 111].

2.6 Functional Foods and Nutraceuticals

With humans living longer, particularly in western society, the prevalence of age-related disease is increasing, especially cardiovascular disease, where dietary habits have a major influence on their development and progression [112]. Diet and its constituents are therefore important and can be exploited in the context of disease. In this regard, functional foods and nutraceuticals are emerging areas in the prevention and treatment of various diseases.

Functional foods are defined as "those [foods] that when consumed regularly exert a specific health-beneficial effect beyond their nutritional properties" [112]. On the other hand nutraceuticals, derived from the words *nutr*ition and pharm*aceutical* and coined by Dr. Stephen Defelice in 1989 [12], are "diet supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods" [112]. Examples of functional foods and their

nutraceutical constituents include: garlic containing allicin and alliin, tomatoes containing lycopene, flaxseed containing alpha-linolenic acid (ALA), fish containing docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) [113], and berries containing polyphenols such as resveratrol [112].

Evidence from epidemiological, *in vivo*, *in vitro*, and clinical studies indicates that plant-based diets and nutraceuticals can reduce the risk of chronic diseases, particularly cancer and cardiovascular diseases [112, 114]. Polyphenols, anthocyanines, proanthocyanidins, flavanones and isoflavanones are classes of compounds which have received increased attention in medical research [112]. Among the classes mentioned above, one of the most studied has been resveratrol (*trans-3,5,4,-trihydroxystilbene*), a polyphenol with tremendous health benefit potential [115, 116].

The effects of functional foods and nutraceuticals on cardiac hypertrophy have been evaluated in a few studies. Duda *et al.* [117] showed that dietary supplementation with omega-3 polyunsaturated fatty acids reversed ventricular remodeling and cardiac dysfunction after abdominal aortic band-induced pressure overload. Duda *et al.* [118] went on to further demonstrate that the omega-3 polyunsaturated fatty acids from fish oil (DHA and EPA), but not flaxseed oil (ALA), contributed to this effect. Other studies have demonstrated that copper supplementation [119] and resveratrol and its analogs (discussed in section 2.7) can also reverse pressure overload-induced cardiac hypertrophy. However, no work has been done to date to evaluate the efficacy of functional foods and nutraceuticals, especially resveratrol, on cardiac hypertrophy induced by volume overload.

2.7 Resveratrol

2.7.1 Occurrence and synthesis

Resveratrol is classified as a phytoalexin, a compound produced by plants in response to environmental stress, such as extreme temperatures, infection and ultraviolet radiation [120]. Resveratrol is found in a wide variety of dietary sources including grapes, berries, plums and peanuts [116]. It is also present in wines, especially red wines, and to a much lesser extent in white wines [121], eucalyptus and spruce trees, and in a few flowering plants, such as *Veratrum grandiflorum* and *Veratrum formosanum* [122, 123]. The synthesis of resveratrol is catalyzed by the enzyme stilbene synthase through a condensation reaction of p-coumaroyl CoA and malonyl CoA precursors in a 1:3 molar ratio [124]. Resveratrol is considered to be a phytoestrogen as its chemical structure has a close resemblance to the synthetic estrogen diethylstilbestrol. Its structure exhibits two phenol rings linked by a styrene double bond to form 3,4,5-trihydroxystilbene [123]. Resveratrol exists in two different isomeric forms, *cis-* and *trans*-resveratrol (Fig. 1) (molecular weight: 228 g/mol), where *trans*-resveratrol is relatively more stable compared to *cis*-resveratrol [125]; both isomers are lipophilic in nature [126].

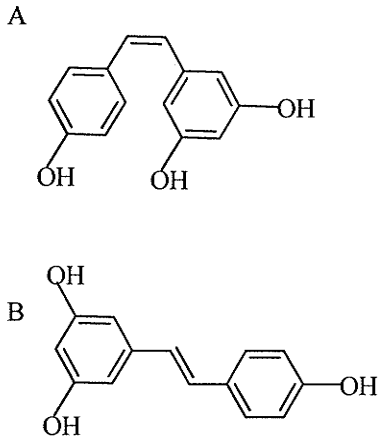

Figure 1. The chemical structure of resveratrol: *cis-3,5,4,-trihydroxystilbene* (A) and *trans-3,5,4,-trihydroxystilbene* (B).

2.7.2 Research History

Resveratrol was first discovered and extracted from the roots of the white hellebore plant [127] and later from *Polygonum cupsidatum*, a plant which has been used in traditional Asian medicine and contains the highest concentration of this polyphenol [128]. The latter plant is known as *Hu-Chang* in Chinese medicine and *Ko-jo-kon* in Japanese medicine [129]. Resveratrol synthesis in grapevines *(Vitis vinifera)* in response to fungal infection was first reported by Langcake and Pryce in 1976 [130].

In 1992, research on resveratrol started getting attention, when its presence was detected in wine for the first time [131]. Thereafter, resveratrol research gained significant momentum due to its association with the "French paradox", a phenomenon wherein the French have been reported to have a low incidence of cardiovascular disease despite a high-fat diet intake. Moderate, but regular consumption of wine, particularly red wine, has been proposed as a reason for cardioprotection [132, 133]. Numerous studies have now shown that resveratrol exhibits positive effects in various diseases such as cancer [134], neurodegenerative and cardiovascular disorders [15, 115, 125].

2.8 Current Pharmacological Treatments for Heart Failure

Treatment for heart failure depends on its severity and ranges from pharmacological to surgical interventions. Pharmacological treatment of heart failure is the most common therapy by far, and includes inhibitors of the renin-angiotensin-aldosterone system, β-adrenergic receptor antagonists and diuretics. Although these therapies have been moderately successful in the treatment of heart failure, they present numerous drawbacks which potentially necessitate newer treatments for heart failure.

2.8.1 Inhibitors of the Renin-Angiotensin-Aldosterone System

These treatments include angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB) and aldosterone antagonists (AA). Renin inhibitors are currently under investigation in clinical trials [25]. It has previously been shown that ACEI do not completely block angiotensin II and aldosterone production, a phenomenon known as "ACE and aldosterone escape", which, in one study, was shown to occur in up to 50% of severe chronic heart failure patients [135]. When high ACE activity is observed, it has been suggested that patient non-compliance may be the culprit as ACEI cause intolerable side-effects, such as ACEI cough [136]. Chronic inhibition with ACEI and AA decreases ACE activity; this may activate a negative feedback mechanism which would result in observed elevated levels of renin and angiotensin I peptides [135]. In the absence of ACE, the activation of non-ACE pathways, such as chymases, may be the reason for elevated angiotensin II levels [136, 137]. Furthermore, addition of an ARB does not fully reverse these effects [138]. Both ACE escape and aldosterone escape, through use of ACEI, ARB and AA, lead to increased levels of angiotensin II and aldosterone. Increased levels of angiotensin II and aldosterone cause hyperkalemia (high potassium serum levels), leading to arrhythmias, increased urinary excretion of zinc, which can cause dysgeusia (an inability to taste), and increased salt and water retention [139]. Lastly, the AA spironolactone has been shown to cause gynecomastia or breast pain in men due to its affinity for the androgen receptors [140].

2.8.2 β-Adrenergic Receptor Antagonists

As in the case with renin-angiotensin-aldosterone system inhibitors, "adrenergic escape" was shown to occur in about one third of stable chronic heart failure patients on

β-blocker therapy; however, the elevated levels of catecholamines could not be thoroughly explained and may be due to more advanced stage of heart failure or lack of treatment effectiveness [141]. Furthermore, many heart failure patients have contraindications to β-blocker therapy. These include reactive airway disease, sinus node or conduction system disease and advanced heart failure with hemodynamic decompensation [142]. Lastly, β-blockers may cause the development of hyperkalemia [139].

2.8.3 Diuretics

Diuretics are used to remove excess fluid volume through excretion of sodium and therefore alleviate hemodynamic overload [143]. However, diuretics do not improve left ventricular remodeling in heart failure patients with systolic dysfunction, which is an important indicator for future survival [144]. In addition, increased volume excretion with diuretic use could activate the renin-angiotensin-aldosterone system, therefore necessitating the use of renin-angiotensin-aldosterone system inhibitors [143]. Diuretics can also cause electrolyte depletion. This includes hypokalemia (decreased potassium levels), which can be exacerbated by secondary increases in aldosterone due to decreases blood volume and sodium levels, hypomagnesemia (decreased magnesium levels) and hypocalcemia (decreased calcium levels), whose reabsorption is dependant on sodium and chloride concentrations in the nephron [139].

2.9 Heart Failure Therapy – Potential Role for Resveratrol

Heart failure continues to be a leading cause of hospitalization and mortality worldwide [18]. Standard guidelines for the treatment of heart failure patients still rely on stabilizing and relieving symptoms, along with prolonging survival. Current

pharmacological treatments have proven to be moderately successful but the incidence of heart failure is on the rise. In this regard, there is an important need for alternative strategies to combat the development of heart failure. One such avenue is the use of functional foods or nutraceuticals that can be administered as dietary supplements [11].

The development of heart failure is secondary to various cardiac pathologies such as coronary artery disease, hypertension, valvular heart disease and cardiomyopathies [18, 145]. The above mentioned diseases promote the development of various aspects of cardiac remodeling including cardiomyocyte hypertrophy, fibrosis and arrhythmias. All these factors culminate in severe impairment of cardiac function and subsequent failure if left untreated. The potential of resveratrol in preventing the development of heart failure is discussed below.

2.9.1 Cardiac hypertrophy

The anti-hypertrophic effects of resveratrol *in vivo* have been documented in different experimental models of pressure overload which mimic clinical situations, such as hypertension and aortic stenosis, as well as certain forms of valvular heart disease. Liu *et al.* [75] reported that daily treatment of partially nephrectomized rats with 10 or 50 mg/kg body weight resveratrol for four weeks immediately after surgery significantly attenuated the increase in systolic blood pressure and the development of cardiac hypertrophy; this effect was dose-dependent and was related to an up-regulation in the levels of nitric oxide (NO), an anti-hypertrophic molecule. In another study, Li *et al.* [146] reported that a daily dose of 50 mg/kg body weight of isorhapontigenin, a resveratrol analog (Fig. 2), for 3 weeks after transverse aortic constriction surgery

attenuated the development of cardiac hypertrophy and improved diastolic function in rats; this effect was linked to a reduction in oxidative stress.

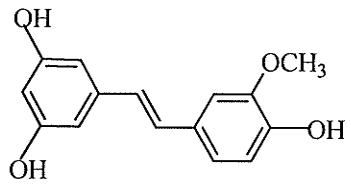

Figure 2. The chemical structure of isorhapontigenin, a derivative of resveratrol.

Lastly, we have reported regression of cardiac hypertrophy and diastolic dysfunction in rats subjected to abdominal aortic banding surgery with resveratrol treatment (at a low dose of 2.5 mg/kg body weight/day) starting two weeks after surgery for a period of two weeks; this effect was also linked to an up-regulation of NO [16].

There is one study which reported anti-hypertrophic effects of resveratrol in an experimental model of autoimmune myocarditis. Yoshida *et al.* [147] showed that treatment of resveratrol (50 mg/kg/day) for 1 day prior to immunization and for 14 days after immunization with cardiac myosin significantly reduced cardiac hypertrophy and improved left ventricular fractional shortening in rats with cardiac myocarditis; the beneficial effect of resveratrol was related to a reduction in the expression of inflammatory proteins and an up-regulation in the expression of the antioxidant genes.

In vitro studies using cardiomyocytes have also reported beneficial effects of resveratrol on cardiomyocyte hypertrophy induced by exposure to potent hypertrophic agents, such as angiotensin II and phenylephrine. Cheng *et al.* [148] showed that treatment with 10 μM resveratrol inhibited angiotensin II-induced cardiomyocyte hypertrophy and that this effect was associated with an attenuation of reactive oxygen species production. Chan *et al.* [149] also showed that treatment with 50 μM resveratrol prevented cardiomyocyte hypertrophy induced by phenylephrine; this effect was linked to

the activation of adenosine monophosphate kinase (AMPK), an energy sensor, and inhibition of Akt, an activator of hypertrophy.

2.9.2 Myocardial Infarction

The effects of resveratrol have also been documented in chronic *in vivo* experimental models of myocardial infarction. Chen *et al.* [150] reported that treatment with resveratrol (5mg/kg body weight/day) for one week before coronary artery ligation surgery and continuing for 14 weeks after surgery prevented the development of myocardial infarction-induced cardiac hypertrophy in rats; this effect was linked with a reduction in infarct size. Lin *et al.* [151] also reported that daily injection of resveratrol (1mg/kg) for four weeks after coronary artery ligation surgery significantly improved left ventricular systolic and diastolic function in rats post-myocardial infarction; this effect was associated with a decrease in infarct size. On the other hand, Burstein *et al.* [152] reported that treatment with resveratrol (17mg/kg body weight/day), for one week before coronary artery ligation surgery and for 12 weeks after surgery, failed to reduce the development of hypertrophy and did not improve cardiac function in rats after myocardial infarction. The discrepancy between the observed results could be attributed to the differences in administration route of resveratrol (oral gavage [150], intraperitoneal injection [151] and in rat chow [152]), dosage and extent of infarct size.

2.9.3 Cardiac fibrosis

Cardiac fibrosis refers to an abnormal thickening of the heart as a result of excess accumulation of extracellular matrix due to inappropriate proliferation of cardiac fibroblasts. As a result, cardiac fibrosis significantly contributes to pathological structural remodeling in the instance of various heart diseases, including myocardial infarction,

hypertension and certain cardiomyopathies [153]. Cardiac fibrosis is a common feature in the advanced stages of heart disease and contributes to impaired systolic and diastolic function.

A few *in vivo* studies have shown beneficial effects with resveratrol treatment in inhibiting cardiac fibrosis. Liu *et al.* [75] showed decreased fibroblast proliferation in partially nephrectomized rats treated with resveratrol. Lin *et al.* [151] also showed reduced expression of cardiac transforming growth factor-β1 (a potent stimulant of cardiac fibrosis) in coronary artery-ligated rats treated with resveratrol. Yoshida *et al.* [147] also showed that treatment with resveratrol significantly reduced cardiac fibrosis in rats which had developed cardiac myocarditis.

A few studies have also reported anti-fibrotic effects of resveratrol *in vitro.* Olson *et al.* [154] showed that treatment with resveratrol (25 μM) limited angiotensin II (a potent inducer of fibrosis) -induced cardiac fibroblast proliferation and differentiation; this was linked with attenuation of extracellular signal-regulated kinase activation. Wang *et al.* [155] also reported inhibition of angiotensin II-induced cardiac fibroblast proliferation in a dose dependant manner upon treatment with resveratrol (25 -100 μM), which was related to the activation of the NO-cGMP pathway.

2.9.4 Ischemia and reperfusion injury - acute models of myocardial damage

Myocardial ischemia results when oxygen and metabolite supply is reduced (low-flow or no-flow ischemia), or the metabolic demands of the cardiac tissues are increased and exceed supply (demand ischemia). These alterations in coronary blood supply are associated with left ventricular hypertrophy, hyperlipidemia and atherosclerosis, diabetes and heart failure [156]. Paradoxically, restoration of blood flow back into the ischemic

myocardium also causes damage, known as reperfusion injury, which includes cardiac dysfunction and arrhythmias [157]. Several studies have shown that resveratrol is effective as a pharmacological preconditioning agent in an *ex vivo* model of ischemia/reperfusion injury. Isolated perfused rat hearts were shown to have protection against apoptosis and reduced infarct size, as resveratrol pretreatment activated pro-survival pathways, via adenosine A_3 receptor [158, 159], and anti-inflammatory actions, via NO [160]. Furthermore, Bradamante *et al.* [161] showed resveratrol improved coronary flow via adenosine-mediated vasodilation and demonstrated delayed protection against ischemia, which is mediated by NO.

In vivo evidence corroborates these *ex vivo* findings. Rats pretreated 15 minutes before left main coronary artery occlusion showed improved cardiac function and reduced infarct size after 30 minutes of ischemia followed by two hours of reperfusion [162]. These effects were mediated by reduced myocardial oxidative stress associated with NO. In addition, Hung *et al.* [163], using a resveratrol pretreatment similar to Shen *et al.* [162] reported the prevention of ischemia/reperfusion-associated arrhythmias and mortality, which was associated with increased production of NO.

However, since pharmacological preconditioning is not always possible, treatment at the time of reperfusion is of more clinical importance. Recently, Xi *et al.* [164] showed that resveratrol administration five minutes prior to reperfusion improved cardiac contractile function and reduced infarct size in isolated perfused rat hearts. In that study, resveratrol prevented mitochondrial permeability transition pore (mPTP) opening, which is involved in mitochondrial swelling and apoptosis, via cGMP/PKG dependent glycogen synthase kinase-3β (GSK-3β) inactivation and translocation to the mitochondria [164].

2.9.5 Cardiac Arrhythmia

Arrhythmias are irregular heartbeats and are central to the development of pathological electrical remodeling of the heart [153, 165]. This remodeling is associated with abnormalities in the function of proteins which are responsible for the electrical activity of the heart. These proteins primarily include the ion channels and gap-junction proteins, which work in coordination with calcium cycling proteins to control cardiac contraction and relaxation.

The effect of treatment with resveratrol on tachycardia (faster heart rate) has been reported in one *in vivo* study. Chen *et al.* [150] showed that treatment with resveratrol reduced the incidence of ventricular tachycardia in rats after myocardial infarction; the beneficial effect was associated with an inhibition in the L-type calcium channel current and an enhancement of the ATP-sensitive potassium current. An *in vitro* study conducted by Zhang *et al.* [166] showed that resveratrol decreased L-type calcium current in ventricular cardiomyocytes which was linked with decreased tyrosine kinase activity. This *in vitro* observation [166] is consistent with reduced L-type calcium current and reduced incidence of ventricular tachycardia upon resveratrol treatment observed *in vivo* by Chen *et al.* [150].

The effect of treatment of resveratrol on bradycardia (slower heart rate) has been reported in two *in vivo* studies. Mayers *et al.* [167] reported that treatment with resveratrol did not affect the incidence of bradycardia in mice subjected to calorie restriction for one week. Furthermore, Yoshida *et al.* [147] observed no effect of resveratrol treatment on the incidence of bradycardia in rats with developed autoimmune myocarditis due to immunization with cardiac myosin.

Table 1 – Evidence for cardioprotection with resveratrol.

Model	Dosage used (start time, end point)	Result	Ref
In vivo			
Transverse aortic (TAC) constriction in rat	* *50 mg/kg/d i.p. injection (24 h post-TAC, 21 d post-TAC)*	Inhibits hypertrophy through antioxidant mechanism	[146]
Partially nephrectomized (PNX) rat	10 & 50 mg/kg/d oral gavage (3 d post-PNX, 4 wk post-PNX)	Inhibits hypertrophy at 50 mg/kg/d with upregulation of NO levels	[75]
Abdominal aortic banding in rat	2.5 mg/kg/d oral gavage (2 wk post-banding, 4 wk post-banding)	Attenuation of hypertrophy & diastolic dysfunction with upregulation of eNOS & iNOS	[16]
LAD coronary artery ligation in rat	0.1-1 mg/kg/d i.p. injection (6 h post-ligation, 4 wk)	1 mg/kg/d significantly limited infarct size & improved left ventricular systolic function, decreased ANP & TGF-β	[151]
LAD coronary artery ligation in rat	5 mg/kg/d orally (7 d pre-treatment, 3 wk post-ligation)	Attenuated ventricular arrhythmias & hypertrophy; improved long-term survival at 14 wk post-ligation	[150]
Left main coronary occlusion in rat	10 μM transjugular infusion (15 min pretreatment, cessation at occlusion)	Improved cardiac function & reduced infarct size via reduced myocardial oxidative stress associated with NO	[162]
Left main coronary occlusion in rat	2.3 $x10^{-7}$,$x10^{-6}$, $x10^{-5}$ g/kg (15 min pretreatment, cessation at occlusion)	Prevented ischemia/reperfusion associated arrhythmias & mortality, with increased production of NO	[163]
aconitine-, oubain- & coronary artery ligation-induced in rat	5-45 mg/kg i.v. injection (one time 10 min prior to measurements)	Dose dependent anti-arrhythmic effect due to shortened action potential duration by enhancement of I_{ks} without change in I_{kr}	[168]
Ex vivo			
Isolated perfused rat hearts	10 μM (15 min pre-ischemia, 2 h post-reperfusion)	Reduced infarct size; Bcl-2 signaling activation of pro-survival pathways via adenosine A_3 receptor activation	[158, 159]
Isolated perfused rat hearts	10 μM (15 min pre-ischemia, 2 h post-reperfusion)	Reduced infarct size with anti-inflammatory actions via NO	[160]
Isolated perfused rat hearts – *ex vivo* & pretreatment *in vivo*	*ex vivo* 10 μM (10 min pre-treatment, 1 h post-ischemia) *in vivo* ~1 mg/kg/d (15 d pre-treatment)	Vasodilation & improved coronary flow via adenosine & NO mechanisms	[161]
Isolated perfused rat hearts	10 μM (5 min pre-reperfusion, 30 min post-reperfusion)	Improved cardiac contractile function & reduced infarct size; reduced mPTP opening via cGMP/PKG dependent GSK-3β inactivation & translocation to the mitochondria	[164]
Isolated perfused rat hearts	1-100 μM (20 min post-occlusion, up to termination of arrhythmia)	Dose dependant anti-arrhythmic effect via ion channel modulation	[169]
Isolated perfused rat hearts	10 μM; 15 min pre-ischemia; 2 h post-reperfusion	Anti-apoptotic effects through Bcl-2 signaling	[158, 159]
Isolated perfused rat hearts	10 μM (15 min pre-ischemia; 2 h post-reperfusion)	Anti-apoptotic effects involving NO	[160]
In vitro			
H9c2 cardiomyocytes	50 μM (30 min pretreatment, 0-48 h post-H_2O_2 treatment)	Anti-apoptotic effects against ROS induced cell death via activation of AMP kinase	[170]
H9c2 cardiomyocytes	20 μM (24 h treatment in ischemic condition)	Anti-apoptotic effects against ischemia via SIRT1-FoxO1 pathway activation	[171]
Phenylephrine induced cardiomyocyte hypertrophy	50 μM (24 h co-treatment)	Inhibits hypertrophy by activating AMP kinase & inhibiting Akt/PKB signaling	[149]
Ang II induced cardiomyocyte hypertrophy	0.1-100 μM (48 h co-treatment)	Dose dependently inhibits hypertrophy via ROS attenuation	[148]
Ang II induced cardiomyocyte hypertrophy	* *10-50 μM (30 min pre-treatment, 72 h)*	Dose dependently inhibits hypertrophy through antioxidant mechanism	[146]
Cardiac fibroblast	25-100 μM (24-72 h co-treatment)	Dose dependently inhibited Ang II induced fibroblast proliferation & hypertrophy; upregulation of NO/cGMP	[155]
Cardiac fibroblast	5-25 μM (30 min pre-treatment, 48 h)	Dose dependently inhibited Ang II-induced fibroblast proliferation & TGF-β-induced differentiation to myofibroblast	[154]

* *isorhapontigenin – an analog of resveratrol was used*; Ang II, angiotensin II; ROS, reactive oxygen species; AMP, adenosine monophosphate; Akt/PKB, protein kinase B; NO, nitric oxide; i.p., intraperitoneal; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; TGF-β, transforming growth factor; LAD, left anterior descending; ANP, atrial natriuretic peptide; Bcl-2, B-cell lymphoma 2; mPTP, mitochondrial permeability transition pore; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; GSK-3β, glycogen synthase kinase 3β; i.v., intravenous; I_{ks}, slow delayed rectifying potassium current; I_{kr}, rapid delayed rectifying potassium current

III. STATEMENT OF HYPOTHESIS

In this study, we hypothesize that resveratrol (*trans-3,5,4,-trihydroxystilbene*), a compound predominantly found in grapes, berries, and red wine, will arrest the development of cardiac hypertophy, as well as regress developed cardiac hypertrophy due to volume overload (VO) and pressure overload (PO). VO will be surgically induced in male Sprague Dawley rats by aortocaval shunt surgery to create eccentric cardiac hypertrophy, while PO will be induced the abdominal aortic banding surgery to create concentric cardiac hypertrophy.

Specifically, this study will test a pre-determined dosage of resveratrol (2.5 mg/kg body weight/day), in two stages of PO and VO-induced cardiac hypertrophy. Stage 1 of the study will address arresting development of PO and VO, with treatment commencing 2 days after surgery for 26 days. Stage 2 of the study will address regression of developed cardiac hypertrophy, with treatment starting 14 days after surgery for 14 days. Echocardiographic analysis of cardiac structure and function will be assessed at 2, 14 and 28 days in stage 1, and 14 and 28 days in stage 2 of the study. This study will be the first to show the effects of resveratrol on cardiac structure and function in VO. It will also be the first to examine whether the development of PO-induced cardiac hypertrophy can be arrested, as well as confirm our earlier brief report of reversal of PO-induced cardiac hypertrophy upon resveratrol treatment [16]. These findings will assess the therapeutic potential of resveratrol in treating clinically relevant conditions of VO, such as valvular regurgitation, septal wall defects and anemia, and PO, such as hypertension and aortic stenosis/coarctation of the aorta.

IV. MATERIALS AND METHODS

The University of Manitoba Animal Care Committee approved the experimental protocols used in this study which conform to the *Canadian Council on Animal Care Concerning the Care and Use of Experimental Animals* (Vol. 1, 2nd Edition, 1993).

4.1 Creation of Pressure Overload (PO) and Volume Overload (VO)

Male Sprague-Dawley rats weighing 150-200 g were subjected to abdominal aortic banding and aortocaval shunt surgeries to induce PO and VO, respectively. These procedures have been described in publications from our laboratory [16, 66]. In general, 5-week old rats were acclimatized within general housing for one week prior to surgery in a temperature- and humidity-controlled room on a 12-hour-light/12-hour-dark cycle. Standard rat chow and tap water were available *ad libitum*. All rats were administered three buprenorphine injections (0.03 mg/kg body weight) at 30 minutes prior to surgery, at 12 hours and 24 hours following surgery. All rats were anesthetized for surgery with 5% isoflurane carried by oxygen at a flow rate of 2 L/min. Rats were then maintained in surgical plane of anesthesia with 2% isoflurane.

In creating PO, a small midline laparotomy (~ 1 inch) was performed and only the stomach exteriorized; blunt dissection was used to expose the suprarenal abdominal aorta. A silk suture was used to tie off the vessel, using a blunt 21-gauge needle as a guide. Successful PO surgeries had bands which were snug while maintaining blood flow to the kidneys and lower extremities. In creating VO, a midline laparotomy (~ 1.5 - 2 inches) was performed and the cecum and small intestines were exteriorized; exposure of the abdominal aorta and inferior vena cava between the renal arteries and the iliac bifurcation was accomplished by blunt dissection. An 18-gauge needle was inserted into the exposed

aorta at an approximate 45° angle and pushed through into the vena cava; cyanoacrylate (Krazy Glue, Elmer's Product Canada, Toronto, ON) was used to seal the external puncture, leaving only the shunt. In successful VO surgeries, oxygenated blood from the abdominal aorta could be seen mixing with deoxygenated blood in the vena cava. In both models, the abdominal musculature and skin incisions were closed by standard techniques using absorbable suture and autoclips. Control rats underwent sham surgeries in which all procedures were identical except for creation of the band or the shunt. Immediately after surgery, all rats were housed in a 37°C incubator for a approximately 12 hours and later returned to general housing. The endpoint for all rats in this study was 28 days after surgery.

4.2 Echocardiography

All rats were weighed and anesthetized using isoflurane as described previously [16, 66]. Transthoracic two-dimensionally (2D)-guided M-mode echocardiography and Pulse Wave Doppler echocardiography were performed using a Sonos 5500 ultrasound system (Agilent Technologies, Andover, MA) equipped with a 12 MHz (s12) transducer. M-Mode echocardiography, using the parasternal short-axis view, was done to obtain a 2-D image of the left ventricle at the level of the papillary muscles with a depth setting of 3 cm. M-mode recordings were then analyzed at a sweep speed of 150 mm/s with the axis of the probe aligned with the middle of the ventricle. Cardiac structure and function parameters were then measured using the leading edge method described by the American Society of Echocardiography [172]. Parameters of cardiac structure included: left ventricular internal dimensions at systole and diastole (LVIDs and LVIDd, respectively), left ventricular posterior wall thickness at systole and diastole (LVPWs and

LVPWd, respectively) and interventricular septal wall thickness at systole and diastole (IVSs and IVSd, respectively). Parameters of cardiac function included: percentage of left ventricular fractional shortening (FS), left ventricular ejection fraction (EF), cardiac output (CO) and heart rate (HR).

Pulse Wave Doppler measurements were taken using the apical 5-chamber view to assess isovolumetric relaxation time (IVRt) and aortic ejection time (AEt). The apical 4-chamber view was used to assess peak early diastolic filling velocity (E wave) across the mitral valve. All echocardiographic data was obtained over three cardiac cycles and averaged for subsequent statistical analysis.

4.3 Resveratrol preparation

Resveratrol (Sigma-Aldrich Canada Ltd, lot number 038K5202) was dissolved in 50% ethanol and administered daily by oral gavage (1 ml per rat) at a dose of ~2.5 mg/kg body weight at the same time each day. Prepared resveratrol was stored at -20°C with new batches made weekly.

4.4 Experimental design

There were two major stages of resveratrol treatment where sham-operated control rats, aortocaval shunt-induced VO rats and abdominal aortic band-induced PO rats were randomly assigned to either resveratrol treated or untreated groups. Stage 1 looked at the effects of resveratrol in arresting the development of cardiac hypertrophy. Resveratrol treatment started 2 days post-surgery and continued for 26 days. Echocardiography analysis of cardiac structure and function were assessed at 2, 14 and 28 days post-surgery. Stage 2 looked at the effects of resveratrol in reversing cardiac hypertrophy. Treatment with resveratrol started 14 days after surgery and continued for

14 days. Cardiac structure and function were analyzed by echocardiography at 14 and 28 days post-surgery.

4.5 General characteristics

After 28 days, all sham, PO and VO rats were weighed and then anesthetized using a cocktail of ketamine (90 mg/kg) and xylazine (10 mg/kg) prior to sacrifice. Hearts were excised, washed in ice-cold saline and weighed. The wet heart weight to body weight ratio (H/BW) was determined. All cardiac tissues were stored at -85°C.

4.6 Statistical analysis

All statistical analysis was performed using the SAS Statistical package (version 9.1; SAS Institute, Inc., Cary, NC, USA). Data was assessed for homogeneity of variance by Levene's test and normality using the Shapiro-Wilk's test. The effect of treatment (± resveratrol) and surgery (PO or VO surgery vs. respective sham surgery) on H/BW ratio, all cardiac structure parameters, blood pressure measurements and TBARS was assessed by 2-way ANOVA in both stages of the study. The effect of treatment, surgery and time on all cardiac function parameters was assessed by 3-way ANOVA in both stages of the study. The significance level was $P<0.05$ for main effects and $P<0.10$ for interactions (to reduce the risk of missing interactions). For post-hoc testing, least-squares means with adjustment for multiple comparison (Tukey-Kramer) was used. Values are expressed as mean ± SEM.

V. RESULTS

5.1 General Observations

VO surgery had a greater H/BW ratio compared to sham surgery in both stages of the study (69% higher - stage 1; 52% higher - stage 2). Resveratrol treatment had no effect on the H/BW ratio in VO rats in both stages (**Figs. 3A** and **3B**). There was a significant interaction of surgery and treatment on the H/BW ratio observed in PO rats in both stages. PO rats had a greater H/BW ratio in both stages of the study compared to sham surgery (29% higher - stage 1; 32% higher - stage 2). Resveratrol treatment prevented an increase in H/BW ratio in PO rats in stage 1 (**Fig. 3C**). Resveratrol treatment also reversed the elevated H/BW ratio in PO rats in stage 2 (**Fig. 3D**). Body weights were unaltered in all groups in this study (Data not shown). Preliminary studies showed that vehicle treatment alone (oral gavage with 50% ethanol) had no effects on any cardiac structure or function parameters compared to untreated rats (Data not shown).

5.2 Cardiac structure in Stage 1: Arresting the development of cardiac hypertrophy with resveratrol treatment commencing 2 days post-surgery

Comprehensive assessment of cardiac structure and function was obtained by echocardiography in each instance. Data for cardiac structure are shown for the diastolic phase of the heart; systolic phase showed similar trends (**Tables 2** and **3** for stage 1; **Tables 6** and **7** for stage 2). In the prevention study (stage 1), M-mode echocardiography showed no significant differences in cardiac structure with respect to wall thickness (IVS and LVPW) and LV internal dimensions in VO (**Fig. 4A**) and PO (**Fig. 4B**) rats compared to sham-operated controls at baseline (2 days after surgery). LVID was greater in VO surgery at 14 days (13% greater) and 28 days (27% greater) with no significant

changes in IVS and LVPW compared with sham surgery; resveratrol treatment failed to prevent the increase in LVID in VO rats (**Figs. 4C** and **4E**). PO surgery showed larger cardiac wall thickness at 14 days (IVS 36% greater; LVPW 25% greater) and 28 days (IVS 47 % greater; LVPW 45% greater) post-surgery without change in LVID compared to sham surgery; resveratrol treatment prevented the increase in wall thickness at 14 and 28 days post-surgery in PO rats (**Figs. 4D** and **4F**) (see **Tables 2** and **3** for all stage 1 cardiac structure values).

5.3 Cardiac function in Stage 1: Arresting the development of cardiac hypertrophy with resveratrol treatment commencing 2 days post-surgery

Baseline measurements 2 days post-surgery showed VO rats had exhibited elevated heart rate (7%) compared to sham surgery. Heart rate in VO rats normalized to sham levels at 14 and 28 days post-surgery, but was significantly decreased at 14 and 28 days compared to 2 day VO rats (**Fig. 5A**). Cardiac output was higher at each time point in VO rats compared to sham rats (26% higher at 2 days; 55% higher at 14 days; 69% higher at 28 days). Resveratrol treatment did not prevent the changes in cardiac output in VO. Furthermore, cardiac output significantly increased over time in VO and sham rats (**Fig. 5A**). Heart rate was not significantly altered by PO surgery and did not change over time while cardiac output did increase over the time of the study (**Fig. 5B**). Throughout the course of stage 1, VO surgery had lower isovolumetric relaxation time compared to sham surgery that was not affected by resveratrol treatment (**Fig. 6A**). VO rats also showed greater early diastolic filling velocities compared to sham rats at each time point (16% higher at 2 days; 29% higher at 14 days; 28% higher at 28 days). Early diastolic filling velocities also increased at 14 and 28 days post-surgery in VO rats compared to 2

day VO rats (**Fig. 6A**). Resveratrol treatment had no effect on early diastolic filling velocity in VO. There was a significant interaction of surgery and treatment, treatment and time and, surgery and time on isovolumetric relaxation time in PO (**Fig. 6B**). Baseline measurements of isovolumetric relaxation time in PO rats were found to not significantly differ from sham rats. Isovolumetric relaxation time was however elevated 16% at 14 days and 37% at 28 days post-surgery in PO rats compared with sham rats. Resveratrol treatment in PO rats produced a trend towards preventing an elevation in isovolumetric relaxation time at 14 days (P=0.0583), which became significant at 28 days (**Fig. 6B**). Early diastolic filling velocity was found not to be significantly altered by PO surgery and did not change over time (**Fig. 6B**). Ejection fraction and fractional shortening were found unchanged in VO (**Fig. 7A**) and PO (**Fig. 7B**) rats compared to respective shams throughout the time course of this study. Similarly, aortic ejection time was also unchanged in both VO (**Table 4**) and PO (**Table 5**) (see **Tables 4** and **5** for all stage 1 cardiac function values).

5.4 Cardiac structure in Stage 2: Regressing cardiac hypertrophy after development with resveratrol treatment commencing 14 days post-surgery

In the reversal study (stage 2), VO surgery produced a 24% larger LVID without changes to IVS and LVPW thickness compared to sham surgery at baseline, 14 days post-surgery (**Fig. 8A**). PO surgery produced an 18% larger IVS and 34% larger LVPW without change to LVID in comparison to sham surgery at baseline (**Fig. 8B**). At 28 days post-surgery, resveratrol treatment did not reverse the increase in LVID in VO rats, which was 36% higher than sham surgery (**Fig. 8C**). PO surgery had 39% larger IVS and 42 % larger LVPW compared with sham surgery at 28 days post-surgery. Resveratrol

treatment had significantly reduced both IVS and LVPW in PO rats (**Fig. 8D**) (see **Tables 6** and **7** for all stage 2 cardiac structure values).

5.5 Cardiac function in Stage 2: Regressing cardiac hypertrophy after development with resveratrol treatment commencing 14 days post-surgery

Heart rate was found to be unchanged in VO (**Fig. 9A**) and PO (**Fig. 9B**) rats compared to respective shams at baseline (14 days) and 28 days post-surgery. Cardiac output was higher at each time point in VO rats compared to sham rats (57% at 14 days; 88% at 28 days). Resveratrol did not prevent the changes in cardiac output in VO. Furthermore, cardiac output significantly increased over time in VO and sham rats (**Fig. 9A**). In PO, cardiac output was found to significantly increase from 14 to 28 days post-surgery (**Fig. 9B**). Throughout the course of stage 2, VO surgery had significantly lower isovolumetric relaxation time and elevated early diastolic filling velocity compared to sham surgery that was not affected by resveratrol treatment (**Fig. 10A**). In PO, there was a significant interaction of surgery, treatment and time on isovolumetric relaxation time. Isovolumetric relaxation time was found to be elevated 17% at baseline (14 days) and 33% at 28 days in PO rats compared with sham rats. Resveratrol treatment was found to significantly decrease the elevated isovolumetric relaxation time in PO rats by 28 days post-surgery (**Fig. 10B**). Early diastolic filling velocity was found not to be significantly altered by PO surgery and did not change over time (**Fig. 10B**). Ejection fraction and fractional shortening were found unchanged in VO (**Fig. 11A**) and PO (**Fig. 11B**) rats compared to respective shams throughout the time course of this study. Similarly, aortic ejection time was also unchanged in both VO (**Table 8**) and PO (**Table 9**) (see **Tables 8** and **9** for all stage 2 cardiac function values).

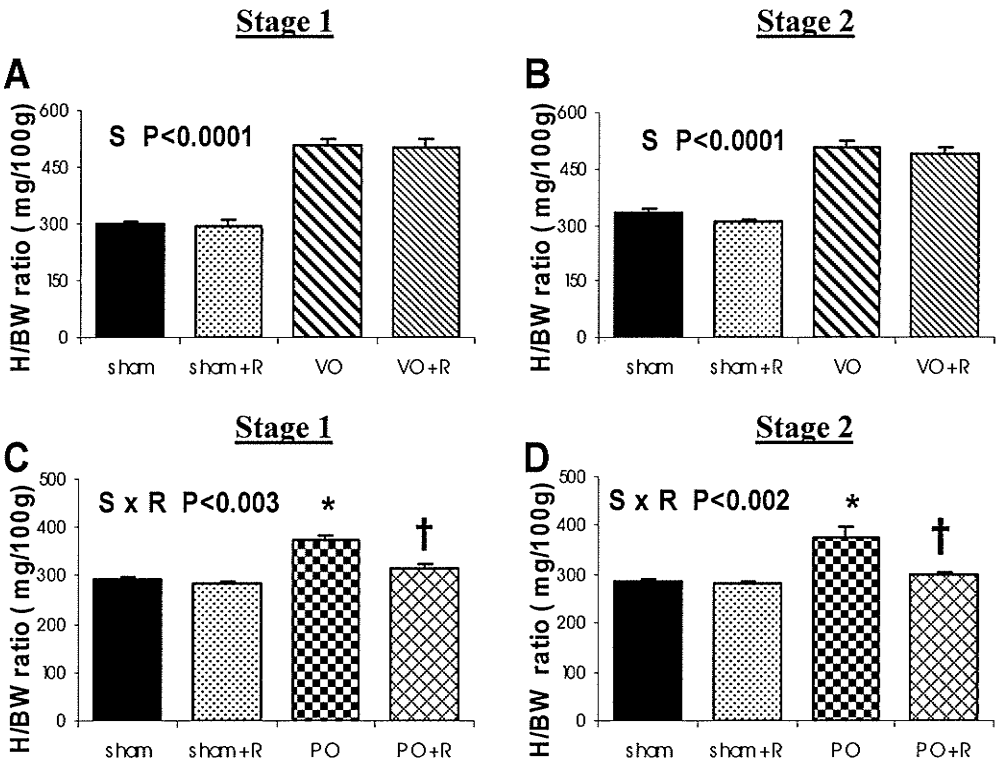

FIGURE 3. The heart-to-body weight ratio (H/BW), an index of cardiac hypertrophy, at 28 days post-surgery for stage 1 of resveratrol treatment in VO rats (*A*) and PO rats (*C*) and for stage 2 of resveratrol treatment in VO rats (*B*) and PO rats (*D*). Stage 1 - resveratrol treatment starting 2 days post-surgery in arresting the development of cardiac hypertrophy. Stage 2 - resveratrol treatment starting 14 days post-surgery in regressing cardiac hypertrophy. R = resveratrol treatment; S = surgery; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 7-11 per group. Significant main effect and interaction indicated in Figure.

* $P<0.05$ vs. sham and sham + R.

† $P<0.05$ vs. PO.

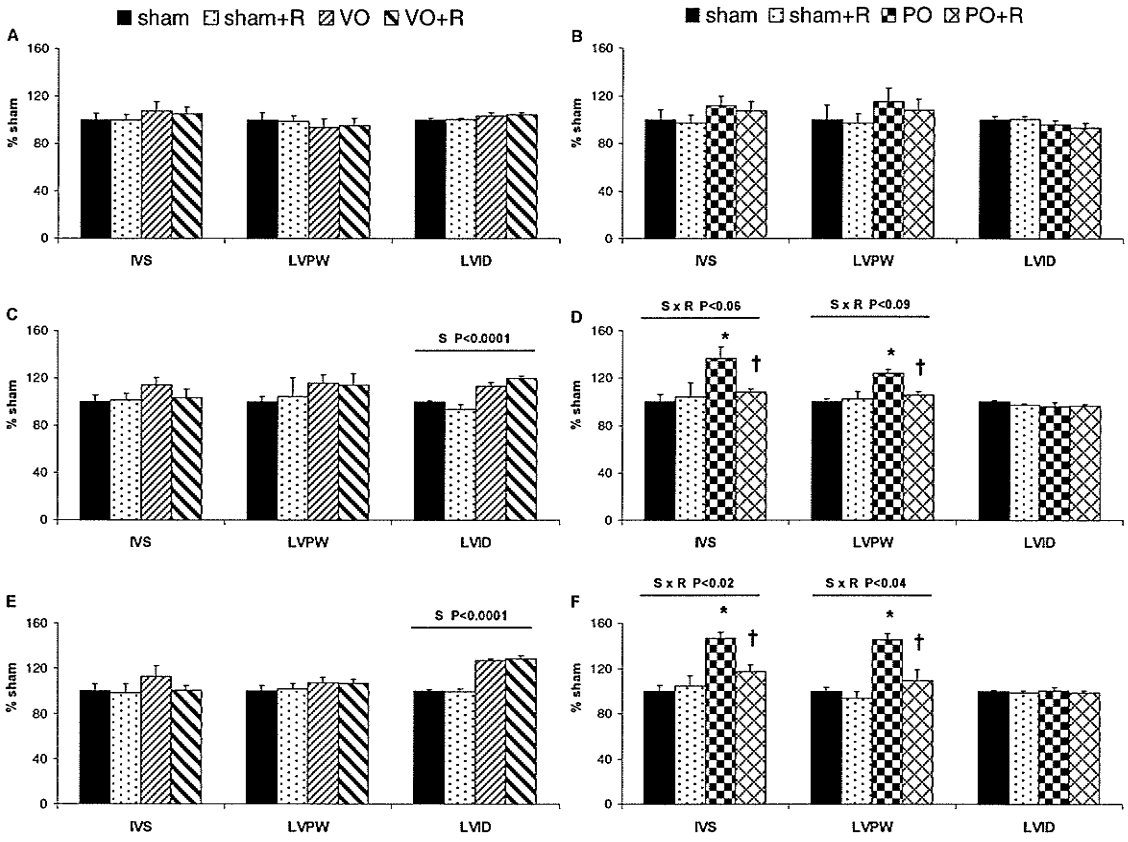

FIGURE 4. Effect of resveratrol treatment in arresting early changes in cardiac structure during the development of VO-induced cardiac hypertrophy (2 days *A*; 14 days *C*; 28 days *E*) and PO-induced cardiac hypertrophy (2 days *B*; 14 days *D*; 28 days *F*). Interventricular septal wall thickness (IVS), LV posterior wall thickness (LVPW), and LV internal dimension (LVID) measured during diastole by M-mode echocardiography. R = resveratrol treatment; S = surgery; VO = volume overload; PO = pressure overload. Data are mean ± SEM (expressed as % sham). n = 7-10 per group. Significant main effect and interaction indicated in Figure.

* $P<0.05$ vs. sham and sham + R.

† $P<0.05$ vs. PO.

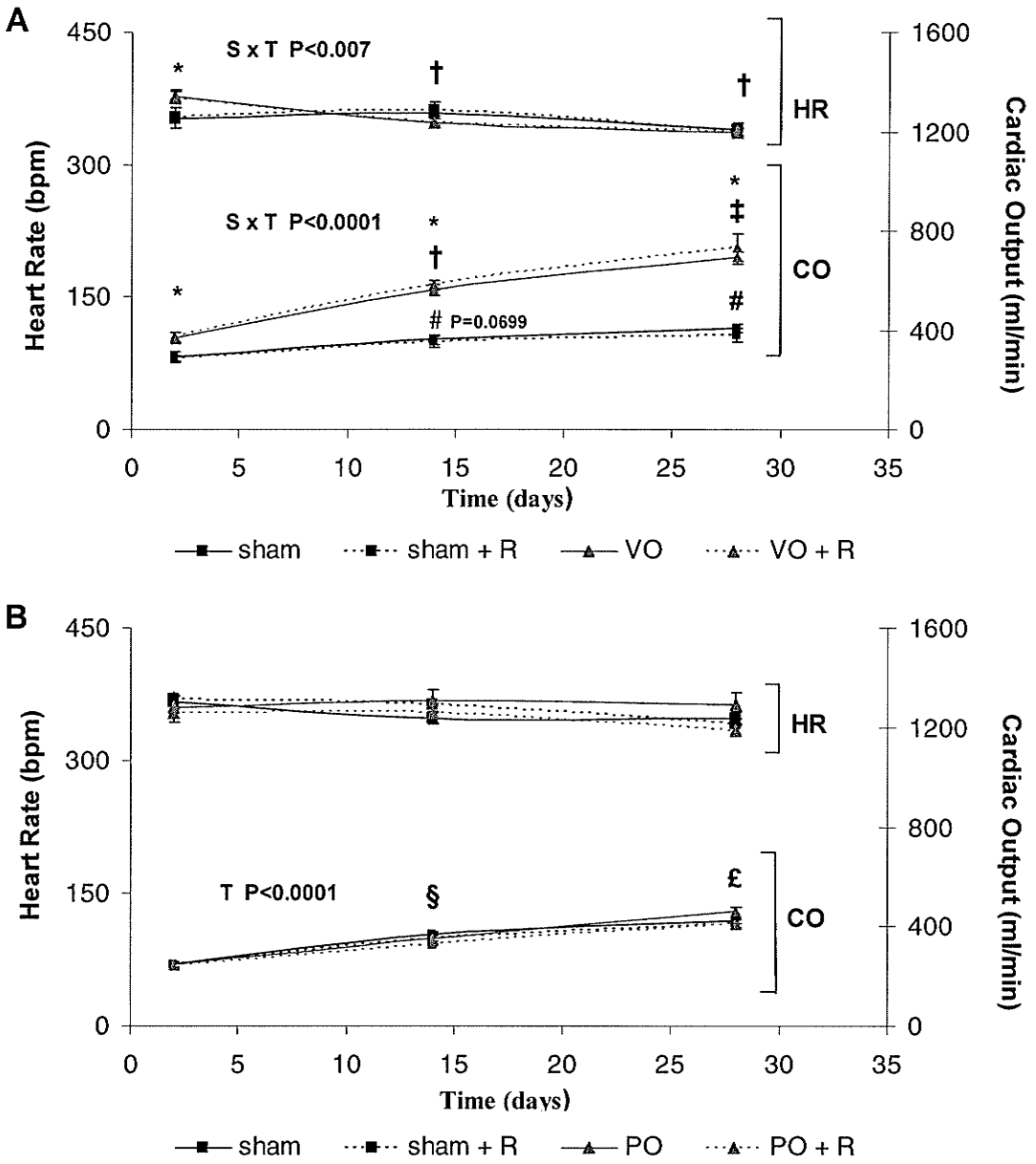

FIGURE 5. Effect of resveratrol treatment in arresting early changes in heart rate and cardiac output during the development of VO-induced cardiac hypertrophy (*A*) and PO-induced cardiac hypertrophy (*B*) at 2, 14 and 28 days post-surgery. R = resveratrol treatment; S = surgery; T = time; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 7-10 per group. Significant main effect and interaction indicated in Figure. All significance are P<0.05, unless otherwise stated.

* sham vs. VO within respective time point.

sham vs. 2 day sham.

† VO vs. 2 day VO.

‡ VO vs. 2 and 14 day VO.

§ 14 day vs. 2 day.

£ 28 day vs. 2 and 14 day.

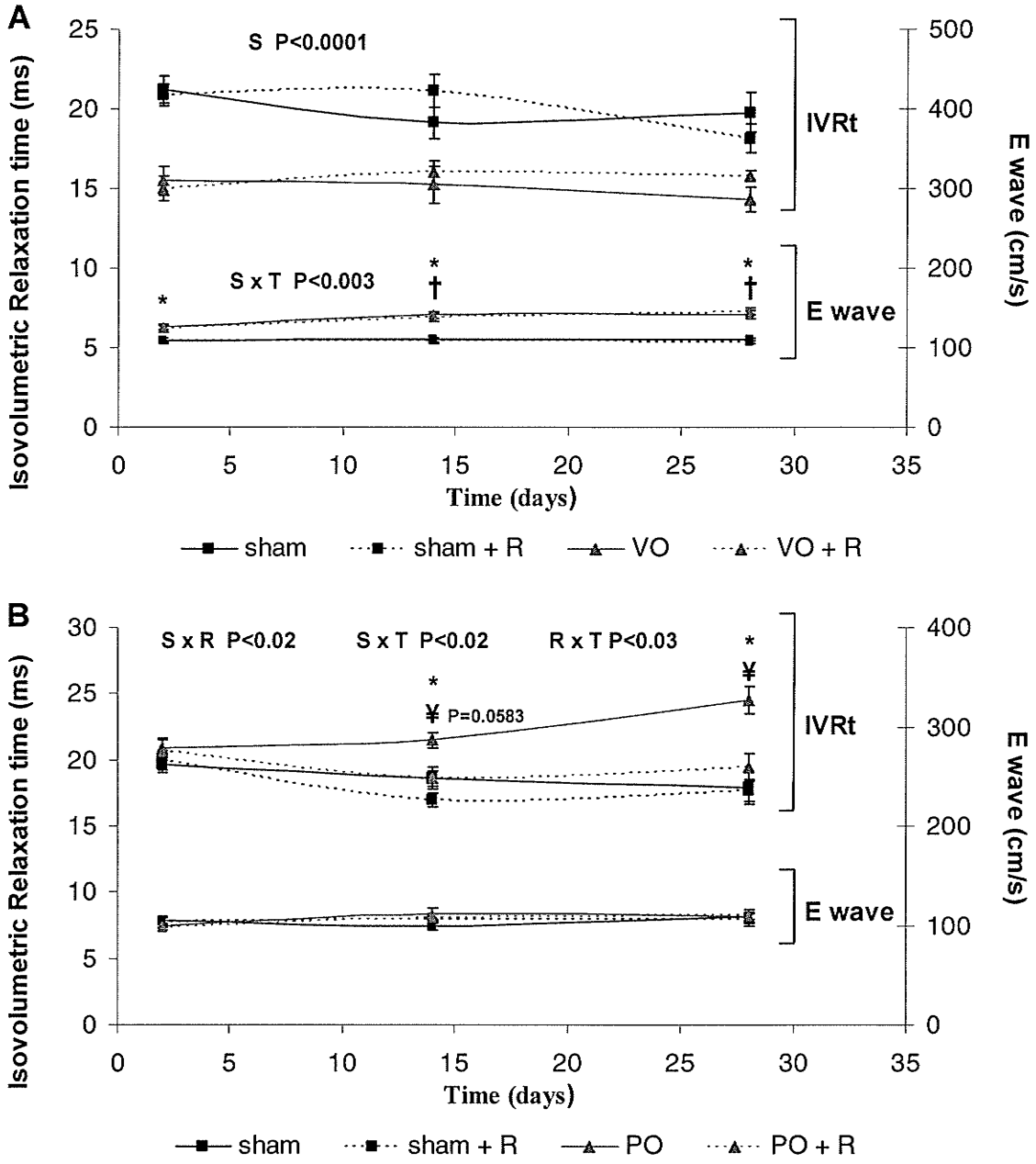

FIGURE 6. Effect of resveratrol treatment in arresting early changes in isovolumetric relaxation time and peak early diastolic filling velocity during the development of VO-induced cardiac hypertrophy (*A*) and PO-induced cardiac hypertrophy (*B*) at 2, 14 and 28 days post-surgery. R = resveratrol treatment; S = surgery; T = time; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 7-10 per group. Significant main effect and interaction indicated in Figure. All significance are $P<0.05$, unless otherwise stated.

* sham vs. VO or PO within respective time point.

† VO vs. 2 day VO.

¥ resveratrol treated vs. untreated within respective time points.

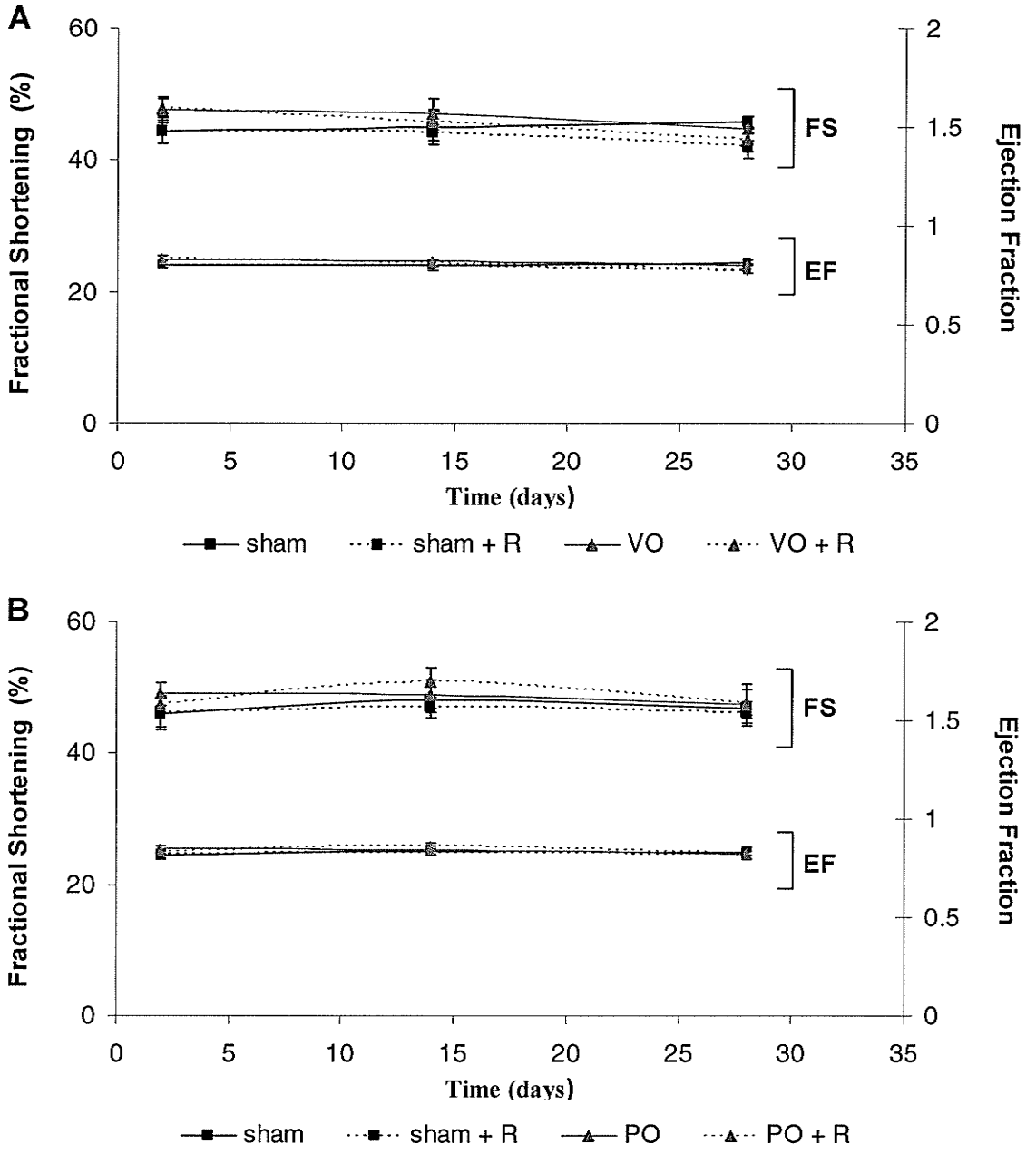

FIGURE 7. Effect of resveratrol treatment in arresting early changes in left ventricular fractional shortening and ejection fraction during the development of VO-induced cardiac hypertrophy (*A*) and PO-induced cardiac hypertrophy (*B*) at 2, 14 and 28 days post-surgery. R = resveratrol treatment; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 7-10 per group.

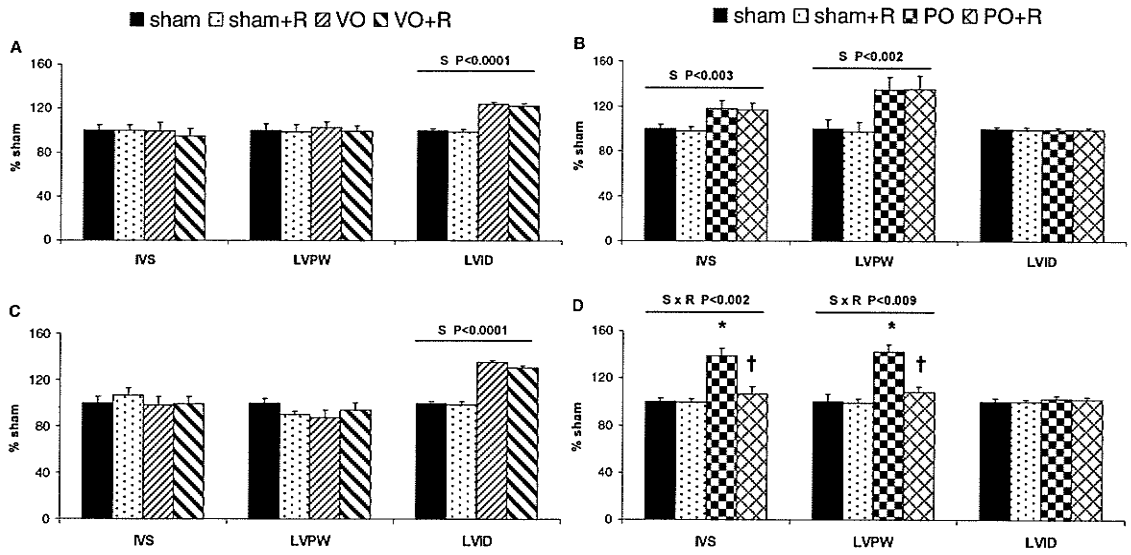

FIGURE 8. Effect of resveratrol treatment in reversing changes in cardiac structure after the development of VO-induced cardiac hypertrophy (14 days *A*; 28 days *C*) and PO-induced cardiac hypertrophy (14 days *B*; 28 days *D*). Interventricular septal wall thickness (IVS), LV posterior wall thickness (LVPW), and LV internal dimension (LVID) measured during diastole by M-mode echocardiography. R = resveratrol treatment; S = surgery; VO = volume overload; PO = pressure overload. Data are mean ± SEM (expressed as % sham). n = 8-11 per group. Significant main effect and interaction indicated in Figure.

* $P<0.05$ vs. sham and sham + R.

† $P<0.05$ vs. PO.

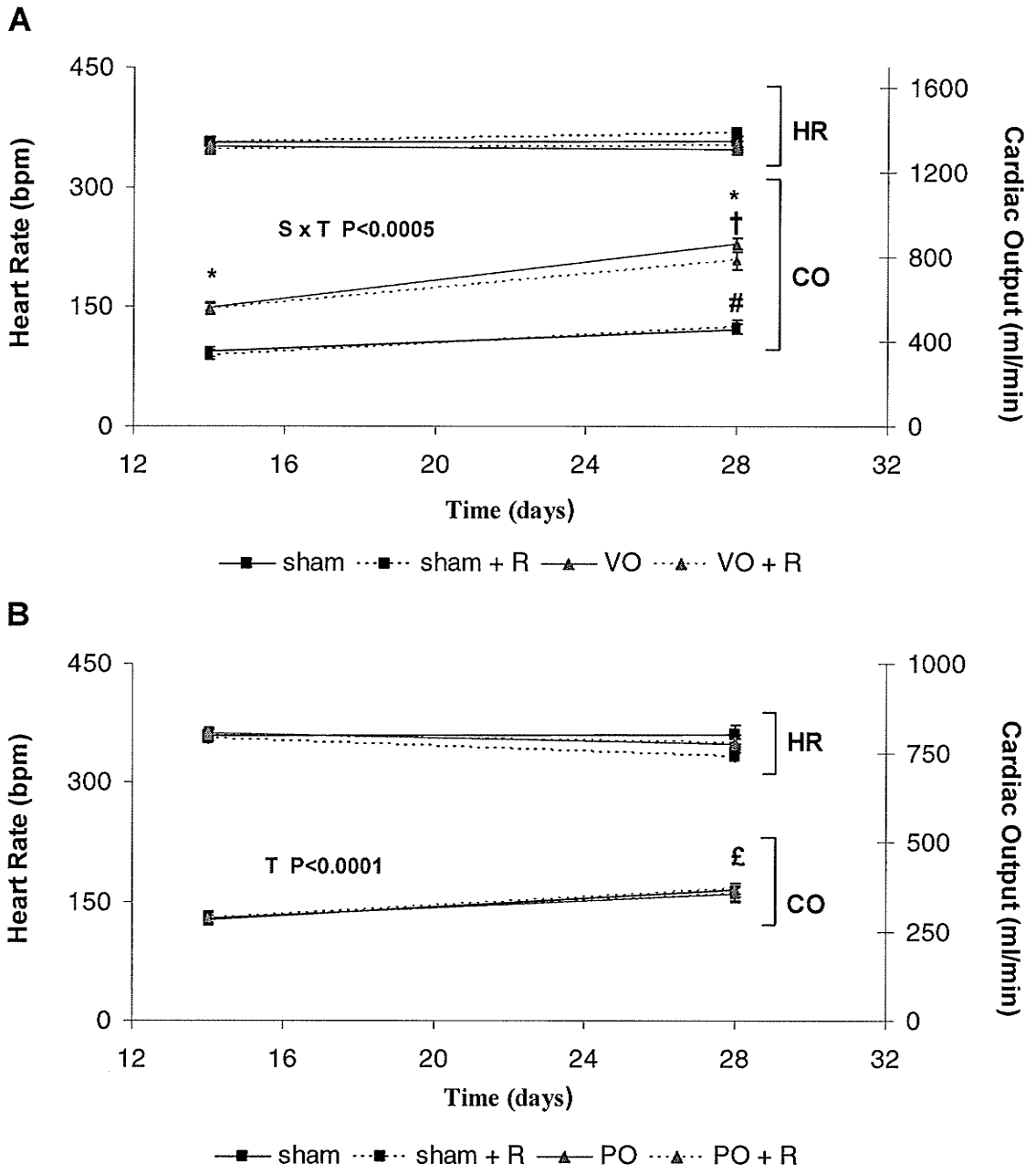

FIGURE 9. Effect of resveratrol treatment in reversing changes in heart rate and cardiac output after the development of VO-induced cardiac hypertrophy (*A*) and PO-induced cardiac hypertrophy (*B*) at 14 and 28 days post-surgery. R = resveratrol treatment; S = surgery; T = time; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 8-11 per group. Significant main effect and interaction indicated in Figure. All significance are $P<0.05$.

* sham vs. VO within respective time point.

sham vs. 14 day sham.

† VO vs. 14 day VO.

£ 28 day vs. 14 day.

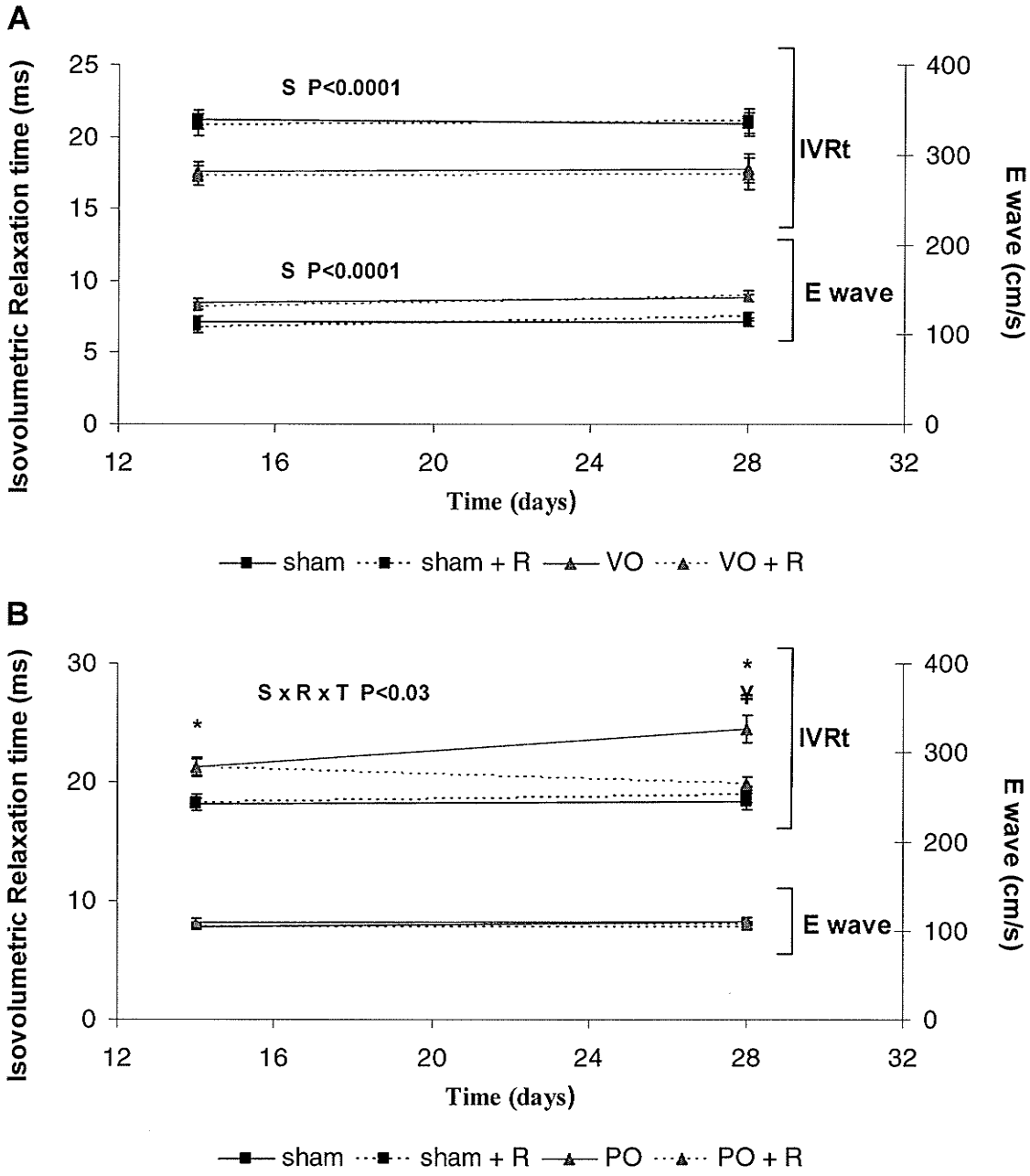

FIGURE 10. Effect of resveratrol treatment in reversing changes in isovolumetric relaxation time and peak early diastolic filling velocity after the development of VO-induced cardiac hypertrophy (*A*) and PO-induced cardiac hypertrophy (*B*) at 14 and 28 days post-surgery. R = resveratrol treatment; S = surgery; T = time; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 8-11 per group. Significant main effect and interaction indicated in Figure. All significance are P<0.05.
* sham vs. PO within respective time point.
¥ PO + R vs. PO within respective time points.

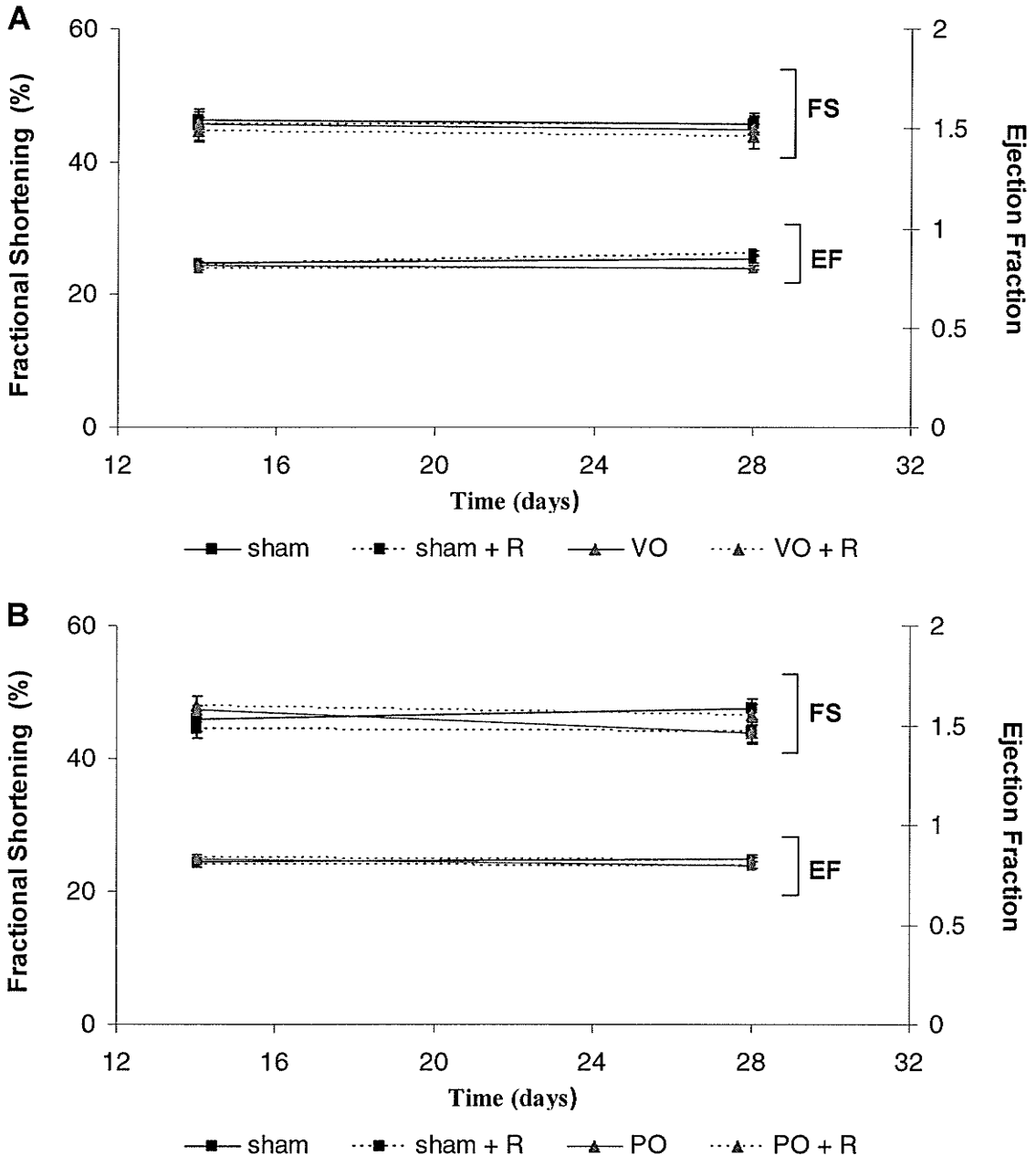

FIGURE 11. Effect of resveratrol treatment in reversing changes in left ventricular fractional shortening and ejection fraction after the development of VO-induced cardiac hypertrophy (*A*) and PO-induced cardiac hypertrophy (*B*) at 14 and 28 days post-surgery. R = resveratrol treated; VO = volume overload; PO = pressure overload. Data are mean ± SEM. n = 8-11 per group.

TABLE 2. Cardiac structure in arresting VO-induced cardiac hypertrophy with resveratrol treatment commencing 2 days post-surgery (stage 1) [1]

Parameter	2 days				14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
IVSd (cm)	0.108± 0.006	0.108± 0.005	0.116± 0.008	0.113± 0.006	0.126± 0.007	0.128± 0.007	0.144± 0.008	0.130± 0.010	0.133± 0.008	0.131± 0.010	0.150± 0.013	0.134± 0.006
IVSs (cm)	0.229± 0.006	0.229± 0.004	0.240± 0.007	0.240± 0.007	0.263± 0.013	0.250± 0.010	0.294± 0.017	0.291± 0.010	0.280± 0.008	0.253± 0.014	0.308± 0.013	0.299± 0.019
LVPWd (cm)	0.115± 0.007	0.114± 0.006	0.108± 0.008	0.110± 0.007	0.126± 0.006	0.132± 0.020	0.146± 0.009	0.144± 0.012	0.144± 0.007	0.147± 0.007	0.155± 0.007	0.154± 0.005
LVPWs (cm)	0.225± 0.010	0.226± 0.009	0.235± 0.014	0.232± 0.012	0.253± 0.010	0.255± 0.019	0.277± 0.010	0.270± 0.018	0.272± 0.006	0.263± 0.008	0.299± 0.011	0.271± 0.008
LVIDd [2] (cm)	0.771± 0.013	0.775± 0.011	0.800± 0.020	0.806± 0.014	0.835± 0.011	0.783± 0.033	0.947± 0.026 *	1.001± 0.017 *	0.879± 0.012	0.875± 0.021	1.118± 0.015 *	1.131± 0.025 *
LVIDs [2] (cm)	0.432± 0.018	0.429± 0.017	0.404± 0.023	0.405± 0.021	0.443± 0.022	0.438± 0.023	0.545± 0.030 *	0.544± 0.023 *	0.467± 0.012	0.506± 0.022	0.609± 0.033 *	0.634± 0.030 *

VO = volume overload; R = resveratrol treatment; IVS = interventricular septal wall thickness; LVPW = left ventricular posterior wall thickness; LVID = left ventricular internal dimension; d = diastole; s = systole. Significant main effects observed are within same row.

[1] Values are expressed as mean ± SEM. n = 7-10 per group.

[2] Significant main effect of surgery was observed at 14 and 28 day time points ($P<0.0001$).

* $P < 0.05$ vs. sham and sham + R within respective time point.

TABLE 3. Cardiac structure in arresting PO-induced cardiac hypertrophy with resveratrol treatment commencing 2 days post-surgery (stage 1) [1]

Parameter	2 days				14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
IVSd [2] (cm)	0.113± 0.009	0.109± 0.008	0.126± 0.009	0.121± 0.009	0.125± 0.008	0.130± 0.015	0.171± 0.012 *	0.135± 0.004 †	0.111± 0.006	0.116± 0.010	0.163± 0.006 *	0.130± 0.007 †
IVSs [3] (cm)	0.226± 0.011	0.226± 0.009	0.249± 0.011	0.243± 0.011	0.255± 0.012	0.245± 0.012	0.313± 0.015 *	0.265± 0.008 †	0.246± 0.011	0.247± 0.009	0.323± 0.015 *	0.269± 0.019 †
LVPWd [4] (cm)	0.102± 0.012	0.099± 0.008	0.117± 0.012	0.110± 0.010	0.128± 0.003	0.131± 0.008	0.159± 0.004 *	0.136± 0.004 †	0.136± 0.005	0.128± 0.008	0.198± 0.007 *	0.149± 0.013 †
LVPWs [5] (cm)	0.201± 0.010	0.198± 0.007	0.209± 0.006	0.208± 0.009	0.247± 0.009	0.255± 0.012	0.310± 0.011*	0.273± 0.004 †	0.271± 0.011	0.255± 0.008	0.332± 0.021 *	0.283± 0.008 †
LVIDd (cm)	0.713± 0.018	0.718± 0.014	0.684± 0.024	0.664± 0.027	0.833± 0.009	0.811± 0.011	0.802± 0.026	0.805± 0.011	0.900± 0.011	0.889± 0.016	0.905± 0.024	0.886± 0.018
LVIDs (cm)	0.388± 0.023	0.387± 0.022	0.367± 0.020	0.370± 0.015	0.405± 0.018	0.409± 0.010	0.390± 0.023	0.380± 0.020	0.477± 0.015	0.479± 0.021	0.479± 0.041	0.485± 0.012

PO = pressure overload; R = resveratrol treatment; IVS = interventricular septal wall thickness; LVPW = left ventricular posterior wall thickness; LVID = left ventricular internal dimension; d = diastole; s = systole. Significant interactions observed are within same row.

[1] Values are expressed as mean ± SEM. n = 7-10 per group.

[2] Significant interaction of surgery and resveratrol treatment was observed at 14 day ($P<0.06$) and 28 day ($P<0.02$) time point.

[3] Significant interaction of surgery and resveratrol treatment was observed at 14 day ($P<0.04$) and 28 day ($P<0.06$) time point.

[4] Significant interaction of surgery and resveratrol treatment was observed at 14 day ($P<0.09$) and 28 day ($P<0.04$) time point.

[5] Significant interaction of surgery and resveratrol treatment was observed at 14 day ($P<0.07$) and 28 day ($P<0.06$) time point.

* $P < 0.05$ vs. sham and sham + R within respective time point.

† $P<0.05$ vs. surgery within respective time point.

TABLE 4. Cardiac function in arresting VO-induced cardiac hypertrophy with resveratrol treatment commencing 2 days post-surgery (stage 1) [1]

Parameter	2 days				14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
FS (%)	44.48± 1.92	44.33± 1.77	47.59± 1.89	47.93± 1.38	45.04± 2.67	44.19± 1.24	46.96± 2.27	45.81± 1.65	45.79± 0.93	42.15± 1.96	44.78± 1.86	43.08± 1.88
EF	0.802± 0.018	0.802± 0.017	0.830± 0.018	0.837± 0.013	0.803± 0.028	0.803± 0.012	0.821± 0.022	0.812± 0.016	0.817± 0.009	0.777± 0.022	0.799± 0.018	0.782± 0.020
Heart rate [2] (bpm)	353±11	353±8	377±8 *	376±8 *	358±13	361±7	347±5 †	348±7	340±8	338±7	336±5 †	339±8
CO [3] (ml/min)	294.5± 19.6	286.3± 14.3	372.0± 22.0 *	373.8± 19.9 *	361.9± 18.7	351.1± 21.4	561.4± 21.7 * †	582.3± 20.8 *	409.4± 17.7 #	379.5± 27.0	693.0± 25.5 * ‡	734.0± 53.8 *
IVRt [4] (ms)	21.18± 0.87	20.90± 0.69	15.50± 0.91 *	15.00± 0.76 *	19.11± 1.01	21.12± 1.04	15.22± 1.18 *	16.00± 0.70 *	19.78± 1.23	18.14± 0.91	14.33± 0.76 *	15.80± 0.33 *
E wave [5] (cm/s)	108.5± 3.3	109.2± 2.8	125.9± 3.8 *	123.7± 4.4 *	109.8± 3.1	109.1± 3.6	141.3± 4.4 * †	137.3± 5.3 *	110.5± 1.8	107.0± 1.2	141.4± 4.8 * †	145.1± 4.3 *
AEt (ms)	64.00± 1.43	64.50± 1.52	64.15± 1.54	64.17± 1.46	63.00± 1.68	61.50± 1.41	64.33± 0.91	63.60± 1.39	67.44± 1.04	66.25± 1.80	67.80± 1.10	67.00± 0.89

VO = volume overload; R = resveratrol treatment; FS = fractional shortening; EF = ejection fraction; CO = cardiac output; IVRt = isovolumetric relaxation time; E wave = early diastolic filling velocity; AEt = aortic ejection time. Significant main effects and interactions observed are within same row.

[1] Values are expressed as mean ± SEM. n = 7-10 per group.

[2] Significant interaction of surgery and time was observed ($P<0.007$).

[3] Significant interaction of surgery and time was observed ($P<0.0001$).

[4] Significant main effect of surgery was observed ($P<0.0001$).

[5] Significant interaction of surgery and time was observed ($P<0.003$).

* $P<0.05$ vs. sham and sham + R within respective time point.

# $P<0.05$ vs. 2 day sham.

† $P<0.05$ vs. 2 day surgery.

‡ $P<0.05$ vs. 2 and 14 day surgery.

TABLE 5. Cardiac function in arresting PO-induced cardiac hypertrophy with resveratrol treatment commencing 2 days post-surgery (stage 1) [1]

Parameter	2 days				14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
FS (%)	45.95± 2.40	46.29± 2.26	49.05± 1.80	47.42± 1.19	48.09± 1.93	46.96± 1.51	48.99± 2.23	50.91± 2.02	46.93± 1.54	46.19± 1.70	47.46± 3.18	47.72± 1.93
EF	0.815± 0.022	0.820± 0.020	0.848± 0.013	0.835± 0.011	0.834± 0.017	0.828± 0.014	0.842± 0.020	0.860± 0.016	0.826± 0.014	0.819± 0.016	0.824± 0.030	0.831± 0.016
Heart rate (bpm)	367±8	370±7	361±12	354±11	348±6	364±13	368±12	354±11	348±7	342±5	363±15	334±6
CO [2 § £] (ml/min)	245.7± 13.9	248.9± 10.5	247.8± 15.9	242.3± 16.6	365.7± 4.1	352.1± 24.3	351.8± 29.0	332.0± 7.9	422.4± 12.8	412.8± 19.8	459.2± 19.1	411.3± 17.2
IVRt [3 ¥] (ms)	19.70± 0.72	20.00± 0.73	20.91± 0.73	20.73± 0.75	18.57± 0.53	17.00± 0.52	21.50± 0.56 *	18.62± 0.84	17.86± 1.18	17.71± 0.81	24.50± 1.06 *	19.43± 1.02
E wave (cm/s)	104.6± 3.8	103.6± 3.4	99.7± 2.3	97.7± 2.7	99.6± 4.7	106.8± 4.9	111.7± 4.9	107.4± 4.4	108.5± 4.7	105.7± 6.5	109.2± 6.5	110.0± 3.7
AEt (ms)	64.90± 1.55	65.00± 1.29	66.73± 1.78	65.73± 2.0	65.82± 1.38	62.86± 1.18	64.10± 1.73	67.25± 1.16	67.14± 1.64	63.29± 1.08	63.86± 2.13	67.40± 1.22

PO = pressure overload; R = resveratrol treatment; FS = fractional shortening; EF = ejection fraction; CO = cardiac output; IVRt = isovolumetric relaxation time; E wave = early diastolic filling velocity; AEt = aortic ejection time. Significant main effects and interactions observed are within same row.

[1] Values are expressed as mean ± SEM. n = 7-10 per group.

[2] Significant main effect of time was observed ($P<0.0001$).

[3] Significant interaction of surgery and resveratrol treatment ($P<0.02$), surgery and time ($P<0.02$), resveratrol treatment and time ($P<0.03$) was observed.

§ 14 day vs. 2 day ($P<0.05$).

£ 28 day vs. 2 and 14 day ($P<0.05$).

* $P<0.05$ vs. sham and sham + R within respective time point.

¥ Resveratrol treated vs. untreated at 14 days ($P=0.0583$) and 28 days ($P<0.05$).

TABLE 6. Cardiac structure in reversing VO-induced cardiac hypertrophy with resveratrol treatment commencing 14 days post-surgery (stage 2) [1]

	14 days				28 days			
Parameter	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
IVSd (cm)	0.131± 0.006	0.131± 0.007	0.130± 0.010	0.124± 0.009	0.128± 0.007	0.137± 0.008	0.126± 0.009	0.127± 0.008
IVSs (cm)	0.252± 0.008	0.251± 0.008	0.265± 0.013	0.259± 0.014	0.275± 0.013	0.286± 0.013	0.298± 0.010	0.283± 0.009
LVPWd (cm)	0.144± 0.009	0.143± 0.010	0.148± 0.008	0.144± 0.007	0.142± 0.006	0.128± 0.004	0.124± 0.010	0.134± 0.009
LVPWs (cm)	0.286± 0.011	0.276± 0.007	0.289± 0.011	0.282± 0.011	0.308± 0.017	0.288± 0.009	0.296± 0.013	0.301± 0.013
LVIDd [2] (cm)	0.804± 0.019	0.798± 0.019	0.999± 0.015 *	0.988± 0.019 *	0.878± 0.013	0.866± 0.025	1.190± 0.016 *	1.148± 0.018 *
LVIDs [2] (cm)	0.438± 0.014	0.430± 0.015	0.550± 0.021 *	0.542± 0.020 *	0.444± 0.019	0.411± 0.024	0.656± 0.021 *	0.638± 0.015 *

VO = volume overload; R = resveratrol treatment; IVS = interventricular septal wall thickness; LVPW = left ventricular posterior wall thickness; LVID = left ventricular internal dimension; d = diastole; s = systole. Significant main effects observed are within same row.

[1] Values are expressed as mean ± SEM. n = 8-11 per group.

[2] Significant main effect of surgery was observed at 14 and 28 day time point ($P<0.0001$).

* $P < 0.05$ vs. sham and sham + R within respective time point.

TABLE 7. Cardiac structure in reversing PO-induced cardiac hypertrophy with resveratrol treatment commencing 14 days post-surgery (stage 2) [1]

Parameter	14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
IVSd [2] (cm)	0.146± 0.005	0.142± 0.006	0.172± 0.010 *	0.170± 0.009 *	0.139± 0.005	0.138± 0.005	0.193± 0.009 *	0.148± 0.008 †
IVSs [3] (cm)	0.264± 0.013	0.258± 0.012	0.312± 0.013 *	0.311± 0.012 *	0.281± 0.010	0.276± 0.011	0.326± 0.013 *	0.290± 0.008 †
LVPWd [4] (cm)	0.125± 0.010	0.122± 0.011	0.168± 0.014 *	0.170± 0.015 *	0.139± 0.009	0.137± 0.005	0.197± 0.009 *	0.150± 0.006 †
LVPWs [5] (cm)	0.251± 0.014	0.249± 0.012	0.297± 0.013 *	0.298± 0.014 *	0.267± 0.009	0.266± 0.007	0.329± 0.010 *	0.290± 0.006 †
LVIDd (cm)	0.758± 0.012	0.754± 0.016	0.751± 0.014	0.753± 0.013	0.826± 0.023	0.827± 0.013	0.847± 0.021	0.843± 0.016
LVIDs (cm)	0.413± 0.018	0.402± 0.018	0.393± 0.016	0.394± 0.014	0.448± 0.022	0.463± 0.017	0.465± 0.018	0.452± 0.018

PO = pressure overload; R = resveratrol treatment; IVS = interventricular septal wall thickness; LVPW = left ventricular posterior wall thickness; LVID = left ventricular internal dimension; d = diastole; s = systole. Significant main effects and interactions observed are within same row.

[1] Values are expressed as mean ± SEM. n = 8-11 per group.

[2] Significant main effect of surgery was observed at 14 day ($P<0.003$); significant interaction of surgery and resveratrol treatment was observed at 28 day ($P<0.002$).

[3] Significant main effect of surgery was observed at 14 day ($P<0.006$); significant interaction of surgery and resveratrol treatment was observed at 28 day ($P<0.003$).

[4] Significant main effect of surgery was observed at 14 day ($P<0.002$); significant interaction of surgery and resveratrol treatment was observed at 28 day ($P<0.009$).

[5] Significant main effect of surgery was observed at 14 day ($P<0.009$); significant interaction of surgery and resveratrol treatment was observed at 28 day ($P<0.01$).

* $P < 0.05$ vs. sham and sham + R within respective time point; † $P<0.05$ vs. surgery within respective time point.

TABLE 8. Cardiac function in reversing VO-induced cardiac hypertrophy with resveratrol treatment commencing 14 days post-surgery (stage 2) [1]

Parameter	14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
FS (%)	46.38± 1.14	45.58± 1.07	45.57± 1.60	44.66± 1.56	45.72± 1.64	45.63± 1.25	44.93± 1.27	43.81± 1.58
EF	0.823± 0.010	0.816± 0.011	0.809± 0.016	0.801± 0.016	0.845± 0.019	0.874± 0.014	0.802± 0.013	0.799± 0.015
Heart rate (bpm)	356±6	356±5	350±4	348±3	357±7	368±1	347±3	352±5
CO [2] (ml/min)	353.55± 21.02	337.82± 23.38	561.33± 27.06 *	558.00± 24.85 *	461.89± 24.11 [#]	470.22± 33.28	866.30± 28.91 *[†]	785.78± 43.31 *
IVRt [3] (ms)	21.18± 0.69	20.82± 0.74	17.58± 0.69 *	17.33± 0.67 *	20.87± 0.85	21.11± 0.86	17.80± 1.00 *	17.44± 1.08 *
E wave [3] (cm/s)	113.6± 6.4	108.0± 6.1	134.9± 4.6 *	131.4± 4.1 *	114.5± 4.8	120.0± 5.1	141.2± 3.7 *	142.8± 5.8 *
AEt (ms)	64.33± 0.10	64.00± 0.76	65.88± 0.93	65.57± 0.81	62.56± 0.96	61.44± 1.46	65.75± 1.50	65.22± 1.50

VO = volume overload; R = resveratrol treatment; FS = fractional shortening; EF = ejection fraction; CO = cardiac output; IVRt = isovolumetric relaxation time; E wave = early diastolic filling velocity; AEt = aortic ejection time. Significant main effects and interactions observed are within same row.

[1] Values are expressed as mean ± SEM. n = 8-11 per group.

[2] Significant interaction of surgery and time was observed ($P<0.0005$).

[3] Significant main effect of surgery was observed at 14 and 28 day time points ($P<0.0001$).

* $P<0.05$ vs. sham and sham + R within respective time point.

[#] $P<0.05$ vs. 14 day sham.

[†] $P<0.05$ vs. 14 day surgery.

TABLE 9. Cardiac function in reversing PO-induced cardiac hypertrophy with resveratrol treatment commencing 14 days post-surgery (stage 2) [1]

Parameter	14 days				28 days			
	Sham	Sham + R	Surgery	Surgery + R	Sham	Sham + R	Surgery	Surgery + R
FS (%)	45.96± 1.94	44.55± 1.52	47.44± 1.90	47.92± 1.48	47.47± 1.56	44.07± 1.53	43.89± 1.55	46.54± 1.53
EF	0.817± 0.017	0.806± 0.015	0.830± 0.018	0.837± 0.013	0.832± 0.015	0.799± 0.017	0.798± 0.016	0.821± 0.013
Heart rate (bpm)	360±7	355±7	362±8	361±8	361±12	334±7	349±11	350±5
CO [2 £] (ml/min)	282.22± 11.94	288.00± 11.91	286.31± 17.48	286.58± 14.55	365.90± 19.72	353.14± 22.25	356.45± 20.11	371.71± 15.08
IVRt [3] (ms)	18.11± 0.56	18.30± 0.72	21.25± 0.78 *	21.27± 0.71 *	18.38± 0.68	19.00± 0.68	24.45± 1.15 *	19.90± 0.60 †
E wave (cm/s)	104.7± 2.5	103.8± 2.9	109.3± 3.6	109.6± 3.2	110.3± 3.9	104.5± 3.7	111.0± 4.2	106.8± 3.4
AEt (ms)	64.60± 1.28	64.33± 1.18	65.92± 0.85	66.08± 0.83	64.56± 1.32	66.50± 1.14	66.73± 1.64	66.43± 1.07

PO = pressure overload; R = resveratrol treatment; FS = fractional shortening; EF = ejection fraction; CO = cardiac output; IVRt = isovolumetric relaxation time; E wave = early diastolic filling velocity; AEt = aortic ejection time. Significant main effects and interactions observed are within same row.

[1] Values are expressed as mean ± SEM. n = 8-11 per group.

[2] Significant main effect of time was observed ($P<0.0001$).

[3] Significant interaction of surgery, resveratrol treatment and time ($P<0.03$) was observed.

[£] 28 day vs. 14 day ($P<0.05$).

* $P<0.05$ vs. sham and sham + R within respective time point.

† $P<0.05$ vs. surgery within respective time point.

VI. DISCUSSION

In response to changes in myocardial load, the heart has the capability to compensate through an increase in size (hypertrophy) as a way to enhance pumping capacity. Cardiac hypertrophy likewise provides a mechanism for the heart to adapt to changes caused by stress and damage. In the latter case, the ensuing ventricular remodeling compensates for the effects of the insult, but prolonged hypertrophy ultimately becomes decompensated leading to heart failure [5]. The transition from hypertrophy to heart failure therefore remains a prospective therapeutic target. It would be advantageous to arrest and regress the development of cardiac hypertrophy, thus preventing its progression to heart failure. The use of nutraceuticals has recently shown great promise in the treatment of cardiac hypertrophy, particularly in the instance of pressure overload [117-119]. We have reported that daily resveratrol treatment of 2.5 mg/kg body weight by oral gavage starting 14 days post-abdominal aortic banding surgery reversed pressure overload-induced cardiac hypertrophy and its deleterious consequences on heart function in rats by the end of 28 days post-surgery [16]. However, no study has evaluated the effects of resveratrol at earlier stages of pressure overload-induced cardiac hypertrophy by abdominal aortic banding. In addition, no study has examined the effects of resveratrol on cardiac hypertrophy due to volume overload in rats. We have therefore examined the effects of resveratrol, at the previously determined dosage of 2.5 mg/kg body weight by oral gavage, in arresting the development of cardiac hypertrophy and regressing developed cardiac hypertrophy through echocardiographic analysis of cardiac structure and function in rats subjected to volume overload and pressure overload.

In order to properly address the objectives of this study, it is important to isolate the various forms of cardiac hypertrophy in their pure states. Therefore, we used consistent, pure and well established models of cardiac hypertrophy: the abdominal aortic banding surgery for pressure overload-induced concentric hypertrophy, and the aortocaval shunt surgery for volume overload-induced eccentric hypertrophy, in Sprague Dawley rats. In our study, four weeks of pressure overload induced cardiac hypertrophy that was concentric in nature producing left ventricular wall thickening without left ventricular chamber dilation and diastolic dysfunction. On the other hand, four weeks of volume overload produced increased venous return allowing for progressively increased cardiac output and enhanced myocardial relaxation; this resulted in eccentric hypertrophy characterized by left ventricular chamber dilation without significant changes to left ventricular wall thickness. The consistency and purity of the left ventricular remodeling found in our animal models is in agreement with the expected left ventricular remodeling due to pressure overload and volume overload [5] and with previous experimental findings [16, 66].

In this study, we established that at baseline for the preventative stage (stage 1), no significant changes in structure were observed in both pressure overload and volume overload rats. Resveratrol treatment arrested the development of concentric hypertrophy in pressure overload rats as evident by preserved IVS and LVPW wall thickness until the study end point. Li *et al.* [146] reported that daily treatment with the resveratrol analog isorhapontigenin starting 24 hours post-transverse aortic constriction surgery prevented an increase in LVPW thickness compared with untreated transverse aortic constriction surgery after three weeks post-surgery; however, the increased LVPW in transverse aortic

constriction was not statistically significant compared to shams, and their model of pressure overload did not produce pure concentric hypertrophy. Nonetheless, these results confirm that in the presence of a band, resveratrol, and its analog, are able to arrest left ventricular wall thickening. In addition, the therapeutic stage (stage 2) confirms our previous findings [16] that resveratrol treatment starting 14 days post-abdominal aortic banding surgery can regress the increased IVS and LVPW thickness seen in pressure overload rats to near sham levels by 28 days post-surgery.

In pressure overload rats, no changes in cardiac function occurred at baseline for the preventative stage, 2 days post-surgery, despite the presence of the band. This allowed us to establish resveratrol treatment before the onset of any functional changes and arrest any subsequent changes. In the therapeutic stage, cardiac function was altered at baseline for resveratrol treatment 14 days post-surgery, allowing for regression of myocardial dysfunction. After four weeks, pressure overload-induced cardiac hypertrophy by abdominal aortic banding typically shows preserved systolic function indicative of compensatory hypertrophy [66, 173]. A previous report by our lab showed that left ventricular ejection fraction was slightly but significantly decreased (~6%) in pressure overload rats compared to their sham groups [16]; this discrepancy in findings may be due to low animal numbers used in that pilot project. In this study, we show that left ventricular ejection fraction and fractional shortening, measures of systolic function are unaltered with pressure overload, which are in agreement with previous reports at similar time points [66, 173]. In pressure overload, only diastolic dysfunction was observed with respects to prolonged isovolumetric relaxation. It is known that concentric hypertrophy induced by pressure overload is accompanied by myocardial fibrosis [173].

Furthermore, the presence of myocardial fibrosis contributes to diastolic dysfunction, a result of impaired filling of the left ventricle [173]. Although we have not looked at cardiac fibrosis in our study, several studies at similar end points have detected the presence of fibrosis in pressure overload through histological examination of the left ventricle [75, 173]. Resveratrol has demonstrated anti-fibrotic properties in both *in vivo* settings of pressure overload [75, 147, 151, 173] and *in vitro* settings induced by angiotensin II [154, 155]. Therefore, we suspect that resveratrol arrests and regresses myocardial fibrosis in pressure overload rats subjected to abdominal aortic banding. This would allow for improved left ventricular relaxation and subsequent amelioration of diastolic dysfunction observed through maintained isovolumetric relaxation time in the preventative stage and improved isovolumetric relaxation time in the therapeutic stage with resveratrol treatment.

Unlike abdominal aortic banding, volume overload induced by the aortocaval shunt produced early changes in cardiac function. Since there is no significant chamber dilation at 2 days post-surgery in the preventative stage, as observed by an unchanged LVID, the increased cardiac output produced by increased venous return must be accomplished by increasing heart rate. This would be expected given that cardiac output is the product of heart rate and stroke volume; stroke volume is dependent on chamber size [66]. Indeed, we observe this compensation for increased venous return at the baseline for the preventative stage of cardiac hypertrophy due to volume overload. Heart rate thereafter normalizes to sham levels once chamber dilation develops, where increasing cardiac output is correlated with increasing chamber dilation. Given that resveratrol treatment did not prevent the development of chamber dilation, cardiac output

ultimately continued to increase. In the transverse aortic constriction model of pressure overload, Li *et al.* [146] showed that chamber dilation with increased left ventricular diastolic diameter was increased after three weeks, and that isorhapontigenin treatment prevented this development. However, transverse aortic constriction is a model of pressure overload dilated cardiomyopathy [70], not volume overload. Therefore, different loading conditions could possibly affect the outcome of treatment. Other systolic functions including left ventricular ejection fraction and fractional shortening were found to be unchanged in both stages of the study and are in agreement with previous reports using aortocaval shunts at similar time points [174]. Furthermore, we observed enhanced diastolic function as early as 2 days post-shunt surgery in our study in volume overload rats. Faster relaxation and greater filling velocities produced greater post-systolic myocardial relaxation allowing the left ventricle to compensate for the increased venous return. Although not analyzed in our study, volume overload is characterized by a decrease in interstitial collagen content and therefore does not typically produce fibrosis [86]. These results would be indicative of the compensatory phase of volume overload-induced cardiac hypertrophy, which is reported to occur in the first four weeks post-shunt surgery [66]. These trends continued in both stages of this study until end point.

Although we have characterized the effects of resveratrol on cardiac structure and function in the instance of pressure overload and volume overload, we have not elucidated the mechanisms by which these differential effects are mediated. We further speculate, in addition to possible anti-fibrotic effects on the heart in the instance of pressure overload, that resveratrol may mediate its effects through other mechanisms.

Abdominal aortic banding results in increased systolic blood pressure contributing to the pressure overload stimulus on the heart [173]. Several studies have shown that pressure overload-induced cardiac hypertrophy was significantly reduced in three week isorhaptogenin (an analog of resveratrol)-treated aortic-banded animals [146] and resveratrol-treated partially nephrectomized rats [75], and that these results have been associated with a decrease in systolic blood pressure. Hemodynamic unloading of the heart through removal of the aortic band has been shown to significantly regress pressure overload-induced cardiac hypertrophy [175]. The presence of the band in resveratrol treated rats was confirmed upon sacrifice in this study. This implies that possible hemodynamic unloading may occur through other means. Resveratrol has been shown to produce vasorelaxation in the aorta [176]. Therefore, it is possible that resveratrol arrests and regresses the development of pressure overload-induced cardiac hypertrophy by acting at the level of blood vessels, relieving blood pressure and thereby alleviating the stimulus onto the heart in the instance of pressure overload.

Four weeks post-aortocaval shunt surgery has been shown to result in increased venous return and resultant volume overload-induced cardiac hypertrophy, without affecting systolic blood pressure [110, 177]. Removal of volume overload by closure of the aortocaval shunt has been reported to significantly regress cardiac hypertrophy [178]. In this study, we observed that resveratrol treatment had no effect on the status of the shunt. Based on the above information, it seems that the ineffectiveness of resveratrol on cardiac hypertrophy due to volume overload may be due to a lack of effect on the stress, in this case, increased venous return.

However, numerous studies have demonstrated the efficacy of resveratrol against cardiac hypertrophy where no external factors such as blood pressure or other hemodynamic stimulus are involved. *In vitro* studies have showed that cardiomyocyte hypertrophy as a result of exposure to angiotensin-II, a potent hypertrophic agent released in response to elevated blood pressure, and phenylephrine, an α-adrenergic agonist, was prevented by treatment with resveratrol [146, 149]. Although several targets have been proposed to mediate the effects of resveratrol in cardioprotection, endothelial nitric oxide synthase (eNOS or NOS3) appears to be a major target [179]. NOS3 serves a vital role in regulating the β-adrenergic transduction system and therefore cardiac function. *In vivo* and *in vitro* studies have demonstrated that chronic activation of cardiac β-adrenergic receptors results in cardiac hypertrophy that is concentric in nature [180-184]. Nitric oxide production by NOS3 signaling in cardiomyocytes has been shown to blunt β-adrenergic stimulation and limit cardiac remodeling and dysfunction due to pressure overload in mice with NOS3 over-expression [180]. We have recently shown that four weeks of abdominal aortic banding decreases NOS3 protein levels in left ventricle myocardial tissue, which is recovered upon treatment with resveratrol [16]. Activation of the NO-cGMP pathway has also been shown to inhibit the calcineurin-nuclear factor of activated T-cell (NFAT) pathway by cGMP-dependent protein kinase type 1 (PKG-1) [185]. Therefore, increased nitric oxide levels via resveratrol-induced up-regulation of NOS3 may play a major role in arresting and regressing the development of pressure overload-induced alterations in cardiac structure and function in aortic-banded animals. Interestingly, NOS3 protein level or activity is unaffected by cardiac hypertrophy due to volume overload [186]; this may partly explain the lack of effect of resveratrol on volume

overload-induced changes in cardiac structure and function in aortocaval-shunted animals.

Conclusion

The novel finding in this study is that treatment with resveratrol (2.5 mg/kg body weight/day) was found to arrest the development of cardiac hypertrophy and regress developed cardiac hypertrophy and its alterations on cardiac function in rats subjected to pressure overload, but not volume overload. This study cannot however rule out the possibility that resveratrol may have a beneficial effect on cardiac abnormalities due to volume overload at a higher dosage. It is interesting to speculate that the anti-hypertrophic effects of resveratrol in pressure overload but not volume overload may, in part, be due to the removal of a specific type of stress placed on the heart and the targeting of specific anti-hypertrophic molecules that are differentially expressed in these two models of cardiac hypertrophy. However, more detailed hemodynamic analysis, especially at the level of the vasculature, and comparative molecular studies will need to be conducted to definitively determine the anti-hypertrophic actions of resveratrol in pressure overload, but not volume overload. Therefore, resveratrol may have potential in the treatment of clinical situations of pressure overload such as hypertension and cardiac valvular stenosis.

VII. REFERENCES

1. Mudd JO, Kass DA. Tackling heart failure in the twenty-first century. Nature 2008; 451: 919-928.
2. Latronico MV, Elia L, Condorelli G, Catalucci D. Heart failure: targeting transcriptional and post-transcriptional control mechanisms of hypertrophy for treatment. Int J Biochem Cell Biol 2008; 40: 1643-1648.
3. http://www.who.int/cardiovascular_diseases/en/. World Health Organization, Accessed May 13, 2009. In.
4. Selvetella G, Hirsch E, Notte A, Tarone G, Lembo G. Adaptive and maladaptive hypertrophic pathways: points of convergence and divergence. Cardiovasc Res 2004; 63: 373-380.
5. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006; 367: 356-367.
6. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. Annu Rev Physiol 2003; 65: 45-79.
7. Frey N, Katus HA, Olson EN, Hill JA. Hypertrophy of the heart: a new therapeutic target? Circulation 2004; 109: 1580-1589.
8. Tunuguntla A. Emerging drug therapies for the management of acute decompensated heart failure. Tenn Med 2007; 100: 33-37.
9. Tavares M, Rezlan E, Vostroknoutova I, Khouadja H, Mebazaa A. New pharmacologic therapies for acute heart failure. Crit Care Med 2008; 36: S112-120.

10. De Luca L, Mebazaa A, Filippatos G, Parissis JT, Bohm M, Voors AA, Nieminen M, Zannad F, Rhodes A, El-Banayosy A, Dickstein K, Gheorghiade M. Overview of emerging pharmacologic agents for acute heart failure syndromes. Eur J Heart Fail 2008; 10: 201-213.

11. Kalantar-Zadeh K, Anker SD, Horwich TB, Fonarow GC. Nutritional and anti-inflammatory interventions in chronic heart failure. Am J Cardiol 2008; 101: 89E-103E.

12. Ramaa CS, Shirode AR, Mundada AS, Kadam VJ. Nutraceuticals--an emerging era in the treatment and prevention of cardiovascular diseases. Curr Pharm Biotechnol 2006; 7: 15-23.

13. Kalra EK. Nutraceutical--definition and introduction. AAPS PharmSci 2003; 5: E25.

14. De Caterina R, Zampolli A, Del Turco S, Madonna R, Massaro M. Nutritional mechanisms that influence cardiovascular disease. Am J Clin Nutr 2006; 83: 421S-426S.

15. Das S, Das DK. Resveratrol: a therapeutic promise for cardiovascular diseases. Recent Pat Cardiovasc Drug Discov 2007; 2: 133-138.

16. Juric D, Wojciechowski P, Das DK, Netticadan T. Prevention of concentric hypertrophy and diastolic impairment in aortic-banded rats treated with resveratrol. Am J Physiol Heart Circ Physiol 2007; 292: H2138-2143.

17. Katz AM. Heart failure: a hemodynamic disorder complicated by maladaptive proliferative responses. J Cell Mol Med 2003; 7: 1-10.

18. Krum H, Abraham WT. Heart failure. Lancet 2009; 373: 941-955.

19. Katz AM. The "gap" between bench and bedside: widening or narrowing? J Card Fail 2008; 14: 91-94.

20. Damasceno A, Cotter G, Dzudie A, Sliwa K, Mayosi BM. Heart failure in sub-saharan Africa: time for action. J Am Coll Cardiol 2007; 50: 1688-1693.

21. Hodges P. Heart failure: epidemiologic update. Crit Care Nurs Q 2009; 32: 24-32.

22. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008; 117: e25-146.

23. Bleumink GS, Knetsch AM, Sturkenboom MC, Straus SM, Hofman A, Deckers JW, Witteman JC, Stricker BH. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J 2004; 25: 1614-1619.

24. Najafi F, Jamrozik K, Dobson AJ. Understanding the 'epidemic of heart failure': a systematic review of trends in determinants of heart failure. Eur J Heart Fail 2009; 11: 472-479.

25. Kaye DM, Krum H. Drug discovery for heart failure: a new era or the end of the pipeline? Nat Rev Drug Discov 2007; 6: 127-139.

26. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352: 1685-1695.

27. Yasue H, Nakagawa H, Itoh T, Harada E, Mizuno Y. Coronary artery spasm--clinical features, diagnosis, pathogenesis, and treatment. J Cardiol 2008; 51: 2-17.

28. Chahine MN, Blackwood DP, Dibrov E, Richard MN, Pierce GN. Oxidized LDL affects smooth muscle cell growth through MAPK-mediated actions on nuclear protein import. J Mol Cell Cardiol 2009; 46: 431-441.

29. Gaddam KK, Verma A, Thompson M, Amin R, Ventura H. Hypertension and cardiac failure in its various forms. Med Clin North Am 2009; 93: 665-680.

30. Manickavasagam S, Merla R, Koerner MM, Fujise K, Kunapuli S, Rosanio S, Barbagelata A. Management of hypertension in chronic heart failure. Expert Rev Cardiovasc Ther 2009; 7: 423-433.

31. Wright JW, Mizutani S, Harding JW. Pathways involved in the transition from hypertension to hypertrophy to heart failure. Treatment strategies. Heart Fail Rev 2008; 13: 367-375.

32. Binder A. A review of the genetics of essential hypertension. Curr Opin Cardiol 2007; 22: 176-184.

33. Finsterer J, Stollberger C. Primary myopathies and the heart. Scand Cardiovasc J 2008; 42: 9-24.

34. Maisch B, Ristic AD, Hufnagel G, Funck R, Alter P, Tontsch D, Pankuweit S. Dilated cardiomyopathies as a cause of congestive heart failure. Herz 2002; 27: 113-134.

35. Towbin JA, Bowles NE. The failing heart. Nature 2002; 415: 227-233.

36. Maisch B, Ristic AD, Portig I, Pankuweit S. Human viral cardiomyopathy. Front Biosci 2003; 8: s39-67.

37. Klatsky AL. Alcohol and cardiovascular diseases. Expert Rev Cardiovasc Ther 2009; 7: 499-506.

38. Oliveira BM, Botoni FA, Ribeiro AL, Pinto AS, Reis AM, Nunes Mdo C, Rocha MO. Correlation between BNP levels and Doppler echocardiographic parameters of left ventricle filling pressure in patients with Chagasic cardiomyopathy. Echocardiography 2009; 26: 521-527.

39. Marian AJ. Genetic determinants of cardiac hypertrophy. Curr Opin Cardiol 2008; 23: 199-205.

40. Newman DB, Quin EM, Winscott JG, Guild C. Hypertrophic cardiomyopathy. J Miss State Med Assoc 2008; 49: 330-334.

41. Afonso LC, Bernal J, Bax JJ, Abraham TP. Echocardiography in hypertrophic cardiomyopathy: the role of conventional and emerging technologies. JACC Cardiovasc Imaging 2008; 1: 787-800.

42. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation 2007; 115: 3213-3223.

43. Aneja A, Tang WH, Bansilal S, Garcia MJ, Farkouh ME. Diabetic cardiomyopathy: insights into pathogenesis, diagnostic challenges, and therapeutic options. Am J Med 2008; 121: 748-757.

44. Kushwaha SS, Fallon JT, Fuster V. Restrictive cardiomyopathy. N Engl J Med 1997; 336: 267-276.

45. Rai TS, Ahmad S, Ahluwalia TS, Ahuja M, Bahl A, Saikia UN, Singh B, Talwar KK, Khullar M. Genetic and clinical profile of Indian patients of idiopathic

restrictive cardiomyopathy with and without hypertrophy. Mol Cell Biochem 2009.

46. Carabello BA, Crawford FA, Jr. Valvular heart disease. N Engl J Med 1997; 337: 32-41.

47. Bekeredjian R, Grayburn PA. Valvular heart disease: aortic regurgitation. Circulation 2005; 112: 125-134.

48. Chambers JB. Aortic stenosis. Eur J Echocardiogr 2009; 10: i11-19.

49. Chan KM, Zakkar M, Amirak E, Punjabi PP. Tricuspid valve disease: pathophysiology and optimal management. Prog Cardiovasc Dis 2009; 51: 482-486.

50. Messika-Zeitoun D, Iung B, Brochet E, Himbert D, Serfaty JM, Laissy JP, Vahanian A. Evaluation of mitral stenosis in 2008. Arch Cardiovasc Dis 2008; 101: 653-663.

51. Carabello BA, Paulus WJ. Aortic stenosis. Lancet 2009; 373: 956-966.

52. Enriquez-Sarano M, Akins CW, Vahanian A. Mitral regurgitation. Lancet 2009; 373: 1382-1394.

53. Rogers JH, Bolling SF. The tricuspid valve: current perspective and evolving management of tricuspid regurgitation. Circulation 2009; 119: 2718-2725.

54. Brickner ME, Hillis LD, Lange RA. Congenital heart disease in adults. First of two parts. N Engl J Med 2000; 342: 256-263.

55. Brickner ME, Hillis LD, Lange RA. Congenital heart disease in adults. Second of two parts. N Engl J Med 2000; 342: 334-342.

56. Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, Cushman SW, Periwal V. Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. PLoS Comput Biol 2009; 5: e1000324.

57. Eghbali M, Wang Y, Toro L, Stefani E. Heart hypertrophy during pregnancy: a better functioning heart? Trends Cardiovasc Med 2006; 16: 285-291.

58. Metivier F, Marchais SJ, Guerin AP, Pannier B, London GM. Pathophysiology of anaemia: focus on the heart and blood vessels. Nephrol Dial Transplant 2000; 15 Suppl 3: 14-18.

59. French BA, Kramer CM. Mechanisms of Post-Infarct Left Ventricular Remodeling. Drug Discov Today Dis Mech 2007; 4: 185-196.

60. McMullen JR, Jennings GL. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. Clin Exp Pharmacol Physiol 2007; 34: 255-262.

61. Molkentin JD. Calcineurin-NFAT signaling regulates the cardiac hypertrophic response in coordination with the MAPKs. Cardiovasc Res 2004; 63: 467-475.

62. Hunter JJ, Chien KR. Signaling pathways for cardiac hypertrophy and failure. N Engl J Med 1999; 341: 1276-1283.

63. Barry SP, Davidson SM, Townsend PA. Molecular regulation of cardiac hypertrophy. Int J Biochem Cell Biol 2008; 40: 2023-2039.

64. Booz GW. Putting the brakes on cardiac hypertrophy: exploiting the NO-cGMP counter-regulatory system. Hypertension 2005; 45: 341-346.

65. McKinsey TA, Kass DA. Small-molecule therapies for cardiac hypertrophy: moving beneath the cell surface. Nat Rev Drug Discov 2007; 6: 617-635.

66. Cantor EJ, Babick AP, Vasanji Z, Dhalla NS, Netticadan T. A comparative serial echocardiographic analysis of cardiac structure and function in rats subjected to pressure or volume overload. J Mol Cell Cardiol 2005; 38: 777-786.

67. Stoyanova V, Zhelev N, Ghenev E, Bosheva M. Assessment of left ventricular structure and function in rats subjected to pressure-overload hypertrophy in time. Kardiol Pol 2009; 67: 27-34; discussion 35.

68. Ling H, Zhang T, Pereira L, Means CK, Cheng H, Gu Y, Dalton ND, Peterson KL, Chen J, Bers D, Heller Brown J. Requirement for Ca2+/calmodulin-dependent kinase II in the transition from pressure overload-induced cardiac hypertrophy to heart failure in mice. J Clin Invest 2009; 119: 1230-1240.

69. Xia Y, Lee K, Li N, Corbett D, Mendoza L, Frangogiannis NG. Characterization of the inflammatory and fibrotic response in a mouse model of cardiac pressure overload. Histochem Cell Biol 2009; 131: 471-481.

70. Hughes WM, Jr., Rodriguez WE, Rosenberger D, Chen J, Sen U, Tyagi N, Moshal KS, Vacek T, Kang YJ, Tyagi SC. Role of copper and homocysteine in pressure overload heart failure. Cardiovasc Toxicol 2008; 8: 137-144.

71. Freeling J, Wattier K, LaCroix C, Li YF. Neostigmine and pilocarpine attenuated tumour necrosis factor alpha expression and cardiac hypertrophy in the heart with pressure overload. Exp Physiol 2008; 93: 75-82.

72. Borges GR, Salgado HC, Silva CA, Rossi MA, Prado CM, Fazan R, Jr. Changes in hemodynamic and neurohumoral control cause cardiac damage in one-kidney, one-clip hypertensive mice. Am J Physiol Regul Integr Comp Physiol 2008; 295: R1904-1913.

73. Gupta RC, Mishra S, Yang XP, Sabbah HN. Reduced inhibitor 1 and 2 activity is associated with increased protein phosphatase type 1 activity in left ventricular myocardium of one-kidney, one-clip hypertensive rats. Mol Cell Biochem 2005; 269: 49-57.

74. Signolet I, Gasser B, Bousquet P, Monassier L. Echocardiography in conscious 1K,1C Goldblatt rabbits reveals typical features of human hypertensive ventricular diastolic dysfunction. Int J Cardiol 2009; 132: 135-137.

75. Liu Z, Song Y, Zhang X, Zhang W, Mao W, Wang W, Cui W, Jia X, Li N, Han C, Liu C. Effects of trans-resveratrol on hypertension-induced cardiac hypertrophy using the partially nephrectomized rat model. Clin Exp Pharmacol Physiol 2005; 32: 1049-1054.

76. Schmitto JD, Doerge H, Post H, Coulibaly M, Sellin C, Popov AF, Sossalla S, Schoendube FA. Progressive right ventricular failure is not explained by myocardial ischemia in a pig model of right ventricular pressure overload. Eur J Cardiothorac Surg 2009; 35: 229-234.

77. Duri S, Molthen RC, Tran CD. Discriminating pulmonary hypertension caused by monocrotaline toxicity from chronic hypoxia by near-infrared spectroscopy and multivariate methods of analysis. Anal Biochem 2009; 390: 155-164.

78. Lamberts RR, Vaessen RJ, Westerhof N, Stienen GJ. Right ventricular hypertrophy causes impairment of left ventricular diastolic function in the rat. Basic Res Cardiol 2007; 102: 19-27.

79. de Visser YP, Walther FJ, Laghmani el H, Boersma H, van der Laarse A, Wagenaar GT. Sildenafil attenuates pulmonary inflammation and fibrin

deposition, mortality and right ventricular hypertrophy in neonatal hyperoxic lung injury. Respir Res 2009; 10: 30.

80. Hsu HH, Ko WJ, Hsu JY, Chen JS, Lee YC, Lai IR, Chen CF. Simvastatin ameliorates established pulmonary hypertension through a heme oxygenase-1 dependent pathway in rats. Respir Res 2009; 10: 32.

81. Cai J, Yi FF, Yang L, Shen DF, Yang Q, Li A, Ghosh AK, Bian ZY, Yan L, Tang QZ, Li H, Yang XC. Targeted expression of receptor-associated late transducer inhibits maladaptive hypertrophy via blocking epidermal growth factor receptor signaling. Hypertension 2009; 53: 539-548.

82. Gao JP, Chen CX, Wang Y, Lu J, Gu WL. Effect of sodium houttuyfonate on myocardial hypertrophy in mice and rats. J Pharm Pharmacol 2009; 61: 677-683.

83. Bonow RO. Aortic Regurgitation. Curr Treat Options Cardiovasc Med 2000; 2: 125-132.

84. Champetier S, Bojmehrani A, Beaudoin J, Lachance D, Plante E, Roussel E, Couet J, Arsenault M. Gene profiling of left ventricle eccentric hypertrophy in aortic regurgitation in rats: rationale for targeting the beta-adrenergic and renin-angiotensin systems. Am J Physiol Heart Circ Physiol 2009; 296: H669-677.

85. Szymczyk E, Wierzbowska-Drabik K, Drozdz J, Krzeminska-Pakula M. Mitral valve regurgitation is a powerful factor of left ventricular hypertrophy. Pol Arch Med Wewn 2008; 118: 478-483.

86. Zheng J, Chen Y, Pat B, Dell'italia LA, Tillson M, Dillon AR, Powell PC, Shi K, Shah N, Denney T, Husain A, Dell'Italia LJ. Microarray identifies extensive downregulation of noncollagen extracellular matrix and profibrotic growth factor

genes in chronic isolated mitral regurgitation in the dog. Circulation 2009; 119: 2086-2095.

87. Carlhall CJ, Nguyen TC, Itoh A, Ennis DB, Bothe W, Liang D, Ingels NB, Miller DC. Alterations in transmural myocardial strain: an early marker of left ventricular dysfunction in mitral regurgitation? Circulation 2008; 118: S256-262.

88. Ishibashi Y, Rembert JC, Carabello BA, Nemoto S, Hamawaki M, Zile MR, Greenfield JC, Jr., Cooper Gt. Normal myocardial function in severe right ventricular volume overload hypertrophy. Am J Physiol Heart Circ Physiol 2001; 280: H11-16.

89. Garcia R, Diebold S. Simple, rapid, and effective method of producing aortocaval shunts in the rat. Cardiovasc Res 1990; 24: 430-432.

90. Deroubaix E, Folliguet T, Rucker-Martin C, Dinanian S, Boixel C, Validire P, Daniel P, Capderou A, Hatem SN. Moderate and chronic hemodynamic overload of sheep atria induces reversible cellular electrophysiologic abnormalities and atrial vulnerability. J Am Coll Cardiol 2004; 44: 1918-1926.

91. Takahashi N, Atsumi H, Nakada S, Takeishi Y, Tomoike H. Alterations in the inotropic responses to forskolin and Ca2+ and reduced gene expressions of Ca2+-signaling proteins induced by chronic volume overload in rabbits. Jpn Circ J 2000; 64: 861-867.

92. Naito Y, Tsujino T, Matsumoto M, Sakoda T, Ohyanagi M, Masuyama T. Adaptive response of the heart to long-term anemia induced by iron deficiency. Am J Physiol Heart Circ Physiol 2009; 296: H585-593.

93. Olivetti G, Quaini F, Lagrasta C, Ricci R, Tiberti G, Capasso JM, Anversa P. Myocyte cellular hypertrophy and hyperplasia contribute to ventricular wall remodeling in anemia-induced cardiac hypertrophy in rats. Am J Pathol 1992; 141: 227-239.

94. Maeba T. Calcium-binding of cardiac sarcoplasmic reticulum and diastolic hemodynamics in volume overloaded canine hearts. Jpn Circ J 1985; 49: 163-170.

95. Christensen LP, Zhang RL, Zheng W, Campanelli JJ, Dedkov EI, Weiss RM, Tomanek RJ. Post Myocardial Infarction Remodeling and Coronary Reserve: Effects of Invabradine and Beta Blockade Therapy. Am J Physiol Heart Circ Physiol 2009.

96. Tiwari R, Mohan M, Kasture S, Maxia A, Ballero M. Cardioprotective potential of myricetin in isoproterenol-induced myocardial infarction in wistar rats. Phytother Res 2009.

97. Wang SB, Tian S, Yang F, Yang HG, Yang XY, Du GH. Cardioprotective effect of salvianolic acid A on isoproterenol-induced myocardial infarction in rats. Eur J Pharmacol 2009.

98. Coatney RW. Ultrasound imaging: principles and applications in rodent research. ILAR J 2001; 42: 233-247.

99. Kono M, Kisanuki A, Takasaki K, Nakashiki K, Yuasa T, Kuwahara E, Mizukami N, Uemura T, Kubota K, Ueya N, Miyata M, Tei C. Left ventricular systolic function is abnormal in diastolic heart failure: Re-assessment of systolic function using cardiac time interval analysis. J Cardiol 2009; 53: 437-446.

100. Zaqout M, Suys B, De Wilde H, De Wolf D. Transthoracic Echocardiography Guidance of Transcatheter Atrial Septal Defect Closure in Children. Pediatr Cardiol 2009.

101. Taneja R, Quaghebeur B, Stitt LW, Quantz MA, Guo LR, Kiaii B, Bainbridge DT. The role of epicardial echocardiography in the measurement of transvalvular flow velocities during aortic valve replacement. J Cardiothorac Vasc Anesth 2009; 23: 292-297.

102. Liodakis E, Al Sharef O, Dawson D, Nihoyannopoulos P. The use of Real Time Three Dimensional Echocardiography for Assessing Mechanical Synchronicity. Heart 2009.

103. Mor-Avi V, Lang RM. The use of real-time three-dimensional echocardiography for the quantification of left ventricular volumes and function. Curr Opin Cardiol 2009.

104. Mureddu GF, Cioffi G, Stefenelli C, Boccanelli A, de Simone G. Compensatory or inappropriate left ventricular mass in different models of left ventricular pressure overload: comparison between patients with aortic stenosis and arterial hypertension. J Hypertens 2009; 27: 642-649.

105. Ristic-Andjelkov A. [Postoperative changes in diastolic function of the left ventricle in patients with aortic valve stenosis]. Srp Arh Celok Lek 2002; 130: 81-86.

106. Lee CH, Hogan JC, Gibson DG. Diastolic disease in left ventricular hypertrophy: comparison of M mode and Doppler echocardiography for the assessment of rapid ventricular filling. Br Heart J 1991; 65: 194-200.

107. Tian JP, Du FH, Cheng LT, Tian XK, Axelsson J, Wang T. Peripheral resistance modulates the response to volume overload in peritoneal dialysis patients. Perit Dial Int 2008; 28: 604-610.

108. Takewa Y, Chemaly ER, Takaki M, Liang LF, Jin H, Karakikes I, Morel C, Taenaka Y, Tatsumi E, Hajjar RJ. Mechanical work and energetic analysis of eccentric cardiac remodeling in a volume overload heart failure in rats. Am J Physiol Heart Circ Physiol 2009; 296: H1117-1124.

109. Varadarajan P, Joshi N, Appel D, Duvvuri L, Pai RG. Effect of Beta-blocker therapy on survival in patients with severe mitral regurgitation and normal left ventricular ejection fraction. Am J Cardiol 2008; 102: 611-615.

110. Kobayashi M, Machida N, Tanaka R, Yamane Y. Effects of beta-blocker on left ventricular remodeling in rats with volume overload cardiac failure. J Vet Med Sci 2008; 70: 1231-1237.

111. Little WC. Heart failure with a normal left ventricular ejection fraction: diastolic heart failure. Trans Am Clin Climatol Assoc 2008; 119: 93-99; discussion 99-102.

112. Espin JC, Garcia-Conesa MT, Tomas-Barberan FA. Nutraceuticals: facts and fiction. Phytochemistry 2007; 68: 2986-3008.

113. Maddi V, Aragade P, VG, Digge, Nitalikar M. Importance of Nutraceuticals in Health Management. Pharmacognosy Reviews 2007; 1: 377-379.

114. Key TJ, Appleby PN, Rosell MS. Health effects of vegetarian and vegan diets. Proc Nutr Soc 2006; 65: 35-41.

115. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 2006; 5: 493-506.

116. Harikumar KB, Aggarwal BB. Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle 2008; 7: 1020-1035.

117. Duda MK, O'Shea KM, Lei B, Barrows BR, Azimzadeh AM, McElfresh TE, Hoit BD, Kop WJ, Stanley WC. Dietary supplementation with omega-3 PUFA increases adiponectin and attenuates ventricular remodeling and dysfunction with pressure overload. Cardiovasc Res 2007; 76: 303-310.

118. Duda MK, O'Shea KM, Tintinu A, Xu W, Khairallah RJ, Barrows BR, Chess DJ, Azimzadeh AM, Harris WS, Sharov VG, Sabbah HN, Stanley WC. Fish oil, but not flaxseed oil, decreases inflammation and prevents pressure overload-induced cardiac dysfunction. Cardiovasc Res 2009; 81: 319-327.

119. Jiang Y, Reynolds C, Xiao C, Feng W, Zhou Z, Rodriguez W, Tyagi SC, Eaton JW, Saari JT, Kang YJ. Dietary copper supplementation reverses hypertrophic cardiomyopathy induced by chronic pressure overload in mice. J Exp Med 2007; 204: 657-666.

120. Wenzel E, Somoza V. Metabolism and bioavailability of trans-resveratrol. Mol Nutr Food Res 2005; 49: 472-481.

121. Pirola L, Frojdo S. Resveratrol: one molecule, many targets. IUBMB Life 2008; 60: 323-332.

122. de Lorgeril M, Salen P, Guiraud A, Boucher F, de Leiris J. Resveratrol and non-ethanolic components of wine in experimental cardiology. Nutr Metab Cardiovasc Dis 2003; 13: 100-103.

123. Pervaiz S. Resveratrol: from grapevines to mammalian biology. FASEB J 2003; 17: 1975-1985.

124. Delaunois B, Cordelier S, Conreux A, Clement C, Jeandet P. Molecular engineering of resveratrol in plants. Plant Biotechnol J 2009; 7: 2-12.

125. Opie LH, Lecour S. The red wine hypothesis: from concepts to protective signalling molecules. Eur Heart J 2007; 28: 1683-1693.

126. Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Forstermann U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. Circulation 2002; 106: 1652-1658.

127. Takaoka MJ. Of the phenolic substances of white hellebore (*Veratrum grandiflorum* Loes. fil.). J. Faculty Sci. Hokkaido Imperial University 1940; 3: 1-16.

128. Nonomura S, Kanagawa H, Makimoto A. Chemical constituents of polygonaceous plants. I. Studies on the components of Ko-jo-kon (Polygonum cuspidatum Sieb. et Zucc.) Yakugaku Zasshi 1963; 83: 988-990.

129. Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. Plant foods and herbal sources of resveratrol. J Agric Food Chem 2002; 50: 3337-3340.

130. Langcake P, Pryce RJ. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. Physiol. Plant Pathol. 1976; 9: 77-86.

131. Siemann EH, Creasy LL. Concentration of the phytoalexin resveratrol in wine. Am. J. Eno. Vitic. 1992; 43: 49-52.

132. Chawla R. Regular drinking might explain the French paradox. BMJ 2004; 329: 1308.

133. Kopp P. Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? Eur J Endocrinol 1998; 138: 619-620.

134. Saiko P, Szakmary A, Jaeger W, Szekeres T. Resveratrol and its analogs: defense against cancer, coronary disease and neurodegenerative maladies or just a fad? Mutat Res 2008; 658: 68-94.

135. van de Wal RM, Plokker HW, Lok DJ, Boomsma F, van der Horst FA, van Veldhuisen DJ, van Gilst WH, Voors AA. Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition. Int J Cardiol 2006; 106: 367-372.

136. MacFadyen RJ, Lee AF, Morton JJ, Pringle SD, Struthers AD. How often are angiotensin II and aldosterone concentrations raised during chronic ACE inhibitor treatment in cardiac failure? Heart 1999; 82: 57-61.

137. Urata H, Nishimura H, Ganten D, Arakawa K. Angiotensin-converting enzyme-independent pathways of angiotensin II formation in human tissues and cardiovascular diseases. Blood Press Suppl 1996; 2: 22-28.

138. McKelvie RS, Yusuf S, Pericak D, Avezum A, Burns RJ, Probstfield J, Tsuyuki RT, White M, Rouleau J, Latini R, Maggioni A, Young J, Pogue J. Comparison of candesartan, enalapril, and their combination in congestive heart failure: randomized evaluation of strategies for left ventricular dysfunction (RESOLVD) pilot study. The RESOLVD Pilot Study Investigators. Circulation 1999; 100: 1056-1064.

139. Dunn SP, Bleske B, Dorsch M, Macaulay T, Van Tassell B, Vardeny O. Nutrition and heart failure: impact of drug therapies and management strategies. Nutr Clin Pract 2009; 24: 60-75.

140. Struthers AD. The clinical implications of aldosterone escape in congestive heart failure. Eur J Heart Fail 2004; 6: 539-545.

141. Frankenstein L, Zugck C, Schellberg D, Nelles M, Froehlich H, Katus H, Remppis A. Prevalence and prognostic significance of adrenergic escape during chronic beta-blocker therapy in chronic heart failure. Eur J Heart Fail 2009; 11: 178-184.

142. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. Circulation 2000; 101: 558-569.

143. Krum H, Cameron P. Diuretics in the treatment of heart failure: mainstay of therapy or potential hazard? J Card Fail 2006; 12: 333-335.

144. Pitt B. Do diuretics and aldosterone receptor antagonists improve ventricular remodeling? J Card Fail 2002; 8: S491-493.

145. Bell DS. Hypertension and diabetes: a toxic combination. Endocr Pract 2008; 14: 1031-1039.

146. Li HL, Wang AB, Huang Y, Liu DP, Wei C, Williams GM, Zhang CN, Liu G, Liu YQ, Hao DL, Hui RT, Lin M, Liang CC. Isorhapontigenin, a new resveratrol analog, attenuates cardiac hypertrophy via blocking signaling transduction pathways. Free Radic Biol Med 2005; 38: 243-257.

147. Yoshida Y, Shioi T, Izumi T. Resveratrol ameliorates experimental autoimmune myocarditis. Circ J 2007; 71: 397-404.

148. Cheng TH, Liu JC, Lin H, Shih NL, Chen YL, Huang MT, Chan P, Cheng CF, Chen JJ. Inhibitory effect of resveratrol on angiotensin II-induced cardiomyocyte hypertrophy. Naunyn Schmiedebergs Arch Pharmacol 2004; 369: 239-244.

149. Chan AY, Dolinsky VW, Soltys CL, Viollet B, Baksh S, Light PE, Dyck JR. Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and Akt. J Biol Chem 2008; 283: 24194-24201.

150. Chen YR, Yi FF, Li XY, Wang CY, Chen L, Yang XC, Su PX, Cai J. Resveratrol attenuates ventricular arrhythmias and improves the long-term survival in rats with myocardial infarction. Cardiovasc Drugs Ther 2008; 22: 479-485.

151. Lin JF, Lin SM, Chih CL, Nien MW, Su HH, Hu BR, Huang SS, Tsai SK. Resveratrol reduces infarct size and improves ventricular function after myocardial ischemia in rats. Life Sci 2008; 83: 313-317.

152. Burstein B, Maguy A, Clement R, Gosselin H, Poulin F, Ethier N, Tardif JC, Hebert TE, Calderone A, Nattel S. Effects of resveratrol (trans-3,5,4'-trihydroxystilbene) treatment on cardiac remodeling following myocardial infarction. J Pharmacol Exp Ther 2007; 323: 916-923.

153. Cutler MJ, Rosenbaum DS, Dunlap ME. Structural and electrical remodeling as therapeutic targets in heart failure. J Electrocardiol 2007; 40: S1-7.

154. Olson ER, Naugle JE, Zhang X, Bomser JA, Meszaros JG. Inhibition of cardiac fibroblast proliferation and myofibroblast differentiation by resveratrol. Am J Physiol Heart Circ Physiol 2005; 288: H1131-1138.

155. Wang S, Wang X, Yan J, Xie X, Fan F, Zhou X, Han L, Chen J. Resveratrol inhibits proliferation of cultured rat cardiac fibroblasts: correlated with NO-cGMP signaling pathway. Eur J Pharmacol 2007; 567: 26-35.

156. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev 2007; 59: 418-458.

157. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? J Clin Invest 1985; 76: 1713-1719.

158. Das S, Cordis GA, Maulik N, Das DK. Pharmacological preconditioning with resveratrol: role of CREB-dependent Bcl-2 signaling via adenosine A3 receptor activation. Am J Physiol Heart Circ Physiol 2005; 288: H328-335.

159. Das S, Tosaki A, Bagchi D, Maulik N, Das DK. Resveratrol-mediated activation of cAMP response element-binding protein through adenosine A3 receptor by Akt-dependent and -independent pathways. J Pharmacol Exp Ther 2005; 314: 762-769.

160. Das S, Falchi M, Bertelli A, Maulik N, Das DK. Attenuation of ischemia/reperfusion injury in rats by the anti-inflammatory action of resveratrol. Arzneimittelforschung 2006; 56: 700-706.

161. Bradamante S, Barenghi L, Piccinini F, Bertelli AA, De Jonge R, Beemster P, De Jong JW. Resveratrol provides late-phase cardioprotection by means of a nitric oxide- and adenosine-mediated mechanism. Eur J Pharmacol 2003; 465: 115-123.

162. Shen M, Jia GL, Wang YM, Ma H. Cardioprotective effect of resvaratrol pretreatment on myocardial ischemia-reperfusion induced injury in rats. Vascul Pharmacol 2006; 45: 122-126.

163. Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res 2000; 47: 549-555.

164. Xi J, Wang H, Mueller RA, Norfleet EA, Xu Z. Mechanism for resveratrol-induced cardioprotection against reperfusion injury involves glycogen synthase kinase 3beta and mitochondrial permeability transition pore. Eur J Pharmacol 2009; 604: 111-116.

165. Jin H, Lyon AR, Akar FG. Arrhythmia mechanisms in the failing heart. Pacing Clin Electrophysiol 2008; 31: 1048-1056.

166. Zhang LP, Yin JX, Liu Z, Zhang Y, Wang QS, Zhao J. Effect of resveratrol on L-type calcium current in rat ventricular myocytes. Acta Pharmacol Sin 2006; 27: 179-183.

167. Mayers JR, Iliff BW, Swoap SJ. Resveratrol treatment in mice does not elicit the bradycardia and hypothermia associated with calorie restriction. FASEB J 2009; 23: 1032-1040.

168. Zhang Y, Liu Y, Wang T, Li B, Li H, Wang Z, Yang B. Resveratrol, a natural ingredient of grape skin: antiarrhythmic efficacy and ionic mechanisms. Biochem Biophys Res Commun 2006; 340: 1192-1199.

169. Chen WP, Su MJ, Hung LM. In vitro electrophysiological mechanisms for antiarrhythmic efficacy of resveratrol, a red wine antioxidant. Eur J Pharmacol 2007; 554: 196-204.

170. Hwang JT, Kwon DY, Park OJ, Kim MS. Resveratrol protects ROS-induced cell death by activating AMPK in H9c2 cardiac muscle cells. Genes Nutr 2008; 2: 323-326.

171. Chen CJ, Yu W, Fu YC, Wang X, Li JL, Wang W. Resveratrol protects cardiomyocytes from hypoxia-induced apoptosis through the SIRT1-FoxO1 pathway. Biochem Biophys Res Commun 2009; 378: 389-393.

172. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978; 58: 1072-1083.

173. Phrommintikul A, Tran L, Kompa A, Wang B, Adrahtas A, Cantwell D, Kelly DJ, Krum H. Effects of a Rho kinase inhibitor on pressure overload induced cardiac hypertrophy and associated diastolic dysfunction. Am J Physiol Heart Circ Physiol 2008; 294: H1804-1814.

174. Hashida H, Hamada M, Hiwada K. Serial changes in sarcoplasmic reticulum gene expression in volume-overloaded cardiac hypertrophy in the rat: effect of an angiotensin II receptor antagonist. Clin Sci (Lond) 1999; 96: 387-395.

175. Ishihara K, Zile MR, Tomita M, Tanaka R, Kanazawa S, Carabello BA. Left ventricular hypertrophy in a canine model of reversible pressure overload. Cardiovasc Res 1992; 26: 580-585.

176. Chen CK, Pace-Asciak CR. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. Gen Pharmacol 1996; 27: 363-366.

177. Tokioka-Akagi T, Fujimori A, Shibasaki M, Inagaki O, Yanagisawa I. Comparison of the angiotensin II type 1-receptor antagonist YM358 and the angiotensin-converting enzyme inhibitor enalapril in rats with cardiac volume overload. Jpn J Pharmacol 2001; 86: 79-85.

178. Gerdes AM, Clark LC, Capasso JM. Regression of cardiac hypertrophy after closing an aortocaval fistula in rats. Am J Physiol 1995; 268: H2345-2351.

179. Das DK, Maulik N. Resveratrol in cardioprotection: a therapeutic promise of alternative medicine. Mol Interv 2006; 6: 36-47.

180. Buys ES, Raher MJ, Blake SL, Neilan TG, Graveline AR, Passeri JJ, Llano M, Perez-Sanz TM, Ichinose F, Janssens S, Zapol WM, Picard MH, Bloch KD, Scherrer-Crosbie M. Cardiomyocyte-restricted restoration of nitric oxide synthase 3 attenuates left ventricular remodeling after chronic pressure overload. Am J Physiol Heart Circ Physiol 2007; 293: H620-627.

181. Colella M, Pozzan T. Cardiac cell hypertrophy in vitro: role of calcineurin/NFAT as Ca2+ signal integrators. Ann N Y Acad Sci 2008; 1123: 64-68.

182. Kiriazis H, Wang K, Xu Q, Gao XM, Ming Z, Su Y, Moore XL, Lambert G, Gibbs ME, Dart AM, Du XJ. Knockout of beta(1)- and beta(2)-adrenoceptors attenuates pressure overload-induced cardiac hypertrophy and fibrosis. Br J Pharmacol 2008; 153: 684-692.

183. Lin Y, Wang LN, Xi YH, Li HZ, Xiao FG, Zhao YJ, Tian Y, Yang BF, Xu CQ. L-arginine inhibits isoproterenol-induced cardiac hypertrophy through nitric oxide and polyamine pathways. Basic Clin Pharmacol Toxicol 2008; 103: 124-130.

184. Zhang S, Rodriguez R, Scholz PM, Weiss HR. Functional interaction of a beta-adrenergic agonist and cyclic GMP phosphodiesterase inhibitor in control and hypertrophic cardiomyocytes. Pharmacology 2006; 76: 53-60.

185. Fiedler B, Lohmann SM, Smolenski A, Linnemuller S, Pieske B, Schroder F, Molkentin JD, Drexler H, Wollert KC. Inhibition of calcineurin-NFAT hypertrophy signaling by cGMP-dependent protein kinase type I in cardiac myocytes. Proc Natl Acad Sci U S A 2002; 99: 11363-11368.

186. Gealekman O, Abassi Z, Rubinstein I, Winaver J, Binah O. Role of myocardial inducible nitric oxide synthase in contractile dysfunction and beta-adrenergic hyporesponsiveness in rats with experimental volume-overload heart failure. Circulation 2002; 105: 236-243.