Effect of Stilbenoid Polyphenols on Resistance Artery Structure and Mechanical Properties in the Spontaneously Hypertensive Heart Failure Rat

by

Danielle I. Lee

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

College of Pharmacy
University of Manitoba
Winnipeg

Abstract:

Small resistance arteries affect total peripheral resistance (TPR). Increased blood pressure (BP) in hypertension is proportionate to changes in cardiac output (CO) and TPR. Thus, it is imperative to examine compounds that affect microvascular parameters. Resveratrol (trans-3,5,4'-trihydroxystilbene), a stilbenoid polyphenol, has gained significant research interest and is purportedly linked to improved longevity and cardiovascular health. Although resveratrol is well-tolerated in humans, it is readily metabolized and exhibits low bioavailability and half-life. I hypothesized that resveratrol analogues with altered pharmacokinetics and pharmacodynamics would produce greater vasculoprotective effects in an experimental model of hypertension and heart failure.

9-week-old Sprague-Dawley (SD; normotensive control) and spontaneously hypertensive heart failure (SHHF) rats were treated for 8 weeks by oral gavage with vehicle (control) or low doses (2.5 mg/kg/d) of resveratrol, pterostilbene and gnetol. Systolic BP increased in the SHHF rat but was unaltered by stilbenoid treatment. SHHF media:lumen (M:L) increased (17.4±1.2 vs. SD 8.9±0.5; $p<0.01$), while media cross-sectional area remained unchanged, indicating eutrophic remodeling, as found in human essential hypertension. All three stilbenoids reduced M:L toward normal (SHHF-R 13.8±0.8, SHHF-P 13.7±0.5, SHHF-G 13.0±0.3; $p<0.01$). Intrinsic stiffness of the arterial wall was comparable between groups, demonstrating unchanged geometry-independent stiffness (SHHF 4.97±0.27 vs SD 4.36±0.18). AMPKα activation was elevated in SHHF small arteries and attenuated by gnetol. No differences in ERK1/2, p38, or AT_1 signaling were detected in mesenteric resistance arteries. Further research on stilbenoid polyphenols as an adjunct to current anti-hypertensive therapy is warranted.

Acknowledgments:

I would like to begin by thanking my advisor, Dr. Hope Anderson. You have been a remarkable mentor and I have learned so much from your advice over the past few years. I am confident that with your guidance and feedback there are many more exciting projects and learning opportunities to be had over my PhD.

To my committee members Dr. Thomas Netticadan and Dr. Heather Blewett. You were always available to answer any questions I had. I could not have asked for a more kind and approachable committee, thank you.

My parents Debbi, Lawrie, Patrick and Terry, this would not be possible without all of you. I thank you for always encouraging me to explore and try new things. Whether it be driving me to the hockey rink at the crack of dawn, sending me off into the woods, or just listening to me talk, you are always there for me. I appreciate all your support.

As the youngest child, I must thank my siblings Amanda, David and Brodie, for putting up with me over the years. To my Uncle John, I am very grateful for your support and assistance throughout my education.

To my partner Anthony, who is with me every step of the way, thank you. In the last five years we have accomplished so much together, and I look forward to seeing what we achieve in the future.

I'd also like to specifically thank my current and past lab mates Crystal, Yan, Bolanle, Caroline and June. Thank you for all your assistance and advice over the past few years. Lastly, to all my friends and colleagues who have provided support, my sincere thanks.

Table of Contents:

List of Tables:

List of Figures:

List of Abbreviations:

AD	Alzheimer's disease
AMPKα	AMP-activated protein kinase
ACE	angiotensin converting enzyme
ARB	angiotensin receptor blocker
AT_1	angiotensin-II receptor type-I
AT_2	angiotensin-II receptor type-II
AUC	area under the curve
BP	blood pressure
BCSC	breast cancer stem cell
BChE	butyrylcholinesterase
CCB	calcium channel blockers
CREB	cAMP response element binding
CHEP	Canadian Hypertension Education Program
CO	cardiac output
CVD	cardiovascular disease
CHF	congestive heart failure
CAD	coronary artery disease
CSA	cross sectional area
DOCA	deoxycortisone acetate
DASH	Dietary Approaches to Stop Hypertension
EM	elastic modulus
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
ERK1/2	extracellular signal-related kinase 1/2
R	flow rate
GSK	GlaxoSmithKline
HR	heart rate
iNOS	inducible nitric oxide synthase
IL6	interleukin 6

JNC	Joint National Committee
L-NAME	L-arginine methyl ester
l	length
MDA	malondialdehyde
MMP-2	matrix-metalloproteinase 2
M:L	media:lumen ratio
MCA	middle cerebral arteries
MI	myocardial infarction
NHP	natural health product
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
L-NNA	nitroarginine
NF-kB	nuclear factor-kappa B
oxLDL	oxidative low-density lipoprotein
p38	p38 mitogen activated protein kinase
PVDF	polyvinylidene difluoride
P	pressure
PPARα	peroxisome proliferator-activated receptor alpha
r	radius
ROS	reactive oxygen species
RAAS	renin angiotensin aldosterone system
SMC	smooth muscle cell
SHHF	spontaneously hypertensive heart failure
SHR	spontaneously hypertensive rat
SD	Sprague-Dawley
SEM	standard error of the mean
s	stress
SV	stroke volume
SOD	superoxide dismutase
SBP	systolic blood pressure

BH4	tetrahydrobiopterin
TPR	total peripheral resistance
TNFα	tumor necrosis factor alpha
UV	ultra violet
VSMC	vascular smooth muscle cells
$\boldsymbol{\eta}$	viscosity
VvROMT	Vitis vinifera resveratrol O-methyl transferase
m	wall thickness
WKY	Wistar Kyoto

CHAPTER 1. INTRODUCTION

1. Hypertension

1.1 Prevalence

Hypertension is one of the top ten leading causes of mortality worldwide and is responsible for 9.4 million deaths every year ("Global Summary Estimates, 20 leading causes of death.," 2012; Lim, 2012). While hypertension can be considered a preventable contributor to disease and death, by the year 2025 it is predicted that 1.56 billion adults will suffer from hypertension (James *et al*., 2014; Kearney *et al*., 2005).

Therefore, global measures need to be implemented to recognize high risk populations, and to initiate population-based prevention, treatment and management protocols (Kearney *et al*., 2005). Hypertension was originally believed to specifically impact the economically developed world, although as the incidence of communicable disease decreases in the developing world, the incidence of hypertension increases (Ibrahim & Damasceno, 2012; Pickering, 1952). With increasing detection and diagnosis of hypertension, especially within the developing world, methods to educate this subset of the population through prevention and management of high BP via affordable and accessible treatments proves necessary. Elevated BP is a major global health burden and risk factor for the development of several disease states including stroke, cardiovascular disease, myocardial infarction, atherosclerosis, and diabetes mellitus ("Kidney Disease Improving Global Outcomes," 2012; Lund-Johansen, 1983; Pickering, 1952).

1.2 Diagnosis

Diagnosis of hypertension occurs after two seated consecutive visits to the doctor yield a BP reading over 140/90 mm Hg (Pickering *et al*., 2005). Over 90% of normotensive adults over the age of 55 will develop hypertension within their lifetimes (Chobanian *et al*., 2003). When

diagnosing hypertension other cardiovascular risk factors, aside from increased hemodynamic load, are also assessed, such as presence of organ damage and cardiac hypertrophy (Ramsay *et al*., 1999). The Joint National Committee (JNC) on the prevention, detection, evaluation, and treatment of high BP outlines the stages of hypertension and forms management guidelines for health care professionals (James *et al*., 2014).

In 2014, the Eighth Joint National Committee (JNC8) published nine recommendations for the management of high BP based on systemic evidence gathered from several hypertensive clinical studies. However, compared to the Seventh Joint National Committee (JNC7) which indicated specific guidelines defining pre-hypertension and hypertension, no such guidelines were supplied by the JNC8 (Chobanian *et al*., 2003; James *et al*., 2014). According to the JNC7 report, hypertension is diagnosed when systolic BP (SBP) is greater than 140 mm Hg and diastolic BP (DBP) is greater than 90 mm Hg. These numeric guidelines remain comparable to the new JNC8 recommendations, who have stated that this definition remains reasonable (Chobanian *et al*., 2003). A key difference between the two panels are new guidelines for patients >60 years old. Per the JNC8's first recommendation, patients who are >60 years old should aim to lower their BP to 150/90 mm Hg even though the SBP is still above the old JNC7 hypertensive definition of 140 mm Hg (James *et al*., 2014). The JNC8 emphasizes that the objective of hypertensive treatment is to reach and maintain a BP goal and forms evidence-based guidelines, while the JNC7 identifies pre-hypertensive, hypertensive stage 1, and stage 2 criteria for patients with specific BP ranges (Table 1).

The Canadian equivalent of the JNC is the Canadian Hypertension Education Program (CHEP). CHEP is similarly evidence-based although it is annually updated with recommendations for diagnosis of hypertension (Leung *et al*., 2016). In 2016 four new

recommendations were made and two were modified from previous reports. Modifications include increasing dietary potassium and extreme lowering of systolic BP to ≤120 mm Hg in select high risk patients to prevent cardiovascular events (Leung *et al*., 2016). Both the JNC and CHEP aim to educate both health care providers and the public via improved methods to measure and treat high BP.

Table 1. Guidelines for diagnosis of hypertension

diagnosis	**SBP (mm Hg)**	**DBP (mm Hg)**
normotensive	<120	<80
pre-hypertensive	120 - 139	80 - 89
hypertension	≥140	≥90
stage 1 hypertension	140-159	90-99
stage 2 hypertension	≥160	≥100

SBP, systolic blood pressure; DBP, diastolic blood pressure (Chobanian *et al*., 2003).

1.3 Forms of hypertension

1.3.1 Essential hypertension

Essential (primary) hypertension occurs in 90% of people with high BP and can occur due to several factors such as genetics, age, sex, race, and environmental influences (Chobanian *et al*., 2003; Folkow, 1982; Staessen *et al*., 2003). There is not one specific cause that leads to the development of essential hypertension, but a cumulative effect of several factors that increase BP (Staessen *et al*., 2003). Essential hypertension is an early sign of development for multiple disease states, including myocardial ischemia, cardiac hypertrophy and heart failure (MacMahon *et al*., 1990). Due to the wide range of hypertensive symptoms, the exact etiology remains largely unknown.

1.3.2 Secondary hypertension

Secondary hypertension has an identifiable cause and is diagnosed in 5-10% of hypertensive cases (Chobanian *et al*., 2003; Rimoldi *et al*., 2014). Secondary hypertension is distinguished from essential hypertension by several indicators, including resistance to antihypertensive treatments, acute onset, and malignant or accelerated hypertension (Calhoun *et al*., 2008). Indirect or direct actions of prescription drugs, illicit drugs, or food products (e.g. black licorice) (Ottenbacher & Blehm, 2015) may cause secondary hypertension or exacerbate hypertension (Saseen & MacLaughlin, 2014). However, most cases of secondary hypertension are a result of a comorbid disease, such as renal dysfunction, chronic kidney disease, and endocrine disorders (Saseen & MacLaughlin, 2014).

1.3.3 White coat and masked hypertension

Patients that demonstrate elevated BP during appointments with health care providers are not always hypertensive and may be experiencing *white coat hypertension* (Pickering *et al*., 2005). This phenomenon occurs when clinical BP readings are elevated but, when measured in a non-clinical setting, are normotensive (Pickering *et al*., 2005). Due to the prevalence of *white coat hypertension,* CHEP 2016 recommended health care providers be absent during automated office BP measurement to prevent patient interaction (Leung *et al*., 2016).

The opposite of white coat hypertension is *masked hypertension,* where patients appear normotensive in a clinical setting even though they are hypertensive in a non-clinical setting (Pickering *et al*., 2005). These two anomalies can be identified through health care providers requesting out-of-office BP measurements either at home or a pharmacy, along with concurrent clinical measurements.

1.4 Pathophysiology

Hypertension is an asymptomatic disease and has been referred to as a *silent killer*. Blood pressure is the pressure exerted against the arterial wall by circulating blood. Symptoms related to the elevated BP are not always experienced in hypertensive patients. When hypertension remains undiagnosed, deleterious effects occur (i.e. resistance artery narrowing and cardiac hypertrophy) (Staessen *et al*., 2003).

Hypertension is characterized by an increase in BP, which is a function of CO and TPR. Where CO is the volume of blood ejected by the heart per minute and in turn is a product of stroke volume (SV) and heart rate (HR) (Lund-Johansen, 1983). However, CO is rarely affected

in a patient diagnosed with essential hypertension (Dustan *et al*., 1972). Therefore, BP is directly related to TPR, which is the resistance to blood flow in the periphery (Figure 1).

$$BP = CO \; x \; TPR$$

$$CO = SV \; x \; HR$$

$$BP \propto TPR$$

Figure 1. BP is proportional to CO and TPR. CO, cardiac output; SV, stroke volume; HR, heart rate.

A major mechanism affecting BP are alterations in the feedback regulation of the Renin-Angiotensin-Aldosterone System (RAAS) (Saseen & MacLaughlin, 2014). Modifications to the RAAS result in vascular complications by increasing angiotensin II through altered renin production in the kidney (Brunner *et al*., 1972; Saseen & MacLaughlin, 2014). Renin catalyzes the conversion of angiotensinogen to angiotensin I, which is converted to angiotensin II that stimulates the adrenal cortex to produce aldosterone (Saseen & MacLaughlin, 2014). Higher incidence of stroke and myocardial infarction are attributed, at least in part, to normal and high levels of renin and aldosterone (Brunner *et al*., 1972). RAAS alterations may explain the initiation of essential hypertension.

2. Microvascular disease in hypertension

2.1 Resistance arteries

Vascular resistance within the arterioles causes a decline in arterial pressure. The arterioles are therefore termed resistance arteries (or small arteries), where lumen diameters are typically less

than 400 μm and greater than 100 μm (Intengan & Schiffrin, 2000; Neves *et al*., 2012). The small resistance vessels regulate TPR through vasodilation and vasoconstriction.

Poiseuille's law is a mathematical equation that describes the fundamental relationship of TPR with vessel flow (R). As stated by Poiseuille's Law, resistance to blood flow is inversely related to the fourth power of the vessel radius (Figure 2) (Ibrahim & Berk, 2009). Several factors are considered in this equation, including resistance to flow rate (R), radius (r), fluid viscosity (η), and length (l). Thus, even small changes in lumen diameter during hypertension result in an exponential increase in TPR.

$$\boldsymbol{R = \frac{8\eta l}{\pi r^4}}$$

$$\boldsymbol{Therefore, r^4 \propto \frac{1}{TPR}}$$

$$\boldsymbol{lumen\ narrowing \rightarrow \downarrow r^4 \rightarrow \uparrow TPR \rightarrow \uparrow BP}$$

Figure 2. Poiseuille's law. Resistance to blood flow is directly related to the fourth power of the radius of the artery. Lumen narrowing, or internal radius decrease, will subsequently increase TPR and BP as they are directly related.

2.2 Vascular remodeling

Vascular disease can be triggered by several different mechanisms and may be caused through varying degrees of resistance artery narrowing within several circulatory systems, including peripheral, renal and cerebral (Schiffrin *et al*., 2000; Virdis *et al*., 2008). The two major forms of small artery remodeling are eutrophic remodeling, which may occur via rearrangement or apoptosis of arterial components, and hypertrophic growth, which is characterized by hypertrophy and hyperplasia of vascular smooth muscle cells (VSMC) (Heagerty *et al*., 1993; Intengan, Thibault, *et al*., 1999). These processes are not mutually exclusive, resistance arteries may undergo a combination of the two processes.

Vascular remodeling increases the severity of hypertension by narrowing the lumen, and increasing TPR (Intengan & Schiffrin, 2000; Schiffrin, 2004). It remains to be determined whether vascular remodeling is a compensatory mechanism resultant from elevated BP or alternatively, whether increased BP is a consequence of TPR changes during vascular remodeling; it is likely that remodeling is bidirectional. Vascular abnormalities occur through geometric, mechanical, and functional modifications to the small resistance arteries (Intengan & Schiffrin, 2000; Neves *et al*., 2012).

2.2.1 Vascular geometry

Structural changes that occur in small arteries during hypertension increase media:lumen (M:L) through thickening of the media and narrowing of the lumen. The media cross sectional area (CSA) is a key property that determines which remodeling process is dominant. Eutrophic remodeling, and more specifically inward remodeling, occurs primarily in human essential hypertension, while hypertrophic growth typically occurs during secondary hypertension

(Mulvany, 1999; Neves *et al*., 2012). Eutrophic remodeling occurs when there is an inward narrowing of the arterial lumen, and media CSA remains unchanged (Intengan & Schiffrin, 2001). Hypertrophic growth involves an increase in the M:L and CSA through media growth (Buus *et al*., 2001; Intengan & Schiffrin, 2001). Both eutrophic and hypertrophic remodeling result in a narrowing of the lumen and increase in the TPR (Figure 3). Different experimental models of hypertension predominantly exhibit eutrophic remodeling, as found in mild and moderate hypertension, or hypertrophic growth, which occurs in severe and secondary hypertension (Table 2) (Korsgaard *et al*., 1993; Lee *et al*., 1995).

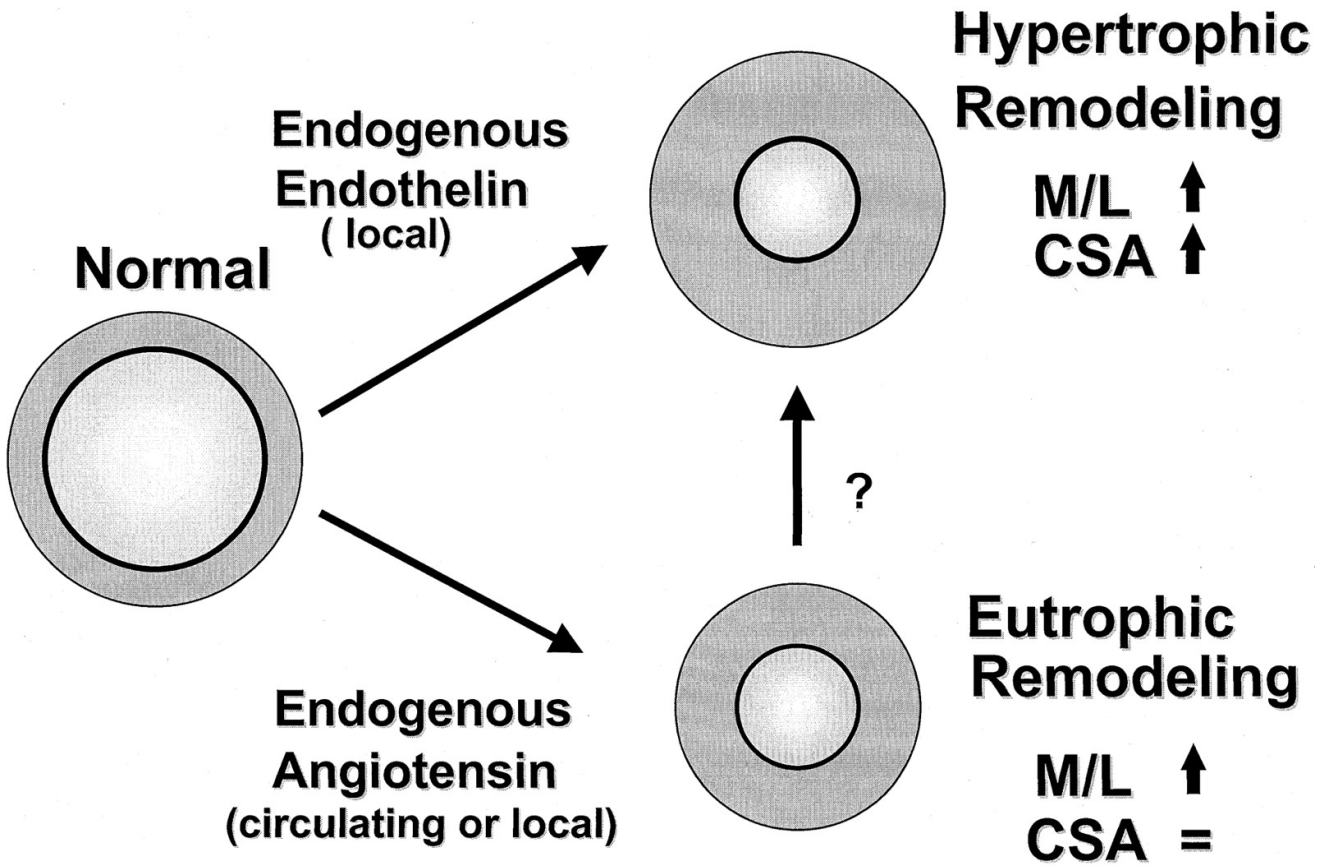


Figure 3. Structural remodeling in small arteries with hypertension. Normotensive vessels may undergo hypertrophic growth or eutrophic remodeling or a combination of both. Hypertrophic remodeling involves increases in both the cross-sectional area (CSA) and media to lumen ratio (M:L). Eutrophic remodeling presents as unchanged media CSA and increased M:L. Reprinted with permission from Wolters Kluwer Health, Inc., (Intengan, H. D., & Schiffrin, E. L, 2000).

Table 2. Hypertensive models and vascular remodeling

eutrophic remodeling	**reference**
spontaneously hypertensive rat (SHR)	(Intengan, Thibault, *et al*., 1999; Mulvany & Halpern, 1977; Mulvany *et al*., 1978)
2-kidney, 1-clip Goldblatt rat	(Li *et al*., 1996)
angiotensin II-induced hypertensive rat	(Dolinsky *et al*., 2013)
mild essential hypertension in humans	(Korsgaard *et al*., 1993)
hypertrophic remodeling	**reference**
deoxycorticosterone acetate (DOCA) salt rat	(Deng & Schiffrin, 1992; Lemkens *et al*., 2012)
1-kidney, 1-clip Goldblatt rat	(Korsgaard & Mulvany, 1988)
Dahl-salt sensitive rats	(d'Uscio *et al*., 1997)
secondary hypertension in humans	(Lee *et al*., 1995)

2.2.2 Vascular mechanics

The mechanical properties of small arteries, such as vascular stiffness and compliance, are altered in hypertension and affect the resistance to blood flow. Stiffness and compliance, or the ability of the artery to buffer changes in pressure, of small resistance arteries may increase through several different mechanisms. These include vascular fibrosis, which can occur due to the rearrangement and change in expression of extracellular matrix components, such as collagen and elastin (Intengan & Schiffrin, 2001). Collagen, for example, is elevated within the arterial wall in hypertensive individuals, thereby increasing vascular stiffness and reducing compliance (Intengan & Schiffrin, 2001).

2.3 Anti-hypertensive treatments

Currently, the JNC8 recommends several pharmacotherapies for the management of high BP including thiazide-type diuretics, calcium channel blockers (CCB), angiotensin-converting enzyme (ACE) inhibitors, or angiotensin receptor blockers (ARB) (James *et al*., 2014). The objective of hypertension management through both pharmacotherapies and lifestyle modifications is to minimize symptoms and prevent progression to target-organ damage, such as heart failure and kidney disease. By the year 2030, it is projected that the cost of treating hypertension will rise to $200.3 billion, from $91.4 billion in 2015 (Heidenreich *et al*., 2011). Even though the cost of treating hypertension is significant, it can be argued that it will remain less than the total cost of treating target-organ failure, which would substantially increase health care cost and burden.

2.3.1 Pharmacotherapies

Initial drug treatment depends on the degree of BP elevation (stage 1 or 2 hypertension) as well as pre-existing comorbidities including diabetes mellitus, stroke, coronary artery disease (CAD) and chronic kidney disease (Saseen & MacLaughlin, 2014). Anti-hypertensive treatment is classified into first line treatments and alternative treatments; all elicit varying degrees of risk reduction for cardiovascular disease. ACE inhibitors, ARB, CCB, and thiazide diuretics are all first line treatments and recommended as monotherapies for stage 1 hypertensive patients and as combination therapies for more severe stage 2 hypertension (Chobanian *et al*., 2003). Current first line treatments exhibit higher rates of reducing cardiovascular events compared to other medications, such as β-blockers, which were previously recommended as first line. However, β-blockers are still considered a beneficial treatment and are recommended for hypertensive cases with specific comorbidities, specifically post-myocardial infarction (MI) or CAD (Saseen & MacLaughlin, 2014).

Beyond BP lowering capabilities, studies indicate concurrent vasculoprotective effects on small resistance arteries. Target-organ damage that occurs in hypertension is likely prefaced by small artery remodeling and it is therefore probable that resistance artery abnormalities are one of the first indicators of human hypertension (Schiffrin, 2001). As mentioned previously, TPR is directly associated with the development of hypertension (Figure 1). Therefore, changes in geometry and mechanics of small resistance arteries are important. Exponential increase in resistance is directly proportional to changes in the radius of the artery (Figure 2). Vascular protection vis-à-vis improving blood vessel structure, mechanics, and function toward normotensive levels are achieved by some, but not all, anti-hypertensive therapies.

Three of the four first line pharmacotherapies suggested by the JNC8 (i.e. ACE inhibitors, ARBs, and CCBs) correct deleterious vascular remodeling in experimental animal models, through decreased collagen deposition, and improved endothelial function (Schiffrin, 2001). ACE inhibitors have been reported by Schiffrin *et al.* to improve vascular structure and endothelial dysfunction in specific vascular beds in humans with stage 1 and 2 hypertension (Schiffrin & Deng, 1995; Schiffrin *et al.*, 1995). Benetos *et al.* have also shown reduced media thickness of radial and carotid arteries after ARB treatment (Benetos *et al.*, 2000). β-blockers do not affect vascular remodeling or endothelial dysfunction even though BP decreases to a level comparable to ACE inhibitors (Schiffrin & Deng, 1995). Incidentally this suggests that β-blockers may not be effective in reversing deleterious vascular remodeling.

Vascular remodeling and improvement of endothelial function are intertwined with BP lowering and it remains challenging to conclude added benefits from anti-hypertensive therapies that remodel small arteries from those that do not (Schiffrin, 2001). β-blockers are less effective at reducing cardiovascular events compared to first line treatments (Saseen & MacLaughlin, 2014) and this may be because they lack the ability to improve small artery geometry, mechanics, and function (Saseen & MacLaughlin, 2014; Schiffrin, 2001). There is evidence to suggest that the regression of vascular remodeling and improvement of endothelial dysfunction associated with ACE inhibitors, ARBs, and CCBs may enhance prevention of cardiovascular events (Saseen & MacLaughlin, 2014).

2.3.2 Lifestyle modifications

Lifestyle and dietary modifications that contribute to lowering BP include, but are not limited to, increased physical activity, salt intake reduction, and adherence to diet plans such as the Dietary

Approaches to Stop Hypertension (DASH), and the Mediterranean diets (Kris-Etherton *et al.*, 2001; Sacks *et al.*, 1999; Sacks *et al.*, 2001; Saseen & MacLaughlin, 2014). Sacks *et al.* reported that low sodium, in combination with the DASH diet, has greater BP lowering effects then the DASH diet alone (Sacks *et al.*, 1999; Sacks *et al.*, 2001). Thus, the JNC has endorsed the DASH diet and recommends it in combination with reduced sodium intake and weight loss (James *et al.*, 2014).

The DASH diet was created to study how dietary factors affect BP and is now known to significantly lower BP in hypertensive individuals (Sacks *et al.*, 1999). This diet stresses fruits, vegetables, low fat dairy products, reduced fats, cholesterol, and sugars. The cumulative effect of the food components in the DASH diet is responsible for BP lowering, rather than just one specific food or nutrient (Appel *et al.*, 2006). The Lyon Heart study examined the effects of the Mediterranean-style diet, which emphasizes bread, root vegetables, fish, and low levels of beef consumption (Kris-Etherton *et al.*, 2001). Olive oils and moderate wine consumption are also encouraged, as this is typical in diets of Greece and Southern Italy (Kris-Etherton *et al.*, 2001). Of the patients participating in the Lyon Heart Study, 50 to 70% had a decreased risk of recurrent cardiovascular disease (Kris-Etherton *et al.*, 2001). The Mediterranean-style diet was popularized when it was discovered that the percent of the population suffering from hypertension and coronary heart disease in French and Mediterranean cultures is lower compared to their surrounding European countries (de Lorgeril *et al*., 2002). The Mediterranean and DASH diets each have their own advantages, and the specific diet recommended for patients is based on preference.

3. Natural health products

3.1 Need for alternatives

Hypertension is an asymptomatic disease that is not always easily managed. A subset of the population will be non-adherent to pharmacotherapies and some may experience resistant hypertension. When a patient is unable to maintain a BP lower then 140/90 mm Hg while prescribed three or more hypertensive therapies of different classes, they are classified as having *resistant hypertension* (Calhoun *et al*., 2008). A study detecting adherence through urine analysis by Pucci *et al*. discovered that only 51% of the cohort were adherent to their prescribed anti-hypertensive medication(s) (Pucci & Martin, 2016). As prescriptions per patient increased, such as the case with resistant hypertension, the more likely a patient became non-adherent or partially-adherent. The most common reasons for non-adherence included adverse side effects of the medication and forgetfulness (Pucci & Martin, 2016). Nonetheless, there are many other reasons for lack of adherence to western medications such as cost, availability and accessibility.

Coinciding with a large percentage of non-adherence to western medications, an increasing number of hypertensive patients seek alternative medications in combination with, or in place of, western medicine. Natural health products (NHP) have gained widespread popularity for treating several morbidities and maladies, including hypertension, diabetes, and cancer. The term NHP includes a broad variety of products including vitamins and minerals, herbal remedies, homeopathic medicine, traditional medicines, probiotics and other products such as fatty acids (Canada, 2015). Currently, the global market for herbal remedies alone totals $60 billion USD annually (WHO, 2008). Approximately 80% of the African population uses traditional medicine as their primary form of health care (WHO, 2008). Reflecting how the prevalence of hypertension in the economically developing world is expected to increase dramatically, an

alternative to western medicine is imperative to potentially reduce this forthcoming global burden (Ibrahim & Damasceno, 2012).

Reasons for NHP use vary, whether they are used in combination with or preferred over western drugs or products, for seasonal illnesses, or improvement of general health. Both Canadians and Americans are turning to these alternatives, with 38% and 40% of the population consuming NHPs respectively (Balluz *et al*., 2000; Troppmann *et al*., 2002). NHPs are widely used and therefore warrant further research.

3.2 Polyphenols and stilbenoids

Polyphenols are highly conjugated compounds that are found in a wide range of plant species such as grapes (*Vitis vinifera*), blueberries (*Vaccinium spp.),* legumes, grasses and peanuts (Aggarwal *et al*., 2004; Chen *et al*., 2002). The most common polyphenols in the grapevine are resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), pterostilbene (*trans*-3,5-dimethoxy-4'-hydroxystilbene), pallidol, and viniferins (Ehrhardt *et al*., 2014; Li *et al*., 2013). Polyphenols may be found naturally or be synthetically produced. The natural isoforms of polyphenols are produced as phytoalexins, secondary metabolites of plants that are produced *de novo* when exposed to stress (Dixon, 2001). Phytoalexins are polyphenolic, and of these compounds many are stilbenoid in structure (Ehrhardt *et al*., 2014). The most important phytoalexin group found in the grapevine is considered to be the stilbenes (Martinez-Marquez *et al*., 2016).

Stilbenes are non-flavonoid compounds that are a subclass of phenolic acids (Rentzsch *et al*., 2009). Stilbenoid polyphenols are structurally similar, consisting of two benzene rings linked by a styrene double bond. Stilbenes are not always monomeric in structure and can exist as viniferins, which are oligomeric and polymeric stilbenes (Rentzsch *et al*., 2009). The stilbenoid

structure has been linked to numerous biological activities including anti-oxidant, anti-angiogenic, anti-carcinogenic and anti-inflammatory actions (Kloypan *et al*., 2012).

3.3 French Paradox

The percentage of the population suffering from hypertension and CAD in French and Mediterranean cultures is lower compared to their surrounding European countries (de Lorgeril *et al*., 2002). In Britain the likelihood of an adult male to develop ischemic heart disease was four times higher than in France (Law & Wald, 1999). This was considered a paradoxical relationship, as the French people at that time consumed a diet high in saturated fats, smoked abundantly, and led sedentary lifestyles. This relationship suggests two notions: the association between CAD and a diet high in saturated fats was invalid, or that there was an external factor that mitigated this risk, and conferred lower CAD risk.

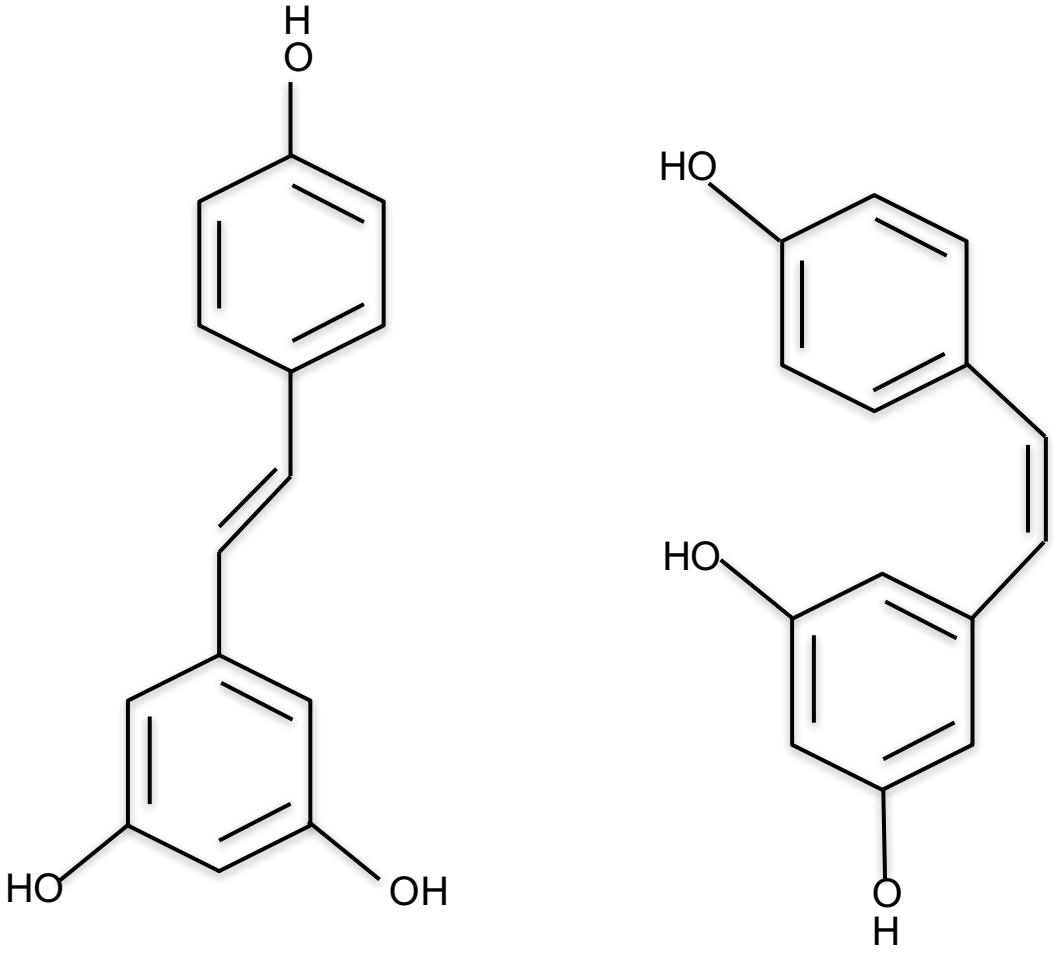
Finland consumes a diet high in saturated fatty acids; however, in comparison to France, this population also has a high percentage of heart disease. The people of Finland consume a diet that is plentiful in fatty red meats, butter and bread, and partake a lifestyle that involves laboring in cold and damp climates (Huang & Sumpio, 2008). The French climate has hot summers and cool winters, which allow an extended growing season for vegetables. The lifestyle in France is suited for a diet rich in olive oils, fresh fruit and vegetables, whole grains, and red wine (Huang & Sumpio, 2008; Perez-Lopez *et al*., 2009). The climate in France has significant periods of sun exposure that allow a high antioxidant content in their plants, which allows protection from reactive oxygen species (ROS) produced during photosynthesis (Huang & Sumpio, 2008). The French Paradox is therefore likely due to an accumulation of several external factors.

The relationship between red wine consumption and lower mortality rates from CAD is one of several theories that attempts to explain the French Paradox (Rodrigo *et al*., 2012).

Despite the significant focus on resveratrol, a polyphenol in red wine, there remains a lack of satisfactory evidence to support that it is solely responsible to explain the French Paradox. Resveratrol exists as two diastereomers, *cis*-resveratrol and *trans*-resveratrol, and the latter is the most common isoform in nature due to increased structural stability (Figure 4) (Ehrhardt *et al*., 2014; Trela & Waterhouse, 1996). The conversion of *trans*-resveratrol to *cis*-resveratrol occurs through photoisomerization. Both *cis* and *trans*-resveratrol are found in red and white wines although the *trans* isomer is readily found in much greater concentrations within dark red wines, such as pinot noir (Rentzsch *et al*., 2009). Red wines that are cultivated in colder climates, such as Ontario and the Bordeaux region of France, also have greater resveratrol concentrations than those from warmer climates, such as California and South America (Rentzsch *et al*., 2009). Other candidate contributors to the French Paradox include vitamin K2, cheese, fresh fruits and vegetables, olive oils, garlic, and alcohol (spirits) (de Lorgeril *et al*., 2002; Law & Wald, 1999; Renaud & de Lorgeril, 1992).

There have been several papers published on other contributing factors such as olive oils and cheeses (Hohmann *et al*., 2015; Huang & Sumpio, 2008; Perez-Lopez *et al*., 2009; Petyaev & Bashmakov, 2012; Zheng *et al*., 2015). France has many olive trees as they are very weather-resistant and resilient. The olive tree root is able to grow in very infertile soil (Huang & Sumpio, 2008). Olive oils contain flavonoids, phenols, and sterols, and the main antioxidants are polyphenols and carotenoids (Huang & Sumpio, 2008). Olive oil antioxidants exert a slight lowering effect on oxLDL cholesterol levels and a moderate effect on lowering SBP (Hohmann *et al*., 2015). Therefore, there is evidence of a positive relationship between cardiovascular risk factors and high phenolic olive oils. Another explanation of the French paradox was revealed through cheese proteomics. This methodology allowed the identification and isolation of novel

peptides that inhibit ACE, and some cheeses also downregulate pro-inflammatory cytokines such as IL-6, C-reactive protein and TNF-α (Petyaev & Bashmakov, 2012). In summary, the French and Mediterranean diets include and allow full year access to a wide variety of foods.



***trans*-resveratrol** ***cis*-resveratrol**

Figure 4. Resveratrol diastereomers. The most common isoform of resveratrol is *trans*-resveratrol (*trans*-3,5,4′-trihydroxy-stilbene). The conversion of *trans*-resveratrol to *cis*-resveratrol (*cis*-3,5,4′-trihydroxy-stilbene) occurs through photoisomerization with UV light.

3.4 Resveratrol and limitations

Resveratrol alters arterial wall components and compliance in the context of hypertension and delays cardiac hypertrophy (Behbahani *et al*., 2010; Dolinsky *et al*., 2013; Raj *et al*., 2014; Thandapilly *et al*., 2010). Resveratrol does not consistently lower BP in animal models (Behbahani *et al*., 2010; Dolinsky *et al*., 2013; Li *et al*., 2006). However, resveratrol exerts cardioprotective effects; heart weight reduction occurs in partially nephrectomised rats, SBP elevation and cardiac hypertrophy is prevented in fructose fed rats, and BP is decreased in high-fat diet rats (Aubin *et al*., 2008; Liu *et al*., 2005; Miatello *et al*., 2005). Resveratrol also increases compliance, and decreases diastolic and systolic dysfunction in SHR (Behbahani *et al*., 2010; Thandapilly *et al*., 2010) (Table 3). *In vivo* studies utilizing *trans*-resveratrol treat hypertension through anti-oxidant and anti-inflammatory pathways that may in turn delay the onset atherosclerosis and heart failure (Dolinsky *et al*., 2013; Thandapilly *et al*., 2010).

These beneficial effects of resveratrol do not seem sufficient to produce the four fold decrease of CAD risk between Britain and France and it is unlikely the sole variable to explain the French Paradox (Law & Wald, 1999). In particular, resveratrol has poor solubility, is readily metabolized, and undergoes rapid glucuronidation during phase II conjugation resulting in low oral bioavailability of 20% and a half-life of 14 minutes in Sprague Dawley rats (Biasutto *et al*., 2010; Hao *et al*., 2015; Kapetanovic *et al*., 2011; McCormack & McFadden, 2012).

Table 3. Cardioprotective effects of resveratrol *in vivo*

animal model	dose	duration	effect	reference
partially nephrectomized rat	50 mg/kg/d	4 weeks	decreased heart weight and SBP	(Liu *et al*., 2005)
fructose fed rat	10 mg/kg/d	45 days	prevented SBP elevation and cardiac hypertrophy	(Miatello *et al*., 2005)
high fat diet rat	20 mg/kg/d	8 weeks	decreased BP and preserved vascular function	(Aubin *et al*., 2008)
SHR	2.5 mg/kg/d	10 weeks	increased compliance, decreased oxidative stress, and diastolic/systolic dysfunction	(Behbahani *et al*., 2010; Thandapilly *et al*., 2010)
CHF – ligation of left coronary artery	5 mg/kg/d	10 months	improved LV systolic function and aortic stiffness	(Ahmet *et al*., 2017)

3.5 Clinical Trials

In clinical trials the bioavailability of resveratrol may be too low to be biologically effective (Goldberg *et al*., 2003). Several trials have used resveratrol, with the highest dose being 5 g/d (Boocock *et al*., 2007; Ghanim *et al*., 2011; Popat *et al*., 2013). This was administered in trials involving healthy individuals, diabetic patients, and patients with multiple myeloma (Boocock *et al*., 2007; Ghanim *et al*., 2011; Popat *et al*., 2013). The long-term effects of daily dosage have not yet been entirely determined for resveratrol. In multiple myeloma patients, 5 of 24 participants developed renal failure, which demonstrated that there is still unknown information about the safety of resveratrol treatment and its side effects (Popat *et al*., 2013). Even though the safety profile has not been readily established, resveratrol supplements are available for purchase with a wide range of doses. In Canada, there are currently 186 natural health products containing resveratrol with market authorization.

The bioavailability of resveratrol is 20% with an absorption of 70% based on urinary excretion data in humans (Biasutto *et al*., 2010; Kapetanovic *et al*., 2011; Walle *et al*., 2004). However, two studies claim bioavailability may be higher than that found in the plasma. Blache *et al*. showed that resveratrol was incorporated into lipoproteins and red blood cells, while Baisutto *et al*. determined incomplete recovery in plasma as some resveratrol is likely retained in whole blood cell pellet (Biasutto *et al*., 2010; Blache *et al*., 1997). The low bioavailability and rapid metabolism through sulfation and glucuronidation of resveratrol is consistent between humans and animals (Walle *et al*., 2004).

Increasing plasma bioavailability has been attempted by changing the structural properties of resveratrol to enhance oral dosage effects. SRT501 is a micronized resveratrol product that was synthesized by Sirtis, a GlaxoSmithKline (GSK) company, to increase oral

bioavailability. Micronized particles were developed and absorption improved through dissolution. The mean plasma concentration of SRT501 was 3.6 fold higher than resveratrol (Boocock *et al*., 2007; Howells *et al*., 2011). SRT501 was used by Howells *et al*. in a phase 1 clinical trial. However, a phase II study utilizing SRT501 was terminated due to adverse side effects (Boocock *et al*., 2007; Popat *et al*., 2013). Sirtis ceased using SRT501 in clinical trials in 2010. Despite the theoretical potential of resveratrol, clinical trials utilizing resveratrol may not produce beneficial results without improved oral bioavailability. The answer for a safer and more bioavailable treatment may be in resveratrol analogues. However, the signaling and effectiveness of resveratrol analogues are not well understood, and there remains a lack of information on *in vivo* effects.

4. Resveratrol analogues

Pterostilbene and gnetol are two structural derivatives of resveratrol that share its characteristic stilbenoid structure, but their minor compositional differences alter their pharmacokinetic and pharmacodynamic profile markedly.

4.1 Pterostilbene

Pterostilbene (*trans*-3,5-dimethoxy-4'-hydroxystilbene) is a dimethoxylated analogue of resveratrol. Blueberries (*Vaccinium spp.)* and certain species of grapes and grape leaves (*Vitis vinifera)* contain pterostilbene (Martinez-Marquez *et al*., 2016; Rimando *et al*., 2004). The natural amount of pterostilbene in *Vitis vinifera* is considerably less compared to its parent compound, *trans*-resveratrol (Martinez-Marquez *et al*., 2016). Within the grapevine resveratrol is converted to pterostilbene or piceatannol via methylation or hydroxylation, respectively

(Martinez-Marquez *et al*., 2016). The conversion of resveratrol to pterostilbene occurs through resveratrol O-methyl transferase, which is specific to *Vitis vinifera* (VvROMT) (Martinez-Marquez *et al*., 2016; Schmidlin *et al*., 2008).

There are approximately 1.5 - 3 mg of resveratrol (per litre of red wine); however, in 100 g of blueberries there is only 15 μg of pterostilbene (McCormack & McFadden, 2012). In contrast, the oral bioavailability of pterostilbene compared to resveratrol is much higher at 80%, with a half-life of 105 minutes (McCormack & McFadden, 2012). The prolonged half-life of pterostilbene is a result of a methoxy group occupying position 3 and 5 of the benzene ring that temporarily prevents glucuronidation. Even though the natural abundance of pterostilbene is not substantial, it is easily attainable through chemical synthesis (Schmidlin *et al*., 2008).

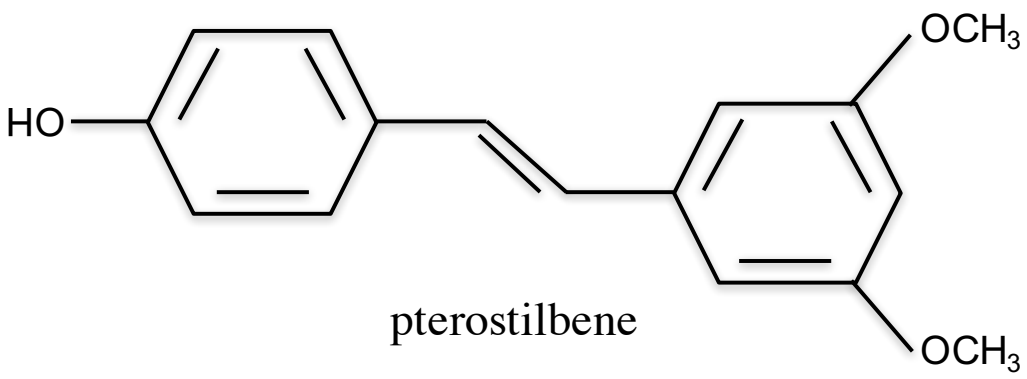


Figure 5. Structure of pterostilbene (*trans*-3,5-dimethoxy-4'-hydroxystilbene). The stilbenoid polyphenol, pterostilbene, is a structural derivative of resveratrol. The increased bioavailability of pterostilbene is due to the addition of two methoxy groups (McCormack & McFadden, 2012).

4.1.1 Traditional uses of pterostilbene

Pterostilbene is an active compound found in several *Vaccinium* berries and plant species, specifically the *Pterocarpus* genus (Manickam *et al*., 1997). Pterostilbene was originally discovered as a constituent compound in *P. santalinus*, more commonly known as red sandalwood (Manickam *et al*., 1997; Schmidlin *et al*., 2008). The heartwood of *P. marsupium* also contains pterostilbene (Manickam *et al*., 1997). Traditionally, the wood of the *P. marsupium* plant was used in Indian Ayurvedic or holistic medicine for the treatment of diabetes (Manickam *et al*., 1997). The Indian herbal preparation called "Darakchasava" is also used in Ayurvedic medicine and consists mainly of dried grape berries (*Vitis vinifera*) that contains pterostilbene and resveratrol (Paul *et al*., 1999). Darakchasava is consumed as both a general health tonic, and more intriguingly, as a cardiotonic (Paul *et al*., 1999).

4.2 Gnetol

The tetrahydroxy stilbene, gnetol (*trans*-2,6,3',5'-tetrahydroxystilbene), is used in NHPs and traditional medicine (Narayanan *et al*., 2015; Xiang *et al*., 2002). However, compared to resveratrol, gnetol has increased solubility, but a low bioavailability of 6.59% (Remsberg *et al*., 2015). The enhanced solubility of gnetol is a result of the increased presence of alcohol groups located in the C2 and C6 position of the stilbene frame (Figure 6).

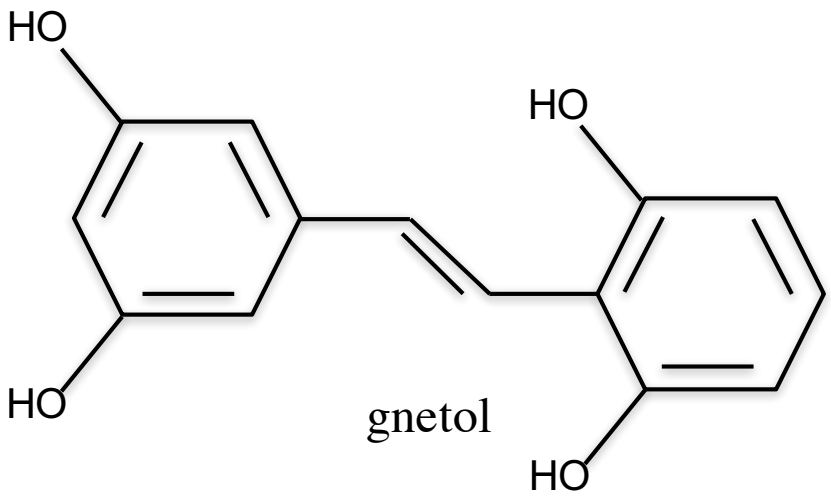


Figure 6. Structure of gnetol (*trans*-2,6,3',5'-tetrahydroxystilbene). The stilbenoid polyphenol, gnetol, is a structural analogue of resveratrol. The tetrahydroxy chemical structure of gnetol allows for increased solubility when compared to resveratrol.

4.2.1 Traditional uses of gnetol

Gnetol has been found in several families of plants including; Gnetaceae and Moraceae, which are commonly located in Southern China, and South East Asia (Ohguchi *et al*., 2003; Sermboonpaisarn & Sawasdee, 2012). Of the family Gnetaceae, the genus *Gnetum* contains several stilbene analogues and is traditionally used in folk medicines as well as traditional Chinese medicines (Xiang *et al*., 2002).

Furthermore, *G. gnetum* and *G. montanum* are traditionally used as treatments for arthritis, bronchitis, and asthma (Ohguchi *et al*., 2003; Xiang *et al*., 2002). Melinjo plants, which are part of the species *G. gnemon,* are fully edible and consumed as a major food item in Indonesia (Narayanan *et al*., 2015). Melinjo seed extract is also sold as a nutritional supplement in Japan (Narayanan *et al*., 2015). The family Moracea*e* contains the rare species *Ficus foveolata*, a medicinal plant located in Northern Thailand (Sermboonpaisarn & Sawasdee, 2012).

The roots of *F. foveolata* are mashed into an alcoholic beverage and consumed for rejuvenating properties, and for enhancing sexual performance (Sermboonpaisarn & Sawasdee, 2012).

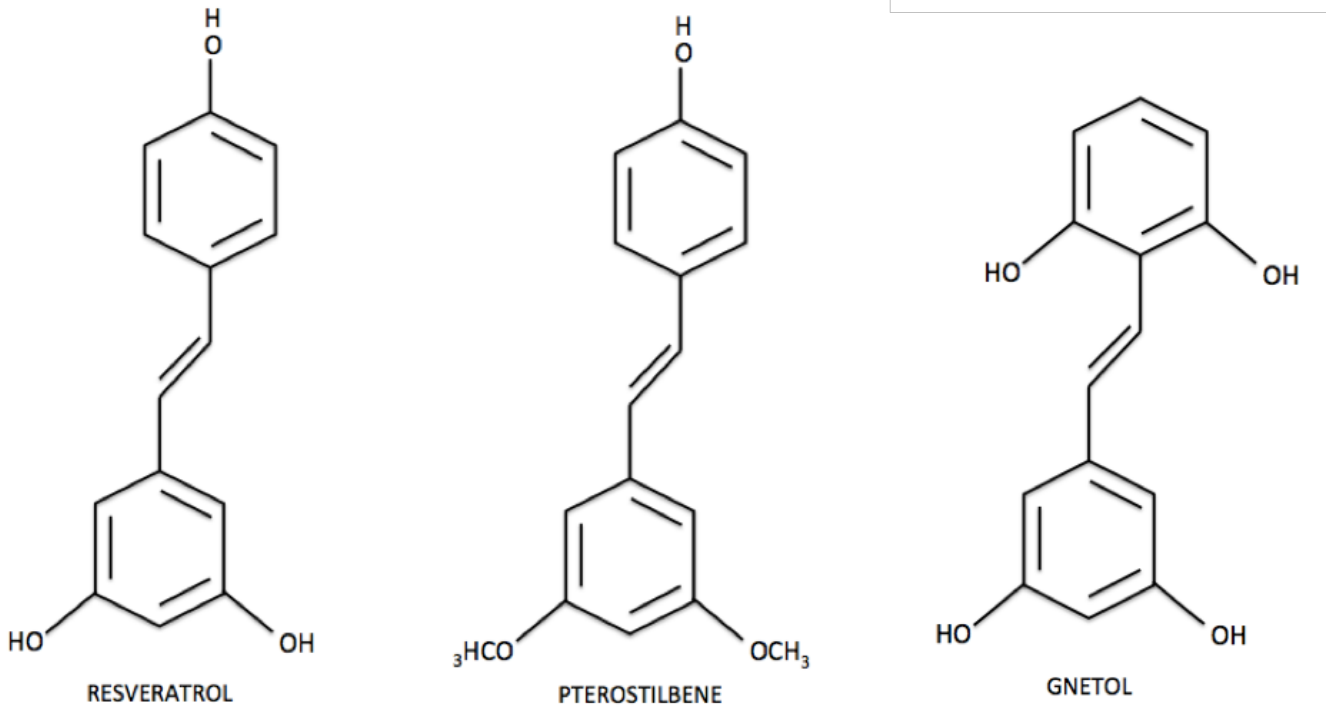


Figure 7. Resveratrol and structural analogues, pterostilbene and gnetol. Stilbenoid polyphenols consist of two benzene rings connected by a styrene double bond. The methoxy groups at C3/C5 of pterostilbene prevent rapid glucuronidation increasing bioavailability, while the tetrahydroxy arrangement on gnetol lowers bioavailability.

5. Disease and stilbenoids

Pterostilbene and *G. gnemon* exert anti-inflammatory, anticancer and antioxidant actions, which overlap with the broad range of effects of resveratrol (Fremont, 2000; McCormack & McFadden, 2012; Narayanan *et al*., 2015).

5.1 Non-cardiovascular diseases

5.1.1 Alzheimer's Disease

Interest in resveratrol has been extended to Alzheimer's disease (AD), perhaps by preventing downregulation of iNOS (Clement *et al*., 1998; Dolinsky *et al*., 2009; Huang *et al*., 2011; Tome-Carneiro, Gonzalvez*, et al*., 2013). Resveratrol also inhibits butyrylcholinesterase (BChE), a causal enzyme of AD (Sermboonpaisarn & Sawasdee, 2012). Sermboonpaisarn *et al*. isolated several polyphenols from *F. faveolata*, including gnetol and resveratrol, and tested anti-cholinesterase activity (Sermboonpaisarn & Sawasdee, 2012). Gnetol exhibited the greatest selectivity and inhibitory effects on BChE (Sermboonpaisarn & Sawasdee, 2012). The tetrahydroxy configuration of gnetol may play an important role as it proved more effective than the trihydroxy structure of resveratrol. The position and number of hydroxyl groups on the stilbenoid frame may influence BChE inhibition. Compared to two commercial drugs for AD, galanthamine and phytostiginine, gnetol exhibited greater BChE inhibition (Sermboonpaisarn & Sawasdee, 2012). Gnetol is therefore considered an attractive therapeutic to treat AD since BChE inhibitors decrease β-amyloid plaques in the brain (Furukawa-Hibi *et al*., 2011).

5.1.2 Melanoma

Gnetol extracted from the roots of *G. gnemon* is a strong inhibitor of tyrosinase activity resulting in decreased melanin biosynthesis (Ohguchi *et al*., 2003). The enzyme tyrosinase plays a role in the accumulation of melanin pigments. Hyperpigmentation occurs when there is an abundance of melanin (Ohguchi *et al*., 2003). Oxyresveratrol, another tetrahydroxy analogue of resveratrol, is also an inhibitor of tyrosinase activity. Both gnetol and oxyresveratrol exhibit greater inhibition compared to resveratrol and again, the quantity and placement of hydroxy groups may affect tyrosinase activity (Ohguchi *et al*., 2003). Currently, kojic acid and albumin are used in beauty products as depigmenting agents; however, gnetol is 30-fold more effective than kojic acid (Ohguchi *et al*., 2003). Gnetol shows potential as a cosmetic for hyperpigmentation or as a pharmacological agent for the treatment of melanomas.

Pterostilbene treatment has inhibited malignant melanomas through several mechanisms, but not specifically through tyrosinase inhibition. Pterostilbene has been attributed to inhibition of NO production and preventing metastasis and apoptosis (McCormack & McFadden, 2012). Pterostilbene is also associated with preclinical anticancer effects. After treatment with pterostilbene, breast cancer stem cells (BCSC) lost tumorigenicity by virtue of effects on cancer stem cell maintenance and reduced CD44 activity (Wu *et al*., 2015). The greater lipophilicity and bioavailability of pterostilbene, compared to resveratrol, increases potential for cellular uptake and promoted apoptosis in several cancer lines including melanoma, breast, bladder, colon, leukemia, lung, pancreas, prostate and stomach (McCormack & McFadden, 2012).

5.1.3 Diabetes

Anti-diabetic effects have also been linked to resveratrol and pterostilbene (Pari & Satheesh, 2006; Szkudelski & Szkudelska, 2011). Pari *et al*. reported that pterostilbene lowered glucose concentration by increasing glycolysis and decreasing gluconeogenesis (Pari & Satheesh, 2006). Pterostilbene-fed diabetic rats also exhibited increased plasma insulin and lowered blood glucose levels (Pari & Satheesh, 2006). A multitude of studies suggest anti-diabetic mechanisms for resveratrol, including reducing blood glucose, preserving beta cells and improving insulin action (Szkudelski & Szkudelska, 2011). Thus, resveratrol, pterostilbene and gnetol may have therapeutic potential through numerous mechanisms.

5.2 Cardiovascular disease

The effect of resveratrol on cardiovascular disease has been studied extensively since the observation of the French Paradox. Resveratrol targets multiple pathways and elicits beneficial cardiovascular effects in several animal models. For example, resveratrol has reduced the size, density, and surface area of atherosclerotic plaques in hypercholesteremic animal models, improved cardiac function and reduced cardiac hypertrophy in SHR, and improved chemotherapy-induced cardiotoxicity by alleviating oxidative stress (Li *et al*., 2012; Thandapilly *et al*., 2012).

Regardless of the low plasma concentration, rapid metabolism and conjugation, pharmacological effects of resveratrol are still detected. Despite resveratrol having low bioavailability it proves to have high bioactivity, leading to a paradoxical relationship (Tome-Carneiro, Larrosa, *et al*., 2013). Resveratrol metabolites that occur through sulfation and glucuronidation are considered potential effectors, and likely contribute to the *resveratrol*

paradox (Tome-Carneiro, Larrosa*, et al*., 2013; Walle *et al*., 2004). However, the promising effects observed pre-clinically do not have comparable outcomes in clinical trials (Bonnefont-Rousselot, 2016).

Stilbenoid analogues such as gnetol and pterostilbene have not been extensively researched within the subject of cardiovascular disease, though pterostilbene has been studied in the context of atherosclerosis. Pterostilbene inhibits VSMC growth and cell cycle progression within rat aorta (Park *et al*., 2010). Matrix-metalloproteinase-2 (MMP-2) and SMC play key roles in the progression of atherosclerosis. Pterostilbene inhibits SMC migration and activation of MMP-2 (Lin *et al*., 2015). Rimando *et al*. also demonstrated that pterostilbene lowered lipid/lipoprotein in hypercholestermic hamsters via PPAR-α agonism (Rimando *et al*., 2005). Stilbenes of the family Gnetaceae, including resveratrol and gnetol, prevent arachidonic acid-induced platelet activation; however, both are less potent then aspirin (Kloypan *et al*., 2012). Structural analogues of resveratrol are gaining interest in cardiovascular research.

6. Rationale and hypothesis

Although resveratrol is well-tolerated in humans, it is readily metabolized and exhibits low oral bioavailability. Resveratrol undergoes phase II conjugation and is readily glucuronidated at the C3 position. Nevertheless, low dose resveratrol reportedly prevents resistance artery abnormalities in the mesenteric arteries of SHR (Behbahani *et al*., 2010). I extended on previous findings using resveratrol analogues, pterostilbene and gnetol. I also utilized a different and possibly more physiological relevant animal model, the SHHF rat. The SHHF is a genetic model of human heart disease with the risk of heart failure superimposed upon hypertension (Heyen *et al*., 2002).

I compared the effects of resveratrol, pterostilbene, and gnetol on the structural and mechanical properties of SD and SHHF mesenteric arteries. Vascular structure was examined through measuring media and lumen diameters of mesenteric resistance arteries (<400 μm), using pressure myography.

I hypothesized, based upon stilbene oral bioavailability found within the literature, that pterostilbene would induce the greatest vasculoprotective effect in the SHHF mesenteric resistance arteries. Gnetol, with the lowest bioavailability compared to resveratrol and pterostilbene, would have a minor, yet positive effect on vascular remodeling in hypertensive resistance arteries.

CHAPTER 2. MATERIALS AND METHODS

1. Animals

This study was conducted per recommendations from the Animal Care Committee of the University of Manitoba and the Canadian Council of Animal Care. Male Sprague Dawley (SD) rats and lean spontaneously hypertensive heart failure (SHHF) rats were obtained from Charles River (Senneville, Quebec, Canada) at 7 weeks of age. Animals were housed under a 12-h light/dark cycle at 22°C and 60% humidity and fed *ad libitum*. The rats were acclimatized for 2 weeks to remove stress induced by their new environment as a variable.

2. Blood pressure and weight

Rats were trained for BP measurement using tail cuff plethysmography (CODA non-invasive blood pressure system; Kent Scientific, Torrington, CT), during the 2 week acclimatization period. Systolic BP was measured on a biweekly basis, for a total of five measurements (Figure 8).

3. Treatments

After 2 weeks of acclimatization, rats (9 weeks of age) were split into four treatment groups within each animal model (i.e. SD, the normotensive animal model, and SHHF, the hypertensive animal model). SD and SHHF rats were treated for 8 weeks by oral gavage with vehicle (SD-C, SHHF-C) or low doses (2.5 mg/kg/d) of resveratrol (SD-R, SHHF-R), pterostilbene (SD-P, SHHF-P), and gnetol (SD-G, SHHF-G) (Sigma Aldrich-Canada, Oakville, ON, Canada; Cayman Chemical, Ann Arbor, MI, USA; and Sabinsa Corporation, East Windsor, NJ, USA).

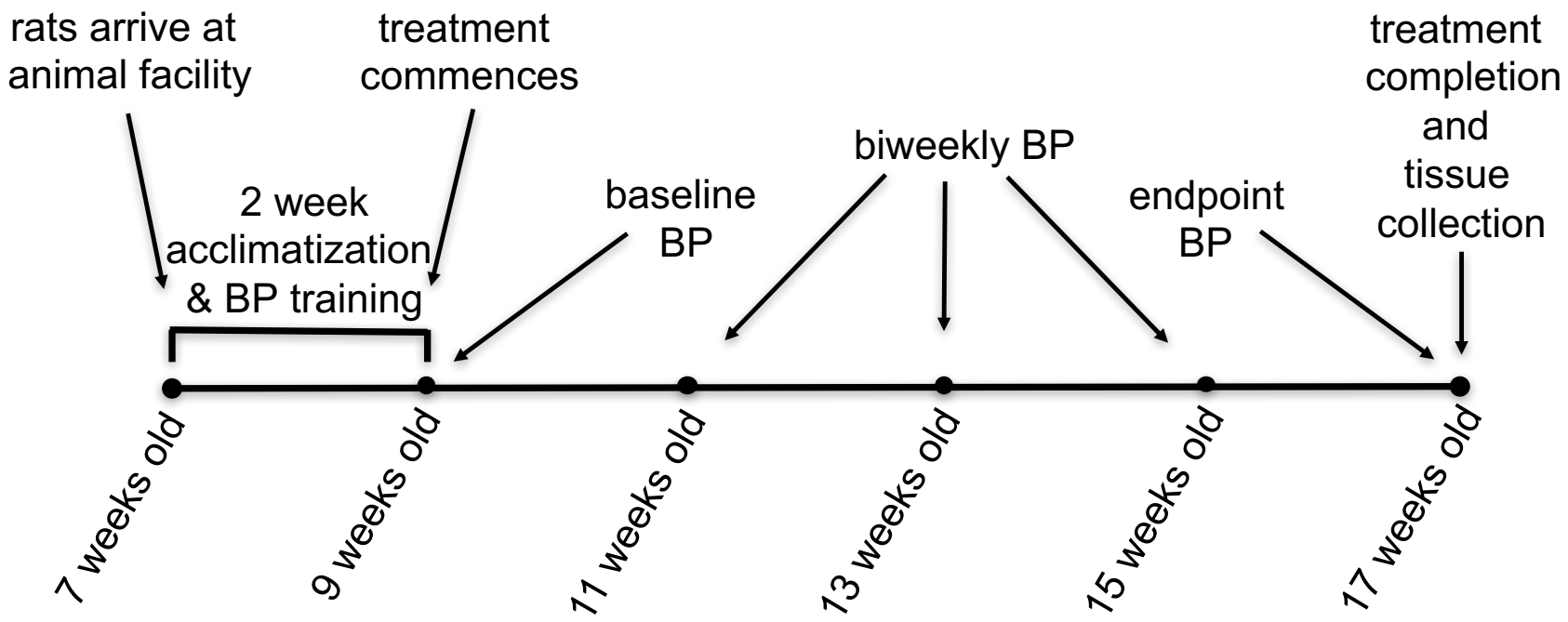


Figure 8. *In vivo* stilbene study timeline. Animals arrived at 7 weeks of age and after 2 weeks of acclimatization, received vehicle, or 2.5 mg/kg/day of resveratrol, pterostilbene, or gnetol via oral gavage for 8 weeks. Blood pressure was measured bi-weekly.

This dose was chosen based on our previous study that showed vascular improvement using low dose resveratrol in the SHR animal model (Behbahani *et al*., 2010). Each treatment group consisted of 8 animals, for a total of 64 animals at the beginning of the study. However, mortality during the eight week treatment period lowered total animals (Table 4).

4. Tissues

At 17 weeks of age, rats were anesthetized by 3% isoflurane and hearts were excised. The mesenteric cascade was isolated and placed in ice-cold Krebs buffer (118 mmol/L NaCl, 4.65 mmol/L KCl, 1.18 mmol/L $MgSO_4$, 1.18 mmol/L $KHPO_3$, 25 mmol/L $NaHCO_3$, 2.5 mmol/L $CaCl_2$, 5.5 mmol/L glucose, 0.026 mmol/L EDTA). As indicated previously (Kopilas *et al*., 2007), the mesenteric artery was chosen for study based on three parameters (Behbahani *et al*., 2010; Thandapilly *et al*., 2013):

1. mesenteric arteries remodel in human hypertensives;
2. the mesenteric circulation contributes to TPR as a large percentage of CO flows through the resistance arteries;
3. mesenteric arteries are more suitable for study than coronary, renal, and femoral arteries, which also remodel and similarly respond to treatment in hypertensive rat models, since branching is less prevalent.

To maintain consistency and ensure unbiased sampling, arterial segments were dissected from 3rd order branches (Figure 9).

5. Pressure myography

Resistance arteries were mounted between two glass micro-cannulas and secured with nylon ties in a pressure myograph chamber (Living Systems Instrumentation, Burlington, VT). Small arteries were adjusted such that the walls were parallel without stretch. Vessels were equilibrated for 1 hour at constant intraluminal pressure (45 mm Hg) in Krebs buffer bubbled with 20% O_2 and 5% CO_2, to pH 7.4 and maintained at 37°C. Vessels were considered viable if KCl (125×10^{-3} mol/L) elicited >50% constriction in lumen diameter.

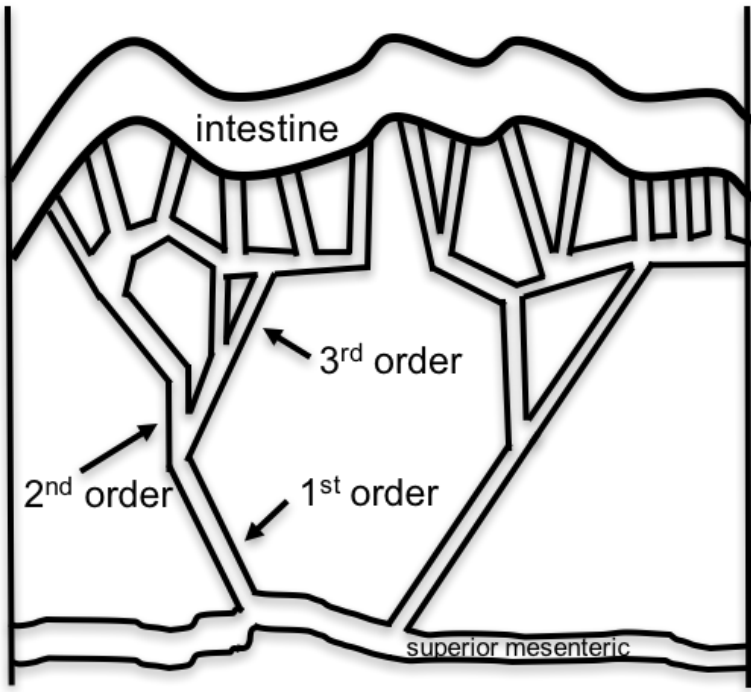


Figure 9. Mesenteric 3rd order arterial segment. Mesenteric resistance arteries are highly branched structures. To maintain consistency and ensure unbiased sampling, all mesenteric resistance arterial segments were isolated from 3rd order branches.

5.1 Vascular geometry

Vessels were deactivated by perfusing with Ca^{2+}-free Krebs solution containing 10^{-3} mol/L EGTA for 30 minutes, thereby removing myogenic tone. Lumen and media dimensions were measured in triplicate along the length of the vessel while the intraluminal pressure was maintained at 45 mm Hg.

5.2 Vascular mechanics

Intraluminal pressure was raised from 3 to 140 mm Hg three times, and arteries were unbuckled by adjusting the cannulas. To obtain pressure-lumen diameter relationships, the intraluminal pressure was increased stepwise (10 increments) from 3 to 140 mm Hg. Lumen and media measurements were measured in triplicate along the length of the vessel at each pressure interval, from which parameters of mechanical properties were calculated.

5.3 Formulas

Media cross-sectional-area (CSA) was calculated by the subtraction of the internal CSA from the external CSA:

$$(1)\ media\ CSA = \frac{\pi}{4} \times \left({D_e}^2 - {D_i}^2 \right)$$

where D_e and D_i are external and internal diameters of the blood vessel, respectively.

Incremental distensibilty was calculated as the product of the fractional change in internal diameter (D) per change in the intraluminal pressure (P):

$$(2)\ incremental\ distensibilty = \left(\frac{1}{\Delta \mathrm{P}} \right) \times \left(\frac{\Delta \mathrm{D}}{\mathrm{D}} \right) \times 100$$

Circumferential strain (ε) was calculated as:

$$(3)\ \varepsilon = \frac{(D - D_o)}{D_o}$$

where D is the internal diameter for a given intravascular pressure, and D_o is the original diameter at 3 mm Hg.

Circumferential stress was determined by:

$$(4)\ \sigma = \frac{(PD)}{(2M)}$$

where P is the intraluminal pressure in dyne/cm^2 (1 mm Hg= 1.334 x 10^3 dyn/cm^2), D is the internal diameter and M is the media thickness.

Elastic modulus (EM) was determined by fitting stress-strain data to the exponential equation ($y=ae^{bx}$) using least squares analysis:

$$(5)\ \sigma = \sigma_o e^{\beta\varepsilon}$$

where σ_o was stress at the original diameter, D_o, and β is a constant related to the rate of increase in the stress-strain curve.

Tangential elastic modulus (ET) was calculated at several values of stress from the derivative of the abovementioned exponential curve (Equation 5):

$$(6)\ ET = \frac{d\sigma}{d\varepsilon} = \beta\sigma_o e^{\beta\varepsilon}$$

The slope of the elastic modulus vs. stress curve, yields the intrinsic stiffness of wall components.

Remodeling index was calculated as:

$$(7)\ remodeling\ index = 100 \times \frac{[(D_i)_n - (D_i)_{remodel}]}{[(D_i)_n - (D_i)_h]}$$

where $(D_i)_n$ and $(D_i)_h$ were lumen diameters of normotensive and hypertensive vessels, respectively. Where $(D_i)_{remodel}$ is calculated as:

$$(7a)\ (D_i)_{remodel} = \left[(D_e)_h{}^2 - \frac{4CSA_o}{\pi}\right]^{0.5}$$

and $(D_e)_h$ is the external diameter of hypertensive vessels.

Growth index was determined as the difference of hypertensive and normotensive cross-sectional area over normotensive cross-sectional area;

$$(8)\ growth\ index = \frac{(CSA_h - CSA_n)}{CSA_n}$$

where CSA_n and CSA_h are media cross-sectional areas of normotensive and hypertensive vessels, respectively.

6. Western blot

The remaining mesenteric cascade was submerged in ice-cold RNAlater immediately after dissection. The mesenteric cascade was cleaned and arteries were dissected as previously described (Rodrigo *et al*., 2002). Protein was isolated from frozen mesenteric resistance arteries using lysis buffer (50 mmol/L Hepes pH 7.4, 150 mmol/L NaCl, 10% glycerol, 1.5 mmol/L $MgCl_2$, 1 mmol/L EGTA, 1% Triton X-100, 1% sodium deoxycholate, and 1% SDS with protease inhibitors). Arteries were cut into smaller pieces and sonicated for 1 minute (10 second sonication/10 second rest), tissues were then centrifuged at 4°C for 20 minutes and supernatant was collected. Protein concentration was determined by BCA assay.

Protein (20 ug) from mesenteric arteries was separated on 10% polyacrylamide gel and transferred to PVDF (polyvinylidene difluoride) membrane. Membranes were blocked for 1 hour using 5% skim milk powder in TBS with 0.1% Tween 20. Membranes were incubated overnight at 4°C with antibodies against phosphorylated and native AMPKα (Thr172) (1:500, Cell Signaling Technology, Danvers, MA), mitogen activated protein kinase 44/42 (ERK1/2), p38,

(1:1500, ERK1/2 and 1:500, p38, Cell Signaling Technology, Danvers, MA), and angiotensin-II type I receptor (1:1000, AT_1, Santa Cruz Biotechnology, Santa Cruz, CA). β-actin antibody (1:50,000, Sigma-Aldrich Canada) was blotted for 1 hour at room temperature and used as a loading control. Amersham ECL Prime Western Blot detection reagent (GE Healthcare Life Sciences, Mississauga, ON) was used to visualize protein bands using an Alpha Innotech FluorChemQ imaging system.

7. Statistics

Data are expressed as mean ± SEM. Statistics are calculated using GraphPad Prism 7 (GraphPad Software Inc., CA, USA). Statistical analysis of data was performed by applying one-way or two-way analysis of variance (ANOVA) for repeated measures, followed by Bonferroni *post-hoc* test for multiple comparisons. Statistical significance was accepted with a p value < 0.05.

CHAPTER 3. RESULTS

1. Blood pressure, weight and treatment groups

Systolic BP was significantly elevated in SHHF rats compared to SD throughout the 8 week treatment period (Figure 10A; $p<0.01$) and remained significantly raised at the end of treatment (195.7±2.9 mm Hg vs. SD 142.6±7.1 mm Hg; $p<0.01$; Figure 10B and Table 6). Stilbenoids did not have a significant BP lowering effect in the SHHF rat. However, it should be noted that compared to the SBP of hypertensive controls (SHHF-C 195.7±2.9 mm Hg, Table 6) there was an approximate drop of 10 mm Hg in both resveratrol- and pterostilbene-treated SHHF animals (SHHF-R 184.9±6.4 mm Hg and SHHF-P 186.2±3.9 mm Hg, Table 6). At the end of the treatment period SHHF rats weighed significantly less than SD (374.7±9.8 vs. SD 564.3±14.5 g; $p<0.01$; Figure 11 and Table 6).

Each treatment group began with 8 rats for a total of 64 rats (32 SD, and 32 SHHF). However, mortality during the eight week treatment period lowered total animals to 55 (Table 4). Furthermore, mortality did not demonstrate any pattern and was random in nature.

Table 4. *In vivo* stilbene study treatment groups.

	control	resveratrol	pterostilbene	gnetol
SD (normotensive)	8	8	7	8
SHHF (hypertensive)	6	6	6	6

Total n=55. SD, Sprague Dawley; SHHF, spontaneously hypertensive heart failure rat.

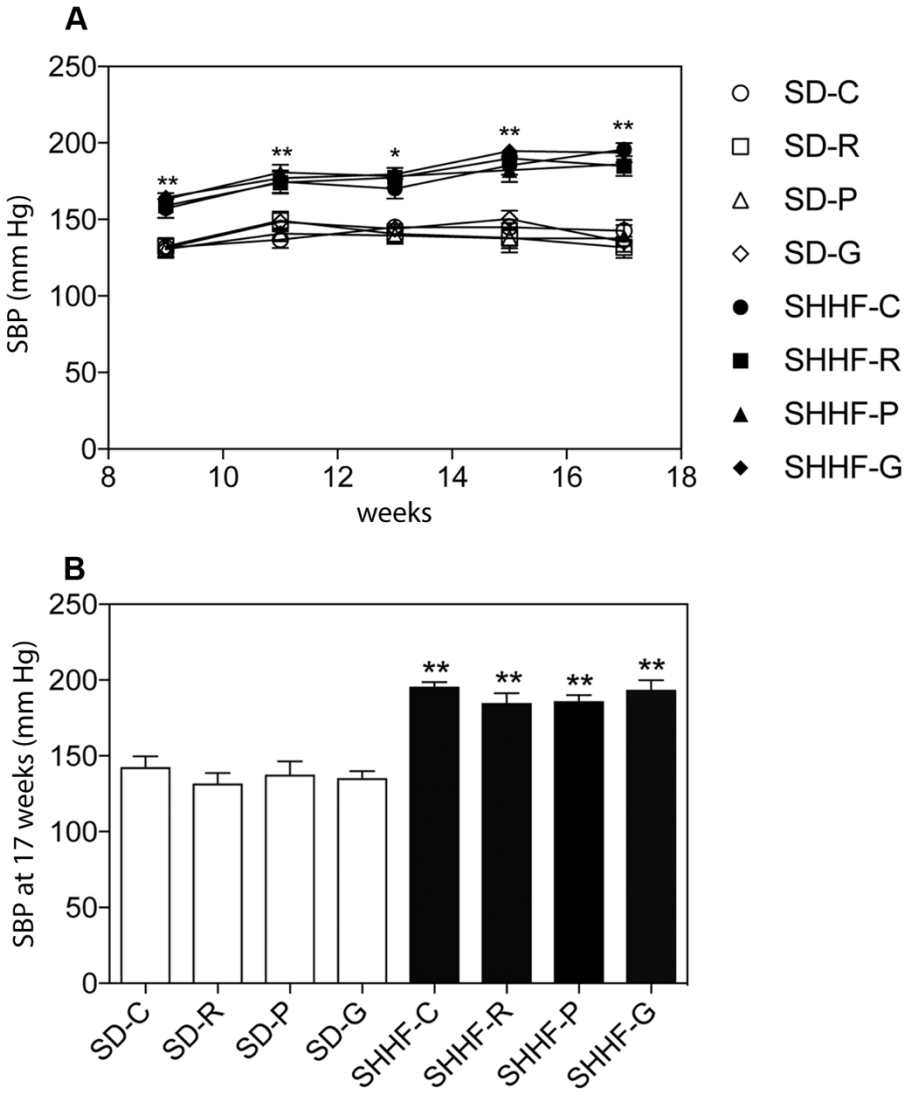


Figure 10. Systolic blood pressure. Blood pressure was measured using tail cuff plethysmography **(a)** biweekly over the 8 week treatment period and for **(b)** endpoint blood pressure of SD and SHHF rats at 17 weeks of age. Blood pressure was significantly higher in SHHF compared to SD animals. (n=5-8). $^{*}p<0.05$ and $^{**}p<0.01$ vs untreated SD.

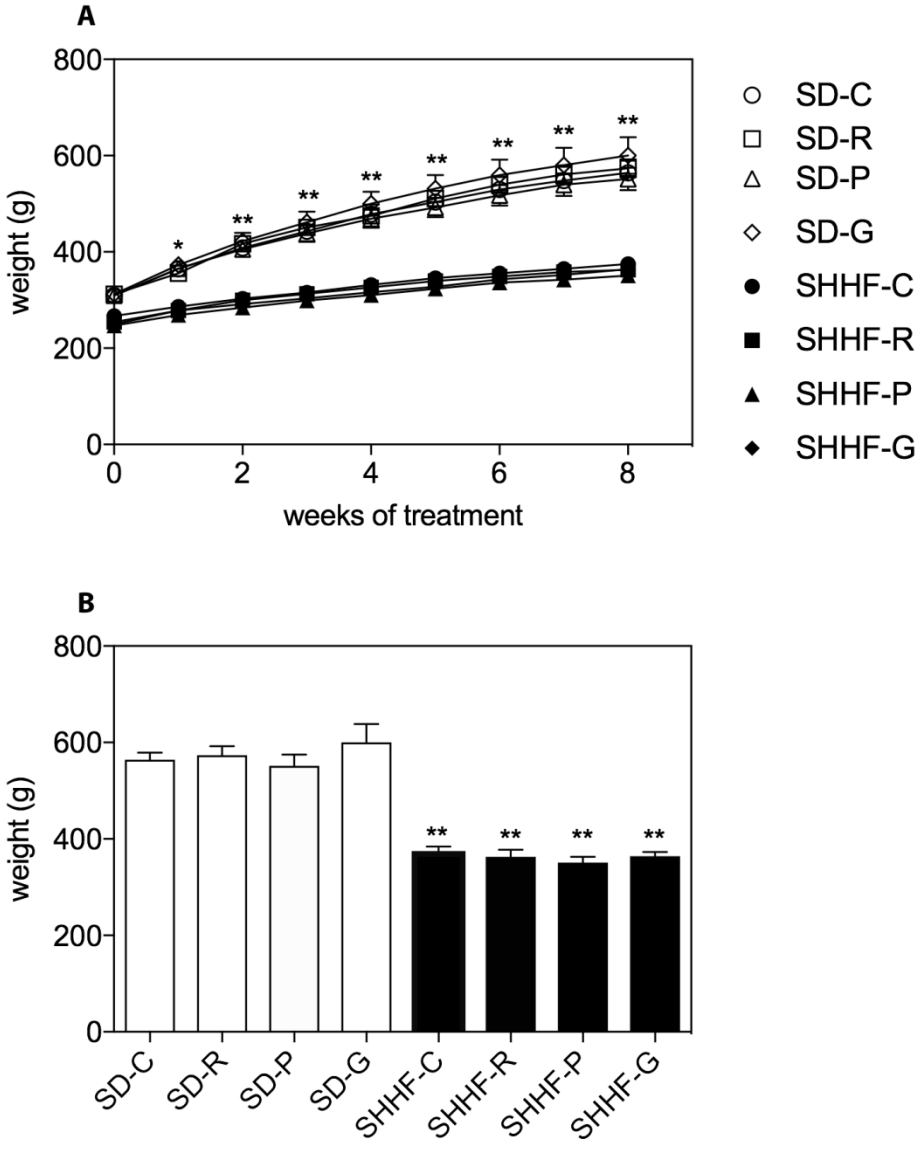


Figure 11. Weight **(a)** over the 8 week treatment period and at **(b)** endpoint (17 weeks) was significantly greater in SD compared to SHHF. (n=5-8). $^{*}p<0.05$ and $^{**}p<0.01$ vs untreated SD.

2. Vascular geometry

The lumen diameter of SHHF arteries was significantly reduced compared to normotensive controls (200.2±5.1 μm vs. SD 317.6±9.5 μm; $p<0.01$; Figure 12A and Table 6). Furthermore, the media thickness of the hypertensive control was increased compared to normotensive control (35.0±2.7 μm vs. SD 28.1±1.5 μm; $p<0.01$; Figure 12B and Table 6). However, stilbene-treated hypertensive arteries did not exhibit a significant increase in media thickening compared to SD arteries (not significant; Table 6). Increased M:L was observed in SHHF control arteries ($p<0.01$ vs. SD-C; Figure 13A). However, 8 week stilbenoid treatment significantly attenuated increased M:L in SHHF arteries ($p<0.01$ vs. SHHF-C; Figure 13A). All three stilbenoids (resveratrol, pterostilbene and gnetol), elicited comparable reduction in M:L within SHHF arteries. Media CSA was similar between animal models and was unaffected by stilbene treatment (not significant; Figure 13B).

The remodeling index of the untreated SHHF mesenteric arteries was 97.4% with a growth index of 3.9% (Table 5). Therefore, eutrophic remodeling was the predominant process in SHHF arteries, whereas hypertrophic growth was minimal.

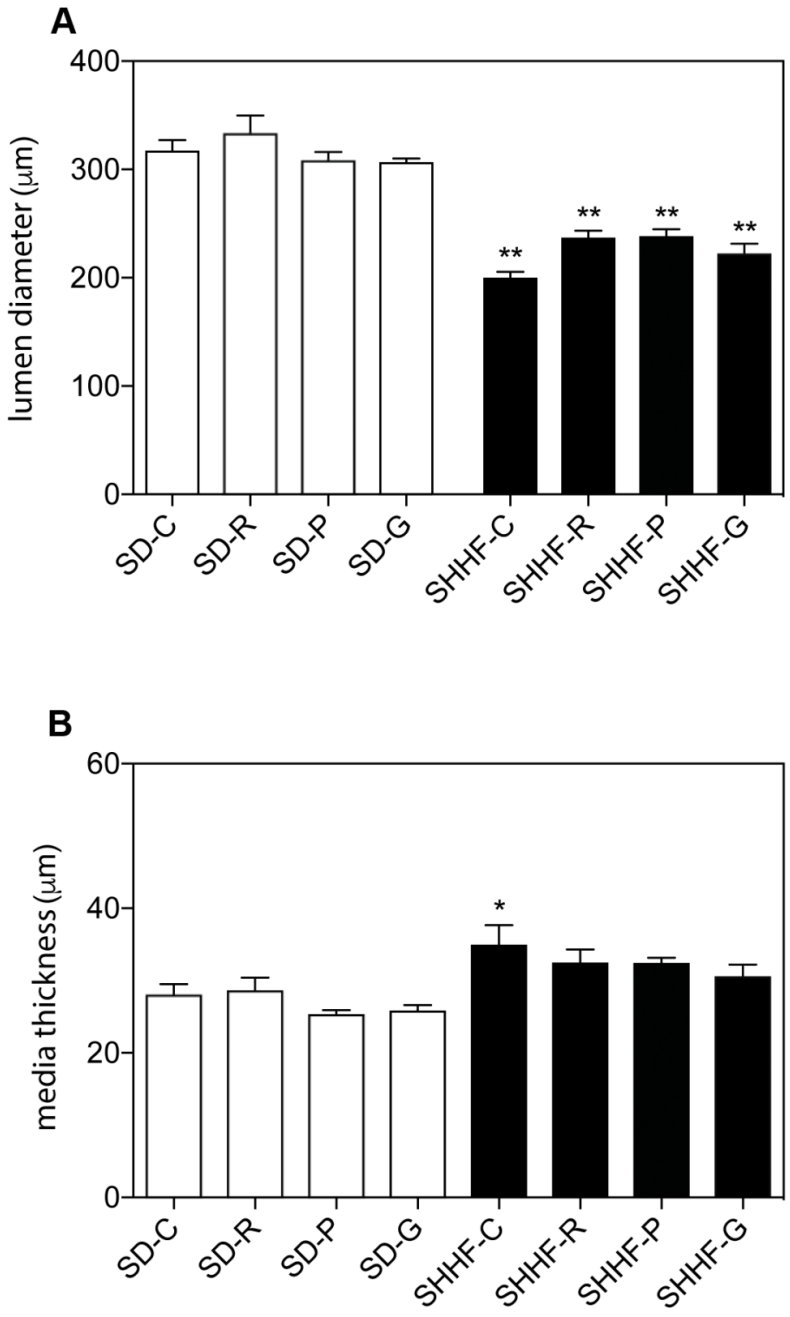


Figure 12. Effect of stilbenoids on lumen diameter and media thickness of SD and SHHF mesenteric arteries. **(a)** Lumen diameter of all SHHF resistance arteries were significantly smaller then SD arteries. However, SHHF arteries treated with stilbenoids did not have increased **(b)** media thickness, while SHHF-C media thickness was significantly greater relative to SD. (n=5-8). $^{*}p<0.05$ and $^{**}p<0.01$ vs. untreated SD.

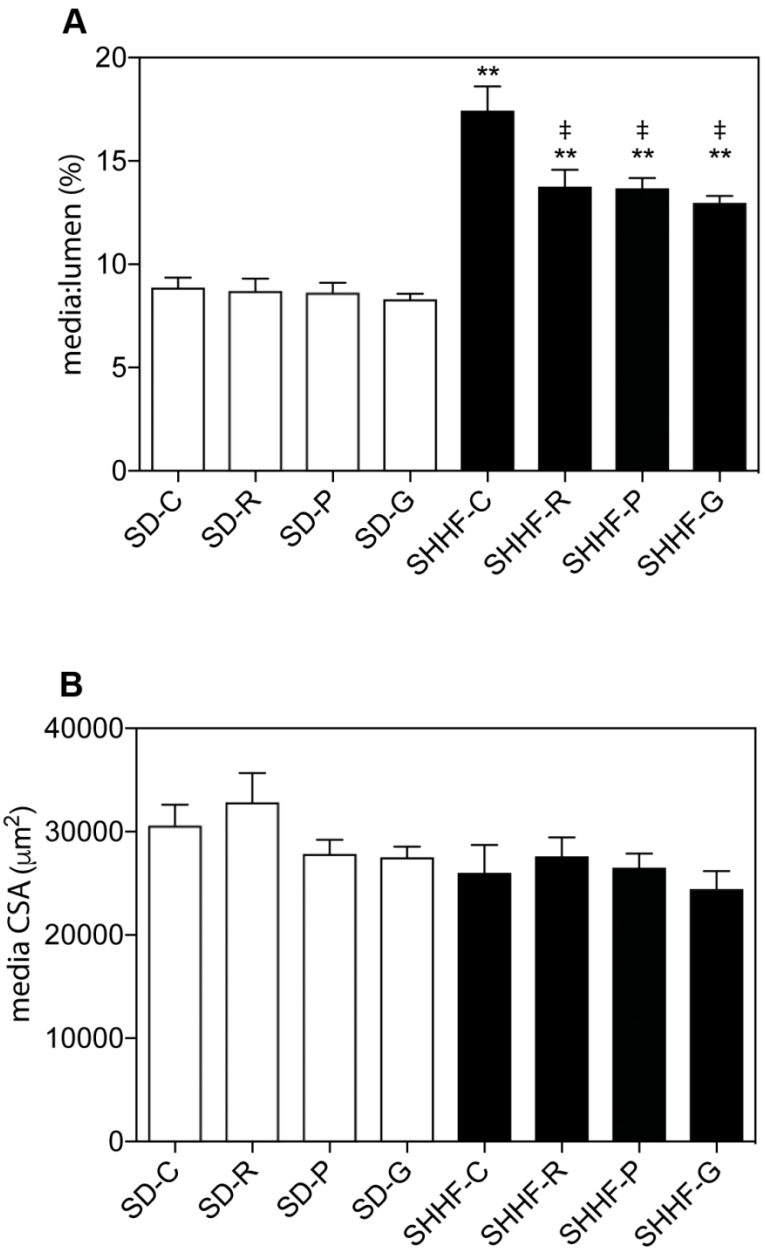


Figure 13. Effect of stilbenoids on vascular geometry. **(a)** Media:lumen was attenuated by 8 week treatment of resveratrol, pterostilbene, and gnetol. No change in **(b)** media CSA was observed between SD and SHHF animals. (n=5-8). $^{*}p<0.05$ and $^{**}p<0.01$ vs. untreated SD. ‡$p<0.01$ vs. untreated SHHF (Lee *et al*., 2017).

Table 5. Growth and remodeling indices in 17 week old SHHF *vs*. SD arterial segments

	control	**resveratrol**	**pterostilbene**	**gnetol**
growth index	3.9%	5.6%	19.4%	14.0%
remodeling index	97.4%	96.2%	79.9%	90.2%

Normalized for body weight $(\text{SHHF BW/SD BW})^{0.05}$ (Lee *et al.*, 2017).

3. Vascular mechanics

3.1 Vascular compliance

The tension in the arterial wall of SHHF was lower than SD arteries at high pressures. SHHF arteries experienced decreased isobaric stress (Figure 14) and strain (Figure 15). When media stress is examined as a function of media strain (i.e. under isometric conditions), vascular compliance is reflected. A leftward shift of the stress *vs*. strain curve indicates a decrease in compliance, or the ability of the artery to buffer changes in pressure. The compliance curve represents geometry and arterial wall components such as elastin and smooth muscle, and less distensible wall components, like collagen and the basement membrane. The SHHF arteries exhibited a leftward shift of the stress vs. strain curve (Figure 16), which indicates a potential decrease in compliance and ability of the artery to buffer changes in pressure. However, when quantified using area under the curve (AUC) there is no significant change between SD and SHHF arteries (Figure 14B). However, there is a trend towards ($p=0.07$) towards reduced compliance in SHHF-C arteries.

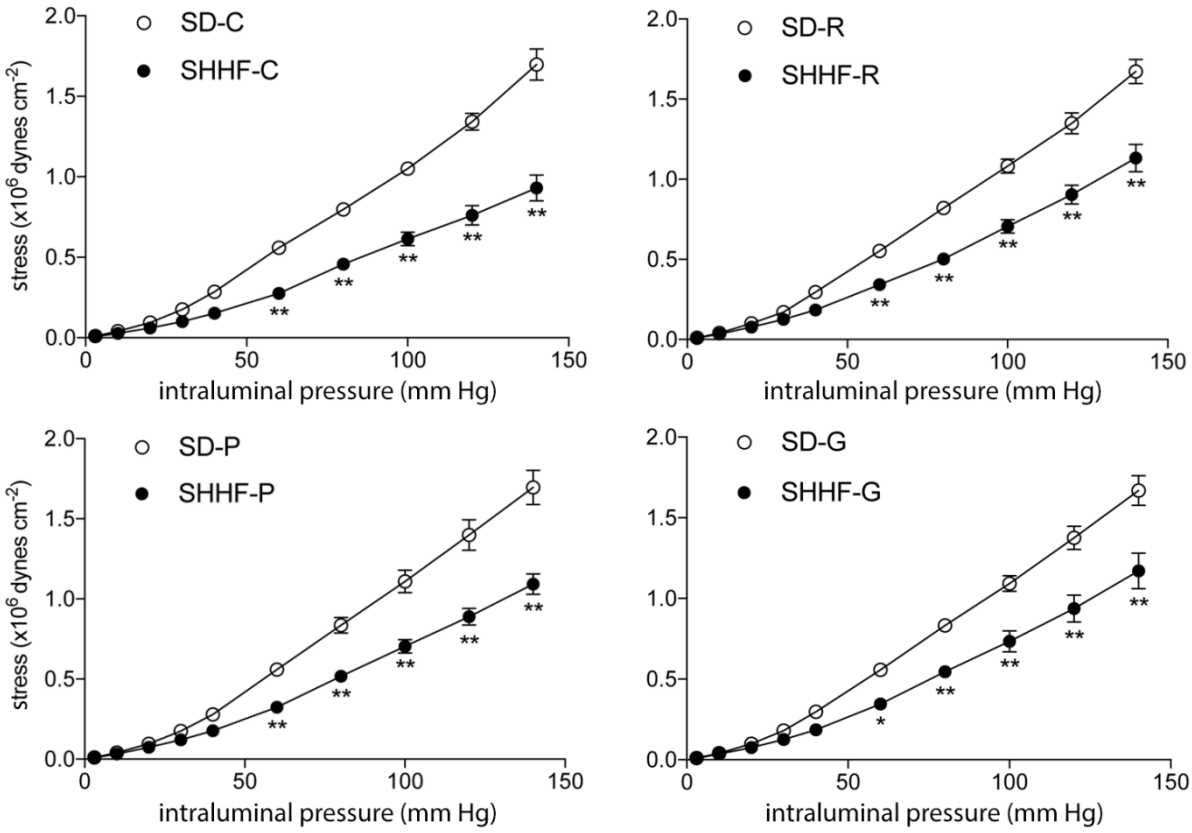


Figure 14. Effect of stilbenoids on isobaric stress. Stilbenoid-treated SHHF arteries experienced less at high pressures compared to SD. Structural changes that occurred in SHHF arteries were beneficial and resulted in protection against pressure-induced tension, these compensatory changes serve to protect the artery at extreme pressures. (n=5-8). $^{*}p<0.05$ and $^{**}p<0.01$ vs. untreated SD. (Lee *et al.*, 2017) **Note:** *The above figure is split into panels for visual clarity, statistical analyses were on all data.*

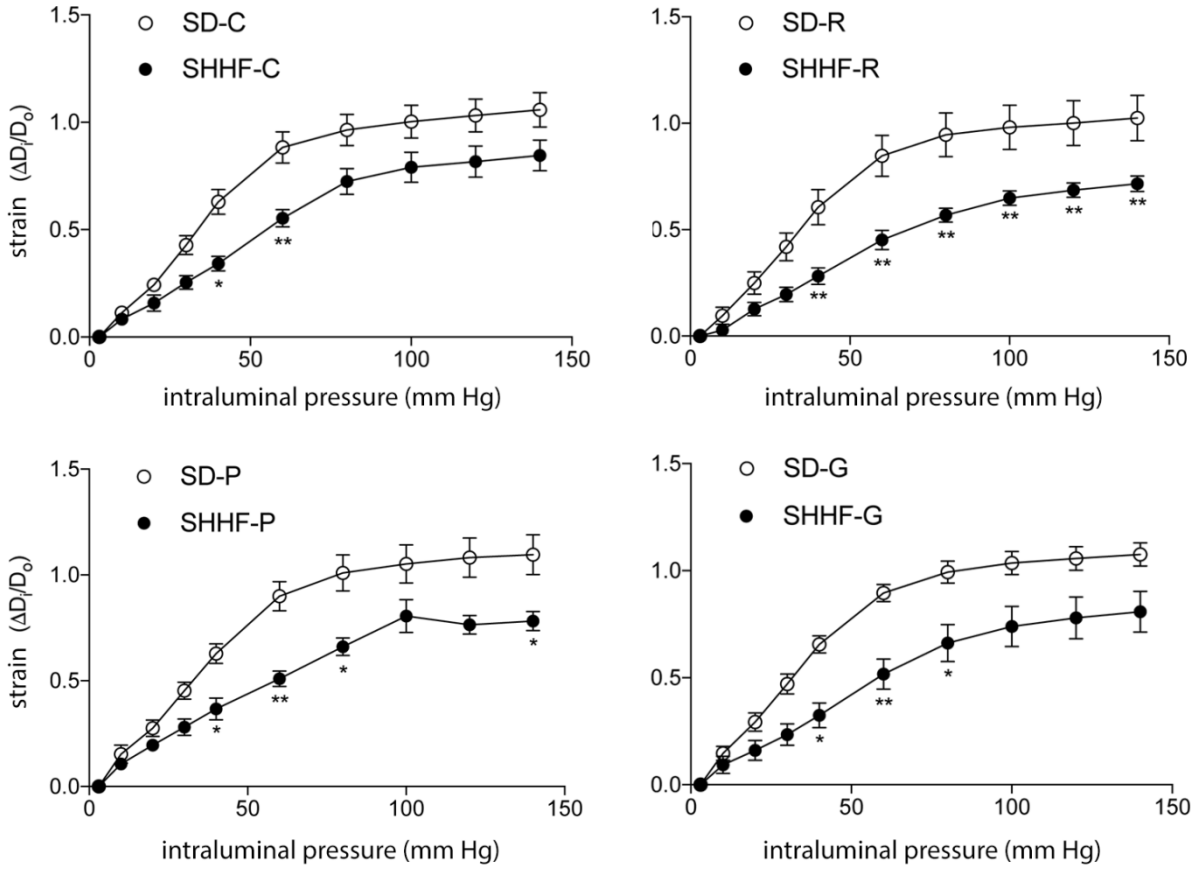


Figure 15. Effect of stilbenoids on isobaric strain. Stilbenoid-treated SHHF arteries experienced less strain at high pressures compared to SD. Geometric changes that occurred in SHHF arteries were beneficial and resulted in protection against pressure-induced tension. Compensatory changes serve to protect the artery at extreme pressures. (n=5-8). $^{*}p<0.05$ and $^{**}p<0.01$ vs. untreated SD. (Lee *et al.*, 2017) **Note:** *The above figure is split into panels for visual clarity, statistical analyses were on all data.*

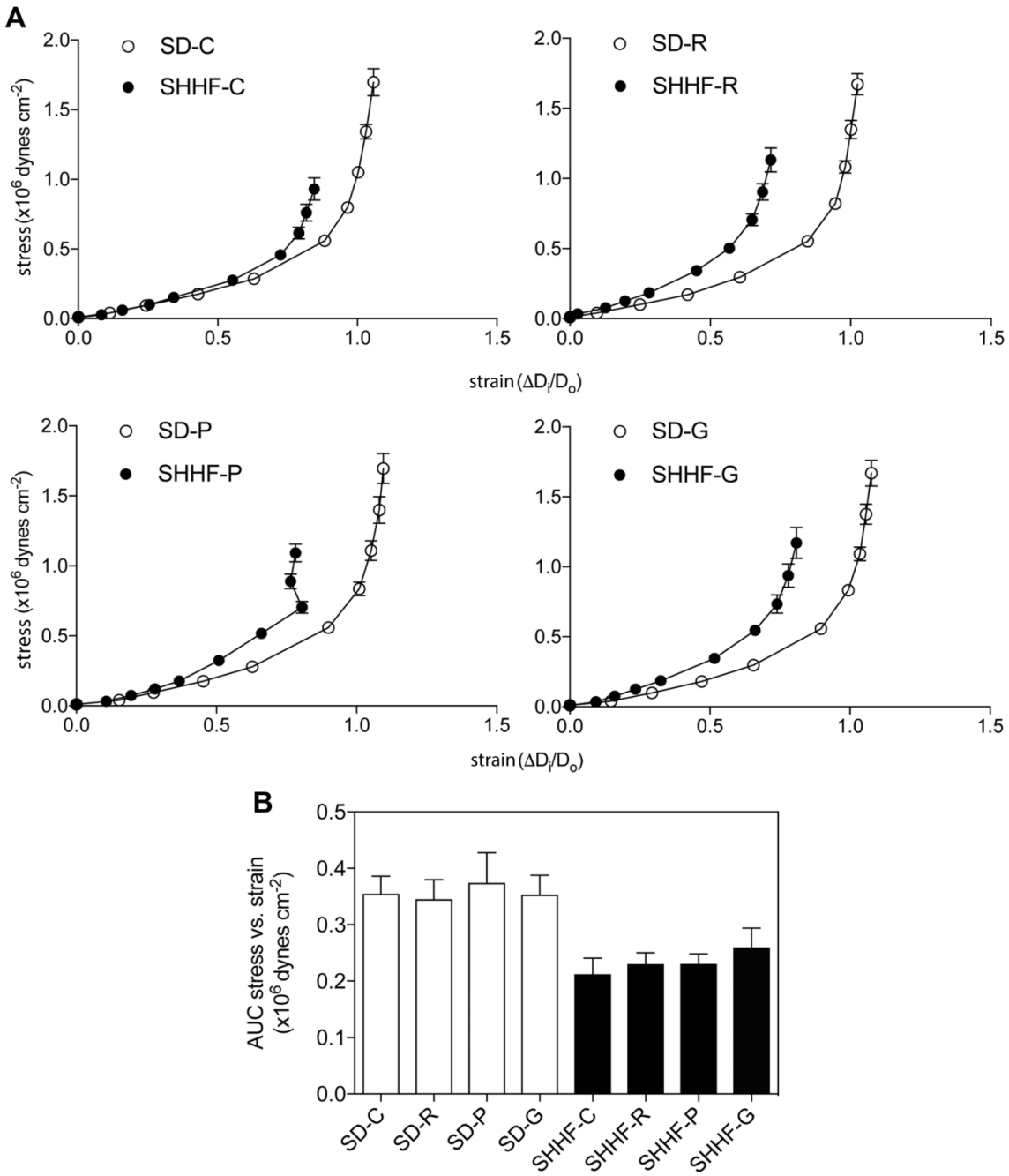


Figure 16. Effect of stilbenoids on vascular compliance. **(a)** The stress vs. strain curve appeared to be shifted to the left in SHHF arteries, but when quantified using **(b)** AUC of stress vs. strain was not statistically significant. However, there is a trend towards reduced compliance (p=0.07, SD-C vs. SHHF-C). (n=5-8). (Lee *et al*., 2017) **Note:** *The above figure is split into panels for visual clarity, statistical analyses were on all data.*

3.2 Wall component stiffness

Isobaric elastic modulus is determined through two factors; wall component stiffness and vessel geometry. Thus, when elastic modulus is plotted against stress, geometry is mathematically removed as a contributor allowing the plot to provide information solely regarding stiffness. The elastic modulus *vs*. stress curve specifically provides information regarding the stiffness of the wall components; i.e. connective tissue, elastin, collagen, smooth muscle and endothelial cells, which is depicted through the slope. There is a slight leftward shift in the elastic modulus vs. stress curve (Figure 17A). However, the slope of the elastic modulus *vs*. stress indicates similar stiffness between animal models and no effect with stilbenoid treatment (not significant; Figure 17B). Therefore, compliance changes in SHHF arteries do not occur through altering the physical elements such as collagen and elastin, and appear to occur predominantly through geometric changes in the artery.

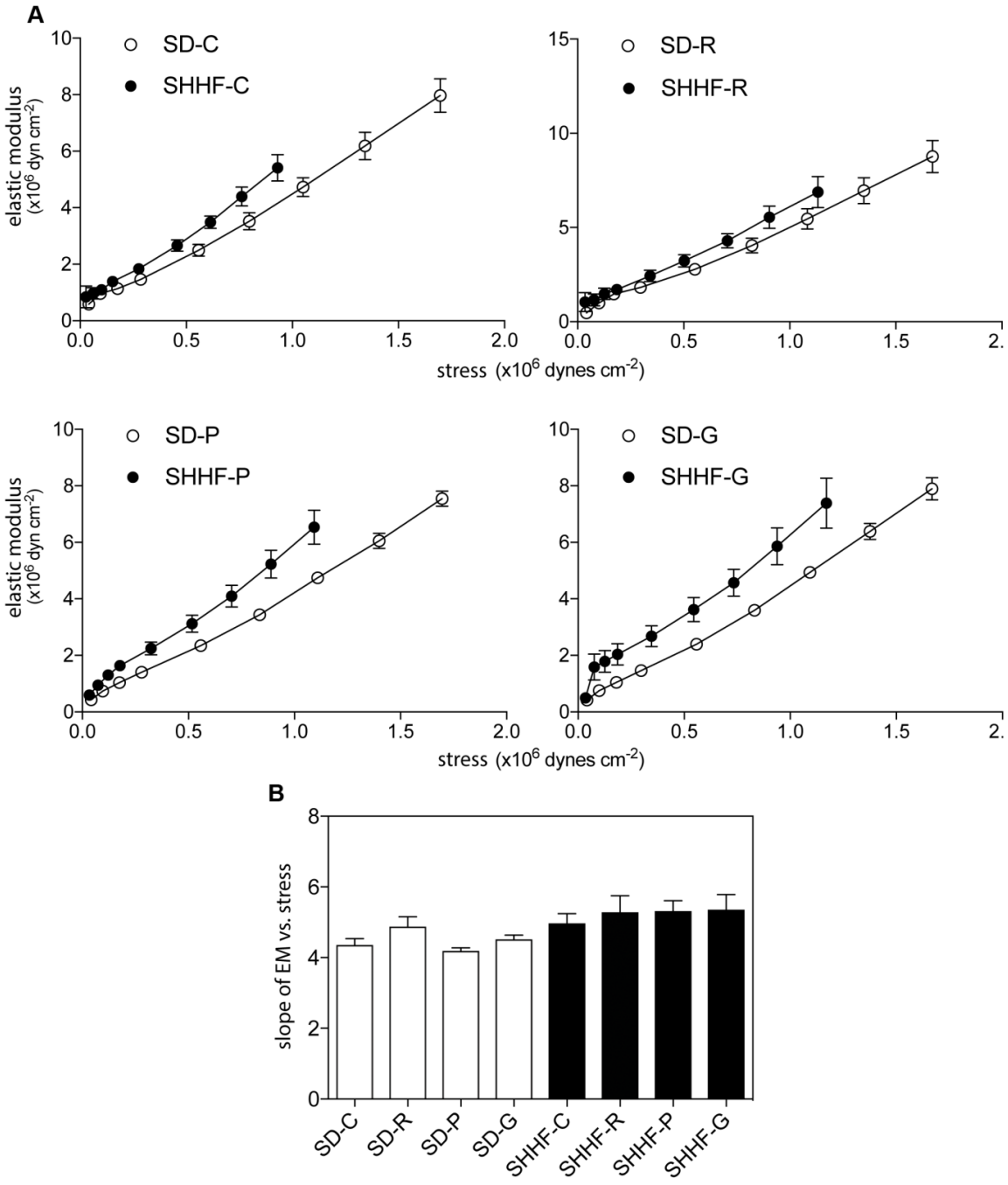


Figure 17. Effect of stilbenoids on vascular wall component stiffness. **(a)** The elastic modulus vs. stress curve of SHHF arteries is not statistically different then SD. This is replicated by **(b)** slope of the EM vs. stress. Wall component stiffness is therefore similar between SD and SHHF arteries with or without stilbenoid treatment. (n=5-8). (Lee *et al*., 2017) **Note:** *The above figure is split into panels for visual clarity, statistical analyses were on all data.*

4. Signaling effectors

4.1 ERK1/2, AMPKα, AT_1 and p38

Four signaling effectors were examined in the mesenteric arteries of SD and SHHF rats: ERK1/2, AMPKα, AT_1 and p38 MAPK. ERK1/2 activation was similar in SHHF rats compared to SD and stilbenoids did not influence arterial ERK1/2 activation (Figure 18). The activation status of AMPK was measured via phosphorylation at Thr172 of AMPKα. SHHF arteries had a significant increase in phosphorylated AMPKα compared to SD arteries (Figure 19A). AMPKα phosphorylation was attenuated in the SHHF mesenteric arteries with gnetol ($p<0.05$, Figure 19A) while resveratrol and pterostilbene had no effect. No detectable difference was determined in angiotensin receptor AT_1 between SD and SHHF or between stilbene groups (Figure 20). No differences in phosphorylated and native p38 were significant in mesenteric small arteries of SHHF (Figure 21).

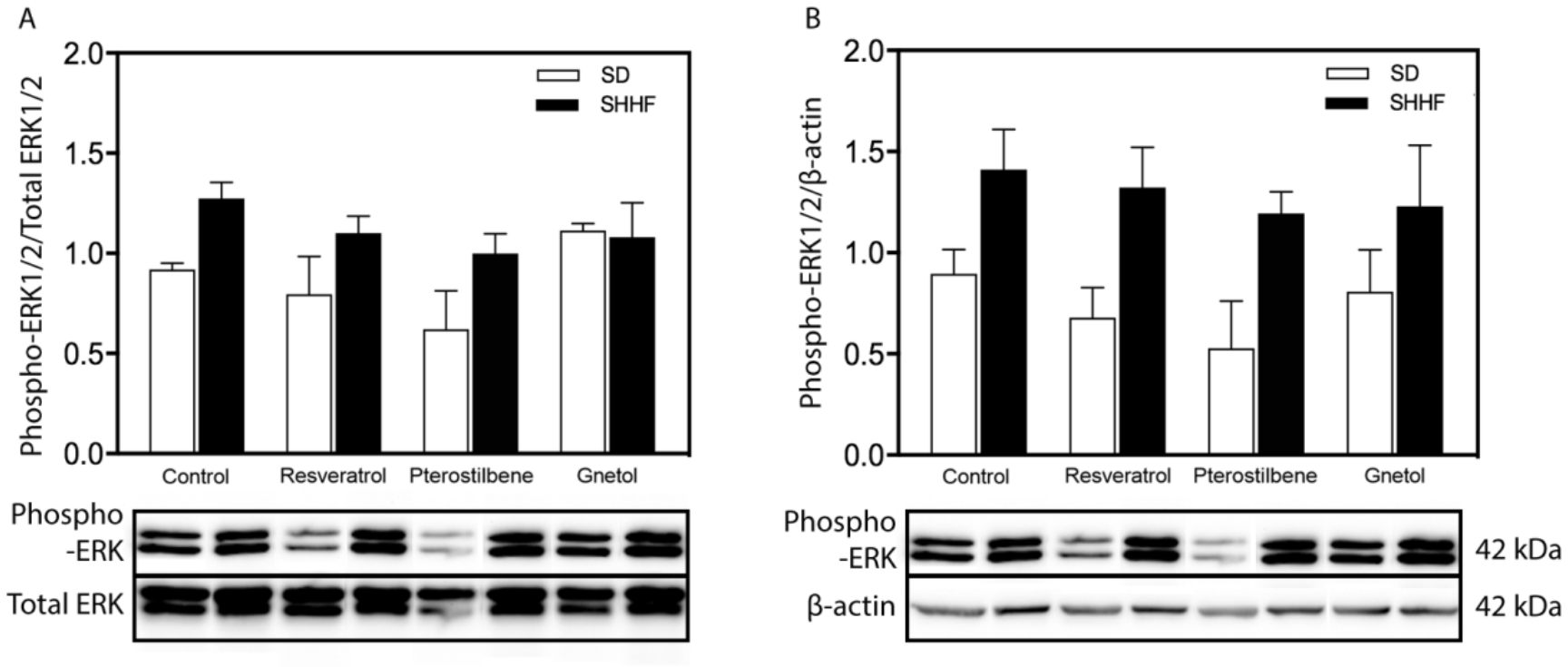


Figure 18. Mesenteric artery signaling - ERK1/2. Effect of stilbenoids on ERK1/2 in mesenteric arteries normalized to **(a)** total ERK and **(b)** β-actin. Stilbenoids did not influence arterial ERK activation, and ERK activation was similar in SHHF rats compared to SD. (n=3-4). (Lee *et al*., 2017)

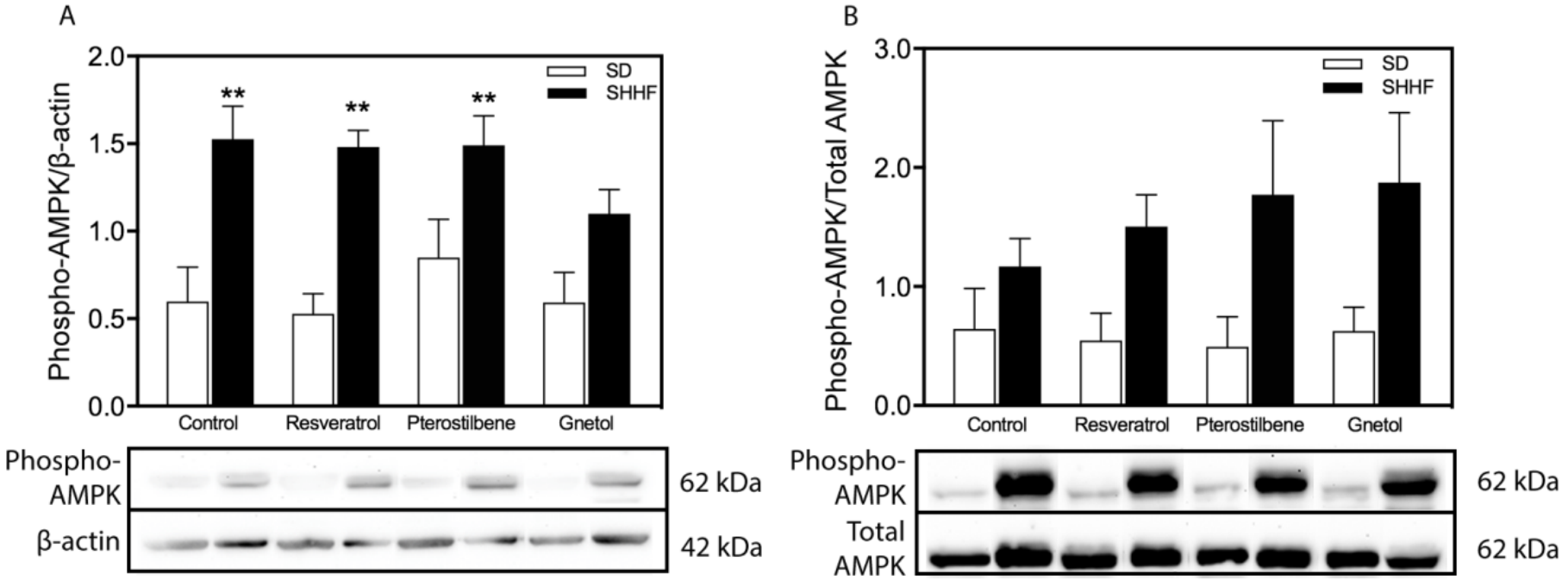


Figure 19. Mesenteric artery signaling - AMPKα. Effect of stilbenoids on AMPKα activation in mesenteric arteries normalized to **(a)** β-actin and **(b)** total AMPK. AMPKα activation through phosphorylation of Thr172 was detected in SHHF arteries, and attenuated by gnetol. (n=3-4). $^{**}p<0.01$ vs. untreated SD. (Lee *et al*., 2017)

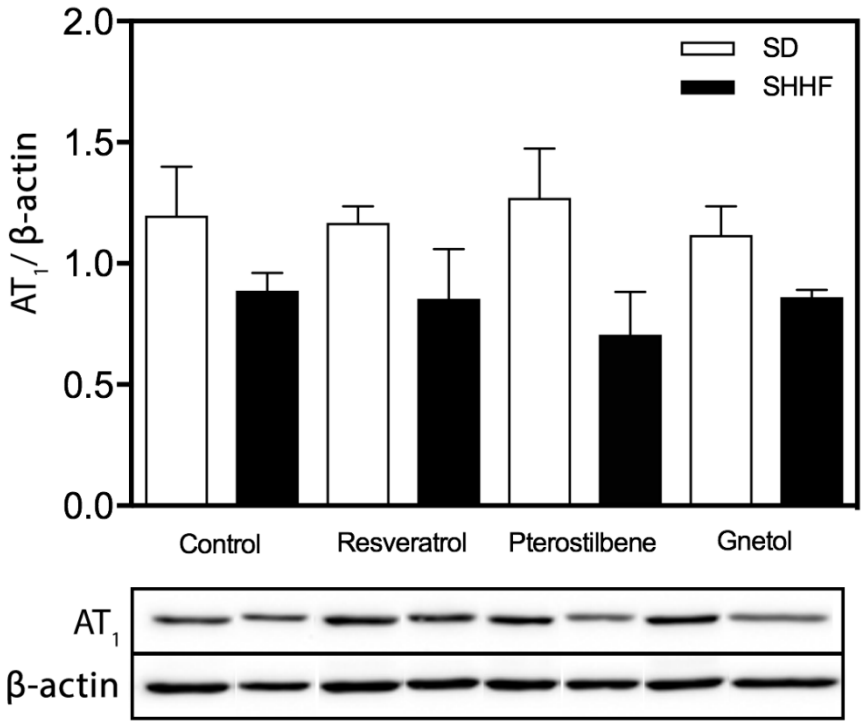


Figure 20. Mesenteric artery signaling – AT_1. Effect of stilbenoids on angiotensin receptor AT_1 activation in mesenteric arteries. Angiotensin receptor AT_1 was similar between SD and SHHF arteries, with or without stilbenoid treatment. (n=3-4).

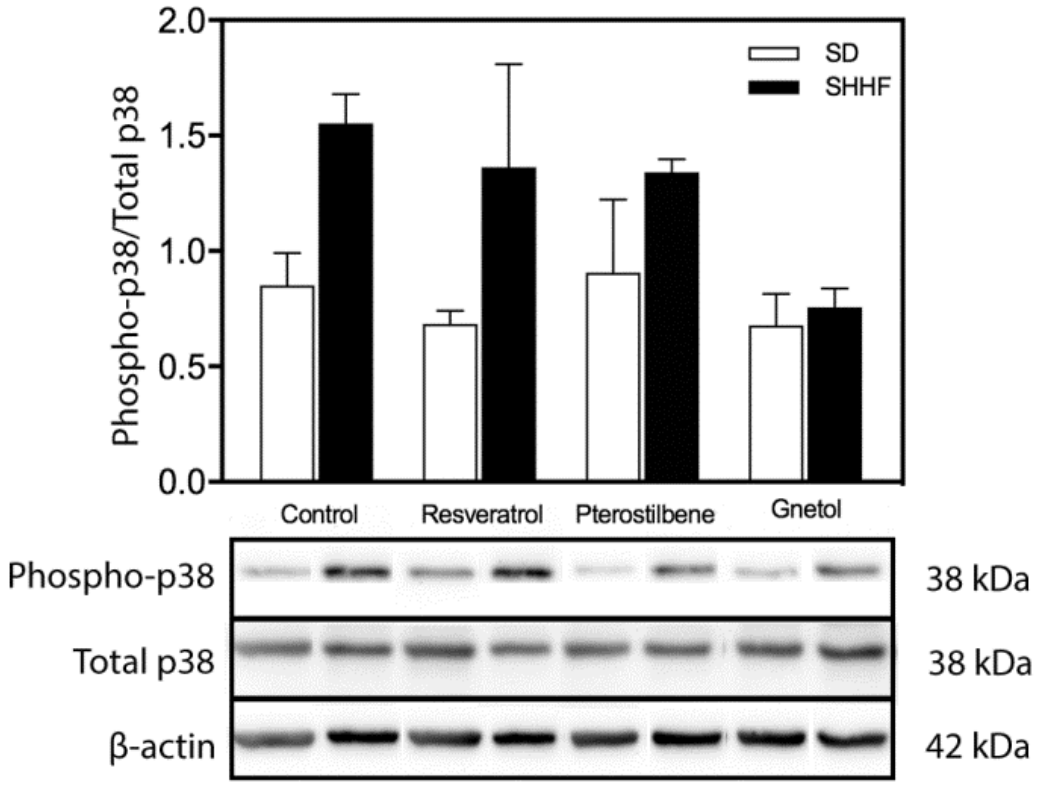


Figure 21. Mesenteric artery signaling – p38. Effect of stilbenoids on p38 MAPK mesenteric arteries normalized to total p38. Stilbenoids did not influence arterial p38 activation. (n=3-4). (Lee *et al*., 2017)

Table 6. Blood pressure and geometry of mesenteric arteries in SD and SHHF rats at 17 weeks of age.

parameter	SD				SHHF			
	C	R	P	G	C	R	P	G
body weight (9 wk old), g	309.6±5.7	312.53±8.5	309.4±7.3	312.3±7.7	267.1±10.5**	254.0±10.3**	246.7±7.2**	250.0±6.4**
body weight, g	564.3±14.5	573.5±18.8	551.5±23.6	600.0±38.1	374.7±9.8**	363.1±14.4**	351.1±12.1**	364.1±8.6**
SBP, mm Hg	142.6±7.1	131.7±6.9	137.5±8.9	135.3±4.5	195.7±2.9 **	184.9±6.4**	186.2±3.9**	193.6±6.3**
baseline lumen diameter (3 mm Hg), µm	186.5±9.6	203.6±17.7	179.0±7.4	178.9±4.6	143.5±5.4	173.6±6.3	173.6±1.8	164.8±13.6
lumen diameter, µm	317.6±9.5	333.5±16.3	308.3±8.0	306.9±3.4	200.2±5.1**	236.9±6.3**	238.4±6.4**	222.6±9.0**
media thickness, µm	28.1±1.5	28.7±1.7	25.4±0.6	25.9±0.7	35.0±2.7*	32.5±1.8	32.5±0.7	30.6±1.6
media:lumen, %	8.9±0.5	8.7±0.6	8.6±0.5	8.3±0.3	17.4±1.2**‡	13.8±0.8**‡	13.7±0.5**‡	13.0±0.3**‡
media CSA, μm^2	30567±2054	32859 ± 2821	27842±1365	27525±1019	26017±2709	27604±1829	26514±1382	24441±1740
slope of EM vs. stress	4.4±0.2	4.9±0.3	4.2±0.1	4.5±0.1	5.0±0.3	5.3±0.4	5.3±0.3	5.4±0.4

Data represent mean ± SEM. Arterial parameters are measured in relaxed mesenteric arteries at intravascular pressure of 45 mm Hg. n = 5-8. *$p<0.05$, **$p<0.01$ vs. untreated SD and ‡$p<0.01$ vs. untreated SHHF. SD, Sprague -Dawley; SHHF, spontaneously hypertensive heart failure; SBP, systolic blood pressure; EM, elastic modulus. C, control; R, resveratrol; P, pterostilbene; G, gnetol.

CHAPTER 4. DISCUSSION

1. Discussion

Total peripheral resistance is a major component of BP regulation (Figure 1). However, this relationship is multi-directional; BP is a vital part of TPR. As blood returns to the heart there is a 50-70% decline in mean arterial pressure within the small arteries (<400 μm), demonstrating the influence these vessels have in regulating TPR (Digne-Malcolm *et al*., 2016; Hanson *et al*., 2016; Kato & Pinsky, 2015). Vascular remodeling of small arteries is related to TPR alterations through Poiseuille's law (Figure 2). This equation demonstrates that minor changes to the vessel diameter will result in exponential changes to the resistance of blood flow. Media thickening that occurs in hypertensive arteries can encroach upon the lumen narrowing the area of blood flow. The Law of Laplace (P=s/rm) describes how media (m) is inversely related to pressure (P), while stress (s) is directly related to pressure (P) and inversely related to radius (r) (Ibrahim & Berk, 2009). Therefore, as BP increases in hypertensive individuals the vessel wall thickens and counterbalances increases in arterial tension. SHHF small arteries had significantly narrower lumens which is a key characteristic that influences eutrophic remodeling. Eutrophic remodeling, and more specifically inward remodeling, is observed in human essential hypertension (Mulvany, 1999).

Stilbene treatment positively affected the geometry of the SHHF resistance arteries at 17 weeks of age. Of the three stilbenes studied, both resveratrol and pterostilbene partially attenuated lumen narrowing, while all three attenuated the M:L increase in hypertensive arteries. Stilbenes had null effects in SD animals. In contrast to my hypothesis that pterostilbene would have the greatest vasculoprotective effect, all three stilbenes lowered M:L to the same extent within hypertensive small arteries. Interestingly, gnetol with the lowest bioavailability of 6.59% showed similar *in vivo* effects as pterostilbene and resveratrol, with 80% and 20% oral

bioavailability, respectively (Kapetanovic *et al*., 2011; McCormack & McFadden, 2012; Remsberg *et al*., 2015). Pterostilbene only lowered M:L 1% more than gnetol and resveratrol treated SHHF (Table 4). All three stilbene compounds demonstrated direct actions on the mesenteric resistance arteries and exerted equal vasculoprotective effects.

Arteries exposed to pterostilbene and resveratrol exhibited very similar remodeling overall, even though bioavailability of pterostilbene is reported to be four-fold greater. This may be a result of comparable chemical structures when metabolized via glucuronidation or sulfation. These processes result in indistinguishable metabolite structures for resveratrol and pterostilbene, while gnetol gives rise to an altered structure due to a fourth hydroxy group on the phenol rings. Studies investigating resveratrol and metabolites showed pleiotropic actions and different biological signaling mediators between metabolite compounds (Calamini *et al*., 2010; Smoliga & Blanchard, 2014). Calimini *et al*. speculated that resveratrol metabolites may have similar or greater physiological activity than the parent compound (Calamini *et al*., 2010). Remsberg *et al*. noted gnetol and pterostilbene are eliminated via non-renal routes, which coincides with Marier *et al*. who reported similar findings for resveratrol (Marier *et al*., 2002; Remsberg *et al*., 2015; Remsberg *et al*., 2008). However, there is possible accumulation of stilbenes in the liver or GI tract (Remsberg *et al*., 2015). Therefore, further research on the bioavailability of stilbenoids in plasma, urine and arterial tissue is necessary, as current findings do not reflect the reported bioavailability from plasma or serum alone. Literature investigating pterostilbene and gnetol on small resistance artery remodeling is currently lacking, despite their structural similarities to resveratrol.

Low-dose stilbene treatment resulted in beneficial vascular remodeling but did not demonstrate significant BP lowering. Nonetheless, the observed changes in BP warrant

discussion. Lewington *et al*. reported two-fold differences in mortality from ischemic heart disease and vascular causes for every 20 mm Hg elevation in SBP in middle-aged populations (Lewington *et al*., 2002). We found premature mortality was approximately eight times higher when BP was elevated (Table 3). However, according to the INTERSTALT study, lowering SBP by 2 mm Hg reduces projected 16-year mortality from cardiovascular disease by 4.6%, while a 10 mm Hg reduction in SBP lowers cardiovascular disease mortality risk by 21.0% (Stamler, 1997). Anti-hypertensive effects of resveratrol and pterostilbene treatment resulted in a SBP decline of 11 mm Hg and 10 mm Hg, respectively (Table 4). Gnetol treatment had the lowest decline in hypertensive SBP with 2 mm Hg reduction. Accordingly, even minor SBP changes, may be sufficient to result in positive long term health benefits.

Previous studies utilizing low-dose resveratrol also showed beneficial vascular remodeling without lowered BP (Behbahani *et al*., 2010), although higher doses of resveratrol, fluctuating from 10 mg/kg/d to 50 mg/kg/d, have demonstrated BP lowering effects (Liu *et al*., 2005; Miatello *et al*., 2005). Long-term low-dose resveratrol treatment has also been investigated by Ahmet *et al*. and again, they did not demonstrate BP lowering (Ahmet *et al*., 2017). Long term dosing spanned 10 months compared to other resveratrol *in vivo* studies ranging from 4 to 10 weeks (Ahmet *et al*., 2017; Behbahani *et al*., 2010; Liu *et al*., 2005). Grape powder consisting of several polyphenols, including resveratrol, has also shown significant BP lowering effects in SHR (Thandapilly *et al*., 2012). Coordinated effects of polyphenols, and not specifically one factor (i.e. resveratrol), may enhance improvement of arterial parameters and BP lowering. Perhaps investigating the synergistic effect of resveratrol, pterostilbene and gnetol could lead to noteworthy findings.

Vascular remodeling that occurred in SHHF is indicative of eutrophic remodeling (Figure 3). There are several studies reporting the remodeling mechanism of the small resistance arteries of SHR, an animal model that specifically develops hypertension (Bakker *et al*., 2014). Bakker *et al*. reported that sublines of SHR differ in several parameters including BP, structure, mechanical properties and endothelial function (Bakker *et al*., 2014). The SHR does not consistently show a decrease in lumen diameter along with media thickening, and therefore does not reliably mimic human essential hypertension through eutrophic inward remodeling (Bakker *et al*., 2014). The SHR model at 20 weeks experiences a combination of hypertrophic and eutrophic remodeling (Bakker *et al*., 2014; Intengan, Thibault*, et al*., 1999). However, the SHHF rat predominately demonstrates eutrophic remodeling, reinforced by a growth index of 3.9% and a remodeling index of 97.4% (Table 3). The structural changes that occurred in the SHHF arteries likely protect the artery against pressure-induced tension.

SHHF and SHR do not only show variance in structural properties, but also demonstrate mechanical differences. The hypertensive resistance arteries experienced less stress and strain at high intraluminal pressures. This is likely due to compensatory mechanisms of the SHHF arteries, which serve to protect the artery in the face of high BP. However, structural changes that occurred in the SHHF arteries did not improve vascular compliance, as demonstrated by the AUC of stress vs. strain (Figure 16). This finding is model specific, SHR mesenteric arteries demonstrate increased compliance (Behbahani *et al*., 2010). Another contrast between SHR and SHHF small arteries is wall component stiffness. While mesenteric resistance arteries in SHR exhibit reduced stiffness, SHHF mesenteric arteries demonstrate unaltered wall component stiffness (Behbahani *et al*., 2010). Vascular changes in SHR and SHHF occur through alterations

in vessel wall composition. Accordingly, the SHHF animal model may be more suitable then SHR to model cardiovascular disease progression (Heyen *et al*., 2002).

Mild human essential hypertension presents with eutrophic remodeling and increased M:L, but is also characterized by increased compliance within the small resistance arteries (Intengan, Deng, *et al*., 1999). Vascular compliance is determined by geometry, and is the ability of the artery to buffer changes in pressure. In contrast to the structural changes indicating that the SHHF models human essential hypertension, the mechanical properties (lack of increased compliance) do not coincide with this finding. In fact, not only do SHHF small resistance arteries show no change in stiffness, there is a trend ($p=0.07$) towards reduced compliance. SHHF rats are characterized initially by spontaneous hypertension at 4 months but further develop symptoms of congestive heart failure (CHF), including compensated left ventricular hypertrophy and cardiac remodeling (Heyen *et al*., 2002). Conceivably, the mechanical properties that are demonstrated by the SHHF small resistance arteries may resemble hypertension alongside progression to CHF. Small resistance arteries of SHHF demonstrate altered mechanical properties compared to SHR but also express regional, bed-specific differences within the model itself (Lee *et al*., 2017).

SHHF mesenteric resistance arteries and the middle cerebral arteries (MCA) yield altered vascular properties. Wall component stiffness of mesenteric small arteries remains unchanged in SHHF, while the MCA demonstrates increased stiffness (Izzard *et al*., 2006; Lee *et al*., 2017). However, both SHHF mesenteric arteries and MCA exhibit reduced vascular compliance, and similar geometrical changes, such as increased M:L (Lee *et al*., 2017). Vascular compliance in SHHF MCA showed a statistically significant decrease, likely occurring through a combination of increased vascular stiffness and changes is vascular geometry. However, SHHF mesenteric

arteries only exhibit geometrical changes and unchanged wall component stiffness, leading to merely a trend in reduced compliance.

SHHF MCA also exhibit both hypertrophic and eutrophic remodeling, with growth and remodeling indices 43.6% and 58%, respectively (Lee *et al*., 2017), whereas the SHHF mesenteric resistance arteries predominantly exhibit eutrophic remodeling. Unlike SHHF mesenteric arteries, the SHHF MCA experience similar remodeling profiles in both SHR and SHHF animals (Izzard *et al*., 2006; Lee *et al*., 2017).

There are several signaling mediators that are modified in the development of hypertension and through polyphenol treatment. ERK1/2 is a mitogen activated protein kinase that is an upstream regulator of angiotensin II induced cAMP response element binding (CREB) and nuclear factor-kappa B (NF-kB) (Sahar *et al*., 2007). Behbahani *et al*. concluded that phosphorylation of ERK1/2 increased in SHR mesenteric arteries, but resveratrol treatment attenuated ERK1/2 upregulation (Behbahani *et al*., 2010). Similar observations were made by Lopez-Sepulveda *et al*. using red wine polyphenols, which were found to inhibit ERK1/2 activation (Lopez-Sepulveda *et al*., 2011). Phosphorylated ERK1/2 signaling was not elevated in SHHF mesenteric arteries, and stilbenoids did not attenuate ERK1/2 signaling. Notably, this shows ERK1/2 signaling abnormalities is a difference between SHR and SHHF mesenteric resistance arteries.

Angiotensin II is a part of the RAAS and binds two receptors, angiotensin-II type-1 (AT_1) and angiotensin-II type-2 (AT_2) (de Gasparo *et al*., 2000). These receptors are major targets for BP lowering drugs. AT_2 receptors are more common in fetal development, but control BP and renal modifications as well, while AT_1 receptors mediate most of the known functions of angiotensin II (de Gasparo *et al*., 2000). In salt-sensitive models of hypertension renin

production within the kidney is affected, causing downstream changes of angiotensin II production and BP increase. *In vivo* studies with resveratrol treatment in C57/B6 mice show inhibitory effects against angiotensin II induced hypertension and production of IL-6 (Inanaga *et al*., 2009). In agreement with Behbahani *et al*. who demonstrated no change in AT_1 or AT_2 receptors in SHR small mesenteric arteries (Behbahani *et al*., 2010), there was also no upregulation or inhibition of AT_1 receptors within SHHF.

AMP-activated protein kinase, a regulator of vascular tone, is elevated in SHR mesenteric arteries (Ford *et al*., 2012). BP lowering effects through AMPK activation are thought to occur via vasorelaxation in small resistance arteries (Ford *et al*., 2012). SHHF mesenteric arteries exhibit exaggerated AMPK signaling which corresponds with SHR model findings (Ford *et al*., 2012). However, resveratrol and pterostilbene failed to attenuate AMPK upregulation in SHHF animals (Figure 21). Only gnetol showed partial attenuation of AMPK signaling. Therefore, it seems unlikely that AMPK is a major signaling mediator involved in stilbene vascular remodeling. Furthermore p38, a MAP kinase, is upregulated during chronic hypertension (Hanson *et al*., 2016). Hanson *et al*. showed amplified phosphorylation of p38 in SHR aorta (Hanson *et al*., 2016). Alterations in phosphorylation of p38 between SD and SHHF stilbene treated mesenteric resistance arteries was not evident (Figure 23).

Although no specific signaling mediator was detected that demonstrated altered effects due to stilbenoid treatment there are other effectors that could potentially be involved. NF-kB is downregulated through the action of resveratrol (Manna *et al*., 2000; Tome-Carneiro *et al*., 2012). However, the mode of action in which resveratrol inhibits NF-kB remains to be concluded. NF-kB inhibitors, such as curcumin, act on IKBα through phosphorylation and degradation of the protein (Singh & Aggarwal, 1995). Resveratrol did not affect IKBα as found

by Manna *et al*. (Manna *et al*., 2000). Caffeic acid phenethyl ester inhibits NF-kB by altering the DNA binding site. However, there was no effect on the NF-kB DNA binding domain by resveratrol (Manna *et al*., 2000). Manna *et al*. therefore reported that resveratrol inhibition of NF-kB may occur through inhibition of TNF-induced translocation, NF-kB p65 subunit, and reporter gene transcription (Manna *et al*., 2000). Inhibition of TNF-α, a pro-inflammatory cytokine, activated the NF-kB pathway in CVD primary prevention (Tome-Carneiro *et al*., 2012). However, the pathway for the downregulation of TNF-α and subsequent NF-kB activation was not concluded. However, it is improbable that NF-kB signaling is responsible for stilbene remodeling as ERK1/2 was not affected.

Another signaling mediator that could play a potential role is eNOS. Under physiological conditions, eNOS produces NO, which is a key molecule in the endothelium for vasoprotection (Li & Forstermann, 2000). When the arterial endothelium experiences oxidative stress the production of NO is rapidly inhibited by the increasing concentration of superoxide radicals (Li *et al*., 2014). Bhatt *et al*. examined eNOS in SHR aortic homogenates using the NOS substrate L-arginine methyl ester (L-NAME) (Bhatt *et al*., 2011). Incubation with L-NAME caused an increase in superoxide generation in SHR but not WKY. When exposed to L-NNA, an eNOS inhibitor, superoxide levels in SHR were re-established to WKY levels (Bhatt *et al*., 2011). Resveratrol treatment had the same effect on SHR as L-NNA, indicating that superoxides were developed through eNOS. However, BH4 addition abolished superoxide development in SHR, resveratrol had equivalent effects on superoxides (Bhatt *et al*., 2011). Bhatt *et al*. report that SHR treated with resveratrol re-established SOD levels, similar to the findings from Cheng *et al*. who found resveratrol increased NO and SOD levels (Bhatt *et al*., 2011; Cheng *et al*., 2014). Resveratrol and red wine polyphenols prevent eNOS uncoupling, re-establish SOD and NO, and

attenuate the onset of hypertension by re-establishing endothelial function or lowering BP in hypertensive animal models.

Lastly, the endothelium-derived contracting factor, endothelin-1 (ET-1) may be a key player in vascular remodeling in hypertension (Lopez-Sepulveda *et al*., 2011). ET-1 is a vasoconstrictor that increases superoxide levels through activation of NADPH oxidase subunits (Jimenez *et al*., 2007). Endothelial dysfunction is common when there are high circulating levels of ET-1, concurrent with excessive lumen narrowing (Lopez-Sepulveda *et al*., 2011). Both red wine polyphenols and resveratrol may inhibit is ET-1, resulting in downregulation NADPH oxidase activity (Jimenez *et al*., 2007; Lopez-Sepulveda *et al*., 2011; Lopez-Sepulveda *et al*., 2008; Sarr *et al*., 2006). Major products of NADPH oxidase are superoxides, which are produced from ROS in the VSMCs (Bendall *et al*., 2007). Increasing ROS would subsequently decrease NO bioavailability (Marchesi *et al*., 2009). Jimenez *et al*. determined that DOCA-salt induced hypertensive rats treated with red wine polyphenols had diminished NADPH oxidase activation and lower p47phox concentration within arteries (Jimenez *et al*., 2007). Rat thoracic aortic rings treated with red wine polyphenols demonstrated similar effects to those found by Jimenez *et al*., resulting in the reduction of ET-1, and improved endothelial function (Lopez-Sepulveda *et al*., 2011). As demonstrated through multiple studies there appears to be an essential relationship between a reduction in NADPH oxidase activity and improving endothelial dysfunction in hypertensive arteries with high circulating ET-1 levels.

Oxidative damage of proteins and lipids alter cell functionality. Cell damage via oxidative stress was quantified via lipid peroxidation using malondialdehyde (MDA), an aldehyde that is an essential end-product of oxidative degradation. SHHF plasma was used to examine MDA concentration, and no detectable changes in oxidative stress from stilbene

treatment were analyzed (Lee *et al*., 2017). Thus, both eNOS and ET-1 are unlikely major signaling effectors within SHHF at 4 months. Perhaps investigating oxidative damage in SHHF at the onset of CHF closer to 16-18 months would show elevated oxidative stress via MDA (Heyen *et al*., 2002).

2. Conclusion

Although BP lowering may seem like the major contributing factor for essential hypertension, vascular remodeling is also noteworthy when it comes to the progression and pathogenesis of this disease. Structural remodeling of mesenteric resistance arteries of the SHHF rat exhibit eutrophic inward remodeling, as found in human essential hypertension (Mulvany, 1999). Mechanical properties of SHHF resistance arteries did not show stilbene induced differences. All stilbenes exerted compensatory structural benefits in hypertensive resistance arteries, resulting in protection against pressure-induced tension at extreme pressures. The ability of the small arteries to buffer changes in pressure, or the vascular compliance, presented a slight leftward shift in the stress vs. strain curve, representative of potential decreased compliance in SHHF. A trend toward reduced compliance ($p=0.07$) was evident when quantified by AUC. Furthermore, wall component stiffness did not appear to be altered in diseased animals. Overall, this study demonstrated that stilbene polyphenols act on small resistance arteries in an analogous manner, regardless of previously reported oral bioavailability.

Clinically, an increase in the M:L of arteries predicts cardiovascular events independently of BP lowering (Mathiassen *et al*., 2007; Rizzoni *et al*., 2003). It is probable that vascular remodeling had already begun before stilbenoid treatment, as BP was already significantly elevated in SHHF at 9 weeks. The lack of stilbene-induced BP lowering was beneficial in

determining how changes to the vasculature were effected. This suggests that vascular improvement was not secondary to BP lowering and reduced hemodynamic stress, but rather a result of direct actions on the arterial wall. These findings could assist in clarifying outcomes related to metabolite function, and insight into future studies investigating stilbene derivatives.

Stilbenes in combination with anti-hypertensive therapy or as part of a healthy diet may assist in the prevention of hypertension. Further studies investigating stilbenes in combination with anti-hypertensive pharmacotherapies or as a component of diet through supplements may yield increased benefits compared to singular stilbene treatment. Thus, further research on optimizing stilbenoid polyphenol dosage, examining stilbenoid pharmacokinetics and pharmacodynamics, and using stilbenoids as an adjunct to current anti-hypertensive therapy is necessary.

3. Strengths and limitations

Overall, this study demonstrated vasculoprotective effects of stilbenoid polyphenols in the mesenteric resistance arteries. Previously, the Anderson lab studied the effects of low-dose resveratrol in the SHR mesenteric arteries, allowing direct comparison between SHR and SHHF vascular remodeling (Behbahani *et al*., 2010). This current study also simultaneously investigated the effects of stilbenes within the failing heart and within the MCA of the SHHF, which demonstrated tissue-specific modifications (Akinwumi *et al*., 2017; Lee *et al*., 2017) Although the use of controls was appropriate for the experimental design (SD for every SHHF stilbene group) this resulted in a large number of groups, decreasing overall statistical power. Perhaps with a lower number of groups or increased n, statistical significance would have emerged within BP, mechanical properties, and signaling effectors.

The dose chosen for pterostilbene and gnetol was based on previous studies using low-dose resveratrol in mesenteric resistance arteries (Behbahani *et al*., 2010). Structural and mechanical changes in the vasculature were indistinguishable between resveratrol, pterostilbene and gnetol, demonstrating this dose was effective for all stilbenes. Investigating higher doses could be noteworthy for identifying dose-dependent remodeling, or if there is a limit of stilbene uptake by the body. This could identify at what dose, volume, and compound structure excess stilbenes are excreted. As noted previously, SHHF and SHR could be directly compared with previous literature on resveratrol treatment. Nevertheless, the effects of pterostilbene and gnetol within the SHR remain unknown. Further experiments in SHR potentially could support and clarify model specific vascular differences. Most importantly further studies examining the metabolomics of urine, plasma or tissue of SHHF and/or SHR animals treated with stilbenes could shed greater insight on bioavailability, metabolites produced and excreted, and accumulation within tissue beds.

Stilbene treatment commenced at 9 weeks of age when SHHF animals were already hypertensive. Perhaps earlier intervention, before hypertension onset within SHHF could further demonstrate how stilbenes interact with the vasculature, i.e whether stilbenes act through genuine attenuation or through remodeling and rearrangement of the vasculature. Before the start of the study there were also several cases of premature mortality, although causes remain unknown. Death of hypertensive animals and the microscopic nature of small resistance arteries resulted in not all animals being included in the study. If resistance arteries were not viable when mounted or if slight errors occurred due to the intricacies of pressure myography, the entire sample could be discarded, leaving some treatment groups with lower sample size. Another limitation of this study was the very small amount of usable tissue acquired from the mesenteric

cascade (~10 mg) for investigating signaling mediators. The aorta has been used to circumvent this limitation (Hanson *et al*., 2016), although it should be noted the aorta is not a resistance artery (<400 μm) and is an imperfect solution.

Lastly, the stilbene dose of 2.5 mg/kg/d equates to a human equivalent dose of 27 mg for a 60 kg person. Resveratrol content in red wine is approximately 1.5 - 3 mg per liter while pterostilbene content in blueberries is 15 μg per 100 g (McCormack & McFadden, 2012). Therefore, to receive the equivalent dose used in our study through diet you would need to consume approximately 10 litres of wine (resveratrol), and 1.8 kg of blueberries (pterostilbene). Accordingly, this further supports that resveratrol alone is unlikely the sole contributor to explain French Paradox.

References:

Aggarwal, B. B., Bhardwaj, A., Aggarwal, R. S., Seeram, N. P., Shishodia, S., & Takada, Y. (2004). Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res, 24*(5A), 2783-2840.

Ahmet, I., Tae, H. J., Lakatta, E. G., & Talan, M. (2017). Long-term low dose dietary resveratrol supplement reduces cardiovascular structural and functional deterioration in chronic heart failure in rats. *Can J Physiol Pharmacol, 95*(3), 268-274. doi:10.1139/cjpp-2016-0512

Akinwumi, B. C., Raj, P., Lee, D. I., Acosta, C., Yu, L., Thomas, S. M., . . . Anderson, H. D. (2017). Disparate Effects of Stilbenoid Polyphenols on Hypertrophic Cardiomyocytes In Vitro vs. in the Spontaneously Hypertensive Heart Failure Rat. *Molecules, 22*(2). doi:10.3390/molecules22020204

Appel, L. J., Brands, M. W., Daniels, S. R., Karanja, N., Elmer, P. J., Sacks, F. M., & American Heart, A. (2006). Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension, 47*(2), 296-308. doi:10.1161/01.HYP.0000202568.01167.B6

Aubin, M. C., Lajoie, C., Clement, R., Gosselin, H., Calderone, A., & Perrault, L. P. (2008). Female rats fed a high-fat diet were associated with vascular dysfunction and cardiac fibrosis in the absence of overt obesity and hyperlipidemia: therapeutic potential of resveratrol. *J Pharmacol Exp Ther, 325*(3), 961-968. doi:10.1124/jpet.107.135061

Bakker, E. N., Groma, G., Spijkers, L. J., de Vos, J., van Weert, A., van Veen, H., . . . VanBavel, E. (2014). Heterogeneity in arterial remodeling among sublines of spontaneously hypertensive rats. *PLoS One, 9*(9), e107998. doi:10.1371/journal.pone.0107998

Balluz, L. S., Kieszak, S. M., Philen, R. M., & Mulinare, J. (2000). Vitamin and mineral supplement use in the United States. Results from the third National Health and Nutrition Examination Survey. *Arch Fam Med, 9*(3), 258-262.

Behbahani, J., Thandapilly, S. J., Louis, X. L., Huang, Y., Shao, Z., Kopilas, M. A., . . . Anderson, H. D. (2010). Resveratrol and small artery compliance and remodeling in the spontaneously hypertensive rat. *Am J Hypertens, 23*(12), 1273-1278. doi:10.1038/ajh.2010.161

Bendall, J. K., Rinze, R., Adlam, D., Tatham, A. L., de Bono, J., Wilson, N., . . . Channon, K. M. (2007). Endothelial Nox2 overexpression potentiates vascular oxidative stress and hemodynamic response to angiotensin II: studies in endothelial-targeted Nox2 transgenic mice. *Circ Res, 100*(7), 1016-1025. doi:10.1161/01.RES.0000263381.83835.7b

Benetos, A., Gautier, S., Lafleche, A., Topouchian, J., Frangin, G., Girerd, X., . . . Safar, M. E. (2000). Blockade of angiotensin II type 1 receptors: effect on carotid and radial artery structure and function in hypertensive humans. *J Vasc Res, 37*(1), 8-15; discussion 68-70. doi:25708

Bhatt, S. R., Lokhandwala, M. F., & Banday, A. A. (2011). Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. *Eur J Pharmacol, 667*(1-3), 258-264. doi:10.1016/j.ejphar.2011.05.026

Biasutto, L., Marotta, E., Garbisa, S., Zoratti, M., & Paradisi, C. (2010). Determination of quercetin and resveratrol in whole blood--implications for bioavailability studies. *Molecules, 15*(9), 6570-6579. doi:10.3390/molecules15096570

Blache, D., Rustan, I., Durand, P., Lesgards, G., & Loreau, N. (1997). Gas chromatographic analysis of resveratrol in plasma, lipoproteins and cells after in vitro incubations. *J Chromatogr B Biomed Sci Appl, 702*(1-2), 103-110.

Bonnefont-Rousselot, D. (2016). Resveratrol and Cardiovascular Diseases. *Nutrients, 8*(5). doi:10.3390/nu8050250

Boocock, D. J., Faust, G. E., Patel, K. R., Schinas, A. M., Brown, V. A., Ducharme, M. P., . . . Brenner, D. E. (2007). Phase I dose escalation pharmacokinetic study in healthy volunteers of resveratrol, a potential cancer chemopreventive agent. *Cancer Epidemiol Biomarkers Prev, 16*(6), 1246-1252. doi:10.1158/1055-9965.EPI-07-0022

Brunner, H. R., Laragh, J. H., Baer, L., Newton, M. A., Goodwin, F. T., Krakoff, L. R., . . . Buhler, F. R. (1972). Essential hypertension: renin and aldosterone, heart attack and stroke. *N Engl J Med, 286*(9), 441-449. doi:10.1056/NEJM197203022860901

Buus, C. L., Pourageaud, F., Fazzi, G. E., Janssen, G., Mulvany, M. J., & De Mey, J. G. (2001). Smooth muscle cell changes during flow-related remodeling of rat mesenteric resistance arteries. *Circ Res, 89*(2), 180-186.

Calamini, B., Ratia, K., Malkowski, M. G., Cuendet, M., Pezzuto, J. M., Santarsiero, B. D., & Mesecar, A. D. (2010). Pleiotropic mechanisms facilitated by resveratrol and its metabolites. *Biochem J, 429*(2), 273-282. doi:10.1042/BJ20091857

Calhoun, D. A., Jones, D., Textor, S., Goff, D. C., Murphy, T. P., Toto, R. D., . . . American Heart Association Professional Education, C. (2008). Resistant hypertension: diagnosis, evaluation, and treatment: a scientific statement from the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research. *Circulation, 117*(25), e510-526. doi:10.1161/CIRCULATIONAHA.108.189141

Canada, H. (2015). Drugs and Health Products. *Natural and Non-prescription Health Products*. Retrieved from http://www.hc-sc.gc.ca/dhp-mps/prodnatur/index-eng.php#share

Chen, R. S., Wu, P. L., & Chiou, R. Y. (2002). Peanut roots as a source of resveratrol. *J Agric Food Chem, 50*(6), 1665-1667.

Cheng, P. W., Ho, W. Y., Su, Y. T., Lu, P. J., Chen, B. Z., Cheng, W. H., . . . Tseng, C. J. (2014). Resveratrol decreases fructose-induced oxidative stress, mediated by NADPH oxidase via an AMPK-dependent mechanism. *Br J Pharmacol, 171*(11), 2739-2750. doi:10.1111/bph.12648

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jr., . . . National High Blood Pressure Education Program Coordinating, C. (2003). The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA, 289*(19), 2560-2572. doi:10.1001/jama.289.19.2560

Clement, M. V., Hirpara, J. L., Chawdhury, S. H., & Pervaiz, S. (1998). Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. *Blood, 92*(3), 996-1002.

d'Uscio, L. V., Barton, M., Shaw, S., Moreau, P., & Luscher, T. F. (1997). Structure and function of small arteries in salt-induced hypertension: effects of chronic endothelin-subtype-A-receptor blockade. *Hypertension, 30*(4), 905-911.

de Gasparo, M., Catt, K. J., Inagami, T., Wright, J. W., & Unger, T. (2000). International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev, 52*(3), 415-472.

de Lorgeril, M., Salen, P., Paillard, F., Laporte, F., Boucher, F., & de Leiris, J. (2002). Mediterranean diet and the French paradox: two distinct biogeographic concepts for one

consolidated scientific theory on the role of nutrition in coronary heart disease. *Cardiovasc Res, 54*(3), 503-515.

Deng, L. Y., & Schiffrin, E. L. (1992). Effects of endothelin on resistance arteries of DOCA-salt hypertensive rats. *Am J Physiol, 262*(6 Pt 2), H1782-1787.

Digne-Malcolm, H., Frise, M. C., & Dorrington, K. L. (2016). How Do Antihypertensive Drugs Work? Insights from Studies of the Renal Regulation of Arterial Blood Pressure. *Front Physiol, 7*, 320. doi:10.3389/fphys.2016.00320

Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature, 411*(6839), 843-847. doi:10.1038/35081178

Dolinsky, V. W., Chakrabarti, S., Pereira, T. J., Oka, T., Levasseur, J., Beker, D., . . . Dyck, J. R. (2013). Resveratrol prevents hypertension and cardiac hypertrophy in hypertensive rats and mice. *Biochim Biophys Acta, 1832*(10), 1723-1733. doi:10.1016/j.bbadis.2013.05.018

Dolinsky, V. W., Chan, A. Y., Robillard Frayne, I., Light, P. E., Des Rosiers, C., & Dyck, J. R. (2009). Resveratrol prevents the prohypertrophic effects of oxidative stress on LKB1. *Circulation, 119*(12), 1643-1652. doi:10.1161/circulationaha.108.787440

Dustan, H. P., Tarazi, R. C., & Bravo, E. L. (1972). Physiologic characteristics of hypertension. *Am J Med, 52*(5), 610-622.

Ehrhardt, C., Arapitsas, P., Stefanini, M., Flick, G., & Mattivi, F. (2014). Analysis of the phenolic composition of fungus-resistant grape varieties cultivated in Italy and Germany using UHPLC-MS/MS. *J Mass Spectrom, 49*(9), 860-869. doi:10.1002/jms.3440

Folkow, B. (1982). Physiological aspects of primary hypertension. *Physiol Rev, 62*(2), 347-504.

Ford, R. J., Teschke, S. R., Reid, E. B., Durham, K. K., Kroetsch, J. T., & Rush, J. W. (2012). AMP-activated protein kinase activator AICAR acutely lowers blood pressure and

relaxes isolated resistance arteries of hypertensive rats. *J Hypertens, 30*(4), 725-733. doi:10.1097/HJH.0b013e32835050ca

Fremont, L. (2000). Biological effects of resveratrol. *Life Sci, 66*(8), 663-673.

Furukawa-Hibi, Y., Alkam, T., Nitta, A., Matsuyama, A., Mizoguchi, H., Suzuki, K., . . . Yamada, K. (2011). Butyrylcholinesterase inhibitors ameliorate cognitive dysfunction induced by amyloid-beta peptide in mice. *Behav Brain Res, 225*(1), 222-229. doi:10.1016/j.bbr.2011.07.035

Ghanim, H., Sia, C. L., Korzeniewski, K., Lohano, T., Abuaysheh, S., Marumganti, A., . . . Dandona, P. (2011). A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. *J Clin Endocrinol Metab, 96*(5), 1409-1414. doi:10.1210/jc.2010-1812

Global Summary Estimates, 20 leading causes of death. (2012). *http://who.int/healthinfo/global_burden_disease/estimates/en/index1.html*.

Goldberg, D. M., Yan, J., & Soleas, G. J. (2003). Absorption of three wine-related polyphenols in three different matrices by healthy subjects. *Clin Biochem, 36*(1), 79-87.

Hanson, M. G., Taylor, C. G., Wu, Y., Anderson, H. D., & Zahradka, P. (2016). Lentil consumption reduces resistance artery remodeling and restores arterial compliance in the spontaneously hypertensive rats. *J Nutr Biochem, 37*, 30-38. doi:10.1016/j.jnutbio.2016.07.014

Hao, J., Gao, Y., Zhao, J., Zhang, J., Li, Q., Zhao, Z., & Liu, J. (2015). Preparation and optimization of resveratrol nanosuspensions by antisolvent precipitation using Box-Behnken design. *AAPS PharmSciTech, 16*(1), 118-128. doi:10.1208/s12249-014-0211-y

Heagerty, A. M., Aalkjaer, C., Bund, S. J., Korsgaard, N., & Mulvany, M. J. (1993). Small artery structure in hypertension. Dual processes of remodeling and growth. *Hypertension, 21*(4), 391-397.

Heidenreich, P. A., Trogdon, J. G., Khavjou, O. A., Butler, J., Dracup, K., Ezekowitz, M. D., . . . Outcomes, R. (2011). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation, 123*(8), 933-944. doi:10.1161/CIR.0b013e31820a55f5

Heyen, J. R., Blasi, E. R., Nikula, K., Rocha, R., Daust, H. A., Frierdich, G., . . . Rudolph, A. E. (2002). Structural, functional, and molecular characterization of the SHHF model of heart failure. *Am J Physiol Heart Circ Physiol, 283*(5), H1775-1784. doi:10.1152/ajpheart.00305.2002

Hohmann, C. D., Cramer, H., Michalsen, A., Kessler, C., Steckhan, N., Choi, K., & Dobos, G. (2015). Effects of high phenolic olive oil on cardiovascular risk factors: A systematic review and meta-analysis. *Phytomedicine, 22*(6), 631-640. doi:http://dx.doi.org/10.1016/j.phymed.2015.03.019

Howells, L. M., Berry, D. P., Elliott, P. J., Jacobson, E. W., Hoffmann, E., Hegarty, B., . . . Gescher, A. J. (2011). Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases--safety, pharmacokinetics, and pharmacodynamics. *Cancer Prev Res (Phila), 4*(9), 1419-1425. doi:10.1158/1940-6207.CAPR-11-0148

Huang, C. L., & Sumpio, B. E. (2008). Olive oil, the mediterranean diet, and cardiovascular health. *J Am Coll Surg, 207*(3), 407-416. doi:10.1016/j.jamcollsurg.2008.02.018

Huang, T. C., Lu, K. T., Wo, Y. Y., Wu, Y. J., & Yang, Y. L. (2011). Resveratrol protects rats from Abeta-induced neurotoxicity by the reduction of iNOS expression and lipid peroxidation. *PLoS One, 6*(12), e29102. doi:10.1371/journal.pone.0029102

Ibrahim, J., & Berk, B. C. (2009). Flow-mediated vascular remodeling in hypertension: relation to hemodyamics. *Stroke, 40*(2), 582-590. doi:10.1161/STROKEAHA.108.529826

Ibrahim, M. M., & Damasceno, A. (2012). Hypertension in developing countries. *Lancet, 380*(9841), 611-619. doi:10.1016/S0140-6736(12)60861-7

Inanaga, K., Ichiki, T., Matsuura, H., Miyazaki, R., Hashimoto, T., Takeda, K., & Sunagawa, K. (2009). Resveratrol attenuates angiotensin II-induced interleukin-6 expression and perivascular fibrosis. *Hypertens Res, 32*(6), 466-471. doi:10.1038/hr.2009.47

Intengan, H. D., Deng, L. Y., Li, J. S., & Schiffrin, E. L. (1999). Mechanics and composition of human subcutaneous resistance arteries in essential hypertension. *Hypertension, 33*(1 Pt 2), 569-574.

Intengan, H. D., & Schiffrin, E. L. (2000). Structure and mechanical properties of resistance arteries in hypertension: role of adhesion molecules and extracellular matrix determinants. *Hypertension, 36*(3), 312-318.

Intengan, H. D., & Schiffrin, E. L. (2001). Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. *Hypertension, 38*(3 Pt 2), 581-587.

Intengan, H. D., Thibault, G., Li, J. S., & Schiffrin, E. L. (1999). Resistance artery mechanics, structure, and extracellular components in spontaneously hypertensive rats : effects of angiotensin receptor antagonism and converting enzyme inhibition. *Circulation, 100*(22), 2267-2275.

Izzard, A. S., Horton, S., Heerkens, E. H., Shaw, L., & Heagerty, A. M. (2006). Middle cerebral artery structure and distensibility during developing and established phases of hypertension in the spontaneously hypertensive rat. *J Hypertens, 24*(5), 875-880. doi:10.1097/01.hjh.0000222757.54111.06

James, P. A., Oparil, S., Carter, B. L., Cushman, W. C., Dennison-Himmelfarb, C., Handler, J., . . . Ortiz, E. (2014). 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA, 311*(5), 507-520. doi:10.1001/jama.2013.284427

Jimenez, R., Lopez-Sepulveda, R., Kadmiri, M., Romero, M., Vera, R., Sanchez, M., . . . Duarte, J. (2007). Polyphenols restore endothelial function in DOCA-salt hypertension: role of endothelin-1 and NADPH oxidase. *Free Radic Biol Med, 43*(3), 462-473. doi:10.1016/j.freeradbiomed.2007.05.007

Kapetanovic, I. M., Muzzio, M., Huang, Z., Thompson, T. N., & McCormick, D. L. (2011). Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother Pharmacol, 68*(3), 593-601. doi:10.1007/s00280-010-1525-4

Kato, R., & Pinsky, M. R. (2015). Personalizing blood pressure management in septic shock. *Ann Intensive Care, 5*(1), 41. doi:10.1186/s13613-015-0085-5

Kearney, P. M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P. K., & He, J. (2005). Global burden of hypertension: analysis of worldwide data. *Lancet, 365*(9455), 217-223. doi:10.1016/S0140-6736(05)17741-1

Kidney Disease Improving Global Outcomes. (2012). *Kidney Int Suppl, 2*, 357–362.

Kloypan, C., Jeenapongsa, R., Sri-in, P., Chanta, S., Dokpuang, D., Tip-pyang, S., & Surapinit, N. (2012). Stilbenoids from Gnetum macrostachyum attenuate human platelet aggregation and adhesion. *Phytother Res, 26*(10), 1564-1568. doi:10.1002/ptr.4605

Kopilas, M. A., Dang, L. N., & Anderson, H. D. (2007). Effect of dietary chromium on resistance artery function and nitric oxide signaling in the sucrose-fed spontaneously hypertensive rat. *J Vasc Res, 44*(2), 110-118. doi:10.1159/000098483

Korsgaard, N., Aalkjaer, C., Heagerty, A. M., Izzard, A. S., & Mulvany, M. J. (1993). Histology of subcutaneous small arteries from patients with essential hypertension. *Hypertension, 22*(4), 523-526.

Korsgaard, N., & Mulvany, M. J. (1988). Cellular hypertrophy in mesenteric resistance vessels from renal hypertensive rats. *Hypertension, 12*(2), 162-167.

Kris-Etherton, P., Eckel, R. H., Howard, B. V., St Jeor, S., Bazzarre, T. L., Nutrition Committee Population Science, C., & Clinical Science Committee of the American Heart, A. (2001). AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step I Dietary Pattern on Cardiovascular Disease. *Circulation, 103*(13), 1823-1825.

Law, M., & Wald, N. (1999). Why heart disease mortality is low in France: the time lag explanation. *BMJ, 318*(7196), 1471-1476.

Lee, D. I., Acosta, C., Anderson, C. M., & Anderson, H. D. (2017). Peripheral and Cerebral Resistance Arteries in the Spontaneously Hypertensive Heart Failure Rat: Effects of Stilbenoid Polyphenols. *Molecules, 22*(3). doi:10.3390/molecules22030380

Lee, R. M., Owens, G. K., Scott-Burden, T., Head, R. J., Mulvany, M. J., & Schiffrin, E. L. (1995). Pathophysiology of smooth muscle in hypertension. *Can J Physiol Pharmacol, 73*(5), 574-584.

Lemkens, P., Nelissen, J., Meens, M. J., Fazzi, G. E., Janssen, G. J., Debets, J. J., . . . De Mey, J. G. (2012). Impaired flow-induced arterial remodeling in DOCA-salt hypertensive rats. *Hypertens Res, 35*(11), 1093-1101. doi:10.1038/hr.2012.94

Leung, A. A., Nerenberg, K., Daskalopoulou, S. S., McBrien, K., Zarnke, K. B., Dasgupta, K., . . . Force, C. G. T. (2016). Hypertension Canada's 2016 Canadian Hypertension Education Program Guidelines for Blood Pressure Measurement, Diagnosis, Assessment of Risk, Prevention, and Treatment of Hypertension. *Can J Cardiol, 32*(5), 569-588. doi:10.1016/j.cjca.2016.02.066

Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R., & Prospective Studies, C. (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet, 360*(9349), 1903-1913.

Li, H., & Forstermann, U. (2000). Nitric oxide in the pathogenesis of vascular disease. *J Pathol, 190*(3), 244-254. doi:10.1002/(SICI)1096-9896(200002)190:3<244::AID-PATH575>3.0.CO;2-8

Li, H., Horke, S., & Forstermann, U. (2014). Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis, 237*(1), 208-219. doi:10.1016/j.atherosclerosis.2014.09.001

Li, H., Witte, K., August, M., Brausch, I., Gödtel-Armbrust, U., Habermeier, A., . . . Förstermann, U. (2006). Reversal of Endothelial Nitric Oxide Synthase Uncoupling and

Up-Regulation of Endothelial Nitric Oxide Synthase Expression Lowers Blood Pressure in Hypertensive Rats. *Journal of the American College of Cardiology, 47*(12), 2536-2544. doi:http://dx.doi.org/10.1016/j.jacc.2006.01.071

Li, H., Xia, N., & Forstermann, U. (2012). Cardiovascular effects and molecular targets of resveratrol. *Nitric Oxide, 26*(2), 102-110. doi:10.1016/j.niox.2011.12.006

Li, J. S., Knafo, L., Turgeon, A., Garcia, R., & Schiffrin, E. L. (1996). Effect of endothelin antagonism on blood pressure and vascular structure in renovascular hypertensive rats. *Am J Physiol, 271*(1 Pt 2), H88-93.

Li, K., Dias, S. J., Rimando, A. M., Dhar, S., Mizuno, C. S., Penman, A. D., . . . Levenson, A. S. (2013). Pterostilbene acts through metastasis-associated protein 1 to inhibit tumor growth, progression and metastasis in prostate cancer. *PLoS One, 8*(3), e57542. doi:10.1371/journal.pone.0057542

Lim, S. S., et al. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet, 380*(9859), 2224-2260. doi:10.1016/S0140-6736(12)61766-8

Lin, H. C., Hsieh, M. J., Peng, C. H., Yang, S. F., & Huang, C. N. (2015). Pterostilbene Inhibits Vascular Smooth Muscle Cells Migration and Matrix Metalloproteinase-2 through Modulation of MAPK Pathway. *J Food Sci, 80*(10), H2331-2335. doi:10.1111/1750-3841.13002

Liu, Z., Song, Y., Zhang, X., Liu, Z., Zhang, W., Mao, W., . . . Liu, C. (2005). Effects of trans-resveratrol on hypertension-induced cardiac hypertrophy using the partially

nephrectomized rat model. *Clin Exp Pharmacol Physiol, 32*(12), 1049-1054. doi:10.1111/j.1440-1681.2005.04303.x

Lopez-Sepulveda, R., Gomez-Guzman, M., Zarzuelo, M. J., Romero, M., Sanchez, M., Quintela, A. M., . . . Jimenez, R. (2011). Red wine polyphenols prevent endothelial dysfunction induced by endothelin-1 in rat aorta: role of NADPH oxidase. *Clin Sci (Lond), 120*(8), 321-333. doi:10.1042/cs20100311

Lopez-Sepulveda, R., Jimenez, R., Romero, M., Zarzuelo, M. J., Sanchez, M., Gomez-Guzman, M., . . . Duarte, J. (2008). Wine polyphenols improve endothelial function in large vessels of female spontaneously hypertensive rats. *Hypertension, 51*(4), 1088-1095. doi:10.1161/hypertensionaha.107.107672

Lund-Johansen, P. (1983). Haemodynamics in early essential hypertension--still an area of controversy. *J Hypertens, 1*(3), 209-213.

MacMahon, S., Peto, R., Cutler, J., Collins, R., Sorlie, P., Neaton, J., . . . Stamler, J. (1990). Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet, 335*(8692), 765-774.

Manickam, M., Ramanathan, M., Jahromi, M. A., Chansouria, J. P., & Ray, A. B. (1997). Antihyperglycemic activity of phenolics from Pterocarpus marsupium. *J Nat Prod, 60*(6), 609-610. doi:10.1021/np9607013

Manna, S. K., Mukhopadhyay, A., & Aggarwal, B. B. (2000). Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol, 164*(12), 6509-6519.

Marchesi, C., Ebrahimian, T., Angulo, O., Paradis, P., & Schiffrin, E. L. (2009). Endothelial nitric oxide synthase uncoupling and perivascular adipose oxidative stress and inflammation contribute to vascular dysfunction in a rodent model of metabolic syndrome. *Hypertension, 54*(6), 1384-1392. doi:10.1161/HYPERTENSIONAHA.109.138305

Marier, J. F., Vachon, P., Gritsas, A., Zhang, J., Moreau, J. P., & Ducharme, M. P. (2002). Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *J Pharmacol Exp Ther, 302*(1), 369-373.

Martinez-Marquez, A., Morante-Carriel, J. A., Ramirez-Estrada, K., Cusido, R. M., Palazon, J., & Bru-Martinez, R. (2016). Production of highly bioactive resveratrol analogues pterostilbene and piceatannol in metabolically engineered grapevine cell cultures. *Plant Biotechnol J, 14*(9), 1813-1825. doi:10.1111/pbi.12539

Mathiassen, O. N., Buus, N. H., Sihm, I., Thybo, N. K., Morn, B., Schroeder, A. P., . . . Christensen, K. L. (2007). Small artery structure is an independent predictor of cardiovascular events in essential hypertension. *J Hypertens, 25*(5), 1021-1026. doi:10.1097/HJH.0b013e32805bf8ed

McCormack, D., & McFadden, D. (2012). Pterostilbene and cancer: current review. *J Surg Res, 173*(2), e53-61. doi:10.1016/j.jss.2011.09.054

Miatello, R., Vazquez, M., Renna, N., Cruzado, M., Zumino, A. P., & Risler, N. (2005). Chronic administration of resveratrol prevents biochemical cardiovascular changes in fructose-fed rats. *Am J Hypertens, 18*(6), 864-870. doi:10.1016/j.amjhyper.2004.12.012

Mulvany, M. J. (1999). Vascular remodelling of resistance vessels: can we define this? *Cardiovasc Res, 41*(1), 9-13.

Mulvany, M. J., & Halpern, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ Res, 41*(1), 19-26.

Mulvany, M. J., Hansen, O. K., & Aalkjaer, C. (1978). Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. *Circ Res, 43*(6), 854-864.

Narayanan, N. K., Kunimasa, K., Yamori, Y., Mori, M., Mori, H., Nakamura, K., . . . Narayanan, B. (2015). Antitumor activity of melinjo (Gnetum gnemon L.) seed extract in human and murine tumor models in vitro and in a colon-26 tumor-bearing mouse model in vivo. *Cancer Med, 4*(11), 1767-1780. doi:10.1002/cam4.520

Neves, M. F., Kasal, D. A., Cunha, A. R., & Medeiros, F. (2012). Vascular dysfunction as target organ damage in animal models of hypertension. *Int J Hypertens, 2012*, 187526. doi:10.1155/2012/187526

Ohguchi, K., Tanaka, T., Iliya, I., Ito, T., Iinuma, M., Matsumoto, K., . . . Nozawa, Y. (2003). Gnetol as a potent tyrosinase inhibitor from genus Gnetum. *Biosci Biotechnol Biochem, 67*(3), 663-665. doi:10.1271/bbb.67.663

Ottenbacher, R., & Blehm, J. (2015). An Unusual Case of Licorice-Induced Hypertensive Crisis. *S D Med, 68*(8), 346-347, 349.

Pari, L., & Satheesh, M. A. (2006). Effect of pterostilbene on hepatic key enzymes of glucose metabolism in streptozotocin- and nicotinamide-induced diabetic rats. *Life Sci, 79*(7), 641-645. doi:10.1016/j.lfs.2006.02.036

Park, E. S., Lim, Y., Hong, J. T., Yoo, H. S., Lee, C. K., Pyo, M. Y., & Yun, Y. P. (2010). Pterostilbene, a natural dimethylated analog of resveratrol, inhibits rat aortic vascular smooth muscle cell proliferation by blocking Akt-dependent pathway. *Vascul Pharmacol, 53*(1-2), 61-67. doi:10.1016/j.vph.2010.04.001

Paul, B., Masih, I., Deopujari, J., & Charpentier, C. (1999). Occurrence of resveratrol and pterostilbene in age-old darakchasava, an ayurvedic medicine from India. *J Ethnopharmacol, 68*(1-3), 71-76.

Perez-Lopez, F. R., Chedraui, P., Haya, J., & Cuadros, J. L. (2009). Effects of the Mediterranean diet on longevity and age-related morbid conditions. *Maturitas, 64*(2), 67-79. doi:10.1016/j.maturitas.2009.07.013

Petyaev, I. M., & Bashmakov, Y. K. (2012). Could cheese be the missing piece in the French paradox puzzle? *Med Hypotheses, 79*(6), 746-749. doi:10.1016/j.mehy.2012.08.018

Pickering, G. W. (1952). The natural history of hypertension. *Br Med Bull, 8*(4), 305-309.

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., . . . Roccella, E. J. (2005). Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation, 111*(5), 697-716. doi:10.1161/01.CIR.0000154900.76284.F6

Popat, R., Plesner, T., Davies, F., Cook, G., Cook, M., Elliott, P., . . . Cavenagh, J. (2013). A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. *Br J Haematol, 160*(5), 714-717. doi:10.1111/bjh.12154

Pucci, M., & Martin, U. (2016). Detecting non-adherence by urine analysis in patients with uncontrolled hypertension: rates, reasons and reactions. *J Hum Hypertens*. doi:10.1038/jhh.2016.69

Raj, P., Louis, X. L., Thandapilly, S. J., Movahed, A., Zieroth, S., & Netticadan, T. (2014). Potential of resveratrol in the treatment of heart failure. *Life Sci, 95*(2), 63-71. doi:10.1016/j.lfs.2013.12.011

Ramsay, L., Williams, B., Johnston, G., MacGregor, G., Poston, L., Potter, J., . . . Russell, G. (1999). Guidelines for management of hypertension: report of the third working party of the British Hypertension Society. *J Hum Hypertens, 13*(9), 569-592.

Remsberg, C. M., Martinez, S. E., Akinwumi, B. C., Anderson, H. D., Takemoto, J. K., Sayre, C. L., & Davies, N. M. (2015). Preclinical Pharmacokinetics and Pharmacodynamics and Content Analysis of Gnetol in Foodstuffs. *Phytother Res, 29*(8), 1168-1179. doi:10.1002/ptr.5363

Remsberg, C. M., Yanez, J. A., Ohgami, Y., Vega-Villa, K. R., Rimando, A. M., & Davies, N. M. (2008). Pharmacometrics of pterostilbene: preclinical pharmacokinetics and metabolism, anticancer, antiinflammatory, antioxidant and analgesic activity. *Phytother Res, 22*(2), 169-179. doi:10.1002/ptr.2277

Renaud, S., & de Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet, 339*(8808), 1523-1526.

Rentzsch, M., Wilkens, A., & Winterhalter, P. (2009). Non-flavonoid Phenolic Compounds *Wine Chemistry and Biochemistry* (pp. 509-527): Springer New York.

Rimando, A. M., Kalt, W., Magee, J. B., Dewey, J., & Ballington, J. R. (2004). Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J Agric Food Chem, 52*(15), 4713-4719. doi:10.1021/jf040095e

Rimando, A. M., Nagmani, R., Feller, D. R., & Yokoyama, W. (2005). Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor alpha-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. *J Agric Food Chem, 53*(9), 3403-3407. doi:10.1021/jf0580364

Rimoldi, S. F., Scherrer, U., & Messerli, F. H. (2014). Secondary arterial hypertension: when, who, and how to screen? *Eur Heart J, 35*(19), 1245-1254. doi:10.1093/eurheartj/eht534

Rizzoni, D., Porteri, E., Boari, G. E., De Ciuceis, C., Sleiman, I., Muiesan, M. L., . . . Agabiti-Rosei, E. (2003). Prognostic significance of small-artery structure in hypertension. *Circulation, 108*(18), 2230-2235. doi:10.1161/01.CIR.0000095031.51492.C5

Rodrigo, M. C., Martin, D. S., Redetzke, R. A., & Eyster, K. M. (2002). A method for the extraction of high-quality RNA and protein from single small samples of arteries and veins preserved in RNAlater. *J Pharmacol Toxicol Methods, 47*(2), 87-92.

Rodrigo, R., Gil, D., Miranda-Merchak, A., & Kalantzidis, G. (2012). Antihypertensive Role of Polyphenols. *Adv Clin Chem, 58*, 225-254. doi:10.1016/b978-0-12-394383-5.00014-x

Sacks, F. M., Appel, L. J., Moore, T. J., Obarzanek, E., Vollmer, W. M., Svetkey, L. P., . . . Karanja, N. (1999). A dietary approach to prevent hypertension: a review of the Dietary Approaches to Stop Hypertension (DASH) Study. *Clin Cardiol, 22*(7 Suppl), III6-10.

Sacks, F. M., Svetkey, L. P., Vollmer, W. M., Appel, L. J., Bray, G. A., Harsha, D., . . . Group, D. A.-S. C. R. (2001). Effects on blood pressure of reduced dietary sodium and the

Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med, 344*(1), 3-10. doi:10.1056/NEJM200101043440101

Sahar, S., Reddy, M. A., Wong, C., Meng, L., Wang, M., & Natarajan, R. (2007). Cooperation of SRC-1 and p300 with NF-kappaB and CREB in angiotensin II-induced IL-6 expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol, 27*(7), 1528-1534. doi:10.1161/ATVBAHA.107.145862

Sarr, M., Chataigneau, M., Martins, S., Schott, C., El Bedoui, J., Oak, M. H., . . . Schini-Kerth, V. B. (2006). Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: role of NADPH oxidase. *Cardiovasc Res, 71*(4), 794-802. doi:10.1016/j.cardiores.2006.05.022

Saseen, J. J., & MacLaughlin, E. J. (2014). Chapter 3. Hypertension. In T. R. DiPiro JT, Yee GC, Matzke GR, Wells BG, Posey L. eds (Ed.), *Pharmacotherapy: A Pathophysiologic Approach* (9 ed.). New York, NY: McGraw-Hill.

Schiffrin, E. L. (2001). Effects of antihypertensive drugs on vascular remodeling: do they predict outcome in response to antihypertensive therapy? *Curr Opin Nephrol Hypertens, 10*(5), 617-624.

Schiffrin, E. L. (2004). Remodeling of resistance arteries in essential hypertension and effects of antihypertensive treatment. *Am J Hypertens, 17*(12 Pt 1), 1192-1200. doi:10.1016/j.amjhyper.2004.05.023

Schiffrin, E. L., & Deng, L. Y. (1995). Comparison of effects of angiotensin I-converting enzyme inhibition and beta-blockade for 2 years on function of small arteries from hypertensive patients. *Hypertension, 25*(4 Pt 2), 699-703.

Schiffrin, E. L., Deng, L. Y., & Larochelle, P. (1995). Progressive improvement in the structure of resistance arteries of hypertensive patients after 2 years of treatment with an angiotensin I-converting enzyme inhibitor. Comparison with effects of a beta-blocker. *Am J Hypertens, 8*(3), 229-236.

Schiffrin, E. L., Park, J. B., Intengan, H. D., & Touyz, R. M. (2000). Correction of Arterial Structure and Endothelial Dysfunction in Human Essential Hypertension by the Angiotensin Receptor Antagonist Losartan. *Circulation, 101*(14), 1653-1659. doi:10.1161/01.cir.101.14.1653

Schmidlin, L., Poutaraud, A., Claudel, P., Mestre, P., Prado, E., Santos-Rosa, M., . . . Hugueney, P. (2008). A stress-inducible resveratrol O-methyltransferase involved in the biosynthesis of pterostilbene in grapevine. *Plant Physiol, 148*(3), 1630-1639. doi:10.1104/pp.108.126003

Sermboonpaisarn, T., & Sawasdee, P. (2012). Potent and selective butyrylcholinesterase inhibitors from Ficus foveolata. *Fitoterapia, 83*(4), 780-784. doi:10.1016/j.fitote.2012.03.009

Singh, S., & Aggarwal, B. B. (1995). Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J Biol Chem, 270*(42), 24995-25000.

Smoliga, J. M., & Blanchard, O. (2014). Enhancing the delivery of resveratrol in humans: if low bioavailability is the problem, what is the solution? *Molecules, 19*(11), 17154-17172. doi:10.3390/molecules191117154

Staessen, J. A., Wang, J., Bianchi, G., & Birkenhager, W. H. (2003). Essential hypertension. *Lancet, 361*(9369), 1629-1641. doi:10.1016/S0140-6736(03)13302-8

Stamler, J. (1997). The INTERSALT Study: background, methods, findings, and implications. *Am J Clin Nutr, 65*(2 Suppl), 626S-642S.

Szkudelski, T., & Szkudelska, K. (2011). Anti-diabetic effects of resveratrol. *Ann N Y Acad Sci, 1215*, 34-39. doi:10.1111/j.1749-6632.2010.05844.x

Thandapilly, S. J., LeMaistre, J. L., Louis, X. L., Anderson, C. M., Netticadan, T., & Anderson, H. D. (2012). Vascular and cardiac effects of grape powder in the spontaneously hypertensive rat. *Am J Hypertens, 25*(10), 1070-1076. doi:10.1038/ajh.2012.98

Thandapilly, S. J., Louis, X. L., Behbahani, J., Movahed, A., Yu, L., Fandrich, R., . . . Netticadan, T. (2013). Reduced hemodynamic load aids low-dose resveratrol in reversing cardiovascular defects in hypertensive rats. *Hypertens Res, 36*(10), 866-872. doi:10.1038/hr.2013.55

Thandapilly, S. J., Wojciechowski, P., Behbahani, J., Louis, X. L., Yu, L., Juric, D., . . . Netticadan, T. (2010). Resveratrol prevents the development of pathological cardiac hypertrophy and contractile dysfunction in the SHR without lowering blood pressure. *Am J Hypertens, 23*(2), 192-196. doi:10.1038/ajh.2009.228

Tome-Carneiro, J., Gonzalvez, M., Larrosa, M., Yanez-Gascon, M. J., Garcia-Almagro, F. J., Ruiz-Ros, J. A., . . . Espin, J. C. (2012). One-year consumption of a grape nutraceutical containing resveratrol improves the inflammatory and fibrinolytic status of patients in primary prevention of cardiovascular disease. *Am J Cardiol, 110*(3), 356-363. doi:10.1016/j.amjcard.2012.03.030

Tome-Carneiro, J., Gonzalvez, M., Larrosa, M., Yanez-Gascon, M. J., Garcia-Almagro, F. J., Ruiz-Ros, J. A., . . . Espin, J. C. (2013). Grape resveratrol increases serum adiponectin and downregulates inflammatory genes in peripheral blood mononuclear cells: a triple-

blind, placebo-controlled, one-year clinical trial in patients with stable coronary artery disease. *Cardiovasc Drugs Ther, 27*(1), 37-48. doi:10.1007/s10557-012-6427-8

Tome-Carneiro, J., Larrosa, M., Gonzalez-Sarrias, A., Tomas-Barberan, F. A., Garcia-Conesa, M. T., & Espin, J. C. (2013). Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. *Curr Pharm Des, 19*(34), 6064-6093.

Trela, B. C., & Waterhouse, A. L. (1996). Resveratrol: Isomeric Molar Absorptivities and Stability. *J Agric Food Chem, 44*(5), 1253-1257. doi:10.1021/jf9504576

Troppmann, L., Johns, T., & Gray-Donald, K. (2002). Natural health product use in Canada. *Can J Public Health, 93*(6), 426-430.

Virdis, A., Ghiadoni, L., Versari, D., Giannarelli, C., Salvetti, A., & Taddei, S. (2008). Endothelial function assessment in complicated hypertension. *Curr Pharm Des, 14*(18), 1761-1770.

Walle, T., Hsieh, F., DeLegge, M. H., Oatis, J. E., Jr., & Walle, U. K. (2004). High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos, 32*(12), 1377-1382. doi:10.1124/dmd.104.000885

WHO. (2008). Traditional Medicine. Geneva: World Health Organization., Fact Sheet No 134.

Wu, C. H., Hong, B. H., Ho, C. T., & Yen, G. C. (2015). Targeting cancer stem cells in breast cancer: potential anticancer properties of 6-shogaol and pterostilbene. *J Agric Food Chem, 63*(9), 2432-2441. doi:10.1021/acs.jafc.5b00002

Xiang, W., Jiang, B., Li, X. M., Zhang, H. J., Zhao, Q. S., Li, S. H., & Sun, H. D. (2002). Constituents of Gnetum montanum. *Fitoterapia, 73*(1), 40-42.

Zheng, H., Yde, C. C., Clausen, M. R., Kristensen, M., Lorenzen, J., Astrup, A., & Bertram, H. C. (2015). Metabolomics investigation to shed light on cheese as a possible piece in the French paradox puzzle. *J Agric Food Chem, 63*(10), 2830-2839. doi:10.1021/jf505878a