University of Nevada, Reno

# The Cardioprotective Actions of Phytochemicals

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Sciences

by

Levi W. Evans

Dr. Bradley S. Ferguson/Dissertation Advisor

December 2020

THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

**Levi W. Evans**

entitled

**The Cardioprotective Actions of Phytochemicals**

be accepted in partial fulfillment of the
requirements for the degree of

**DOCTORATE OF PHILOSOPHY**

Dr. Bradley Ferguson
*Advisor*

Dr. Stanley Omaye
*Committee Member*

Dr. Wei Yang
*Committee Member*

Dr. Silvia Stan
*Committee Member*

Dr. Robert Harvey
*Graduate School Representative*

David W. Zeh, Ph.D., Dean
*Graduate School*

December, 2020

**Abstract:**

We found that phytochemicals similarly and differentially regulated pathways and signal transduction cascades and that these actions were associated with an attenuation in agonist-induced cardiomyocyte hypertrophy (**Chapter 2**). Indeed, phenylephrine (PE) as an $\alpha_1$-adrenergic agonist observably increased phosphorylation of the mitogen activated protein kinases (MAPKs), extracellular regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38, as well as protein kinase C/D (PKC/D). These actions of PE were associated with an increase in cardiomyocyte hypertrophy, which is a common hallmark of heart failure. In contrast, Apigenin, Baicalein, Berberine Hydrochloride (BHCl) and Emodin blocked PE-induced cardiomyocyte hypertrophy, but not via cytotoxicity. Moreover, the overlapping and divergent effects of Apigenin, Baicalein, BHCl and Emodin on the MAPKs and PKC/D highlight our hypothesis in that phytochemicals work through several mechanisms, both similar and different and at different magnitudes (e.g., Emodin reduced phosphorylated-PKD more than Apigenin). Interestingly, Emodin completely abolished PKD phosphorylation while Apigenin, Baicalein andBHCl only attenuated PKD phosphorylation.

Four phytochemicals, Apigenin, Baicalein, BHCl and Emodin, out of 18 were shown to be cardioprotective in **Chapter 2**. As our lab had previously found that these dietary compounds inhibited histone deacetylase (HDAC) activity, we next tested the postulate that Apigenin, Baicalein, BHCl and Emodin would attenuate HDAC activity in our cardiomyocyte experiments. Of the four, Emodin reduced HDAC activity the most. As HDAC inhibition is cardioprotective in experimental models of cardiovascular disease

(CVD), our next task sought to elucidate the effects of Emodin on HDAC activity in the heart and if an Emodin-rich plant, rhubarb, behaved similarly to Emodin (**Chapter 3**). Firstly, our data showed that Emodin and rhubarb inhibited HDAC activity similarly in a test tube, as both inhibited HDAC activity in a fast-on slow-off manner. Additionally, rhubarb blocked intracellular- and receptor-mediated cardiomyocyte hypertrophy in a comparable manner to Emodin. Of interest, Emodin reduced HDAC activity concomitant with increased histone acetylation. As these epigenetic modification data suggest differential gene expression, we next compared and contrasted the effects of Emodin to a well-known HDAC inhibitor, Trichostatin A (TSA), on gene expression in cardiomyocytes. Our data showed that Emodin and TSA affected similar pathways but also different. These data make sense as phytochemicals, per **Chapter 2**, indeed act in multi-faceted manners, including via HDAC inhibition. Finally, we found that the effects of Emodin in cardiomyocytes were reflected in hypertensive mice. Thus, we found Emodin to be cardioprotective both *in vitro* and *in vivo*.

Finally, we found it interesting that Emodin blocked hypertension-induced hypertrophy in mice despite lacking distribution to the heart. The microbiome has been implicated in heart failure. For example, germ-free mice infused with angiotensin II do not develop hypertension, hypertrophy nor fibrosis to the extent of their conventionally raised counterparts. Thus, we sought to characterize the effects of Emodin on hypertensive and healthy mice(**Chapter 4**). Our data linked the cardioprotective actions of Emodin in hypertensive mice with enrichment of *Akkermansia* (**Chapter 4**), a microbe that had previously been shown to be efficacious for diabetes and atherosclerosis. Intriguingly, healthy mice given Emodin via oral gavage also had enriched *Akkermansia*

which suggests Emodin increases microbial abundance of *Akkermansia* independent of administration route. Other notable microbes that were enriched in healthy mice supplemented with Emodin included *Roseburia* and *Allobaculum*.

**Table of Contents with Tables and Figures**

**Chapter 1. Literature Review: which contains excerpts from my previously published review articles:**

- **"Food Bioactive HDAC Inhibitors in the Epigenetic Regulation of Heart Failure" (2018)** ***Nutrients.*** **doi: 10.3390/nu10081120.**
- **"Dietary natural products as epigenetic modifiers in aging-associated inflammation and disease" (2020)** ***Nat Prod Rep*** **2020 May 1;37(5):653-676. doi: 10.1039/c9np00057g. Epub 2020 Jan 29. - Reproduced by permission of The Royal Society of Chemistry.**

## 1. Cardiovascular disease (CVD)

Heart disease remains the leading cause of death around the world.[1] This is concerning beyond the financial and mortality issues, as the heart supplies oxygen and nutrients to and removes toxins from the human system. Thus, the pumping function of the heart (i.e., systole (contraction) and diastole (relaxation)) is particularly important to human life. Essential to this function are cardiomyocytes, the muscle cells that make-up 70-80% of the heart's mass but only 30% of the heart's cells.[2] In response to the sinus node and conducting cells, cardiomyocytes force nutrient-rich blood out of the heart to the body, with ventricular cardiomyocytes generating this force more so than atrial cardiomyocytes. However, injury or insult to cardiomyocytes results in cardiomyocyte death, remodeling such as enlargement, and cardiac fibrosis as well as dysfunction.

### 1.1. Hallmarks of CVD Involving the Myocardium

Cardiomyocyte death is oftentimes associated with post-myocardial infarction.[3] The death of cardiomyocytes is particularly concerning as cardiomyocytes are one of the few types of cells in mammals that cannot regenerate.[4] This is because cardiomyocytes experience terminal differentiation and lose their ability to proliferate three days after birth.[5] Several signaling pathways, including extracellular signal-regulated kinase (ERK), cyclic AMP-dependent protein kinase (PKA)-activated p38, necrosis factor kappa B (NF-κB), and protein kinase B (Akt), regulate cardiomyocyte death.[6] Of note, the signaling transduction cascades that regulate cardiomyocyte death redundantly regulate cardiac hypertrophy.[7] Thus, by inhibiting these signaling cascades, heart cell death and hypertrophy may be attenuated.

In response to stressors, such as hypertension, toxins or myocardial infarction, the heart will enlarge which is known as cardiac hypertrophy.[7] However, an enlarged heart is not the result of newly synthesized cardiomyocytes as, again, these cells are unable to proliferate or divide.[5] Instead, cardiomyocytes themselves enlarge which then translates to the phenotype of cardiac hypertrophy. Cardiac hypertrophy can be adaptive, which is a compensatory response to acute stress allowing physiological functions to maintain, or maladaptive, which results in pathological cardiac dysfunction and the deadly clinical endpoint of heart failure. The signaling transduction cascades associated with cardiac hypertrophy are complex.[7] Though, some common pathways are: the mitogen-activated protein kinases (MAPKs) such as ERK, c-Jun N-terminal kinases (JNK) and p38; protein kinase C (PKC); insulin-like growth factor 1/phosphoinositide-3-kinase/protein kinase B (IGF1/PI3K/Akt); and Janus kinases/signal transducer and activator of transcription proteins (JAK/STAT).[7] These pathways are often therapeutic targets, yet, few have shown clinical efficacy for CVD. This is not because of the lack of clinical relevance but rather because of the off-target negative side-effects.

Cardiac fibrosis is an excessive accumulation of tissue made up of extracellular matrix proteins (ECM), such as collagen, matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs), which interfere with physiological cardiac function.[8] Cardiac fibrogenesis, which can be characterized by a deposition of fibroblasts and fibroblast maturation into myofibroblasts, occurs in response to cardiomyocyte death and other stressors as to maintain the integrity of the heart. Inflammation and oxidative stress will also induce cardiac fibrogenesis.[9] Cardiac hypertrophy and dysfunction, subsequently resulting in heart failure, can result from

cardiac fibrosis as fibrotic tissue will impair heart contraction and relaxation as well as cardiomyocyte conductivity.[8,10] Fibrotic cells secrete pro-inflammatory and pro-hypertrophic stimuli, thus, exacerbating cardiac pathogenesis.[8] Finally, cardiac fibrosis is found in most forms of CVD.[8]

As with the rest of the pathological processes in the heart, cardiac dysfunction is a complex issue with several mechanisms involved. These mechanisms include molecules involved in calcium handling, sodium/potassium pump regulation and phosphoinositide 3 kinase (PI3K) signaling. At the forefront of cardiac dysfunction is calcium handling and its regulators, ryanodine receptor 2 (RyR2), sarco/endoplasmic reticulum $Ca^{+}$-ATPase (SERCA2a), $Ca^{2+}$/ calmodulin-dependent protein kinase II (CaMKII), phospholamban, protein kinase A (PKA), sodium/calcium exchanger (NCX), L-type Calcium Channels (LTCC), T-tubules and protein phosphatases. For example, a disbalance in phosphorylation status of RyR2, SERCA2a or phospholamban can result in calcium mishandling and subsequent cardiac dysfunction and heart failure.[11] Additionally, dysfunctional sodium pumps in the heart also results myocardial pathologies.[12]

Important particularly to the next section of Chapter 1, are the roles of oxidative stress and inflammation in the pathogenesis of cardiovascular diseases. Many well-characterized pathogeneses of the heart manifest from oxidative stress and inflammation, and pro-inflammatory markers in circulation are used to (strongly) predict CVD risk.[13] One of the most common of these oxidative stress/inflammation-induced heart diseases is atherosclerosis. Chronic, low grade inflammation results in endothelium insult that upregulates the inflammatory response around the affected site.[14] There, macrophages

take up oxidized apolipoprotein B (ApoB)-containing lipoproteins that accumulate in the subendothelial space. The macrophages then transform into foam cells, which are primary constituents of atherosclerotic plaques. Intriguingly, rupture or erosion of atherosclerotic plaques initiate myocardial infarction (MI), both of which mediate an ongoing inflammatory response. Following MI, cardiomyocytes suffer injury or death, which further exacerbates inflammation and cardiac remodeling,[15,16] a common hallmark of heart failure. Cytokines produced from aforementioned cardiac insults activate free radical producing signaling cascades involved in heart failure pathology, including cardiac hypertrophy, fibrosis and dysfunction.[17,18] These data suggest oxidative stress and inflammation drive cardiac pathologies and are prime targets for CVD therapy.

### 1.2. Diet and CVD

Diet and nutrition play a key role in health and disease, in which dietary intervention can ameliorate type II diabetes, cancer progression and CVD.[19] Poor dietary habits attribute 13.2% to overall CVD mortality in the U.S.[20] Similarly, hyper-caloric intake is linked to the development of hypertension and type II diabetes, two major risk factors for CVD and HF.[21] By reducing calories and body weight, low-density lipoproteins (LDL), a classic marker and target of heart disease, have been shown to additionally reduce.[22] Conversely, saturated fat intake correlates with plasma LDL.[23] The American Heart Association, World Health Organization and Academy of Nutrition and Dietetics have stressed that the consumption of fruits, vegetables and other plant-based foods should compose the majority of ones diet to reduce the risk of developing CVD and other morbidities.[24–28] These foods are high in vitamins, minerals and phytochemicals that actively participate in biological processes that govern health and are also low in

calories. Indeed, implementation of the dietary approaches to stop hypertension (DASH) diet and the Mediterranean diet, which emphasize plant-based foods, substantially improved cardiac function in patients at risk of HF.[29] Unfortunately, plant-based foods that contain beneficial nutrients and phytochemicals are, for the most part, under-consumed in the U.S.[20,24] Recent data suggest that plant-based foods confer health independently of their essential nutrients.[30] Thus, phytonutrients may play the primary role of protecting heart health and deterring heart disease.

## 2. Epigenetic Modifications

### 2.1.The Basics

Traditionally, epigenetic modifications regulate differential gene expression independently from altering DNA sequences. Well-known epigenetic modifications occur on DNA and histone proteins and include DNA methylation as well as histone methylation, acetylation and acylation. Moreover, there are well-described catalytic enzymes that add/remove these epigenetic modifications, which include: DNA methyltransferases (DNMTs) for DNA methylation; histone methyltransferases for histone methylation; histone demethylases (HMDs) for histone demethylation; histone acetyltransferases (HATs) for histone acetylation; and histone deacetylases (HDACs) for histone deacetylation. DNMTs, such as DNMT1, promote gene repression by forming hypermethylated-induced heterochromatin (chromatin condensation),[31] inhibiting transcription factors from binding at promoter sites on genomic DNA[32] as well as coupling with other gene repressing proteins such as HDACs.[33] Histone deacetylation is regulated by HDACs, which mediate histone hypoacetylation towards heterochromatin and gene suppression, and HATs, which mediate histone hyperacetylation towards

euchromatin and gene upregulation. In contrast to histone acetylation, which primarily occurs on lysine residues, histone methylation can occur on lysine, arginine and several other amino acid residues on histone protein tails, subsequently resulting in either gene expression or suppression.

### 2.3. Epigenetic Modifications and CVD

Epigenetic modifications have been characterized in CVD. A recent epigenome-wide association study (EWAS) in myocardial infarct patients associated DNA methylation at several gene promoter regions with CVD risk including associated blood lipid levels that contribute to atherosclerotic events.[34] Further studies have also linked hypertension and inflammation with methylation status.[35] While these studies focused on methylation, others have reported changes in acetylation. For example, sirtuin levels were reduced in CVD patients, linking changes in acetylation to CVD.[36] Much like in age-associated diseases described in above sections, sirtuin deacetylase activity has been shown to have cardioprotective effects.[37] Consistent with these findings, histone acetyltransferase activity of p300 was shown to be necessary for agonist-induced cardiac hypertrophy.[38] Conversely, HDAC inhibition and histone hyperacetylation have been shown to attenuate cardiac/cardiomyocyte hypertrophy and associated fetal gene re-expression,[39,40] cardiac fibrosis[41] and cardiac dysfunction.[42] Class I HDACs seem to be necessary for the pathological cardiac hypertrophy as well as inflammatory signaling.[43,44] Likewise, recent reports demonstrate an important role for HDACs in age-related diastolic heart failure, where HDAC inhibition improved diastolic function.[45] These data combined, demonstrate an epigenetic role for DNA methylation and histone modification

in CVD risk and development, yet further studies are needed to determine the various molecular targets of the HAT and HDAC enzymes that relay different outcomes.

**3. Microbiome**

The human body hosts trillions of microorganisms, with the highest densities found in the gastrointestinal (GI) tract. Indeed, microbial densities in the colon can reach $10^{11}$ colony forming units per gram of content (cfu/g).[46] Here, the density of microbiota populations varies along the small intestine, cecum and large intestine (colon) as each GI region houses distinct microbial habitats that are affected by chemical and nutrient gradients. Specifically, the small intestine is more acidic and contains more antimicrobial compounds, which results in reduced microbial densities.[47] Moving down the GI tract, pH and, with it, microbial densities increase. In fact, microbial densities in the colon reach $10^{11}$ colony forming units per gram of content (cfu/g) compared to $10^{2}$ cfu/g in the small intestine.[46] These observations demonstrate that the gut microbiome is considerably larger than the genome of its host, and not surprisingly has been heavily investigated for its role in regulating health and disease. The gut microbiome has been suggested to play a key role in cardiovascular health and disease. Consistent with this postulate, changes in microbial communities have been linked to CVD-related events and risks,[48] including atrial fibrillation,[49] heart failure[50] and elevated blood lipids and cholesterol.[51,52] In addition, the gut microbiota has also been shown to modulate HDAC activity in the gut[53] and may serve as a critical bridge between the beneficial effects of polyphenols and improved cardiac function.

The human gut consists of a variety of bacteria, mainly species that belong to *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Cerrucomicrobia phylum*.

Among them, *Bacteroidetes* and *Firmicutes* are the most prominent species[54] and often used as a ratio to mark pathological gut dysbiosis.[55] Gut microbiota regulate, in part, host immunity, energy metabolism, hormonal balance and fermentation and synthesis of metabolites such as short-chain fatty acids (SCFA), amino acids and vitamins.[46] The composition of the gut microbiome of a healthy person can vary considerably from one person to another. This variation between individuals is due to a number of factors including age,[56] environment,[57] prescription drug use[58] and, of interest, diet.[59] As the gut microbiome is considerably larger than the genome of its host, a shift in focus has emerged towards understanding and targeting the microbiome in health and disease.

### 3.1. Microbiome and CVD

A growing body of evidence suggests gut microbiota and its metabolites are involved in cardiovascular health and disease. For example, Jie et al. found distinct differences in microbial taxa between 187 healthy controls and 218 patients diagnosed with atherosclerotic cardiovascular disease (ACVD).[60] Here, *Streptococcus* and *Escherichia coli*, which are associated with inflammation, were enriched while *Roseburia intestinalis* and *Faecalibacterium cf. prausnitzii*, which synthesize short chain fatty acids (SCFAs), were depleted in ACVD patients.[60] The Bogalusa Heart Study linked *Alloprevatella* and *Catenibacterium* enrichment with low lifetime CVD risk and *Prevotella 2*, *Prevotella 7*, *Tyzzerella* and *Tyzzerella 4* enrichment with high lifetime CVD risk,[61] while others have shown that changes in the human microbiome are linked to CVD-related events and risks,[48] including atrial fibrillation,[49] heart failure,[50] elevated blood lipids and cholesterol[51,52] and hypertension.[62]

Indeed, clear differences in microbial communities and metabolites, e.g., SCFAs, were observed between normotensive and hypertensive patients.[62] Additionally, Kim et al. showed that *Ruminococcus torques*, *Eubacterium siraeum* and *Alistipes finegoldii* were positively associated with systolic blood pressure and intestinal inflammation while *Bacteroides thetaiotaomicron*, which protects intestinal junctions, was negatively associated with systolic blood pressure.[63] Animal experiments have strengthened the microbiome-hypertension postulate, as germ-free mice exposed to angiotensin II-induced hypertension experienced attenuated cardiac fibrosis, inflammation and systolic dysfunction compared to their conventionally-raised counterparts.[64] Finally, Adnan et al. showed an increase in the *Firmicutes:Bacteroidetes* ratio, a commonly used indicator of gut dysbiosis, and systolic blood pressure in rats gavaged microbiota from spontaneously hypertensive rodents.[65] As hypertension is one of the major risk factors for heart disease, these aforementioned studies yield promise in targeting the microbiome and particular metabolite-producing microbiota to alleviate CVD.

Gut microbiota are involved in second-phase metabolism of phytochemicals, specifically through xenobiotic reactions of ring-cleavage, reduction, decarboxylation, demethylation and dihydroxylation reactions, which synthesize phytochemical metabolites.[66] These phytochemical metabolites are then either excreted via feces or transported into circulation as compounds that may or may not be more bioavailable and/or bioactive than their parent compound.[66,67] Thus, one interaction between phytochemicals and the microbiome that may influence host health is by synthesizing metabolites via xenobiotic metabolism. A second phytochemical-microbiome interaction

that may influence host health is microbiota recomposition driven by phytochemical exposure.[68,69]

## 4. Phytochemicals

Phytochemicals are secondary plant metabolites that are synthesized to help a plant thrive or deter competitors, predators and pathogens.[70,71] Phytochemicals can further interact in human biological processes after ingestion to promote health. In fact, phytochemicals classically reduce oxidative stress and attenuate inflammation which benefit the heart.[72] Fruits, vegetables, nuts, seeds, legumes, whole grains, herbs and natural spices are common dietary items that contain phytochemicals in varying concentrations. Moreover, phytochemicals and their parent plants have been used in traditional medicines for centuries. Thousands of phytochemicals have been identified to date with more likely to be discovered and described.[73] Currently, phytochemicals are characterized into one of six different classes: polyphenols/phenolics, alkaloids, N-containing compounds, organosulfur compounds, phytoesterols, and carotenoids.[74] Following is a brief description of the different phytochemical groups as well as some compounds within these groups and their observed mechanisms in the heart. Eighteen of these phytochemicals we identified to inhibit HDAC activity,[75] an action that has been associated with blocking agonist-induced cardiomyocyte hypertrophy,[39] which is why we further investigated their cardioprotective actions in Chapter 2.

### 4.1. Polyphenols

The structure of polyphenols have been intensively reviewed.[76–78] Polyphenols are highly abundant in the plant kingdom and comprise a family of molecules with more than 8,000 structural variants. These secondary metabolites contain many aromatic rings with

one or more hydroxyl moieties.[79] Hydroxyl groups are classically recognized in oxidation-reduction reactions. Thus, many studies have focused on the anti-oxidant role for polyphenols in CVD.[70] As polyphenols are among the most abundant bioactive molecules in the plant kingdom, it is not surprising that polyphenols are among the most abundant phytochemicals consumed in the human diet. For this reason, polyphenols are important compounds to study in human health and disease. While oxidative stress and inflammation are the classical targets for polyphenol health protection, recent research indicates an important role for polyphenols in diet-gene regulation.[80,81]

Polyphenols are divided by chemical structure into two primary groups: phenolic acids and flavonoids. Moreover, polyphenols are distinguished by their hydroxyl moiety and their aromatic, phenyl rings. Phenolic acids contain the subgroups: hydroxycinnamic acids and hydroxybenzoic acids, while flavonoids contain the subgroups: flavanols, flavonols, flavones, flavanones, anthocyanidins, isoflavonoids, and proanthocyanidins. Other polyphenol groups include: lignans, stilbenes and quinones. Below, we highlight the role for these polyphenol subgroups and their compounds as epigenetic regulators in the heart.

### 4.1.1. Phenolic acids

Studies suggest that phenolic acids are inversely correlated with coronary heart disease mortality and heart attack incidence.[82] Phenolic acids contain two subgroups, hydroxycinnamic acids and hydroxybenzoic acids, which differ in carbon backbone length, with hydroxycinnamic acids containing an additional carbon bond. Both hydroxycinnamic acids and hydroxybenzoic acids contain a functional carboxyl group with potent metal chelation properties.[83] This would imply that hydroxycinnamic acids

and hydroxybenzoic acids can chelate zinc in order to inhibit zinc-dependent HDAC activity. Indeed, docking studies using HDAC8 confer that the carboxylic group of phenolic acids strongly interacts with the zinc ion, resulting in high HDAC inhibition potency.[83] Below we discuss recent findings regarding phenolic acid HDAC inhibitors in the heart.

#### 4.1.1.1. Hydroxycinnamic acids

Caffeic acid is one of the most abundantly consumed hydroxycinnamic acids.[84] Caffeic acid is found in most fruits, particularly the skin of ripened fruit.[85] However, the largest source for caffeic acid consumption is coffee.[86] Coffee has been linked to improvements in CVD, where coffee consumption was inversely correlated with death after acute myocardial infarction.[87] These epidemiological findings suggest that coffee and its phytochemicals have cardioprotective effects. Further *in vitro* and *in vivo* reports demonstrated efficacy for caffeic acid in CVD models.[88] Indeed, caffeic acid ethanolamide, a caffeic acid derivative, ameliorated cardiac oxidative stress in isoproterenol-induced HL-1 cells as well as in isoproterenol-induced cardiac diseased mice.[88] Additionally, caffeic acid attenuated cardiac dysfunction and fibrosis through HDAC regulation.[88] Similar to the pan-HDACi Vorinostat, caffeic acid phenethyl ester attenuated cardiac hypertrophy and ameliorated cardiac dysfunction in I/R-injured rabbits.[89] These therapeutic actions occurred in part by inhibiting MAPK activation.[90] As HDACs have been shown to regulate MAPK activity,[44] these data suggest that caffeic acid-mediated inhibition of HDACs protect the heart via MAPK inactivation. More recently, caffeic acid was shown to inhibit class I, IIa and IIb HDAC activity in cardiac lysate.[75] Unfortunately, no other studies have further examined the role for caffeic acid as

a zinc-dependent HDAC inhibitor in heart failure. Further delineation of the cardioprotective actions of caffeic acid and its derivatives would be of great interest due to their high intake through coffee consumption. Additionally, other dietary hydroxycinnamic acids such as coumaric acid and ferulic acid should be examined as regulators of HDAC activity in the heart. Both coumaric acid and ferulic acid have been reported to attenuate pathological cardiac remodeling. In addition, studies suggest that ferulic acid inhibits HDAC activity.[91–95] Combined, these studies would suggest that hydroxycinnamic acids protect the heart in part via direct changes in gene expression; hydroxycinnamic acids inhibit HDAC activity, leading to hyper-acetylation of nucleosomal histones.

#### 4.1.1.2. Hydroxybenzoic acids

Compared to foods containing their subgroup relative, those that contain hydroxybenzoic acids are consumed less and have lower phytochemical concentrations.[96] However, berries such as blackberries and strawberries are commonly consumed and contain substantial amounts of the hydroxybenzoic acids, gallic acid and ellagic acid. Black tea is also a good source of gallic acid and is of particular interest due to its large consumption and its correlation with reduced risk for coronary heart disease as well as stroke.[97,98] In addition, these compounds have been examined as nutraceuticals that can protect the heart.[99–102] For example, gallic acid has been shown to repress cardiac remodeling via inhibition of genes involved in advanced glycation end products (AGE) in rats.[99] Moreover, Umadevi et al.[99] reported that gallic acid attenuated cardiac fibrosis via inhibition of matrix melloproteinase (MMP) gene expression of MMP-2 and MMP-9. Inhibition of MMP gene expression was linked to decreased inflammation and the

intracellular signaling cascades, nuclear factor kappa beta (NF-κB) and extracellular signal-regulated kinase (ERK). HDACs have been reported to regulate both NF-κB and ERK signaling, where HDAC inhibition attenuated NF-κB and ERK activity.[41,44,103] These data suggest that cardioprotective actions of gallic acid are partially mediated through HDAC inhibition. Indeed, gallic acid was shown to dose-dependently inhibit class IIa and IIb HDAC activity resulting in cardiac protection.[100] While this study supports that postulate that hydroxybenzoic acid HDAC inhibitors protect the heart via changes in gene expression, the evidence is far from conclusive. Thus, further studies are warranted to examine the role for gallic acid and other hydroxybenzoic acids on global changes in histone acetylation and gene expression.

### 4.1.2. Flavonoids

The largest polyphenolic group, the flavonoids, are aglycone structures that contain two active phenyl rings, which vary in hydroxylation between its subgroups: flavanols, flavonols, flavones, flavanones, anthocyanidins, isoflavonoids and proanthocyanidins. Currently there are approximately 6,000 flavonoids that are found in fruits, vegetables, herbs and medicinal plants. Research has shown that diets high in flavonoids reduced a person's risk for developing CVD as well as reduced CVD mortality rates. [104,105] Moreover, a meta-analysis of 15 cohort studies with 386,610 individuals and 16,693 deaths showed flavonoid intake was inversely correlated with CVD mortality in a dose-dependent manner.[106] Such findings confirm the importance of, and validate policies directed towards, consuming more fruits and vegetables. Notably, reports have shown that flavonoids have metal-binding chelating properties,[107,108] and thus suggest potential roles for flavanoids as HDAC inhibitors for cardio-protection.

### 4.1.3. Flavonols

Flavonols are 3-hydroxy derivatives of flavones and contain a number of commonly studied phytochemicals that include quercetin. Quercetin is the most consumed flavonol and is abundant in tea, apples, onions and berries.[109,110] Quercetin intake is inversely correlated with ischemic heart disease mortality in a dose-dependent manner.[111] In addition, quercetin has been shown to protect against ischemia/reperfusion injury, isoproterenol-induced cardiac injury, aortic constriction-induced cardiac remodeling and diabetic cardiomyopathy.[112–116] Of interest, two independent double-blind, placebo-controlled trials demonstrated that quercetin ameliorated hypertension in patients at risk for CVD and reduced plasma oxidized low-density lipoproteins (oxLDLs) responsible for atherosclerotic disease. [117,118] Few reports, however, have shown quercetin's mechanistic action of cardioprotection through acetyl-lysine regulation. Hung et al. showed that quercetin attenuated oxLDL-induced atherosclerotic injury by increasing the class III HDAC Sirt-1.[119] Our lab demonstrated that quercetin inhibited class I and II HDACs in bovine cardiac tissue.[75] Others have reported that quercetin can inhibit class I HDACs in cancer cell models and that these actions are in part responsible for the anti-carcinogenic actions associated with quercetin [120,121]. As an HDAC inhibitor, quercetin would alter the electrostatic interactions between DNA and histone proteins, directly impacting gene expression and therefore effecting cellular fate. While the role for quercetin in cardio-protection is undeniable, studies examining the epigenetic impact for quercetin remain underexplored. Thus, further investigation for quercetin as an HDAC inhibitor in cardiac biology is warranted.

Kaempferol is a flavonol found in a variety of foods like teas, tomatoes, hops, grapes, grapefruit, strawberries, broccoli, honey, apples and beans.[122] Kaempferol is the second-most consumed flavonol in the U.S. behind quercetin, mostly in the form of green and black tea.[109] Similar to quercetin, kaempferol intake is inversely correlated with ischemic heart disease mortality[111] and kaempferol treatment is efficacious in *in vitro* and *in vivo* CVD models.[123–127] Indeed, I/R-induced cardiac injury was ameliorated with kaempferol treatment; this was linked to inhibition of the MAPK pathway.[125,126] As HDAC inhibitors have previously been shown to attenuate MAPK signaling in the heart, these data would suggest a potential role for kaempferol as an HDAC inhibitor.[41,44] Kaempferol has also been shown to attenuate cardiac injury and oxidative stress in I/R-injured rats by inhibiting glycogen synthase kinase-3β activation (GSK-3β).[127] As the class I HDAC, HDAC2 was recently shown to regulate GSK-3β signaling,[128] these data support the postulate that kaempferol protects the heart in an HDAC-dependent manner. Consistent with this postulate, kaempferol was recently shown to inhibit HDAC activity, which led to increased histone acetylation.[129] Berger et al.[129] further showed that kaempferol docked to class I HDACs 2 and 8 as well as class IIa HDACs 4 and 7, suggesting that this binding may inhibit HDAC activity. Finally, we reported that kaempferol inhibited HDAC activity and increased histone acetylation in cardiac lysate.[75] As a next step, experiments are underway to determine if the cardioprotective effects of kaempferol are mediated through HDAC-dependent inhibition. These studies would also examine the impact for green and black tea extracts in the regulation of HDAC inhibition and cardiac disease, although additional tea compounds would likely impart additive or synergistic actions towards HDAC activity (e.g. EGCG). As others have shown that anti-

carcinogenic actions for kaempferol are regulated, in part, through changes in lysine acetylation,[130] we anticipate promising findings that would demonstrate that kaempferol-dependent HDAC regulation links diet-gene interactions in an epigenetic-dependent manner in the heart.

Myricitrin and its aglycone, myricetin, are two naturally occurring flavonols that were first isolated in the early 1900s from bark of the bayberry tree (Myrica nagi).[131] Bayberry has been a cultural staple in Asian countries for over 2,000 years[132] and the tree's therapeutic properties in traditional medicines have led to current studies of these two flavonols. Myricitrin is primarily synthesized in the bayberry tree's fruit, bark and leaves,[133] while myricetin is also found in a variety of other foods including tea, wine, berries and vegetables. The majority of myricetin consumption is from tea; however, its intake is quite low in comparison to other flavonoids like kaempferol and quercetin.[109] The bioactivity of myricetin and myricitrin are very similar to each other due to sharing of functional groups. Both phytochemicals exhibit anti-inflammatory and anti-oxidant properties,[132,133] which have been suggested as a major mechanism for their cardioprotective actions.[134–136] However, additional studies have reported cardio-protection for myricitrin and myricetin that involve regulation of intracellular signaling cascades and gene expression. For instance, myricetin was shown to attenuate I/R-induced cardiac injury by inhibiting signal transducer and activator of transcription 1 (STAT1) activation;[137] inhibition of JAK/STAT signaling would be expected to alter gene expression in the heart. Two other reports showed that myricitrin attenuated diabetic cardiomyopathy as well as hyperglycemia-induced cardiomyocyte apoptosis via changes in PI3K/Akt and MAPK signaling.[138,139] Cardiac myocytes exposed to hyperglycemic

conditions and treated with myricitrin had reduced apoptosis via Akt-nuclear factor erythroid 2-related factor 2 (Nrf2) inhibition.[138] Similarly, myricitrin attenuated diabetic cardiomyopathy by inhibiting ERK phosphorylation, Nrf2 expression and NF-κB.[139] As Nrf2 and NF-κB are transcription factors, these data would suggest that myricitrin regulates cardiac gene expression via regulation of intracellular signaling cascades. HDAC inhibitors have previously been shown to regulate Akt,[140] MAPK phosphorylation[41,44] and NF-κB.[103] Only one report to date, however, has shown myricetin and myricitrin regulated lysine acetylation through HDAC inhibition.[75] Thus, investigation into the role for these two compounds as bioactive HDAC inhibitors in the heart is warranted.

#### 4.1.4. Flavones

Flavones are synthesized from flavanones via flavone synthases. These polyphenols distinctly contain a double bond between carbons two and three on the heterocyclic pyran ring (also known as the C ring), which is further attached to an aromatic phenyl ring.[141] Multiple hydroxyl groups that are attached to this phenyl ring provide flavones with their function, especially regarding redox reactions.[141] Flavone consumption is less than flavonols, but are well-represented in research studies. Apigenin and luteolin, as well as their glycosides, are two of the major flavones currently being investigated in the heart. Apigenin is found in citrus fruits, onions, parsley and chamomile.[142] Several reports have shown that apigenin is cardioprotective.[143–146] Similar to other flavonoids, apigenin was shown to attenuate I/R-induced cardiac injury via inhibition of MAPK signaling[143,145] and Nrf2 transcriptional activation.[144] These reports are interesting as they suggest that apigenin protects the heart through intracellular

signaling and gene expression. Again, inhibition of HDACs has been linked to MAPK inactivation and control of transcription factor activation.[41,44,103] In addition, we and others have shown that apigenin inhibits class I HDACs activity.[75] Inhibition of HDAC activity by apigenin has been linked to hyper-acetylation of histone proteins in cancer models that contributes to cancer cell death.[147,148] Collectively, these data would suggest that cardio-protective actions for apigenin is controlled in part via HDAC-dependent mechanisms that necessitate epigenome wide changes in gene expression.

Luteolin is commonly found in celery, parsley, broccoli, onions, carrots, peppers, cabbages and apples.[149] These foods and other plants, such as the chrysanthemum flower, have been used in traditional Chinese medicine for the treatment of hypertension as well as to fight microbial infections.[150] Unlike other flavonoids, epidemiological studies examining the cardio-protective role for luteolin remains unclear;[151,152] this may partly be explained by the low intake of this flavone in the diet [111]. In cell culture and rodent models however, luteolin has shown clear cardio-protection. Mechanistic actions for luteolin generally involve regulation of sarcoplasmic reticulum $Ca^{2+}$-ATPase 2a (SERCA2a).[153–155] SERCA2a is decreased in the failing heart, which leads to impaired calcium-re-uptake and cardiac contractile dysfunction.[156] Post-translational modification of SERCA2a has been suggested as critical for SERCA2a function; modifications from small ubiquitin-related modifier 1 (Sumo1) and phosphorylation via MAPK activation appear important for SERCA2a-dependent calcium re-uptake into the sarcoplasmic reticulum.[156,157] Recent findings showed that class I HDAC inhibition promoted SERCA2a SUMOylation;[158] this would be expected to improve cardiac contractility. Notably, luteolin was reported to inhibit class I HDAC activity as well as increase lysine

acetylation on histone H3 in cardiac myoblasts.[75] Further, docking studies demonstrated that luteolin binds within the catalytic domain of class I HDACs to inhibit HDAC activity.[159] Finally, luteolin was reported to attenuate cardiac dysfunction through regulation of Akt and MAPK signaling.[155,160] Similar to other flavonoids, these data would suggest that luteolin attenuates MAPK phosphorylation via inhibition of HDAC activity and that this attenuates cardiac remodeling and dysfunction. This postulate is currently being tested.

*Scutellaria baicalensis* was used as an herbal remedy in traditional medicine to treat bacterial and viral infections, specifically hepatitis, but has since shown efficacy for the treatment of hypertension, inflammation, oxidative stress and cancer.[161] While over 50 flavonoids have been isolated from this mint plant for traditional Chinese and Japanese medicine, baicalin and baicalein constitute its major phytochemicals.[162] These two phytochemicals only differ in that baicalein has a distinguishable aglycone.[163] With regards to the heart, baicalein[164–166] and baicalin[167–173] have shown efficacy in ischemia- and isoproterenol-induced cardiac dysfunction. Similar to other flavonoids, baicalein and baicalin elicit cardo-protection by inhibiting oxidative stress, inflammation, as well as attenuation of MAPK signaling.[159–161,164,166–169] Baicalein was also reported to inhibit cardiac hypertrophy and fibrosis in mice exposed to aortic constriction,[174] this was partly explained by inhibition of ERK phosphorylation.[174] Similar results were shown for baicalin, in which baicalin-mediated ERK inactivation improved isoproterenol-induced cardiac dysfunction,[169] bleomycin-induced pulmonary hypertension[171] and myocardial infarction.[173] These studies did not examine the epigenetic actions for baicalein or baicalin in the regulation of heart function. Others have reported that baicalein inhibited

HDAC4 and HDAC5 expression contributing to monocyte chemoattractant protein-1 (MCP-1) gene suppression in retinal ganglion cells.[175] Baicalin was shown to inhibit HDAC2 activity leading to suppression of inflammatory genes and improved pulmonary function in mice exposed to cigarette smoke.[176] Other reports showed that baicalin attenuated HDAC1 expression, which suppressed pain sensation in a rodent model of spinal cord injury.[177] These findings demonstrate that baicalein and baicalin act as potential HDAC inhibitors for the treatment of various disease. Coupled with our more recent findings that baicalein and baicalin inhibited HDAC activity in cardiac tissue,[75] these data would suggest that future studies for these two phytochemicals as epigenetic regulator of cardiac function is warranted.

### 4.1.5. Flavanols

Flavanols, or catechins, are structurally similar to flavonols but differ in the heterocyclic C ring; flavanols do not contain a double carbon bond that thus allows four diastereoisomers to form from two chiral centers.[178] These phytochemicals are commonly found in chocolate, in the skins of apples and berries as well as in teas. Notably, epigallocatechin gallate (EGCG) is a flavanol that is abundant in the leaves of the green *Camellia sinensis* plant.[179] The compounds in these leaves are mostly consumed as the beverage green tea, and have been used in traditional medicines for thousands of years around the world. Epidemiological research has shown that tea consumption is cardio-protective, particularly in overweight and obese individuals.[180] Indeed, evidence supports that EGCG is cardio-protective, and is particularly thought to improve heart function via inhibition of oxidative stress and inflammation; these actions have been attributed to the eight hydroxyl groups in EGCG.[181–188] In these reports, EGCG was shown to inhibit

diabetic cardiac dysfunction[182,183] and chemotherapy-induced cardiotoxicity.[184,186] In addition to its actions as an antioxidant and anti-inflammatory, EGCG acts as a chelator.[189,190] This suggests that EGCG can interact with and chelate zinc within the catalytic domain of HDACs. In support of this, EGCG has been reported to inhibit HDAC activity, although docking studies have yet to be performed.[75] In addition, EGCG was shown to attenuate age-related cardiac dysfunction, in part, through increased acetylation of histone H3 at the cardiac troponin I promoter; this increased troponin's expression and improved muscle function.[191] Increased histone acetylation was likely due to inhibition of class I HDAC activity.[191] Additional reports have shown that EGCG inhibited HDAC3 activity in the heart, which led to FoxO1 hyper-acetylation and attenuation of hyperglycemia-induced apoptosis;[192] FoxO1 plays an important role in apoptosis.[193] Based on these findings and considering that tea is heavily consumed worldwide, it would be interesting to elucidate HDAC activity in the peripheral blood mononuclear cells (PBMCs) of patients before and after green tea consumption. PBMCs have been used as indirect read-outs for disease states in patients with type II diabetes and CVD.[194,195]

### 4.1.6. Flavanonols

Flavanonols are 3-hydroxy derivatives of flavanones and are also known as dihydroflavonols.[178] Phytochemicals identified as flavanonols are sparse within the literature. However, dihydromyricetin is a flavanonol that has been implicated in health and disease.[196] In regards to the heart, reports have shown that dihydromyricetin is protective in I/R-induced cardiac injury,[197] angiotensin II-induced cardiac fibrosis,[198,199] diabetic cardiomyopathy[200] and lipopolysaccharide (LPS)-induced cardiac injury.[201]

Dihydromyricetin elicited its cardio-protective effects in part by acting as an anti-oxidant, anti-inflammatory and an inhibitor of the NF-κB pathway.[198–201] While no study has examined the role for dihydromyricetin in the epigenetic regulation of gene expression, recent findings from our lab showed that dihydromyricetin inhibited HDAC activity.[75] These data, while preliminary, highlight the potential for dihydromyricetin as an epigenetic modifier of gene expression for the prevention and or treatment of cardiac disease.

### 4.1.7. Proanthocyanidins

Proanthocyanidins are abundant in the diet as they are found in fruits such as grapes, peaches, apples, pears and berries as well as wine, tea and beer.[202] These compounds are the subsequent products of catechins and form dimer, oligomer and polymer complexes that promote their bioactivity.[203] Studies show that proanthocyanidins protect the heart; many of these studies reported anti-oxidant and anti-inflammatory properties for proanthocyanidins.[203] For example, grape seed procyanidin (GSP) was shown to improve cardiac function by inhibiting inflammation and oxidative damage.[204,205] A systematic review/meta-analysis examined GSP intake in the regulation of blood pressure, heart rate, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, total cholesterol, triglycerides and C-reactive protein.[206] This report demonstrated that GSP extract lowered systolic blood pressure and heart rate but did not significantly affect other cardiac markers. Other reports have shown proanthocyanidins are efficacious for the treatment of human hypertension.[207] Consistent with these reports, experimental rodent models of cardiac disease demonstrated that GSP extract protected the heart in response to high fat diet,[204,205,208] doxorubicin-induced cardiotoxicity,[209–213]

heavy metal-induced cardiac stress,[214–216] isoproterenol-induced HF[217–219] and I/R injury.[220–224] Additional studies reported that GSP extract lowered liver and blood cholesterol and triglycerides;[225–228] this would suggest CVD protection. Moreover, GSP extract was shown to inhibit HDAC activity, specifically HDACs 2 and 3, and increase histone acetylation in the liver;[228] this was suggested to impact nuclear hormone receptor expression and lower serum triglycerides.[228] These results are interesting and suggest that cardio-protective actions of GSP result from HDAC inhibition. This postulate is currently under investigation.

### 4.2. Stilbenes

Stilbenes are a small group of phytochemicals that are derived from the phenylpropanoid pathway via stilbene synthase.[229] While stilbene concentrations are low in the diet, resveratrol is an exception. Resveratrol is found in wine as well as grapes and berries[230] and has been credited for the "French Paradox;" CVD rates in France are lower than the rest of the world despite their high intake of saturated fats.[231] Indeed, studies suggest that resveratrol is cardio-protective.[230,232–234] Resveratrol was reported to attenuate cardiac damage in response to myocardial infarction,[235–239] pressure overload[240–244] and hypertension.[245–250] These reports demonstrated resveratrol inhibited oxidative stress and upregulated AMP-activated protein kinase (AMPK) expression and activity.[238] Other reports have confirmed resveratrol improves AMPK levels in the heart.[244] AMPK senses energy needs and stress in the heart. In response to cardiac remodeling, compensatory mechanisms activate AMPK;[251] AMPK activation has been shown to improve cardiac dysfunction.[251] Thus, resveratrol-mediated activation of AMPK is considered cardio-protective. In addition, resveratrol has been shown stimulate

class III sirtuin HDAC activity. This topic has been thoroughly reviewed elsewhere.[252] Notably, the class III HDAC, Sirt1, regulates AMPK, thus leading to a mechanism by which resveratrol-mediated activation of Sirt1 stimulates AMPK expression and activity.[238] Sirt1 is a deacetylase that has been shown to deacetylate lysine residues on histone tails.[253] Thus, most studies have shown that unlike the phytochemicals discussed above, resveratrol attenuated diabetic cardiac remodeling concomitant with histone H3K9 deacetylation and changes in gene expression. This would suggest that class III HDAC inhibition has negative consequences in the heart. It should be noted that recent proteomics studies have shown that mitochondrial proteins are hyper-acetylated in failing hearts. Moreover, hyper-acetylation of mitochondrial proteins likely results from down-regulation of class III HDACs, which predominantly localize to the mitochondria.[254,255] While these data support a role for resveratrol in the "French Paradox," doses of resveratrol used in these studies significantly exceed concentrations found in the diet.[230] Nutraceutical companies, however, have developed supplements for human consumption. These nutraceutical may impart benefits as a recent double-blind, randomized control demonstrated that patients who received 500 mg resveratrol had reduced histone H3K56 acetylation, increased anti-oxidant activation in peripheral blood mononuclear cells (PBMCs) and reduced body fat.[256] While resveratrol activates class III HDACs, its role with zinc-dependent HDACs remains less well-studied. Resveratrol was shown to inhibit class I, II and IV HDACs in hepatoma cells.[257] This would suggest that resveratrol can stimulate the activity of class III $NAD^+$-dependent HDACs and also inhibit zinc-dependent HDACs. Thus, bioactive food compounds may serve multiple epigenetic roles in the control of human health and disease.

### 4.3. Other Polyphenols

Turmeric is a yellow-pigmented spice that has been used in several cultures, including Indian and Southeast Asian for centuries. Tumeric was traditionally used to treat inflammation and flu-like illnesses.[258] Tumeric is isolated from rhizomes of the plant *Curcuma longa* and contains several phytochemicals known as curcuminoids, including the well-studied curcumin.[259] Curcumin is a polyphenol that has several hydroxyl groups and two aromatic phenyl rings, each of which contain a functional methoxy group.[259] Curcumin has been studied for the treatment of many diseases including cancer, Alzheimer's disease, rheumatoid arthritis and cardiac disease.[260] In the heart, curcumin has been shown to attenuate free fatty acid-induced injury,[261] I/R-induced injury,[262] chemo-induced cardiotoxicity,[263,264] hypertension-induced cardiac remodeling,[265] diabetes-induced cardiac injury[266,267] and trauma-induced cardiac dysfunction.[268] Moreover, reports suggest that curcumin's cardioprotective effects are translatable to humans.[269–274] Of these, curcumin was shown to reduce circulating triglycerides[272–274] and improve cholesterol status,[272] which are two known risk factors in the development of heart disease. Recently, curcumin was shown to inhibit p300/cAMP response element binding protein (p300/CBP)-mediated GATA4 acetylation via inhibition of HAT activity.[275,276] GATA4 acetylation by p300/CBP stimulates GATA4 transcriptional activation and thus promotes pathological cardiac gene expression leading to cardiac hypertrophy.[277] Moreover, adrenergic-agonist-induced cardiac myocyte hypertrophy was attenuated with curcumin treatment concomitantly with GATA4 deacetylation as well as inhibition of GATA4-DNA binding in hypertensive rats.[278] In addition to its inhibitory actions on HATs, curcumin was shown to act as a pan-HDAC

inhibitor targeting zinc-dependent HDACs in cancer.[279] Similar to resveratrol, these data suggest multiple levels of epigenetic regulation for curcumin in the regulation of diet-gene interactions. These data also highlight curcumin as a promising nutraceutical for CVD and HF; although continued work on curcumin bioavailability is warranted.[280,281]

### 4.3.1. Alkaloids

Dietary alkaloids are widely consumed. Alkaloids are precursor compounds that can be derived from ornithine, lysine, tyrosine, tryptophan, nicotinic acid and purine.[282] For example, berberine is an isoquinoline alkaloid derived from tyrosine that naturally occurs in edible and herbal plants including *Hydrastis canadensis, Coptis chinensis, Berberis aquifolium, Berberis vulgaris, and Berberis aristata*. Moreover, traditional Indian and Chinese medicines have used berberine-rich plants for the treatment of viral and bacterial infections.[283] More recently, berberine was shown to attenuate diabetes and improve metabolic function.[284,285] In these studies, berberine improved insulin sensitivity through AMPK activation[284] as well as reduced LDL, total cholesterol and circulating triglycerides and increased HDL in the blood.[285] This is of interest, as diabetes and metabolic dysfunction are major risk factors for the development of cardiac disease. In regard to the heart, berberine was shown to attenuate myocardial fibrosis in diabetic rats; these actions were mediated through inhibition of TGF-β1/Smad.[286] In addition, it has been reported that berberine improved cardiac function in hypertensive rats by inhibiting STAT3 binding and promoting STAT5a binding to the promoter region of the relaxin gene; this increased relaxin gene expression and subsequently attenuated cardiac fibrosis.[287] Switching of STAT3 for STAT5a at the relaxin gene promoter is controlled by histone H3 acetylation.[288] This is critical, as we published that berberine hydrochloride

inhibited class I and II HDAC activity.[75] Combined, these data would suggest that berberine-mediated HDAC inhibition would increase histone H3 acetylation at the relaxin gene promoter to inhibit cardiac fibrosis. Further examination of this hypothesis in the heart would be interesting and provide epigenetic mechanisms by which berberine regulates gene expression.

Danggui Longhui Wan is an active alkaoloid that has been used for over 4,000 years. Danggui Longhui Wan was the customary treatment for chronic myelocytic leukemia and has had moderate success in leukemic disorders without major side effects.[289] The primary bioactive phytochemical in the medicinal recipe, indirubin, has since been isolated and characterized with several aromatic rings. The role for indirubin in cancer has been intensively reviewed.[290] In regard to the heart, indirubin and its derivatives protect against hyperglycemia-induced cardiac injury, aortic constriction-induced hypertrophy, I/R injury, hyperlipidemia-induced cardiac injury and diabetes-induced cardiomyopathy.[291–296] Cardiac protection was shown to be mediated in part through attenuation of c-Jun-N-terminal kinase (JNK) signaling, caspase-3-directed apoptosis and NF-κB expression.[291] Others have reported that indirubin regulated GSK-3β signaling in order to protect cardiac function.[292–296] These results are interesting as class I HDACs have been shown to regulate GSK-3β signaling,[128] JNK phosphorylation[44] and NF-κB activation.[103] As we reported that indirubin inhibited HDAC activity in cardiac tissue,[75] these data would suggest that cardio-protection is mediated in part through HDAC-dependent actions. Further investigation is needed to elucidate the epigenetic role for indirubin in diet-gene regulation within the heart.

### 4.3.2. Isothiocyanates

Many foods contain phytochemicals with one or more sulfur groups and are commonly known as organosulfur compounds. Of these, isothiocyanates have been linked to attenuation of cancer, diabetes and CVD. Sulforaphane is an isothiocyanate that is found in cruciferous vegetables like broccoli and cauliflower. Early studies showed that sulforaphane inhibited zinc-dependent HDAC activity and thus blocked cancer proliferation and induced cancer cell death.[297–302] Importantly, these studies showed that sulforaphane blocked HDAC activity in cell culture, rodents and humans fed broccoli sprouts.[297–302] In experimental models of heart disease, sulforaphane attenuated chemotherapy-induced cardiotoxicity,[303,304] I/R injury, [305,306] angiotensin II-induced hypertrophy,[307] myoblast apoptosis,[308] diabetes-induced cardiomyopathy [309,310] and aortic constriction-induced HF. [311] These studies consistently showed that cardio-protective effects of sulforaphane were via inhibition of oxidative stress. This likely resulted from Nrf2 upregulation,[309,310] a transcription factor that regulates genes involved in the oxidative stress response. As previously mentioned, class III HDACs regulate Nrf2.[103,312,313] In addition to its actions directed at Nrf2 induction, sulforaphane was shown to block oxidative stress-induced AMPK inhibition.[309] AMPK is downstream of Nrf2 and upstream of the class III HDAC, Sirt1.[238] In addition to its role in the regulation of zinc-dependent HDACs and sirtuins, sulforaphane was also shown to attenuate cardiac hypertrophic gene expression by inhibiting GATA4/6 transcriptional activation; this was likely mediated by inactivation of the MAPKs.[314] HDAC inhibition has previously been shown to inhibit MAPK activity.[44] Conversely, HAT inhibition controls GATA4 acetylation and subsequent activation.[275,276] However, no report examined the role for

sulforaphane in the HDAC-dependent regulation of CVD or HF. This is interesting considering its historical role as a pan-HDAC inhibitor in cancer. Moreover, sulforaphane has been translated from bench to bedside demonstrating efficacy for this compound as an HDAC inhibitor.[301,302] Combined, these studies suggest further investigation of sulforaphane as an epigenetic regulator of gene expression and cardiac function are warranted. Similar to curcumin and resveratrol, sulforaphane likely regulates many epigenetic pathways in the control of human health and disease, and these studies too should be performed. Lastly, other isothiocyanates, including phenethyl isothiocyanate (PEITC), should be investigated in the heart as preliminary evidence suggest a cardio-protective role for PEITC[315] as well as a potential role for PEITC as an HDAC inhibitor.[316,317]

### 4.4. Other Food Bioactives

Butyrate is a short-chain fatty acid metabolized from bacteria within the large intestine and is a well-known short-chain fatty acid HDAC inhibitor.[318] Recent data suggests that gut bacteria play an important role in biological function that governs human health and disease.[319] For example, these bacteria, or gut microbiota, synthesize butyrate from consumed fibrous, plant-based foods and, once synthesized, butyrate has been shown to inhibit cancer,[318] diabetes[320] and CVD.[321] While no epidemiological studies were found linking butyrate to heart health, there is no doubt that consuming fruits, vegetables and other fibrous, plant-based foods are cardio-protective. Moreover, experimental studies have shown that butyrate is cardio-protective; these studies demonstrated that butyrate protects the heart in an HDAC-dependent manner.[322,323] Butyrate was shown to improve cardiac function via HDAC inhibition in diabetic

mice.[322] Moreover, GLUT1 and GLUT4 were upregulated via GLUT1 acetylation and p38 phosphorylation, leading to improvements in glucose uptake.[322] Similarly, butyrate improved serum cholesterol and left ventricle function via HDAC inhibition in diabetic mice.[323] Like butyrate, valproic acid has been shown to improve cardiac function by acting as an HDAC inhibitor.[324] As valproic acid is currently approved for the treatment of epilepsy, these data would suggest that short-chain fatty acid HDAC inhibitors are safe and tolerated in humans. Thus, investigation of HDAC activity in the PBMCs of patients treated with short-chain fatty acids would be of keen interest. However, it should be cautioned that millimolar doses of short-chain fatty acids are required for HDAC inhibition, and thus these compounds likely elicit off-target actions that may contraindicate their therapeutic use for the treatment of CVD/HF.

**5. Emodin**

Plants contain enzymes like polyphenol oxidase that catalyze a multitude of reactions such as oxidation-reduction. Quinones are one product of these reactions and are synthesized from organic, aromatic compounds.[325] Quinones are not aromatic but conjugated, and contain at least one benzene-like ring with redox functionality.[326] Anthraquinones are a subgroup of quinones that participate in redox reactions, in particular the regulation of hydrogen peroxide.[326] Emodin is an anthraquinone found in plants often used in traditional Chinese medicines, such as buckthorn, knotweed, rhubarb and Da Huang, which is a primary ingredient for the decoction, Dahuang Fuzi,[327] as well as other plant-based foods like beans and cabbage.[328,329] As herbal remedies, emodin-rich plants have been used for viral and bacterial insults, kidney disorders and gastrointestinal distress.

### 5.1. Emodin and heart health

Due to its strong redox function and recently discovered anti-inflammatory properties, emodin has been investigated in the heart. Reports showed that emodin inhibited I/R-induced cardiac damage via improvements in mitochondrial redox regulation.[330,331] Emodin was also reported to attenuate cardiac dysfunction in left coronary artery ligated mice, in part, though inhibition of NF-κB signaling and subsequently inflammation.[332] However, emodin is a strong metal chelator,[333] suggesting that emodin can bind to and inhibit zinc-dependent HDACs. Consistent with this hypothesis, our lab published that emodin inhibited class I and II HDACs and increased histone acetylation in cardiac myoblasts.[75] Further data from our lab suggest that emodin inhibits cardiac myocyte hypertrophy, in part, through HDAC-dependent mechanisms. Combined, these observations would suggest an epigenetic function for emodin via HDAC inhibition. Our lab is currently investigating the *in vitro* and *in vivo* epigenetic implications for emodin and emodin-rich foods like rhubarb to delineate its role in diet-gene interactions.

### 5.2. Emodin as an epigenetic modifier

In relation to epigenetics, early reports showed emodin was efficacious in cancer models by regulating DNA methylation.[334–336] However, recent evidence showed that emodin was able to inhibit the removal of histone H3K27 trimethylation in macrophages exposed to inflammatory stress.[337] This was particularly apparent at promoter regions of inflammatory signaling molecules, inducible nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNFα), interleukin 6 (IL6), interferon regulatory factor 4 (IRF4), arginase 1 (Arg1) and chitinase 3-like protein 3 (YM1) in M1 or M2 macrophage

phenotypes.[337] Further, emodin inhibited nuclear translocation of interferon regulatory factor 5 (IRF5), nuclear factor kappa B p65 (NF-κBp65) and signal transducer and activator of transcription 1 (STAT1) in M1 macrophages as well as STAT6 and IRF4 in M2 macrophages. [337] Activation of macrophages by pro-inflammatory signaling molecules is important to the anti-inflammatory response.[338] However, macrophage hyperpolarization into either M1 or M2 phenotypes is deleterious to health.[338,339] The M1 hyperpolarized macrophage is of particular interest in neurodegenerative disease and has been suggested as a target for phytochemicals.[340] Indeed, emodin has been shown to be protective in multiple neurodegenerative disease models, including cerebral ischemic stroke,[341] traumatic brain injury [342] and AD.[343] In the above report, emodin restored the balance away from M1 and M2 hyperpolarization phenotypes[337] and thus suggests that emodin may provide protection against brain pathologies through epigenetic balancing of macrophage activation versus hyperpolarization.

Emodin was recently shown to dose-dependently attenuate the NOD-, LRR- and pyrin-domain containing protein 3 (NLRP3) inflammasome pathway in hypoxic-exposed hearts and heart cells.[344] The NLRP3 inflammasome synthesizes pro-inflammatory byproducts and mediates inflammation-induced cell death, or pyroptosis.[345] Indeed, this report showed emodin blocked pro-inflammatory byproduct signaling, namely NF-κB, and pyroptosis concomitantly with reduced scar tissue formation in the heart.[344] As NF-κB activity is regulated by HDACs, emodin may have blocked NF-κB activity through HDAC inhibition. In this regard, our lab recently reported that emodin dose-dependently inhibited cardiac-based HDAC activity and increased histone acetylation in cardiac myoblasts.[75] HDAC inhibition is well-known to prevent and treat cardiac dysfunction in

pre-clinical animal models of heart failure, as described above.[39–42] Emodin likely inhibits HDAC activity by chelating zinc ions within HDAC catalytic domains due to its chelating properties.[346] Furthermore, other well-characterized HDAC inhibitors have been shown to block NF-κB signaling,[347] thus, further suggesting emodin is an HDAC inhibitor that regulates pro-inflammatory signaling cascades. Finally, histone acetylation mediates differential gene expression.[39,75,348] This suggests that emodin may reverse stress-induced changes in the transcriptome. Experiments aimed at elucidating the epigenetic-dependent actions of emodin in CVD models are currently underway. Nevertheless, these data collectively suggest emodin inhibits pro-inflammatory signaling and downstream insults; these actions potentially depend on emodin's role as an HDAC inhibitor.

# Chapter 2: Divergent and Overlapping Roles for Phytochemicals in the Regulation of Pathological Cardiac Hypertrophy

## Abstract

Plant-based foods, like fruits, vegetables, whole grains, legumes, nuts, seeds and other foodstuffs, have been deemed as heart-healthy. The chemicals within these plant-based foods, i.e., phytochemicals, are credited protecting the heart. However, the mechanistic actions of phytochemicals which prevent clinical endpoints, such as cardiac hypertrophy, are still being elucidated. Thus, we sought to characterize the overlapping and divergent mechanisms by which 18 phytochemicals prevent agonist-induced cardiomyocyte hypertrophy. Of the tested 18 compounds, six attenuated PE- and PMA-mediated enlargement of neonatal rat ventricular myocytes. Cell viability assays showed that Apigenin, Baicalein, Berberine HCl, Emodin, Luteolin and Quercetin Dihydrate did not reduce cell size through cytotoxicity. Four of the six phytochemicals, Apigenin, Baicalein, Berberine HCl and Emodin, robustly inhibited stress-invoked hypertrophy and for which similar and differing anti-hypertrophic activities were analyzed further. For example, western blot revealed the four phytochemicals differentially regulated mitogen-activated protein kinases and protein kinase C/D. Further pathway analysis via RNA-sequencing is expected soon. In closing, Apigenin, Baicalein, Berberine HCl and Emodin blocked agonist-induced cardiomyocyte hypertrophy and did so through overlapping and divergent mechanisms as representative by the differential regulation of mitogen-activated protein kinase and protein kinase C/D phosphorylation.

## 1. Introduction

Plants, both edible and non, have been shown to benefit the heart.[349] In fact, several meta-analyses have reported that diets rich in fruits, vegetables, whole grains, nuts, seeds and other plant-based foods found in vegan/vegetarian diets, the Mediterranean Diet or the DASH diet (Dietary Approaches to Stop Hypertension) reduce the risk of heart diseases including heart failure.[350–352] Certainly, a heart-healthy diet is one consisting of a wide variety of plants.[352] Additionally, plant-based remedies have been used in Traditional Medicine practices for centuries; food bioactives, or phytochemicals, contained in these traditional medicines and plants have been shown to attenuate pathological cardiac hypertrophy[353] and are thus often credited for cardioprotection.

Phytochemicals are synthesized by plants to aid in protection against UV rays, pathogens and predators but can also interact in human biological processes after consumption. Historically, biomedical research focused on the actions of phytochemicals in reduction-oxidation reactions and immunological signaling.[72] In these early reports, phytochemicals offered cardioprotection through attenuated pathological cardiac hypertrophy by reducing oxidative damage and attenuating inflammation.[72] More recent evidence however, demonstrates that phytochemicals can regulate other mechanisms to inhibit pathological cardiac hypertrophy that includes regulation of signal transduction (e.g., mitogen activated protein kinases (MAPK)), contractile function (e.g., $Ca^{2+}$ handling) and epigenetic modifications (e.g., histone deacetylase (HDAC) inhibition).[354] As such, phytochemicals may act as natural pharmacological agents that prevent pathological enlargement of the heart through a myriad of cellular changes.

In this report, we examined the inhibitory actions of eighteen phytochemicals in a cellular based model of pathological cardiac hypertrophy. Of these 18 screened phytochemicals, six: Apigenin; Baicalein, Berberine Hydrochloride (BHCl); Emodin; Luteolin; and Quercetine Dihydrate (QD) inhibited pathological cardiac hypertrophy in response to two distinct agonists (pheneylepherine (PE) and phrobol 12-myristate 13-acetate (PMA)). Potent inhibition (i.e. >80%) was noted for four of these phytochemicals: Apigenin, Baicalein, BHCL and Emodin. As mentioned above, phytochemicals are pleiotropic. Consistent with this, we report that these four phytochemicals potently inhibited pathological cardiac hypertrophy by regulating overlapping as well as divergent changes in global gene expression and signal transduction pathways.

## 2. Results

### *2.1. Six phytochemicals attenuated pathological cardiac hypertrophy.*

Pathological cardiac hypertrophy is characterized by enlarged and weakened cardiomyocytes and has been shown to impair heart function and result in heart failure. Likewise, inhibition of pathological cardiomyocyte hypertrophy has been shown to improve heart function.[355,356] Here, we sought identify phytochemicals that could inhibit pathological cardiac hypertrophy. As such, we screened 18 phytochemicals in a model of pathological cardiac hypertrophy: Apigenin; Baicalein, Baicalin; BHCl; Caffeic acid; Dihydromyrcetin; Emodin; Epigallocatechin Gallate; Gossypol; Hematoxylin; Indirubin; Kaempferol; Luteolin; Morin hydrate; Myricetin; Myricitrin; Palmatine and QD. For these studies, we induced hypertrophy in neonatal rat ventricular myocytes (NRVMs) by stimulating with either an α1-adrenergic receptor agonist (phenylephrine; PE) or an

intracellular agonist (phorbol 12-myristate 13-acetate; PMA) prior to co-spiking with one of 18 dietary compounds. Baicalin, Caffeic Acid and Dihydromyricetin did not attenuate cardiomyocyte hypertrophy under any condition (**Table 1**). Gossypol and Myricetin appeared toxic as NRVMs treated with these two compounds died in culture. Hematoxylin, Kaempferol, Myricitrin and Morin hydrate blocked PMA-induced hypertrophy with no effect noted for PE-induced hypertrophy; Hematoxylin was the only compound with considerable inhibitory actions (**Table 1**). In contrast, Indirubin and Palmatine only blocked PE-induced cardiomyocyte hypertrophy (**Table 1**). Epigallocatechin (EGCG) showed little effect on either hypertrophic agonist (**Table 1**).

Of the 18 compounds screened, Apigenin, Baicalein, BHCl, Emodin, Luteolin and QD were the only compounds that attenuated pathological cardiac hypertrophy in NRVMs exposed to PE or PMA (**Table 1; Figure 1**). It is important to note that inhibition of hypertrophy was not due to cytotoxicity, as these six phytochemicals did not decrease cell viability (**Figure 2**). While Luteolin and QD did inhibit pathological hypertrophy (>40% inhibition), Apigenin (>90%), Baicalein (>90%), BHCl (>50%) and Emodin (>75%) demonstrated the strongest anti-hypertrophic actions (**Table 1; Figure. 1**). As such, we sought to further characterize the overlapping and divergent mechanistic actions of these four phytochemicals in the cardiomyocyte.

*2.2. Phytochemicals regulate overlapping and divergent changes in gene expression.*

As our data showed that Apigenin, Baicalein, Berberine HCl and Emodin affected agonist-induced hypertrophy differently, we next sought to characterize the divergent mechanisms of these compounds in NRVMs via RNA-sequencing and subsequent

pathway analyses. As shown in **Figure 3A**, Apigenin, Baicalein, Berberine HCl and Emodin had clearly affected gene expression of the stressed cardiomyocyte differently from and similar to one another. For example, 10 genes were normalized (away from PE-treated cells) and shared between these four compounds while five were affected only by Emodin, 24 only by Baicalein, 59 only by Apigenin and 39 only by Berberine (**Figure 3B**). Looking more closely at the primary pathways that were affected by the four phytochemicals, inflammation (e.g., inflammatory response, cellular response to interleukin-1 or response to lipopolysaccharide) was the leading or the second leading pathway (**Figure 3C**). These data highlight the overlapping and divergent mechanisms of phytochemicals. Indeed, clear overlap and divergence of mechanistic actions by Apigenin, Baicalein, Berberine HCl and Emodin in the cardiomyocyte did occur. For example, the RNA-sequencing data showed that one of the primary pathways affected by Emodin was histone H3 lysine residue 9 acetylation. Berberine affected the drug response. Finally, Apigenin affected extracellular regulated kinase (ERK) 1 and 2.

*2.3. Phytochemicals regulate overlapping and divergent changes in signal transduction.*

Several signal transduction pathways participate in causing the cardiomyocyte to hypertrophy. The pathways of the mitogen activated protein kinases (MAPKs), protein kinase D (PKD) isoforms and Janus kinase-signal transducer and activator of transcription (JAK/STAT) through phosphorylation cascades will all drive heart enlargement. Thus, we postulated Apigenin, Baicalein, BHCl and Emodin would regulate MAPKs, PKD and JAK/STAT in NRVMs exposed to PE. Our data showed that in NRVMs spiked with vehicle and PE, phosphorylation increased in all the MAPKs (i.e.,

JNK, ERK and p38), PKD and STAT3 (**Figure 3**). Apigenin reduced pJNK and pERK but not phosphorylated p38 (**Figure 3**). Baicalein reduced JNK and p38 phosphorylation but had little effect on ERK phosphorylation (**Figure 3**). Both BHCl and Emodin reduced phosphorylation of all MAPKs (**Figure 3**). In relation PKD isoforms, all phytochemicals reduced pPKD; however, Emodin completely abolished pPKD (**Figure 3**). Apigenin, Baicalein, BHCl and Emodin minimally reduced STAT3 activation. Finally, while these four compounds affected MAPK and PKD signaling, they often did so differently. For example, Emodin and Baicalein reduced JNK and ERK phosphorylation, but Baicalein likely did more so. Coomassie stain showed that changes in protein expression from our immunoblots were not due to loading irregularities (**Figure 4**). These data, indeed, show the overlapping and divergent mechanisms that Apigenin, Baicalein, BHCl and Emodin prevent agonist-induced cardiomyocyte hypertrophy.

**3. Discussion**

In this study, we screened 18 phytochemicals to identify compounds that would inhibit pathological cardiomyocyte hypertrophy (**Table 1**). We report that six phytochemicals: Apigenin, Baicalein, BHCl, Emodin, Luteolin and QD inhibited cardiomyocyte hypertrophy in response to two distinct agonists, PE or PMA (**Table 1, Figure 1**). Further examination of these compounds demonstrated overlapping and divergent regulation of genome-wide changes in gene expression and signal transduction. These findings are consistent with the fact that phytochemicals elicit a myriad of changes within cells that involve changes in oxidation-reduction, inflammation, gene expression and intracellular signaling.[166,344,357] Thus, our compounds likely regulate similar and divergent pathways to

improve cardiac myocyte size that can ultimately improve cardiac function; this likely contributes to the multi-faceted actions of fruits and vegetables in myocardial disease protection.

Maladaptive cardiac hypertrophy is a complex event that exacerbates cardiovascular disease (CVD) and contributes to heart failure and death. In response to physiologic conditions like exercise, the heart enlarges to compensate for the increase in peripheral vascularization and blood pressure changes.[358] In contrast, chronic pathological stimuli promote non-compensatory heart enlargement that results in a weakened myocardium contributing to heart failure.[359] While compensatory and non-compensatory cardiac hypertrophy can share similar intracellular signal transduction pathways,[7] they also show unique intracellular signaling signatures.[360] For instance, G protein-coupled receptors (GPCRs) and their interaction with heterotrimeric G proteins, such as $G\alpha_i$, $G\alpha_q$ and $G\alpha_s$, have been reported to determine pathogenesis.[2] Indeed, agonizing α1-adrenergic receptors, such as with PE, activates this pathological GPCR response.[361] PE-induced activation can further contribute to activation of mitogen-activated protein kinase (MAPK) signaling and protein kinase D (PKD) signaling through activation of GPCRs.[361–363]

Not surprisingly, inflammation was amongst the primary pathways by which phytochemicals protected the stressed cardiomyocyte in our experiments. This is important as inflammation, via C-reactive protein (CRP), predicts CVD which suggests that targeting inflammation is efficacious to the pathological heart.[364] Certainly, atherosclerosis, which is the formation of plaque within arteriole walls, is partially driven

by inflammation.[365] Further, inflammatory T-lymphocytes and macrophages were shown to localize within the cardiomyocyte, upregulate fibrogenic genes and cause cardiac fibrosis.[366] More importantly, inflammation has been shown to drive cardiac hypertrophy.[367] In fact, the pro-inflammatory cytokine, interleukin 6 has been shown in several models to be critical for pathological cardiac remodeling.[368–370] Of interest, signal transducer and activator of transcription 3 (STAT3) may be important for interleukin 6-mediated cardiac hypertrophy,[368] which we showed both to be downregulated by the majority of these phytochemicals. Thus, our data are consistent with the majority of science which shows that phytochemicals are cardioprotective by attenuating deleterious inflammation.

The three most well-studied MAPKs are ERK, JNK and p38, in which activation has been implicated in pathological cardiac hypertrophy.[371] Phosphorylation of MAPKs on their constitutive TxY motif is necessary and sufficient for MAPK activation.[372] Interestingly, recent reports have shown that threonine 188 (T188) auto-phosphorylation of ERK drives PE-induced nuclear ERK compartmentalization necessary for pathological hypertrophy and fibrosis *in vitro* and *in vivo*.[373] While we did not look at this site, it is likely that several of our compounds would target this T188 phosphorylation for inhibition. What's less clear is if phosphorylation of the TxY motif of ERK regulates compensatory vs. non-compensatory hypertrophy;[373] although our data would suggest that inhibition of ERK is one mechanism by which phytochemicals can inhibit hypertrophy. Similar to ERK, there are conflicting reports for p38 phosphorylation of the TxY motif. Indeed, p38 was shown to regulate the pro-hypertrophic transcription factor, nuclear factor of activated T-cells (NFAT),[374] and inhibiting p38 activity with small

molecule inhibitors (e.g., SB203580) can attenuate pathological cardiomyocyte hypertrophy whereas p38 activation was shown to promote cardiomyocyte hypertrophy.[375] On the other hand, *in vivo* transgenic activation of p38 did not alter cardiac hypertrophy.[376] Again, this is interesting to note, as some of our compounds (i.e. ) attenuated p38 signaling while others did not change p38 phosphorylation (i.e. ). These data might suggest that p38 plays an indirect role in regulating cardiac hypertrophy but is not the major driver of this process. Not surprising, there are also contrasting reports regarding JNK phosphorylation in cardiac hypertrophy, where JNK activation can inhibit NFAT activity, while other reports have shown that JNK is activated in models of cardiomyocyte hypertrophy.[377,378] Again, we report that some of our compounds inhibited JNK phosphorylation (i.e. ), while others showed no effect (i.e. ). Future studies will need to be performed to determine if phytochemical-mediated inhibition of cardiomyocyte hypertrophy is a direct result from changes in MAPK signaling, or if changes in MAPK signaling are an indirect effect due to changes in cardiomyocyte hypertrophy.

The protein kinase C (PKC) and D (PKD) families have also been implicated in cardiac hypertrophy.[360] For example, PKC-induced activation of PKD1 was shown to phosphorylate class II histone deacetylases, which led to HDAC translocation out of the nucleus that allowed for induction of pro-hypertrophic genes.[379] Apigenin, Baicalein, BHCl and Emodin attenuated PKD phosphorylation at serine 744/748 (S744/748). It should be noted that S744/748 phosphorylation of PKD confers activity.[380] These data are consistent with other reports showing that Emodin can inhibit PKC activity.[328]

Lastly, we should note that several of these compounds are derivatives of each other. For example, Baicalein is the aglycone of Baicalin and both are found in the *Scutellaria baicalensis* Georgi plant. This as interesting as some studies report that Baicalin prevents pathological cardiac hypertrophy, fibrosis and dysfunction in transverse aortic constricted mice,[167] and others report that Baicalin in combination with other phytochemicals is anti-hypertrophic.[381] These data contrast our findings which suggest that Baicalein inhibits hypertrophy whereas Baicalin does not block pathological hypertrophy in primary cardiomyocytes. This could be for a variety of reasons that includes metabolism *in vivo* of Baicalin, or as mentioned by Wu et al. Baicalin-induced changes in the microbiome that would not take place in cell culture.[381] The microbiome participates in second phase metabolism of phytochemicals, which would subsequently synthesize systemically bioactive compounds and metabolites that could inhibit cardiac hypertrophy and not the parent compound. With the emergence of the microbiome in nutrition research, future research will likely yield new findings for phytochemical metabolism in cardioprotection.

## 4. Conclusion

In conclusion, Apigenin, Baicalein, BHCl and Emodin all blocked PE- and PMA-induced cardiomyocyte hypertrophy. Transcriptomic and signal transduction analysis demonstrated that four of these dietary compounds impacted muscle cell enlargement through overlapping and divergent pathways. Combined, these data suggest that phytochemicals can regulate cardiac function through a myriad of actions within the cell and are not limited to one mechanistic outcome (e.g. oxidation-reduction).

## 5. Methods

### *5.1. Chemicals and Reagents:*

The following dietary compounds were purchased from Selleckchem: Apigenin (Selleckchem, Cat#- S2262), Baicalein (*Selleckchem*, Baicalin (Selleckchem, Cat#- S2269), Berberine Hydrochloride (Selleckchem, Cat#- S2271), Cat#- S2268), Caffeic Acid (Selleckchem, Cat#- S2277), Dihydromyricetin (Selleckchem, Cat#- S2399), Emodin (Selleckchem, Cat#- S2295), (-)-Epigallacatechin Gallate (Selleckchem, Cat#- 2250), Gossypol Acetate (Selleckchem, Cat#- S2303), Hematoxylin (Selleckchem, Cat#- S2384), Indirubin (Selleckchem, Cat#- S2386), Kaempferol (Selleckchem, Cat#- S2314), Luteolin (Selleckchem, Cat#- S2320), Morin Hydrate (Selleckchem, Cat#- S2325), Myricetin (Selleckchem, Cat#- S2326), Myricitrin (Selleckchem, Cat#- S2327), Palmatine Chloride (Selleckchem, Cat#- S2397) and Quercetin Dihydrate (Selleckchem, Cat#- S2347).

### *5.2. Neonatal Rat Ventricular Myocyte (NRVM) culture:*

Cardiac ventricles from one- to three-day old Sprague-Dawley rats (Charles River) were excised and minced before being digested in a calcium- and bicarbonate-free Hanks HEPES (CBFHH) solution containing trypsin (Gibco Life Technologies) and DNaseII from bovine (Sigma-Aldrich). Isolated ventricular myocytes were then plated overnight in gelatin-coated (0.2%, Sigma-Aldrich) cell culture dishes (6-wells and 100 mm dishes) containing Minimum Eagles Medium (MEM, Genesee Scientific) with 10% calf serum, 2 mM L-glutamine and penicillin-streptomycin. The next day, NRVMs were washed with Dulbecco's Modified Eagles Medium (DMEM) before adding DMEM media containing

Nutridoma-SP (Roche Applied Science). Finally, NRVMs were spiked with vehicle (DMSO) or one of two hypertrophic agonists, phenylephrine (PE, 10 $\mu$ M, Tocris Bioscience) or phorbol 12-myristate 13-acetate (PMA, 50 nM, Sigma-Aldrich), and co-spiked with one of the 18 dietary compounds listed above at 10 $\mu$ M, except for Apigenin which was dosed at 50 $\mu$ M. NRVM data, both in immunoblotting and gene expression (e.g., qPCR and RNA-sequencing), use treatments and samples from same NRVM prep.

*5.3. Immunohistochemistry:*

For immunohistochemistry experiments, a previously characterized protocol was followed which will be briefly described here.[382] After experimental treatment periods, NRVMs plated in 6-well dishes were washed with phosphate buffered saline (PBS, 7.6 pH) and then fixed in 4% paraformaldehyde at room temperature for 20 minutes. Next, a PBS solution containing bovine serum albumin (BSA, 3%, Fisher Bioreagents, BP1605) and Nonidet NP-40 (0.1%, Sigma-Aldrich IGEPAL CA-630) was used to permeabilize NRVM membranes. NRVMs were then incubated for two hours in a primary antibody cocktail containing antibodies for the hypertrophic marker, atrial natriuretic factor (ANF, 1:1000, Phoenix Pharmaceuticals, H-005-24) and the sarcomeric protein, α-actinin (1:750, Sigma-A7811). NRVMs were then incubated for one hour in a secondary antibody cocktail (goat anti-rabbit Cy3, Jackson ImmunoResearch; donkey anti-mouse FITC, Jackson ImmunoResearch) before being washed in PBS and briefly exposed to Hoechst (10 μM, Invitrogen H3570). The EVOS FL Cell Imaging System (Thermo Fisher Scientific) with DAPI, GFP and RFP imaging cubes were used to image nuclei, cardiomyocyte sarcomeres and the perinuclear expression of ANF, respectively, at a 20x

objective. Twenty images were captured per well, resulting in 60 images per treatment, which were then used to analyze cell area via ImageJ software.

*5.4. Cell Viability:*

NRVMs in gelatin-coated 96-well plates were spiked with either dimethyl sulfoxide (DMSO (veh), Pharmco-AAPER) or phenylephrine (PE, 10 μM, Tocris Bioscience) and then co-treated with either Apigenin, Baicalein, Berberine Hydrochloride, Emodin, Luteolin or Quercetin Dihydrate before incubated at 37 degree Celsius for 48-hours per the experimental treatment period. Next, Invitrogen alamarBlue™ HS Cell Viability Reagent, a resazurin-based reagent that is reduced to the highly fluorescent resorufin in healthy cells, was used as described by the manufacturer instructions. Briefly, alamarBlue™ was added to each well at a ratio of 10:1 media:alamarBlue™ and NRVMs were incubated for one hour. BioTek Synergy was then used to measure fluorescence with a 530 nm excitation filter and a 590 nm emission filter. Results were normalized to DMSO-spiked NRVMs.

*5.5. Immunoblotting:*

After experimental treatments and incubation periods, NRVMs were washed with ice cold PBS and lysed with PBS containing 300 mM NaCl, 0.5% Triton-X and HALT™ protease/phosphatase inhibitors. NRVM lysates were sonicated and centrifuged to then separate the supernatant and pellet. The supernatant was isolated and used for immunoblotting experiments. BCA reagents were used to measure protein concentration of all samples as to normalize to 10 ug per sample for SDS-PAGE. Samples were resolved with SDS-PAGE and transferred onto a nitrocellulose membrane which was

then incubated overnight in a primary antibody cocktail of BSA (2.5%) in 1x Tris-Buffered Saline and Tween® (TBST). Used antibodies were for JNK (Santa Cruz, Sc-571), phosphorylated JNK (Cell Signaling, 4668), ERK (Santa Cruz, Sc-153), phosphorylated ERK (Cell Signaling, 4370), p38 (Santa Cruz, Sc-7149), phosphorylated p38 (Cell Signaling, 4511), STAT3, phosphorylated STAT3, phosphorylated PKC α/βII (Cell Signaling, 9375), phosphorylated PKC pan (Cell Signaling, 9371), phosphorylated PKD (Cell Signaling, 2054) and PKD (Cell Signaling, 90039). The next day, membranes were washed in TBST prior to being exposed to horseradish peroxidase-conjugated secondary antibodies (Southern Biotech). Finally, SuperSignal West Pico Chemiluminescence System (Thermo Fisher Scientific) was used on membranes prior to exposure on a ChemiDoc XRS+ Imager (BioRad). Coomassie Staining was used for internal controls of immunoblotting; SDS-PAGE gels were stained with BIO-RAD's Bio-Safe Coomassie G-250 Stain following manufacture's protocol.

*5.6. RNA sequencing:*

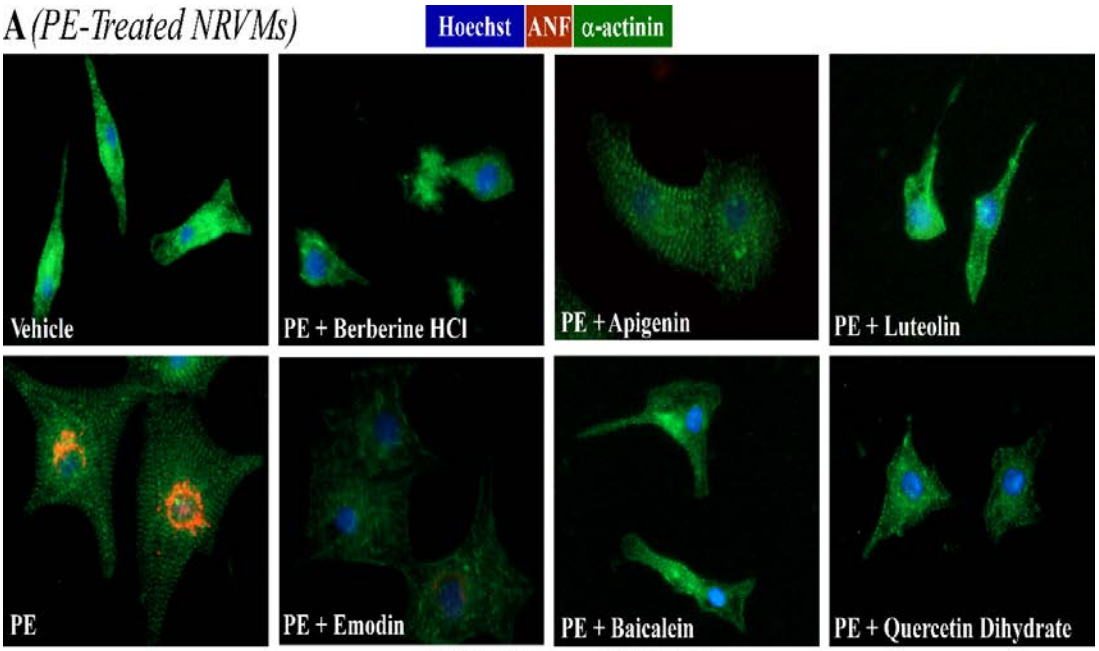
NRVMs were lysed with QIAzol (Qiagen) and RNA was isolated from treatment groups (n=5) per the manufacturer's instructions. Prior to synthesizing cDNA libraries, RNA integrity (RIN) and concentrations were analyzed by the Nevada Genomics Center using an RNA Agilent Bioanalyzer which confirmed RIN values were greater than 8.0. Following the Illumina's protocol, the TruSeq Stranded Total RNA containing Ribo-Zero Human/Mouse/Rat kit (Illumina) was used to synthesize 1000 ng RNA into cDNA libraries. Libraries were then validated using the DNA 7500 chip and then sequenced using the NextSeq 500.

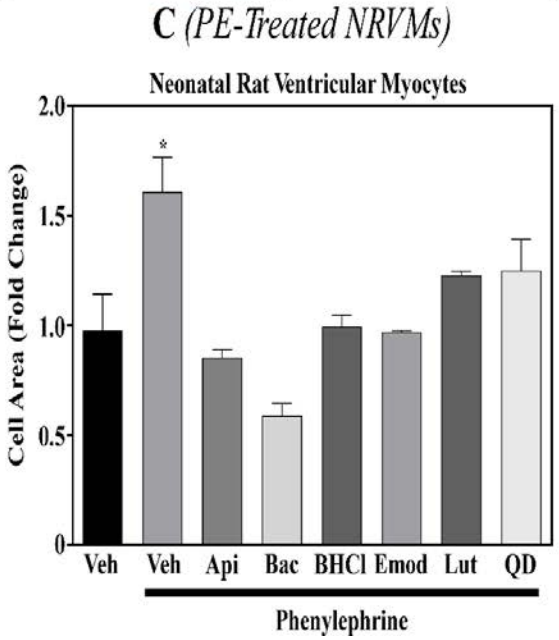
*5.8. Statistical analysis:*

A minimum of three experiments with an n = 3 per experimental treatment group was performed and data quantified. One-way ANOVA with Tukey's post-hoc was performed unless otherwise specified using GraphPad7 (GraphPad Software, La Jolla, CA). $p < 0.05$ was considered significant.

**Table 1. Phytochemical inhibitors of cardiac hypertrophy**

| | % Inhibition Cell Size | |
|---|---|---|
| **Bioactive Food Compound** | **PE** | **PMA** |
| **Apigenin** | **100** | **91** |
| **Baicalein** | **100** | **100** |
| Baicalin | N.I. | N.I. |
| **Berberine Hydrochloride** | **100** | **52** |
| Caffeic acid | N.I. | N.I. |
| Dihydromyricetin | N.I. | N.I. |
| **Emodin** | **100** | **78** |
| Epigallocatechin Gallate | 48 | 14 |
| Gossypol | Toxic | Toxic |
| Hematoxylin | N.I. | 76 |
| Indirubin | 38 | N.I. |
| Kaempferol | N.I. | 30 |
| **Luteolin** | **62** | **40** |
| Morin hydrate (Aurantica) | N.I. | 26 |
| Myricetin (Cannabiscetin) | Toxic | Toxic |
| Myricitrin (Myricitrine) | N.I. | 40 |
| Palmatine | 81 | N.I. |
| **Quercetin dihydrate** | **45** | **42** |


A (PE-Treated NRVMs)
Hoechst
ANF
α-actinin
Vehicle
PE + Berberine HCl
PE + Apigenin
PE + Luteolin
PE
PE + Emodin
PE + Baicalein
PE + Quercetin Dihydrate


C (PE-Treated NRVMs)
Neonatal Rat Ventricular Myocytes
Cell Area (Fold Change)
2.0
1.5
1.0
0.5
0
*
Veh
Veh
Api
Bac
BHCl
Emod
Lut
QD
Phenylephrine

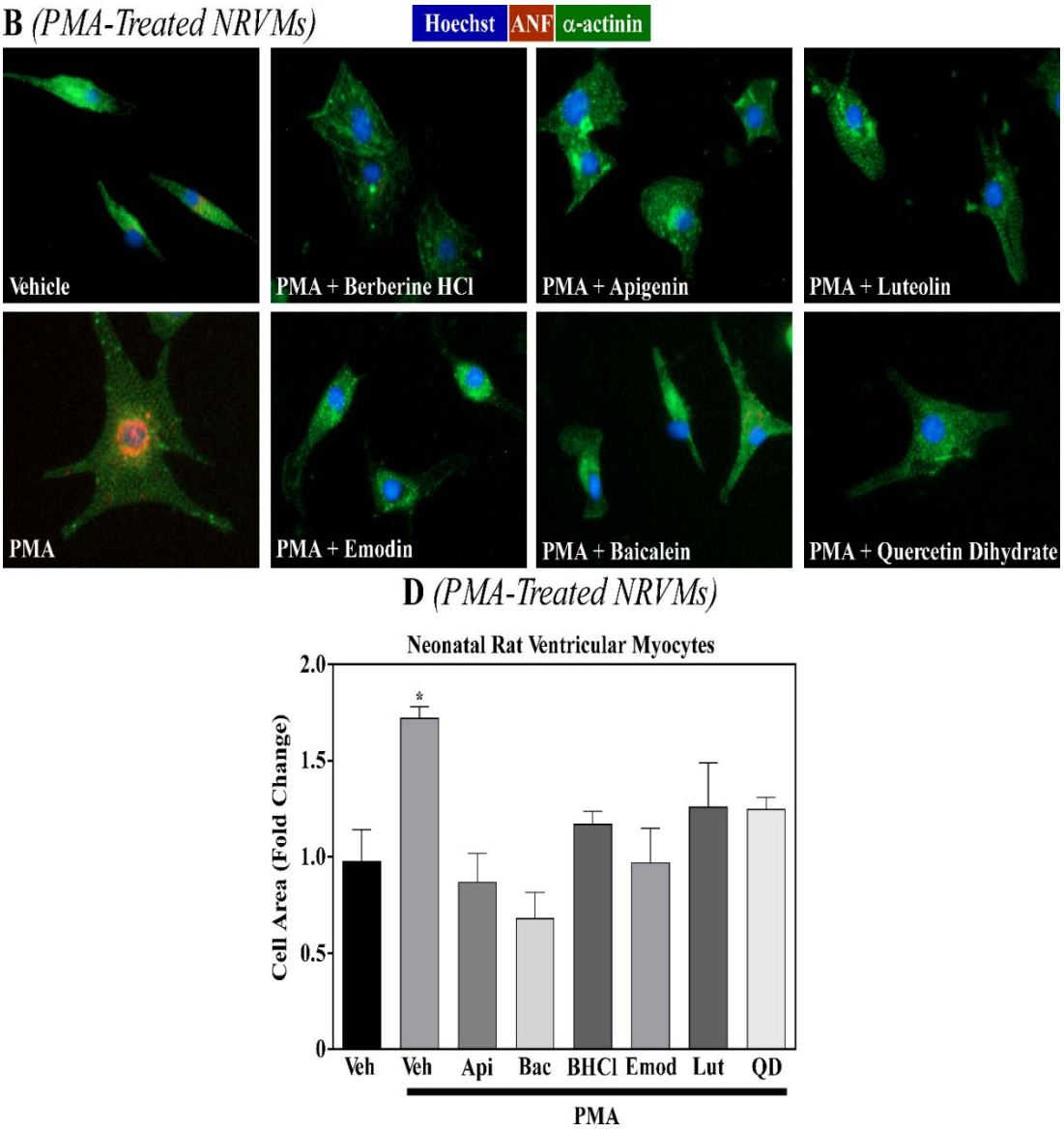



**Figure 1: Six phytochemicals significantly inhibited cardiac hypertrophy under two different conditions.** A) Neonatal rat ventricular myocytes (NRVMs) were spiked with or without phenylephrine (10 $\mu$ M; PE) and then co-spiked with one of six phytochemicals: Apigenin, Baicalein, Berberine Hydrochloride, Emodin, Luteolin or Quercetin Dihydrate. All compounds were treated at 10 $\mu$ M except Apigenin, which was treated at 50 $\mu$ M, for 48-hours. Cells were fixed and immunostained with antibodies

**Figure 1 cont.** directed against α-actinin or atrial natruetic factor (ANF). Cell nuclei were stained with Hoechst. Cells were visualized with EVOS microscopy. Ten pictures were taken per well and cell size was calculated using Image J software. GraphPad Prism was used to examine statistical significance. One-way ANOVA with Tukey's post-hoc analysis was used. Significance was set at $p<0.05$. B) NRVMs were spiked and treated with six phytochemicals as described above except phorbol myristate acetate (50 nM; PMA) was used in place of PE. Cell area was quantified for PE spiked cells C) and for PMA spiked cells D).

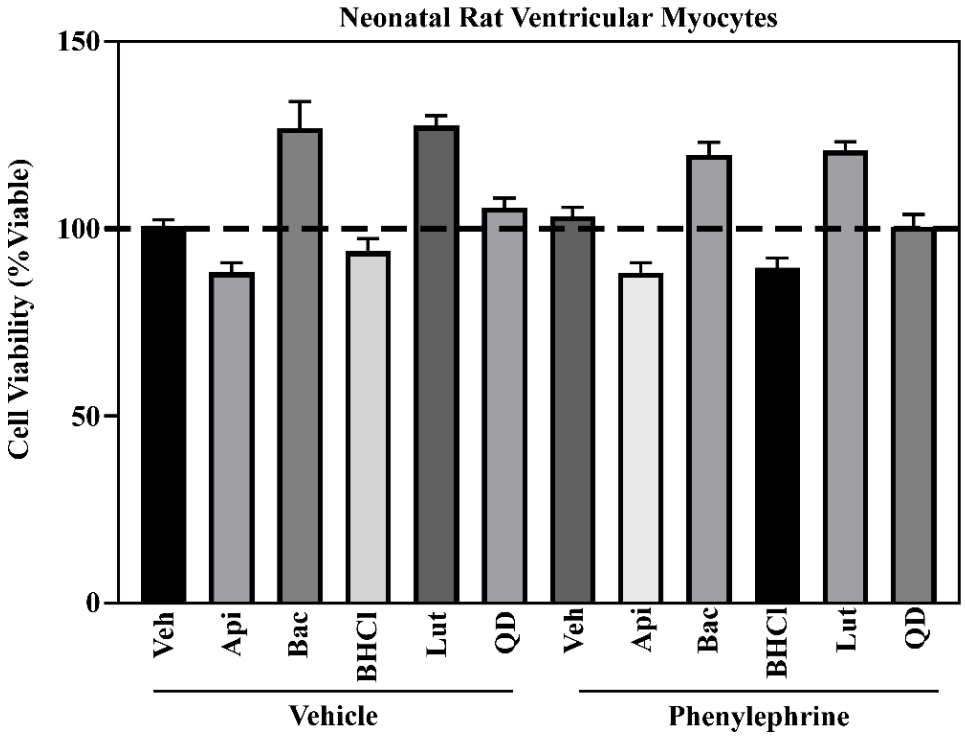
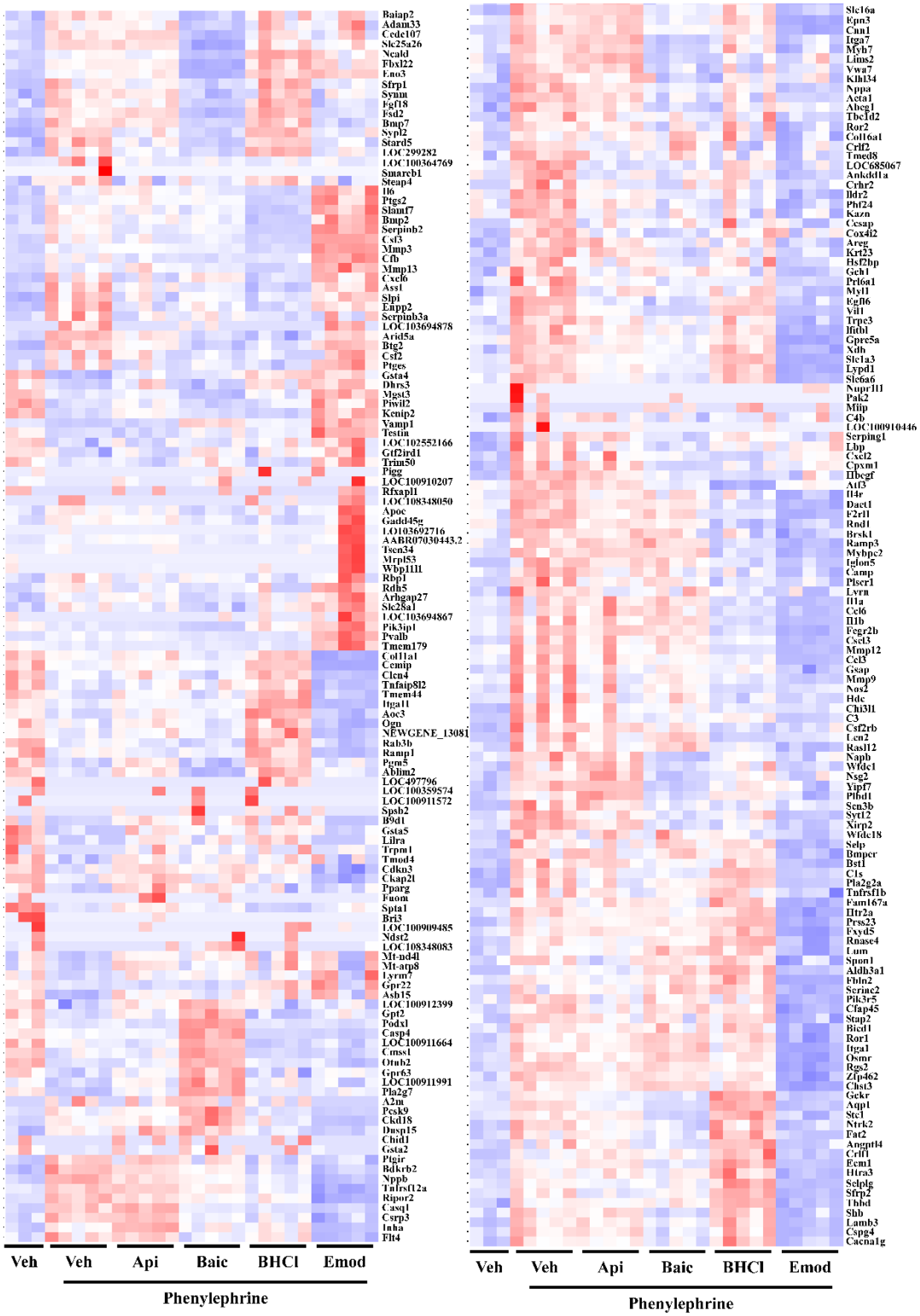
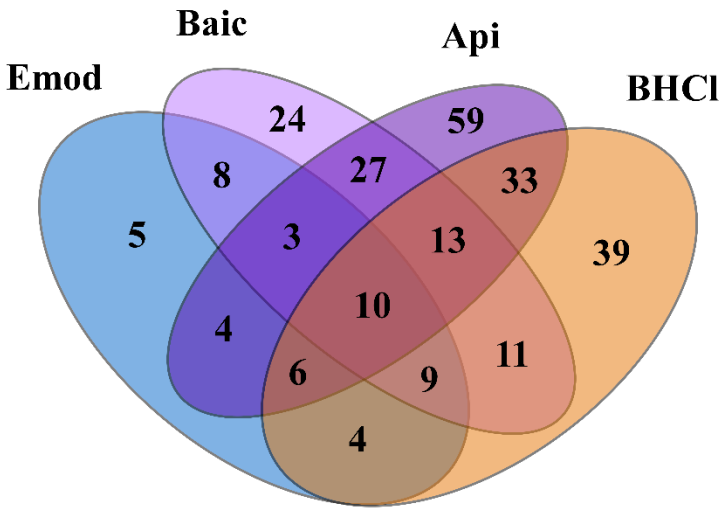



**Figure 2: Phytochemical treatment did not induce cell death.** Neonatal rat ventricular myocytes (NRVMs) were plated in 96-well plates, spiked with or without phenylephrine (10 $\mu$ M; PE) and co-treated with one of five phytochemicals: Apigenin (50 $\mu$ M), Baicalein (10 $\mu$ M), Berberine Hydrochloride (10 $\mu$ M), Luteolin (10 $\mu$ M) or Quercetin Dihyrate (10 $\mu$ M). An n=8 was used per treatment group. After a 48-hour treatment period, alarmaBlue™ was added at 1:9 (alarmaBlue™:media) ratio and incubated for 1 hour before fluorescence was measured at 560/590 Excitation/Emission. One-way ANOVA with Tukey's post-hoc analysis was used to assess differences in cell viability. Significance was set at $p<0.05$.

**A** *(NRVM-Gene Expression)*



**B** *(Genes Normalized in Response to PE)*



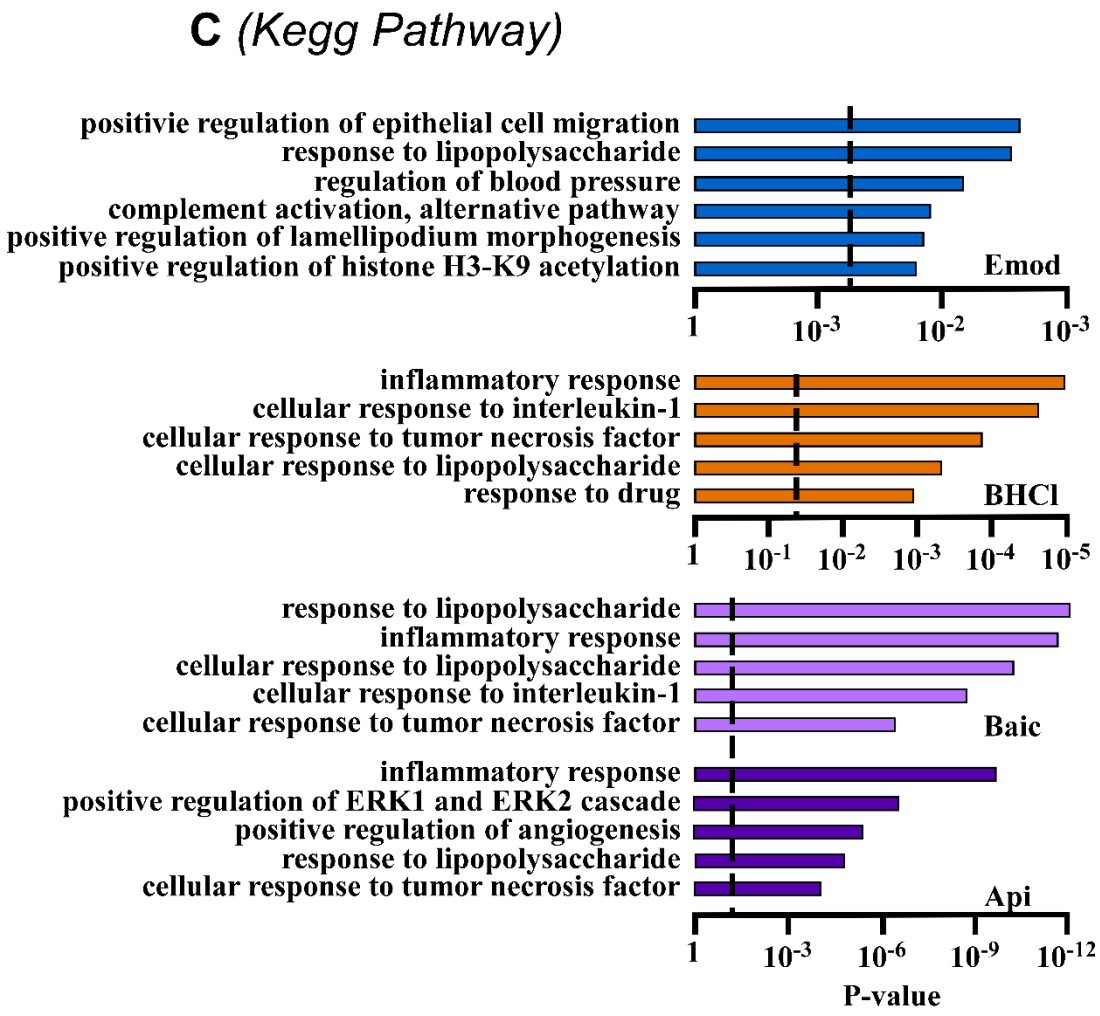



**Figure 3: Phytochemicals share similar but also regulate divergent gene expression.**

NRVMs were co-spiked with Phenylephrine (10 μM, PE) and Apigenin, Baicalein, Berberine HCl and Emodin and incubated for 48-hours before isolating RNA via QIAzol. RNA-sequencing libraries were prepared (n=5) using Illumina's TruSeq Total RNA Prep. A) The heatmap of gene expression data. B) Venn diagram of overlapping and divergent gene. C) Kegg pathway analysis of the primary pathways affected by Apignein, Baicaelin, Berberin HCl and Emodin in the cardiomyocyte.

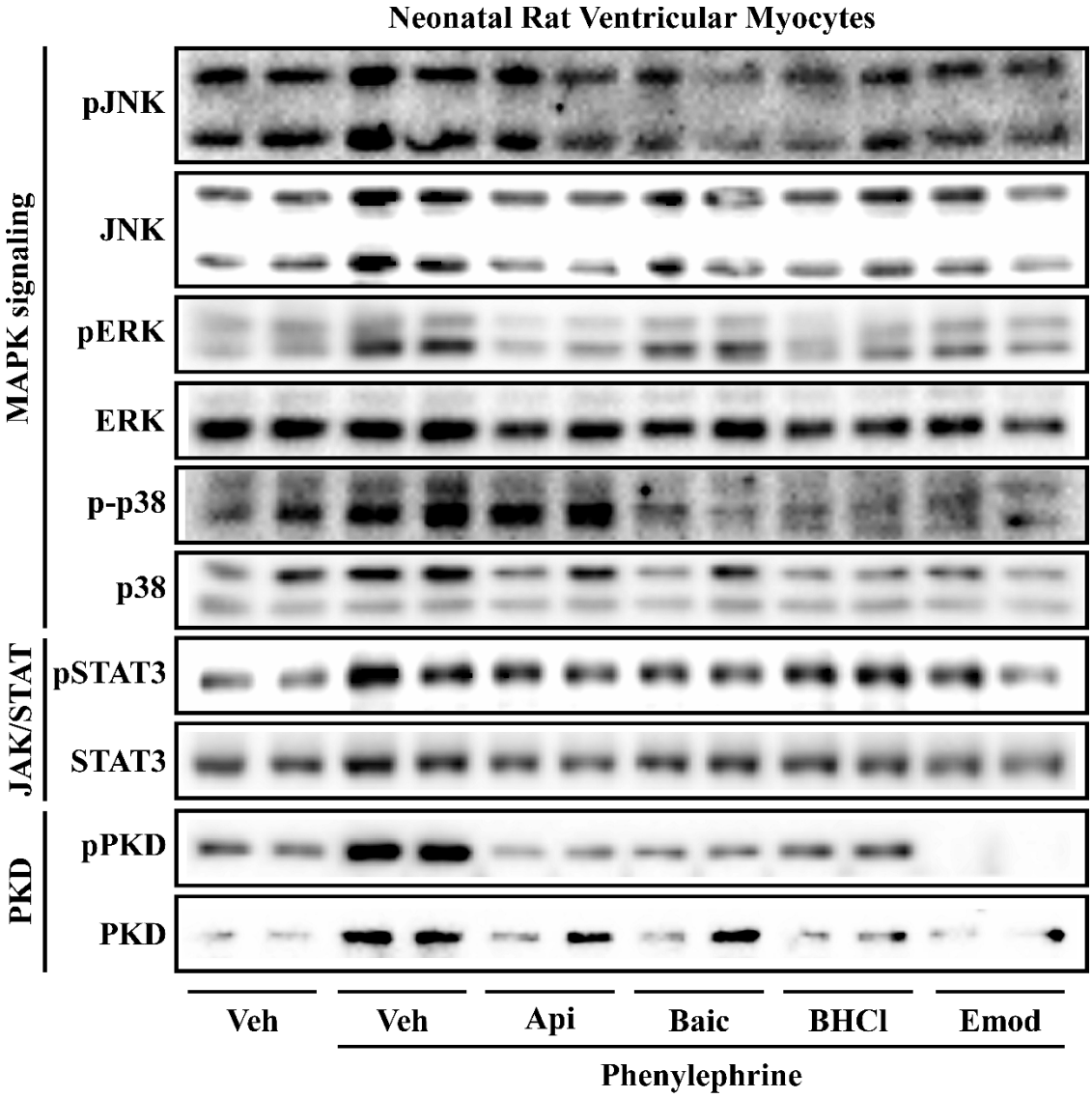



**Figure 4: Phytochemicals regulated signaling pathways of cardiomyocyte hypertrophy.** Neonatal rat ventricular myocytes (NRVMs) were spiked with or without phenylephrine (10 μM; PE) in the absence or presence of one of four phytochemicals – Apigenin (50 $\mu$ M), Baicalein (10 $\mu$ M), Berberine Hydrochloride (10 $\mu$ M; BHCl) or Emodin (10 $\mu$ M) – for 48 hours prior to protein lysis. Protein lysates were prepared for sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). Samples were incubated with antibodies against phosphorylated-JNK, total JNK, phosphorylated-

**Figure 4 cont.** ERK, total ERK, phosphorylated-p38, total p38, phosphorylated STAT3, total STAT3, phosphorylated-PKD and total PKD

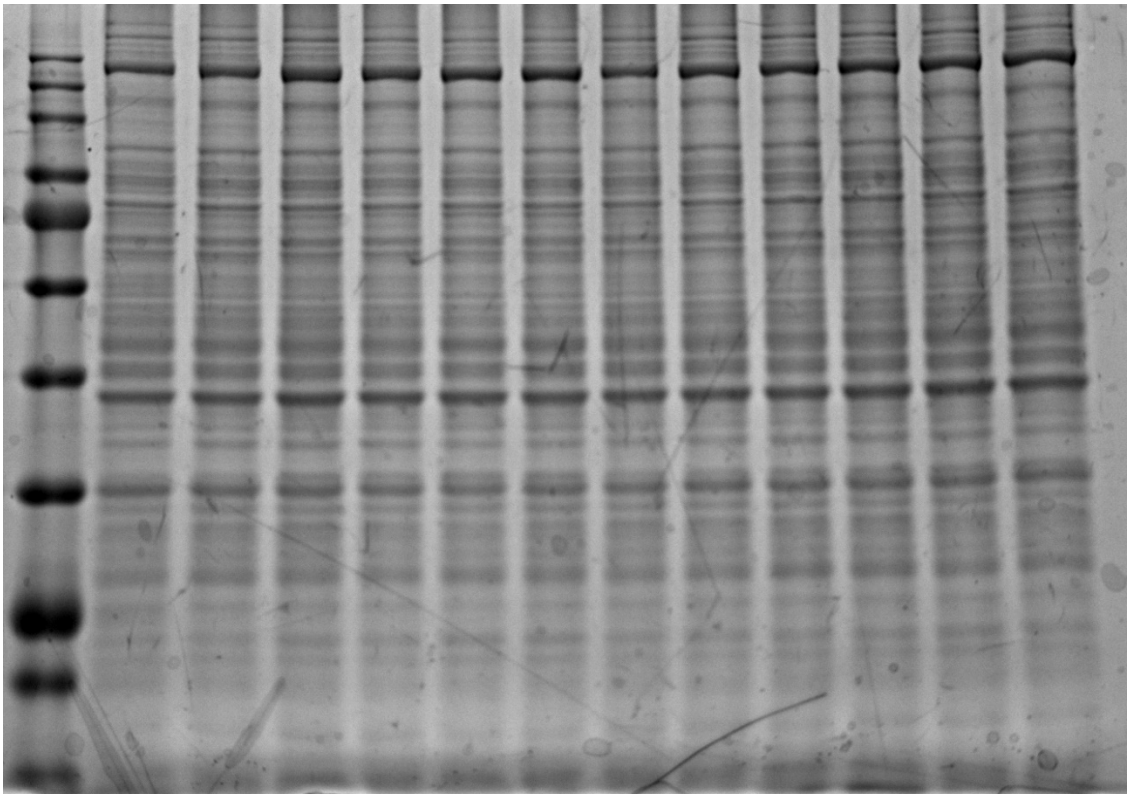


**Figure 5: Coomassie Stain of samples.** Protein lysate from samples treated as Vehicle, Phenylephrine (PE), PE + Apigenin, PE + Baicalein, PE + BHCl and PE + Emodin (n=2) was ran via SDS-PAGE before stained with BIO-RAD's Bio-Safe Coomassie for 1 hour.

**Chapter 3. Emodin and emodin-rich rhubarb inhibits histone deacetylase (HDAC) activity and cardiac myocyte hypertrophy.**

Levi W. Evans[1,2], Abigail Bender[3], Leah Burnett[3], Luis Godoy[1], Yiqui Shen[1,3], Dante Staten[2], Tong Zhou[3], Jeffrey E. Angermann[2], Bradley S. Ferguson[1,2,4]

[1]Department of Nutrition, University of Nevada, Reno

[2] Environmental Sciences, University of Nevada, Reno

[3] Department of Biochemistry and Molecular Biology, University of Nevada, Reno

[4]Center of Biomedical Research Excellence for Molecular and Cellular Signal Transduction in the Cardiovascular System, University of Nevada, Reno

**This work was published May 2020 in *The Journal of Nutritional Biochemistry*.**

**doi: 10.1016/j.jnutbio.2019.108339**

**Abstract:**

Pathological cardiac hypertrophy is a classical hallmark of heart failure. At the molecular level, inhibition of histone deacetylase (HDAC) enzymes attenuate pathological cardiac hypertrophy *in vitro* and *in vivo*. Emodin is an anthraquinone that has been implicated in cardiac protection. However, it is not known if the cardio-protective actions for emodin are mediated through HDAC-dependent regulation of gene expression. Therefore, we hypothesized that emodin would attenuate pathological cardiac hypertrophy via inhibition of HDACs, and that these actions would be reflected in an emodin-rich food like rhubarb. In this study, we demonstrate that emodin and Turkish rhubarb containing emodin inhibit HDAC activity *in vitro*, with fast-on, slow-off kinetics. Moreover, we show that emodin

increased histone acetylation in cardiomyocytes concomitant to global changes in gene expression; gene expression changes were similar to the well-established pan-HDAC inhibitor trichostatin A (TSA). We additionally present evidence that emodin inhibited phenylephrine (PE) and phorbol myristate acetate (PMA)-induced hypertrophy in neonatal rat ventricular myocytes (NRVMs). Lastly, we demonstrate that the cardioprotective actions of emodin are translated to an angiotensin II (Ang) mouse model of cardiac hypertrophy and fibrosis and are linked to HDAC inhibition. These data suggest that emodin blocked pathological cardiac hypertrophy, in part, by inhibiting HDAC-dependent gene expression changes.

**Keywords:** Emodin, histone deacetylase, HDAC, cardiac hypertrophy, food bioactives, heart failure

## 1. Introduction

Pathological cardiac hypertrophy is a hallmark of heart failure that affects millions of people and costs billions of dollars each year.[20] In response to stress, muscle cells of the heart, or cardiomyocytes, enlarge which leads to cardiac dysmorphism and subsequent dysfunction, resulting in heart failure. Poor dietary habits such as the Western Diet are associated with cardiac hypertrophy.[383] Conversely, cardiac hypertrophy can be prevented through proper dietary management.[384]

A heart-healthy diet is defined by leading experts as one consisting mainly of plant-based foods.[24] Indeed, reports from cellular studies [70] as well as human [29] and epidemiology analyses[19,21] suggest that plant-based foods are beneficial to overall heart health and deter heart disease. Plant-based foods like fruits, vegetables, whole grains and legumes contain essential macronutrients and micronutrients including fiber, vitamins and minerals. However, a building body of evidence suggests that benefits of these plant-based foods are independent of their essential nutrients.[30] This hints at the idea that other chemicals in these foods, i.e., phytochemicals, drive their efficacy. Indeed, phytochemicals have been shown to be cardioprotective; many of these early reports demonstrate protection via inhibition of oxidative stress, inflammation and shifts in intracellular signaling.[118,385–387] However, more recent evidence suggests that phytochemicals can regulate epigenetic modifications contributing to global changes in gene expression.[354]

Epigenetic modifications differentially regulate gene expression independent of changes in DNA sequence. Lysine acetylation on histone tails is a reversible epigenetic modification that is regulated by two enzymes: histone deacetylases (HDACs) and

histone acetyltransferases (HATs).[388] HDACs remove acetyl groups from lysine residues on histones leading to nucleosome compaction and transcriptional repression. Eighteen identified mammalian HDACs have been separated into classes I (HDACs 1,2 3, 8), II (HDACs 4, 5, 6, 7, 9, 10), III (Sirt1-7) and IV (HDAC 11). Class II is further divided into sub-classes IIa and IIb. Of interest, inhibiting class I and II HDAC activity is efficacious in experimental models of HF[39,40] and several phytochemicals have been characterized as HDAC inhibitors.[354] However, it remains unclear if phytochemicals protect the heart through HDAC-dependent regulation of gene expression.

Emodin is an anthraquinone phytochemical found in plant-based foods like rhubarb, cabbage and beans.[328,329,389] Additionally, emodin-rich plants, including buckthorn and knotweed, have been used in traditional medicines for centuries against viral, bacterial and bowel abnormalities. In the heart, emodin has been reported to reduce mitochondrial oxidative stress[331] and attenuate inflammation.[332] Recently, we showed that emodin inhibited HDAC activity in a test tube.[75] In this report, we sought to elucidate the cardioprotective actions of emodin and emodin-rich rhubarb. We report that emodin inhibited HDAC activity and increased histone acetylation in cardiomyocytes concomitant to global changes in cardiac gene expression. Moreover, we report that emodin normalized cardiac gene expression changes similar to the well-known HDAC inhibitor TSA. Lastly, we demonstrate that emodin blocked pathological cardiac hypertrophy *in vitro* and *in vivo*, consistent with its role as an HDAC inhibitor.

## 2. Materials and Methods

### 2.1 Reagents

Emodin was purchased from SelleckChem (S2295) and Turkey rhubarb purchased through Prescribed for Life (Sb15-H091519). Phenylephrine (PE; 10 µM) was purchased through Tocris Bioscience, phorbol-12-myristate-13-acetate (PMA; 50 nM) and trichostatin A (TSA; 200 nM) were purchased through Sigma-Aldrich. Emodin, turkey rhubarb and TSA were prepared in dimethyl sulfoxide (DMSO, Pharmco-AAPER).

**2.2 HDAC Activity Assays**

Bovine heart tissue was procured from the University of Nevada, Wolf Pack Meats. Animal care and handling was approved by the University of Nevada, Reno, Institutional Animal Care and Use Committee. HDAC activity assays were completed as previously described [390]. Each substrate is based on ε-N-acylated lysine, derivatized on the carboxyl group with amino methylcoumarin (AMC).[391] Heart tissue lysate was prepared in PBS (pH 7.4) containing 0.5% Triton X-100, 300 mM NaCl and protease/phosphatase inhibitor cocktail (ThermoFisher Scientific) using a Bullet Blender homogenizer (Next Advance). Tissue was clarified by centrifugation prior to determination of protein concentration using a BCA Protein Assay Kit (Pierce). Tissue (30 µg protein/well) was diluted in PBS for a total volume of 100 µl/well in a 96-well plate. For concentration-response determination, tissue was dosed with increasing semi-log scale concentrations of emodin (SelleckChem) or Turkey Rhubarb (Prescribed for Life) for 2 hrs. For kinetic analysis, heart lysate was treated with 50 µM emodin or 100 mg/L turkey rhubarb at the prescribed time points. Class-specific HDAC substrates were added (5 µl of 1 mM DMSO stock solutions), and plates returned to the 37 °C incubator for 2 hrs. HDAC substrates for HDAC activity experiments were as follows: ZLPA

(Class I, GeneScript custom peptide), I-1985 (Class IIa, Bachem, #4060676) and I-1875 (Class IIb, Bachem, #4033792). Developer/stop solution was added (50 µl per well of PBS with 1.5% Triton X-100, 3 µM TSA, and 0.75 mg/ml trypsin) and plates incubated at 37 °C for 20 min. Subsequent to deacetylation, trypsin is used to release AMC, resulting in increased fluorescence. AMC fluorescence was measured via BioTek Synergy plate reader, with excitation and emission filters of 360 nm and 460 nm, respectively. Background signals from buffer blanks were subtracted, and GraphPad Prism used to calculate $IC_{50}$ values for each compound. In addition, NRVMs were treated with PE (10 µM) and co-stimulated with either emodin (10 µM) or TSA (200 nM) for 48 hrs prior to cell lysis in PBS (0.5% Triton X-100, 300 mM NaCl and protease/phosphatase inhibitors). Protein concentrations were determined via BCA and HDAC activity assessed via class-selective HDAC substrates as described above. Fluorescence was measured via a BioTek Synergy plate reader (excitation filter at 360 nm and emission filter at 460 nm). Finally, male and female C57BL/6 mice were randomly assigned into groups to receive sham with vehicle (DMSO:PEG-300), angiotensin II (1.5 µg/kg/min) with vehicle or angiotensin II with emodin (30 mg/kg/day) for 14 days. On day 14, left ventricles were dissected and flash frozen, later to be lysed in PBS (0.5% Triton X-100, 300 mM NaCl and protease/phosphatase inhibitors). Protein concentrations were determined via BCA and HDAC activity assessed via class-selective HDAC substrates as described above. Fluorescence was measured via a BioTek Synergy plate reader (excitation filter at 360 nm and emission filter at 460 nm).

**2.3 HPLC**

An Agilent 1100 high performance liquid chromatography system, including a programmable solvent delivery pump, autosampler, and diode-array UV detector, was used for determination of emodin. Detection was set at 437 nm for emodin (6-methyl-1,3,8trihydroxyanthraquinone) analysis. Emodin separation was carried out using a Kinetex 5 um XBC18 100A 250 x 4.6mm column (Phenomenex, Torrance, CA). HPLC-grade methanol and water were used as reagents (Fisher Scientific). Isocratic separation was performed with a methanol/water (70:30, v/v) mobile phase at a flow rate of 0.8 mL/min. A stock emodin standard of 100 μg/mL was used to develop a seven-point calibration curve following serial dilution with methanol. Rhubarb extract was prepared in methanol at 100 mg/L. Each sample or standard was then sonicated for 30 minutes prior to triplicate injection via HPLC. A 30 μL aliquot of all samples or standards were injected directly into the HPLC system for quantitation. Emodin was identified in unknown samples by retention time matching between standards and unknowns, and the data was expressed in μg/mL.

**2.4 Neonatal rat ventricular myocyte (NRVM) isolation and culture**

NRVMs were prepared as previously described.[392] Briefly, hearts from 1-3-day-old Sprague-Dawley neonates were collected and digested in a solution containing trypsin (Gibco Life Technologies) and DNaseII from bovine (Sigma-Aldrich). Ventricular myocytes were isolated and then cultured in 100-mm dishes or 6-well plates that were coated with gelatin (0.2%, Sigma-Aldrich). Cardiomyocytes were placed in Minimum Eagles Medium (MEM, Genesee Scientific) with 10% calf serum, 2 mM L-glutamine and penicillin-streptomycin and incubated overnight. Media was replaced the next morning with Nurtidoma-SP (Roche Applied Science) and Dubelco's Modified

Eagles Medium (DMEM) prior to experimental treatments. Cells were co-spiked with either hypertrophic agonist, PE (10 μM) or PMA (50 nM), and emodin (10 μM), turkey rhubarb (100 mg/L) or TSA (200 nM) and incubated for 48 hours prior to being lysed or fixed for experiments described below.

**2.5 Immunoblotting**

Cells were lysed in PBS containing 300 mM NaCl, 0.5% Triton-X and HALT™ protease/phosphatase inhibitors. Cell lysate was then sonicated and centrifuged (16,000g for 5min) prior to BCA for protein quantification. Samples were resolved with SDS-PAGE and transferred to a nitrocellulose membrane prior to overnight incubation with primary antibodies for acetyl histone H3 lysine residues 9/14 (H3K9/14; Cell Signaling Technology, 9677), H3K18 (Cell Signaling Technology, 13998s) and H3K27 (Cell Signaling Technology, 8173s) as well as Total histone H3 (Cell Signaling Technology, 4499), phosphorylated ERK (Cell Signaling Technology, 4370), total ERK (Santa Cruz Biotechnology; Sc-1647) and atrial natriuretic factor (ANP, Santa Cruz Biotechnology, Sc-515701). The next day, horseradish peroxidase-conjugated secondary antibodies (Southern Biotech) were used prior to exposing with SuperSignal West Pico Chemiluminescence System (Thermo Fisher Scientific) on a ChemiDoc XRS+ Imager (BioRad).

**2.6 Immunostaining**

NRVMs were plated in 6-well dishes and treated as described above. Plates were fixed with 4% paraformaldehyde at room temperature for 20 minutes and prepared for immunostaining as previously described.[382] After fixation, cells were permeabilized with PBS containing bovine serum albumin (3%, Fisher Bioreagents, BP1605) and Nonidet

NP-40 (0.1%, Sigma-Aldrich IGEPAL CA-630) prior to being incubated for two hours with a primary antibody containing ANF (1:1000, Phoenix Pharmaceuticals, H-005-24) and α-actinin (1:750 Sigma A-7811). Cells were then incubated with a secondary antibody cocktail (donkey anti-mouse FITC, Jackson ImmunoResearch; goat anti-rabbit Cy3, Jackson ImmunoResearch) for one hour and briefly washed and incubated with Hoechst (10 μM, Invitrogen H3570) in PBS. Cells were washed with PBS and imaged via the EVOS FL Cell Imaging System (Thermo Fisher Scientific) at 20x. Twenty images of cells were taken per well. Cells were then analyzed for cell area and ANF expression via Image J (NIH Software).

**2.7 Cell viability**

NRVMs, spiked with either DMSO (veh), emodin (Emod, 10μM), phenylephrine (PE, 10μM) or PE + Emod, were exposed to Invitrogen alamarBlue™ HS Cell Viability Reagent (Thermo Fisher Scientific) for cell viability analyses. Following manufacturer instructions, NRVMs were incubated with Invitrogen alamarBlue™ HS Cell Viability Reagent (10:1, media with NRVMs:Invitrogen alamarBlue™ HS Cell Viability Reagent) in gelatin-coated 96-well plates for 1 hour. Invitrogen alamarBlue™ HS Cell Viability Reagent is resazurin based, which upon entering an NRVM will be reduced to the highly fluorescent resorufin. After 1 hour incubation, fluorescence was measured via BioTek Synergy plate reader, with excitation and emission filters of 530 nm and 590 nm, respectively. Results were normalized to DMSO control and expressed as % cell viability.

**2.8 RNA-sequencing**

To analyze transcriptome-wide changes, RNA was isolated from NRVMs using QIAzol (Qiagen). RNA integrity was assessed via RNA Agilent Bioanalyzer; all samples achieved a RIN value >8. 500 ng of RNA was used for cDNA library preparation with the Ribo-Zero Human/Mouse/Rat TruSeq Stranded Total RNA kit from Illumina. Set-B Adapters (Illumina) were used for the cDNA library prep. Validation of library prep was performed with the Agilent Bioanalyzer and sequencing performed in the Genomics Center at the University of Nevada Reno via the NextSeq 500.

To assure the sequencing performance and library quality, we applied the RNA-SeQC[393] tool to assess the data quality of each sequencing dataset. We used the *Sailfish* pipeline[394] to quantify the mRNA expression from the raw sequencing data, using the *Ensembl*[395] rat gene annotation (Rnor_6.0). Transcript per million reads (*TPM*) was used as the unit of human gene expression level. We used the *edgeR* algorithm[396] to compare the groupwise gene expression pattern. The *TMM* algorithm implemented in the *edgeR* package was applied for reads count normalization and effective library size estimation. Groupwise differential expression was estimated by the likelihood ratio test implicated in the *edgeR* package. The genes were false discovery rate < 5% were deemed differentially expressed. **Supplementary Table 1** illustrates genes examined via RNA-sequencing.

**2.9 Real-time qPCR**

RNA was isolated as described above and RNA quantity determined via NanoDrop Spectrometry ND1000. 500 ng of RNA was reverse transcribed to cDNA via Verso cDNA Synthesis Kit (ThermoFisher Scientific). Quantitative real-time polymerase chain reaction (qPCR) was used to determine mRNA expression for select genes. In short, cDNA underwent qPCR with Apex qPCR GREEN Master Mix (Genesee

Scientific, 42–120) and the following IDT Primers were used: rat atrial natriuretic peptide/factor (ANP, forward- GCC GGT AGA AGA RGA GGT CAT, reverse- GCT TCC TCA GTC TGC TCA CTC A); rat b-type natriuretic peptide (BNP, forward- GGT GCT GCC CCA GAT GAT T, reverse- CTG GAG ACT GGC TAG GAC TTC); rat skeletal muscle alpha actin (Kcnc3, forward-, reverse-); and 18s ribosomal RNA (forward- GCC GCT AGA GGT GAA ATT CTT A, reverse- CTT TCG CTC TGG TCC GTC TT). Fluorescence was detected in real-time using the BioRad CF96X qPCR instrument.

**2.10 Experimental animals**

Nine-week old C57BL/6 mice were housed at the University of Nevada, Reno. All mice were maintained on standard chow and housed under standard 12h light/dark cycle. Animal care and use was approved by the Institutional Animal Use and Care Committee at the University of Nevada, Reno. At ten-weeks of age, C57BL/6 male and female mice were randomized to receive vehicle control (Sham) or angiotensin II (Ang) for 14 days. 14-day micro-osmotic pumps (Alzet, model 1002) were subcutaneously implanted in mice under isoflurane anesthesia; pumps contained angiotensin II (Bachem) at 1.5 μg/kg/min or vehicle control. Mice in both groups were further randomized to receive: 1) sham dosed with vehicle (1:1 DMSO:PEG-300 (Acros Organics)), 2) Ang osmotic pump dosed with vehicle or 3) Ang osmotic pump dosed with emodin (30 mg/kg/day). Mice received intraperitoneal (IP) injection of vehicle or emodin every day for 14 days. Three days prior to the end of the study, systolic blood pressure measurements were taken via tail cuff using the Coda High Throughput System (Kent Scientific); the first two days were used for acclimation and third day for data collection.

At the end of the study, whole hearts and left ventricles (LVs) were weighed and compared to tibia length for morphology analyses. LVs were also dissected for histology and HDAC activity experiments.

### 2.11 Histology

Left ventricles (LVs) were fixed in 4% paraformaldehyde for 24 hours prior to 70% ethanol housing. LVs were then processed using the Leica ASP300S and paraffin embedded using Leica EG1160. PicroSirius Red for collagen staining was performed on LVs cross-sectioned at 5 μm as previously described [41]. Stained LVs were imaged using the Keyence BZ-X700, and collagen staining was quantified using ImageJ software.

### 2.12 Statistical Methods

A minimum of three experiments with an n = 3 per experimental treatment group was performed and data quantified. One-way ANOVA with Tukey's post-hoc was performed unless otherwise specified using GraphPad7 (GraphPad Software, La Jolla, CA). $p < 0.05$ was considered significant.

## 3. Results

*3.1 Emodin and emodin-rich rhubarb inhibited HDAC activity in a dose-dependent, fast-on/slow-off manner*

Emodin is a phytochemical commonly found in rhubarb.[328,389] Our lab previously identified emodin as a class I, IIa and IIb HDAC inhibitor *in vitro* (**Fig. 1A** and [75]). As such, we postulated that emodin-rich rhubarb would phenocopy HDAC inhibition. Indeed, rhubarb inhibited class I and II HDAC activity in a dose-dependent manner ($IC_{50}$=100 mg/L, **Fig. 1A**). Similar to emodin, rhubarb inhibited recombinant class I, IIa and IIb HDACs (**Supplemental Figure 1**), only HDACs 2 and 6 differed between

emodin and rhubarb. These experiments demonstrated that both emodin and rhubarb inhibited HDAC activity at a single point in time. To determine HDAC inhibition kinetics, we incubated cardiac tissue with emodin (50 μM) or rhubarb (100 mg/L) for 0.5, 1, 2, 4, 8, 12 or 24 hours prior to analyzing class I, IIa and IIb HDAC activity. Emodin rapidly inhibited HDAC activity (0.5 hours), with prolonged HDAC inhibition out through 24-hours (**Fig. 1B**). Rhubarb HDAC inhibition kinetics phenocopied emodin (**Fig. 1B**). As the phytochemical profile of a plant can be affected by soil content, climate and other environmental factors, we examined emodin content via high-performance liquid chromatography (HPLC). We confirmed that emodin is a key component of a turkey rhubarb extract (P4L, **Fig. 2**). Surprisingly, 100mg/L of turkey rhubarb contained 2.67 μg/ml of emodin which approximated to 10 μM, further supporting the postulate that emodin-rich rhubarb inhibited HDAC activity. These results collectively suggest that emodin is a fast-on, slow-off pan-HDAC inhibitor that is likely responsible for rhubarb-dependent HDAC inhibition.

*3.2 Emodin attenuated HDAC activity concomitant with increased histone acetylation in cardiac myocytes*

*In vitro* analysis above demonstrated that emodin inhibited HDAC activity, however, these findings do not demonstrate inhibitory actions for this compound within cells or tissue. Therefore, we sought to elucidate the actions of emodin on HDAC activity and histone acetylation in neonatal rat ventricular myocytes (NRVMs). We postulated that emodin would inhibit HDAC activity in NRVMs. To test this postulate, NRVMs were co-spiked with phenylephrine (PE, 10 μM) in the absence or presence of vehicle control, emodin (10 μM) or the well-established pan-HDAC inhibitor Trichostatin A

(TSA, 200 nM). Cells were lysed after 48 hours for protein to assess HDAC activity. We report that emodin significantly inhibited class I, IIa and IIb HDAC activity, similar to TSA (**Fig. 3A**). As HDACs catalytically reduce histone acetylation and we've shown that emodin inhibits HDAC activity, we postulated that emodin would increase histone acetylation in cardiomyocytes. To test this, we treated NRVMs with PE in the absence or presence of emodin as described above. As hypothesized, emodin increased histone H3 acetylation on lysine residues 9/14 (Ac-H3K9/14), 18 (Ac-H3K18) and 27 (Ac-H3K27) in NRVMs (**Fig. 3B** and **3C**). Finally, a cell viability assay to verify that HDAC inhibition and cardiac hypertrophy described below were not secondary to cell death. NRVMs were dosed with either vehicle (DMSO), emodin, PE or PE + emodin at concentrations used in experiments above. No significant differences were observed between treatments (**Fig. 3D**), suggesting that emodin at 10 μM is not cardiotoxic.

*3.3 Emodin and rhubarb blocked receptor- and intracellular-mediated cardiomyocyte hypertrophy*

HDAC inhibitors have been shown to block cardiomyocyte hypertrophy [39]. Thus, we postulated that emodin and emodin-rich rhubarb (P4L) would attenuate receptor- and intracellular signaling-mediated cardiac hypertrophy in NRVMs. Cells were co-spiked with either emodin (10 μM) or rhubarb (P4L, 100 mg/L) and either PE (10 μM) or PMA (50 nM) and incubated for 48 hours prior to being fixed and stained with antibodies against the sarcomere protein α-actinin (green), the hypertrophic marker atrial natriuretic factor (ANF, red) and DAPI (nuclear stain, blue). Cardiomyocytes co-spiked with emodin and either agonist were significantly smaller than those spiked without emodin (**Fig. 4A, 4B**, **4D** and **4E**). Rhubarb (P4L, 100 mg/L) similarly attenuated agonist-induced cardiac

hypertrophy (**Fig. 4A, 4B**, **4D** and **4E)**. Moreover, as emodin-treated NRVMs co-spiked with or without phenylephrine showed no significant differences in cell viability (**Fig. 3D**), cardiotoxicity did not drive these observed differences in NRVM size. Therefore, emodin blocked cardiomyocyte hypertrophy that was induced either by receptor- or intracellular-mediated agonists, suggesting that emodin elicits cardioprotective actions within the cell.

Activation of intracellular signaling cascades such as the mitogen-activated protein kinase (MAPK) pathway as well as re-activation of fetal genes are common features involved in pathological cardiac hypertrophy. Moreover, HDAC inhibition has been shown to attenuate MAPK activation[44] and the fetal gene program.[39,40] We thus hypothesized that emodin would suppress MAPK activation and attenuate the fetal gene ANF in NRVMs. To test this hypothesis, PE treated NRVMs were co-spiked with emodin as described above. Cells were then lysed for immunoblotting experiments or fixed and stained for ANF protein expression. Similar to cardiac hypertrophy, emodin significantly attenuated the MAPK, extracellular signal-regulated kinase (ERK) phosphorylation (**Supp. Fig. 2A and 2B**). In addition, emodin attenuated ANF (part of the fetal gene program) protein expression (**Fig. 4A, 4C, 4D, 4F, Supp. Fig. 2A and 2C**). Similar to emodin, rhubarb (P4L) also inhibited ANF protein expression (**Fig. 4A, 4C, 4D, 4F**).

*3.4 Emodin reversed stress-induced changes in the cardiomyocyte transcriptome similar to TSA*

HDAC inhibitors such as the well-established inhibitor TSA regulate differential gene expression in cardiomyocytes.[397] Moreover, HDAC inhibitors attenuate the fetal

gene program. We thus postulated that emodin and TSA would similarly reverse PE-induced differential gene expression in NRVMs. To test this postulate, cells were treated with vehicle or PE. PE-treated NRVMs were co-spiked with either vehicle control, emodin (10 μM) or TSA (200 nM), incubated for 48 hours and then lysed for RNA-sequencing. We report that emodin normalized 54 genes that were upregulated with PE, 30 of which overlapped with TSA (**Fig. 5A and 5B**). Furthermore, 18 genes were normalized with emodin that were downregulated in PE-treated NRVMs, 12 genes overlapped with TSA (**Fig. 5A and 5B**). As mentioned above, pathological cardiac hypertrophy is linked to re-activation of the fetal gene program and HDAC inhibitors attenuate this re-activation.[39,40] In keeping with these reports, emodin reversed agonist-induced mRNA expression of the fetal genes atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (**Fig. 5C**), as examined by qPCR. Finally, our heat map showed that emodin also upregulated genes that were suppressed by PE. Indeed, qPCR demonstrated that emodin increased potassium voltage-gated channel subfamily C member 3 (Kcnc3) mRNA (**Fig. 5C**). Combined, these data demonstrate that emodin reverses stress-induced differential gene expression in cardiomyocytes, similar to the pan-HDAC inhibitor TSA.

### *3.5 Emodin attenuated pathological cardiac hypertrophy and fibrosis in angiotensin II-infused mice*

To elucidate the role of emodin in regulating cardiac hypertrophy *in vivo*, we treated angiotensin II (Ang)-infused male and female C57BL/6 mice with or without 30 mg/kg/day of emodin (Emod). After 14-days with Ang treatment in the absence or presence of Emod, heart weight (HW) and left ventricle (LV) weight was examined and

date normalized to tibia length (TL). As anticipated, Ang significantly increased systolic blood pressure (**Table 1**), HW and LV weight in male and female mice compared to vehicle control mice (**Fig. 6A and 6C**). However, HW and LV weight were significantly attenuated with Emod treatment for male or female mice, Emod did not differ from Veh control (**Fig. 6A and 6C**). Interestingly, differences in cardiac hypertrophy were not due to attenuation of systolic blood pressure, as no significant difference was observed between Ang and Ang+Emod male and female mice (**Table 1**). HDAC inhibitors have also been shown to block angiotensin II-induced cardiac fibrosis.[41] Consistent with this, emodin significantly attenuated Ang-induced cardiac fibrosis in male and female mice (**Fig. 6B and 6D**). Of note, treatment with emodin significantly inhibited class I and IIa HDAC activity compared to Ang-treated male and female mice (**Supplemental Fig. 3**). Combined, these data suggest that emodin protects the heart from pathological cardiac hypertrophy and fibrosis *in vivo,* in part, by inhibiting HDAC activity.

**4. Discussion**

In this study, we showed that emodin attenuated pathological cardiac hypertrophy *in vitro* and *in vivo,* with *in vivo* findings further demonstrating that emodin attenuated cardiac fibrosis. A rhubarb extract rich in emodin, as confirmed via HPLC (**Fig. 2**), similarly attenuated agonist-induced cardiomyocyte hypertrophy. These cardioprotective events correlated with increased histone acetylation and attenuated HDAC activity in NRVMs treated with emodin. Additionally, cardiac lysate spiked with either emodin or rhubarb inhibited HDAC activity in a dose-dependent, fast-on, slow-off kinetic manner. Emodin also inhibited HDAC activity in the hearts of mice exposed to angiotensin II. Finally, PE induced differential changes in the cardiomyocyte transcriptome; emodin

reversed these changes similar to the well-established pan-HDAC inhibitor, TSA. Combined, our data support our postulate that dietary food bioactive HDAC inhibitors like emodin attenuate cardiac hypertrophy via transcriptome-wide changes in gene expression.

Class I and II HDAC inhibition is efficacious in primary cell culture and animal models of pathological cardiac hypertrophy. Antos and colleagues[39] first reported the pan-HDAC inhibitor, TSA dose-dependently attenuated cardiomyocyte hypertrophy; this was concomitant to increased histone acetylation and inhibition of the fetal gene program, which is a set of genes (e.g., ANF and BNP) that are re-activated in hypertrophic models *in vitro* and *in vivo* as well as in human HF.[398–400] Later reports further demonstrated that TSA increased histone acetylation and attenuated cardiac hypertrophy and fetal gene program re-activation in mice exposed to pressure-overload-induced hypertrophy.[40] Consistent with these reports, our data showed that emodin inhibited cardiac myocyte hypertrophy concomitant to increased histone acetylation and inhibition of HDAC activity and the fetal gene program. HDACs regulate the removal of acetyl marks from nucleosomal histones and as such control DNA accessibility leading to global changes in gene expression.[401] Not surprisingly, TSA has been shown to alter the transcriptome in NRVMs, normalizing gene expression changes in response to pathological stress. [39,348] Consistent with these findings, emodin reversed stress-induced changes in the cardiomyocyte transcriptome similar to TSA, supporting the postulate that emodin inhibits cardiac enlargement via epigenetic regulation of HDAC activity. It should be noted however, that complete overlap was not observed between emodin and TSA, suggesting that emodin potentially regulates gene expression through other diet-

gene mechanisms. Of interest, some of these overlapping genes were involved in pathological cardiac hypertrophy and muscle contraction including the myosin light and heavy chains and cardiac troponin as well as inflammatory mediators like interleukin 6. Despite the non-complete overlap in gene expression, these are the first reports, to our knowledge, demonstrating transcriptome wide changes in cardiac myocytes in response to the dietary HDAC inhibitor emodin.

In addition to pan-HDAC inhibitors, class I selective HDAC inhibitors have been shown to increase histone acetylation and inhibit cardiomyocyte hypertrophy in NRVMs.[402] Moreover, class I selective HDAC inhibition was shown to block transverse aortic constriction-induced cardiac hypertrophy[402] as well as angiotensin II-induced fibrosis in mice.[41] Unlike class I selective inhibitors, inhibition of the class II HDAC, HDAC6 was shown to improve cardiac contractile function in a mouse model of hypertension, independent of changes to cardiac hypertrophy.[42] Combined, these reports would suggest that targeting class I and II HDACs contribute to cardioprotection via improvements in cardiac enlargement and contractile function. In this study, we report that emodin and emodin-rich rhubarb inhibited class I and II HDAC activity, suggesting that cardioprotection noted for fruit and vegetable intake is likely mediated through a myriad of epigenetic and non-epigenetic mechanisms that contribute to normalization of the transcriptome and improvements in muscle function.

Early reports involving non-epigenetic regulation showed that emodin ameliorated oxidative stress and inflammation in the heart.[344,403,404] For example, emodin attenuated inflammation and apoptosis in cardiac myocytes in response to

ischemia/reperfusion (I/R).[344] Here, Ye and colleagues[344] showed that emodin dose-dependently reduced the nuclear factor kappa B (NF-κB) inflammasome pathway. Many intracellular signaling cascades play fundamental roles in regulating pathological cardiac hypertrophy and contribute to cardiac dysfunction, including NF-κB and the MAPKs.[373,405–407] For example, nuclear ERK activation has been shown sufficient to drive cardiac enlargement and fibrosis in mice.[373,407] Of significance, HDAC inhibitors, including TSA, have been shown to down-regulate NF-κB[103] and MAPK activation.[44] In particular, class I inhibition of HDACs was shown to attenuate ERK activation in cardiac myocytes; this partially contributed to inhibition of pathological cardiac hypertrophy.[44] Consistent with this report, we showed that emodin inhibited ERK phosphorylation in NRVMs. Combined, these data would suggest that emodin elicits cardioprotection via regulation of intracellular signaling cascades that are dependent on HDAC activity (e.g. ERK and NF-κB).

Emodin is found in many plants, with high concentrations noted in rhubarb.[328,329] We were unable to find human studies that examined rhubarb consumption or emodin bioavailability from rhubarb consumption, yet would speculate low circulating levels of the compound after a meal. While it is unlikely that rhubarb is consumed frequently or in large amounts, it should be noted that our dose of emodin approximated 10 μM in 100 mg/L of rhubarb, our dose used in these studies. This dose of rhubarb would be considered low in a meal. In addition, we reported fast-on and slow-off HDAC inhibition with rhubarb or emodin. From these kinetic data, we would speculate that rhubarb need not be ingested frequently for HDAC inhibition. Lastly, we would argue that an emodin dietary supplement could also be considered for HDAC inhibition. Despite these

speculations, however, emodin bioavailability remains low,[328,408] suggesting the need for research examining its interactions with the microbiome on heart health. Others have taken a more direct approach by examining ways to improve emodin bioavailability e.g. with nanoparticle encapsulation.[409]

In this study we focused on emodin within rhubarb and neglected the actions for the other phytochemicals present. These additional phytochemicals likely played a role in the discrepancy observed against the recombinant HDACs, in which emodin inhibited all HDACs but HDAC4 while rhubarb inhibited all HDACs except for HDACs 2, 4 and 6 (**Supplemental Fig. 1**). It should be noted, however, that emodin and rhubarb similarly attenuated stress-induced cardiac hypertrophy in NRVMs, suggesting that emodin is the primary compound in rhubarb that epigenetically alters the transcriptome contributing to anti-hypertrophic actions in the heart.

Lastly, reports have reviewed the therapeutic potential of HDAC inhibition in pre-clinical models of HF;[410,411] however, no study or trial has been published (to our knowledge) that cites HDAC inhibition as the primary or secondary target of any pharmacological agent in the human heart. Several FDA-approved HDAC inhibitors, such as Vorinostat (i.e. SAHA), are currently on the market to treat human T-cell lymphoma, with several more in clinical trials for various cancers (clinicaltrials.gov). It should be noted however that Xie and colleagues[412] showed efficacy for Vorinostat in I/R-induced cardiac dysfunction; this study was important as it showed that HDAC inhibitors could reverse stress-induced cardiac damage in a large animal model (i.e. rabbit) and therefore has established efficacy for moving SAHA into clinical trials for HF

patients. However, clinical trials are expensive and the duration from pre-clinical experiments to FDA-approval is lengthy. Dietary compounds provide intriguing preventative or therapeutic options for their current lack-of-oversight from the FDA, per the Dietary Supplement Health and Education Act of 1994 (DSHEA) and can reach the market without human study in a timely manner.

**Funding:** This work is supported by the USDA NIFA (Hatch-NEV00727, Hatch-NEV00767), the Dennis Meiss & Janet Ralston Fund for Nutri-epigenetic Research, the National Institute for General Medical Sciences (NIGMS) of the NIH (P20 GM130459) and the National Heart, Lung, and Blood Institute of the NIH (R15 HL143496) and NSF EPSCOR Track II (OIA-1826801) to B.S.F. Core facilities used for Research were supported by NIGMS of the NIH (P20 GM103554).

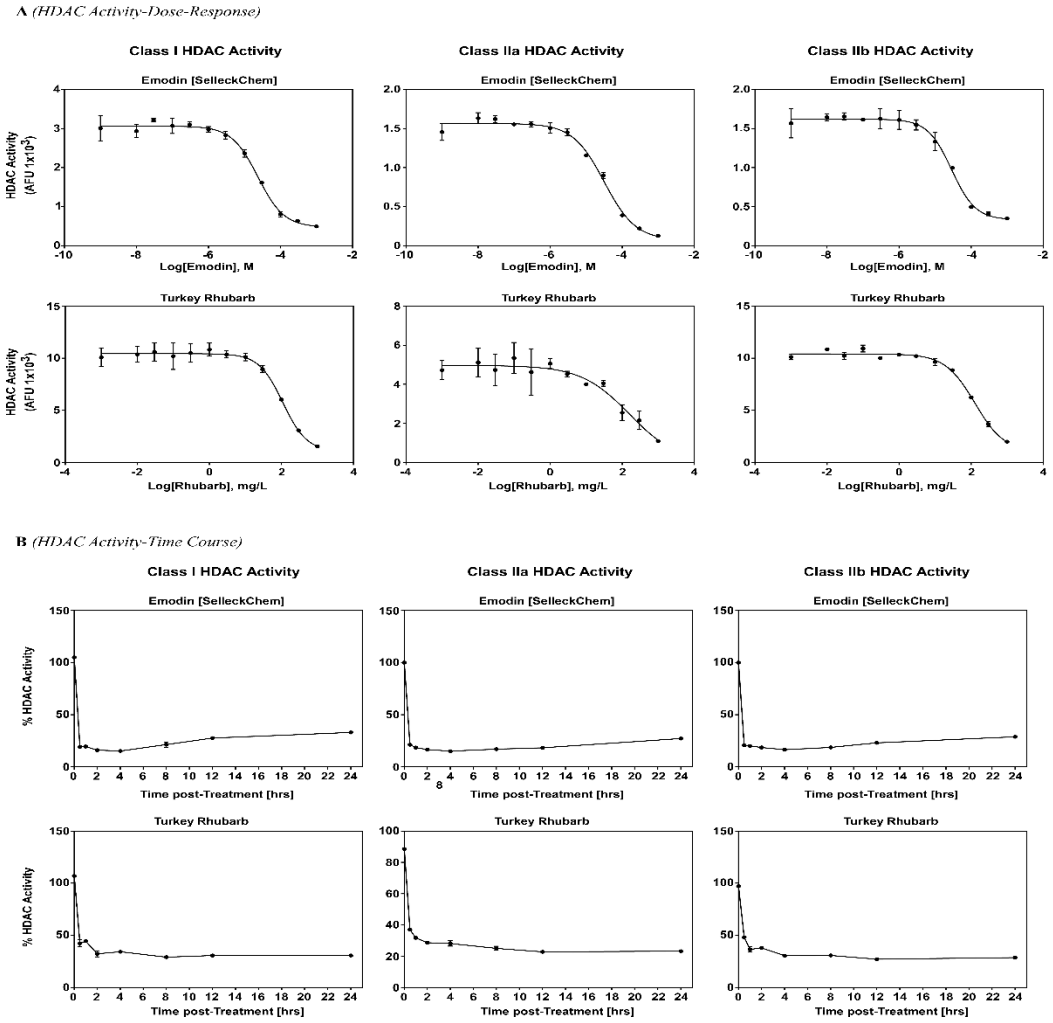
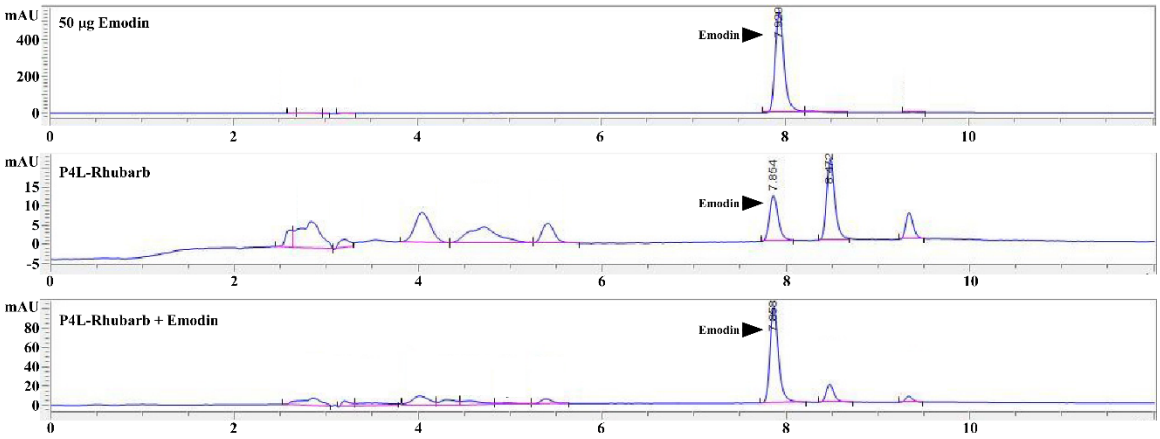



**Figure 1. Emodin and rhubarb inhibit HDAC activity in bovine cardiac tissue with fast-on, slow-off kinetics.** A) Bovine cardiac tissue was treated for 2 hours with increasing doses of emodin (Top Panel) or turkey rhubarb (bottom panel) prior to incubation with cell permeable fluorogenic HDAC substrates for 2 hours and developer solution for 20 minutes. B) Bovine cardiac lysate was treated with emodin (10 µM; Top Panel) or turkey rhubarb (100 mg/L) over time (24 hrs max) prior to incubation with the cell permeable fluorogenic HDAC substrates followed by developer solution. Fluorescence was assessed via BioTek Synergy plate reader with excitation/emission set at 360/460 nm.

**A** *(P4L-Rhubarb HPLC)*




**B** *(Emodin Quantification)*

| | µg/ml | 95% CI | p-Value | S.D | S.E.M | RSD% |
|---|---|---|---|---|---|---|
| P4L-Rhubarb | 2.67 | (2.65,2.69) | $3.38 \times 10^{-8}$ | 0.0085 | 0.0049 | 0.32% |

**Figure 2. Turkey rhubarb contains appreciable emodin as determined by HPLC.** High Performance Liquid Chromatography (HPLC) was used to determine emodin concentrations within turkey rhubarb, 50 µg emodin standard was used to determine emodin peaks and compare emodin concentrations within turkey rhubarb. Turkey rhubarb spiked with emodin was used to verify the emodin peak. B) Emodin concentration was quantified.

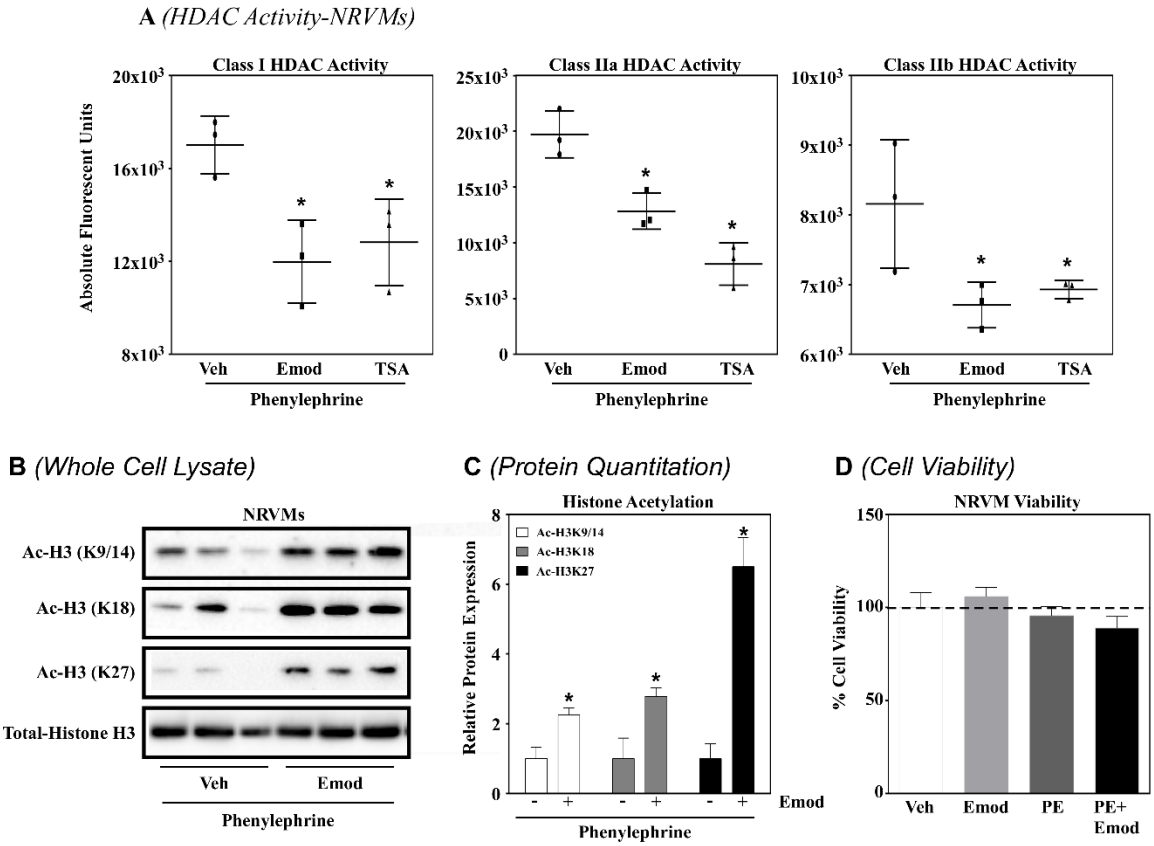



**Figure 3. Emodin inhibited HDAC activity in cardiac myocytes concomitant to increased histone acetylation.** A) Neonatal rat ventricular myocytes (NRVMs) were incubated with phenylephrine (10 µM; PE) with or without emodin (10 µM; Emod) or trichostatin A (200 nM; TSA) for 48 hours prior to protein lysis. Cells were lysed and incubated against the cell permeable fluorogenic HDAC substrates for 2 hours and developer solution for 20 minutes. Fluorescence was assessed via BioTek Synergy plate reader with excitation/emission set at 360/460 nm. B) NRVMs were incubated with phenylephrine (10 µM; PE) with or without emodin (10 µM; Emod) or trichostatin A (200 nM; TSA) for 48 hours prior to protein lysis. Cell lysate was then incubated against antibodies for acetylated histone 3 at lysine (K) residues K9/14 (Ac-H3K9/14), K18 (Ac-H3K18), K27 (Ac-H3K27) as well as against total histone H3 (Total-Histone H3) prior to

**Figure 3 cont.** analysis via immunoblot. C) Acetyl Histone H3 proteins were normalized to total H3 and quantitation performed via Image J software. D) NRVMs were treated with DMSO or emodin (10 μM; Emod) with or without phenylephrine (10 μM; PE) for 48 hours prior to incubation with Invitrogen alamarBlue™ HS Cell Viability Reagent (10:1, media with NRVMs:Invitrogen alamarBlue™ HS Cell Viability Reagent) for 1 hour. Fluorescence was measured via BioTek Synergy plate reader, with excitation and emission filters of 530 nm and 590 nm, respectively. All statistical analyses were run in GraphPad Prism Software. One-way ANOVA with Tukey's Post-hoc analysis was used for HDAC activity assays, while student's t-test with Welch's Correction used for immunoblot analysis.

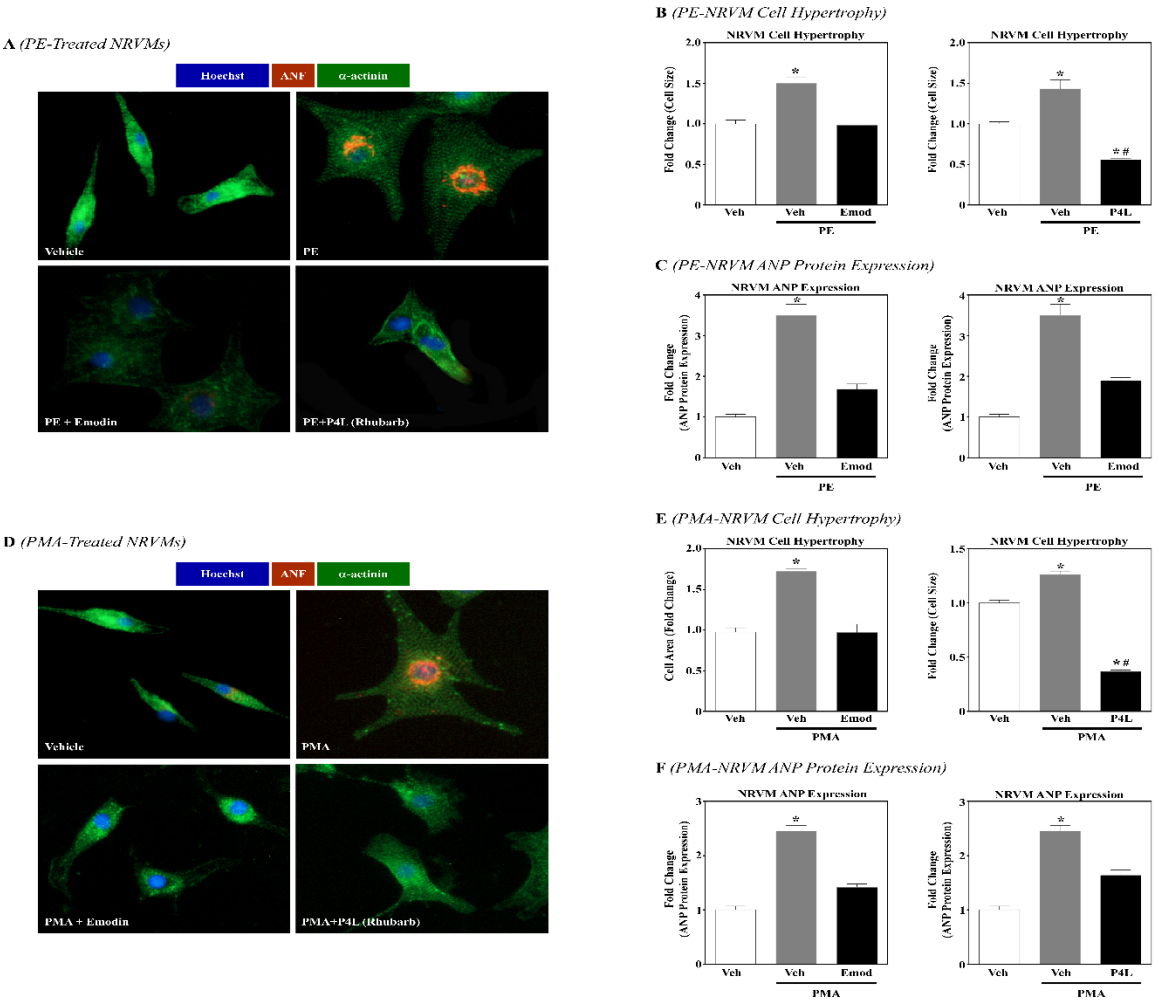
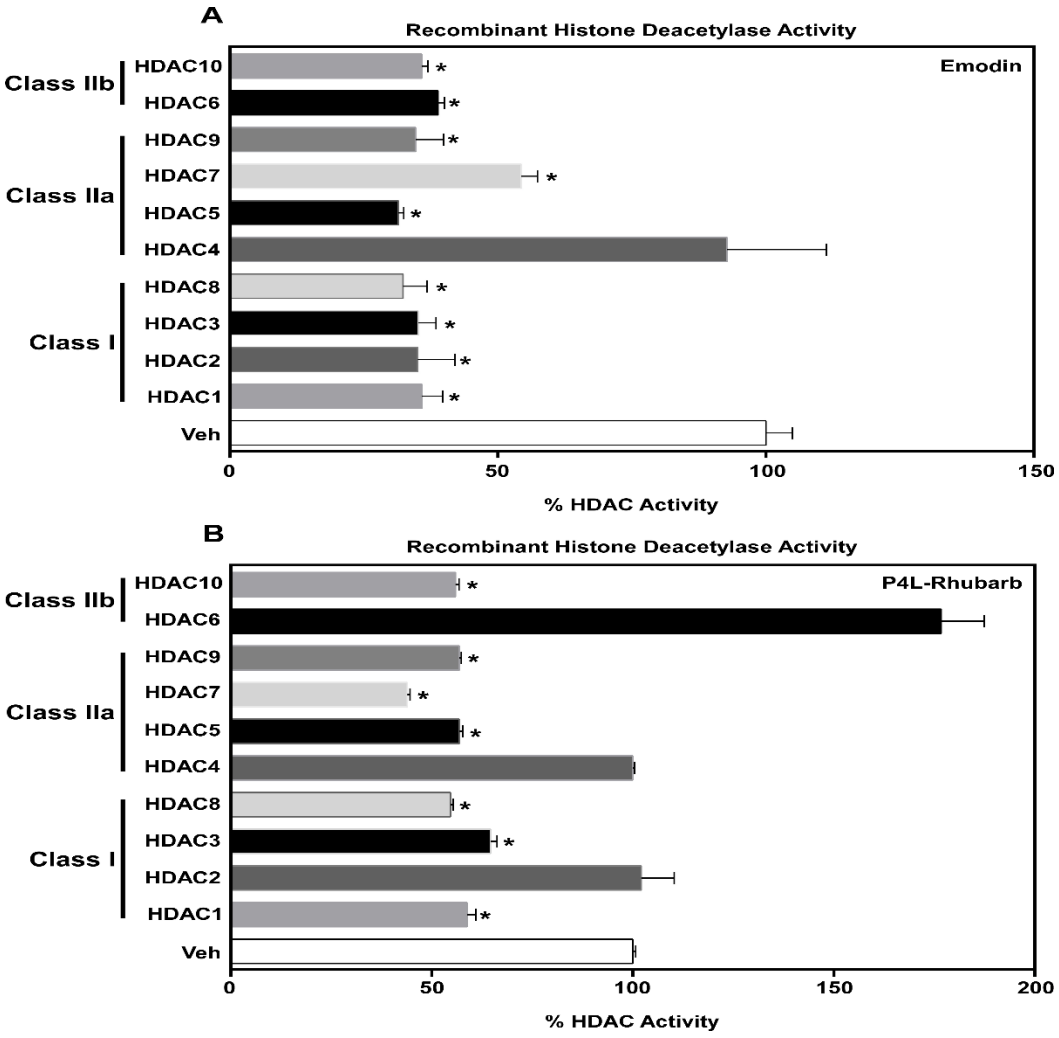



**Figure 4. Emodin and rhubarb inhibit cardiomyocyte hypertrophy.** A) Neonatal rat ventricular myocytes (NRVMs) were stimulated to hypertrophy with phenylephrine (10 µM; PE) in the absence or presence of Emodin (10 µM) or rhubarb (100 mg/L; P4L) for 48 hrs. Cells were fixed and immunostained with antibodies directed against α-actinin or atrial natruetic factor (ANF). Cell nuclei were stained with Hoechst. Cells were visualized with EVOS microscopy. Ten pictures were taken per well and cell size (area) and ANF expression (pixels) were calculated using Image J software. GraphPad Prism was used to examine statistical significance. One-way ANOVA with Tukey's post-hoc analysis was used. Significance was set at $p<0.05$. B) Cell area and C) ANF expression

**Figure 4 cont.** was quantified. D) NRVMs were stimulated to hypertrophy with phorbol myristate acetate (50 nM; PMA) in the absence or presence of Emodin (10 μM) or rhubarb (100 mg/L; P4L) for 48 hrs. Cells were fixed and immunostained with antibodies directed against α-actinin or atrial natriuretic peptide (ANP). Cell nuclei were stained with Hoechst. Cells were visualized with the Invitrogen EVOS FL microscope. Ten pictures were taken per well and cell size (area) and ANP expression (pixels) were calculated using Image J software. GraphPad Prism was used to examine statistical significance. One-way ANOVA with Tukey's post-hoc analysis was used. Significance was set at $p<0.05$. E) Cell area and F) ANP expression was quantified.




**Supplementary Figure 1. Emodin and rhubarb inhibit recombinant HDAC activity.** Recombinant HDACs were incubated with A) emodin (10 µM) or B) turkey rhubarb (100 mg/Ll P4L) for 2 hours prior to the addition of cell permeable HDAC substrates. Developer solution was added for 20 minutes fluorescence assessed via BioTek Synergy plate reader with excitation/emission set at 360/460 nm. GraphPad Prism software was used to visualize data and determine significance ($p<0.05$). One-way ANOVA with Bonferroni's Post-hoc was used to determine significance

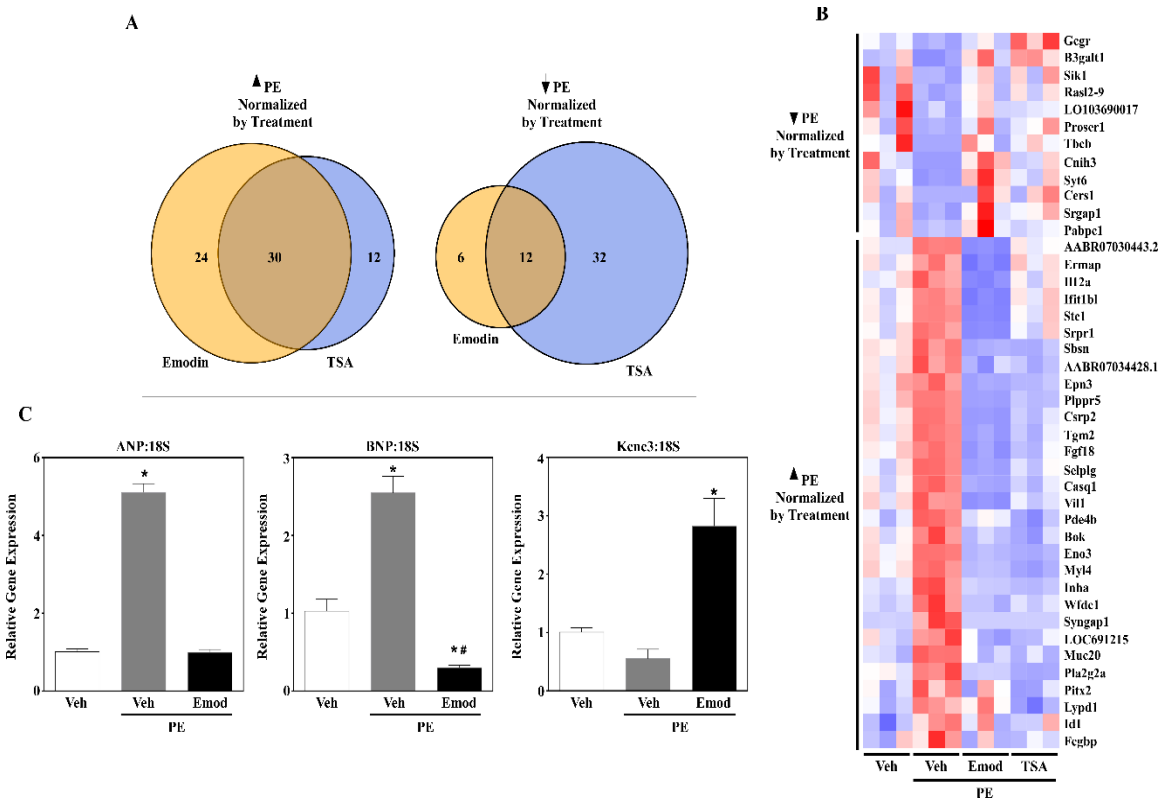
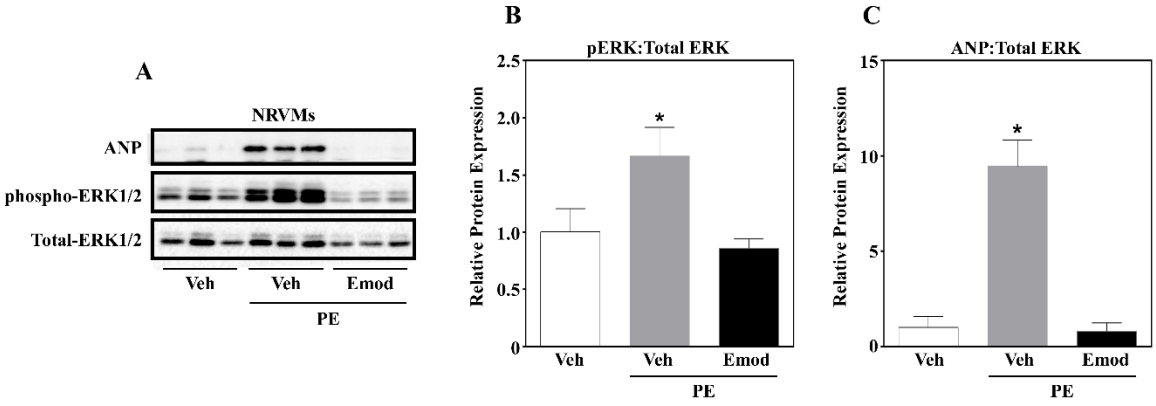



**Figure 5. Emodin and TSA normalized PE-mediated cardiac gene expression changes.** Neonatal rat ventricular myocytes (NRVMs) were stimulated to hypertrophy with phenylephrine (10 µM; PE) in the absence or presence of Emodin (10 µM) or Trichostatin A (200 nM; TSA) for 48 hrs. RNA was isolated via Trizol prior to RNA-sequencing analysis. A) Venn diagrams demonstrate gene expression overlap for emodin and TSA. TSA and emodin normalized PE-induced cardiac gene (A; Left Panel) as well as PE-suppressed (A; Right Panel) gene expression. B) A heat map representing overlap for TSA and emodin normalization of PE-mediated genes. C) Pathological hypertrophy is linked to re-activation of fetal genes. NRVMs stimulated as described above were lysed for RNA. Quantitative PCR was used to examine fetal gene profiles for atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and potassium voltage-gated channel subfamily C member 3 (Kcnc3). GraphPad Prism was used to examine statistical

**Figure 5. cont.** significance via one-way ANOVA with Tukey's post-hoc. Significance was set at $p<0.05$.




**Supplementary Figure 2. Emodin inhibited ANP protein expression and ERK phosphorylation in cardiac myocytes.** A) Neonatal rat ventricular myocytes (NRVMs) were incubated with emodin (10 µM; Emod) for 48 hours prior to protein lysis. Cells were lysed and incubated against antibodies for atrial natriuretic peptide (ANP), phosphorylated ERK1/2 (phospho-ERK1/2) and total-ERK1/2 prior to analysis via immunoblot. B) Phospho-ERK1/2 and C) ANP were normalized to total-ERK1/2 and quantitation performed via Image J software. All statistical analyses were run in GraphPad Prism Software. One-way ANOVA with Tukey's Post-hoc analysis was used to determine significance set at $p<0.05$.

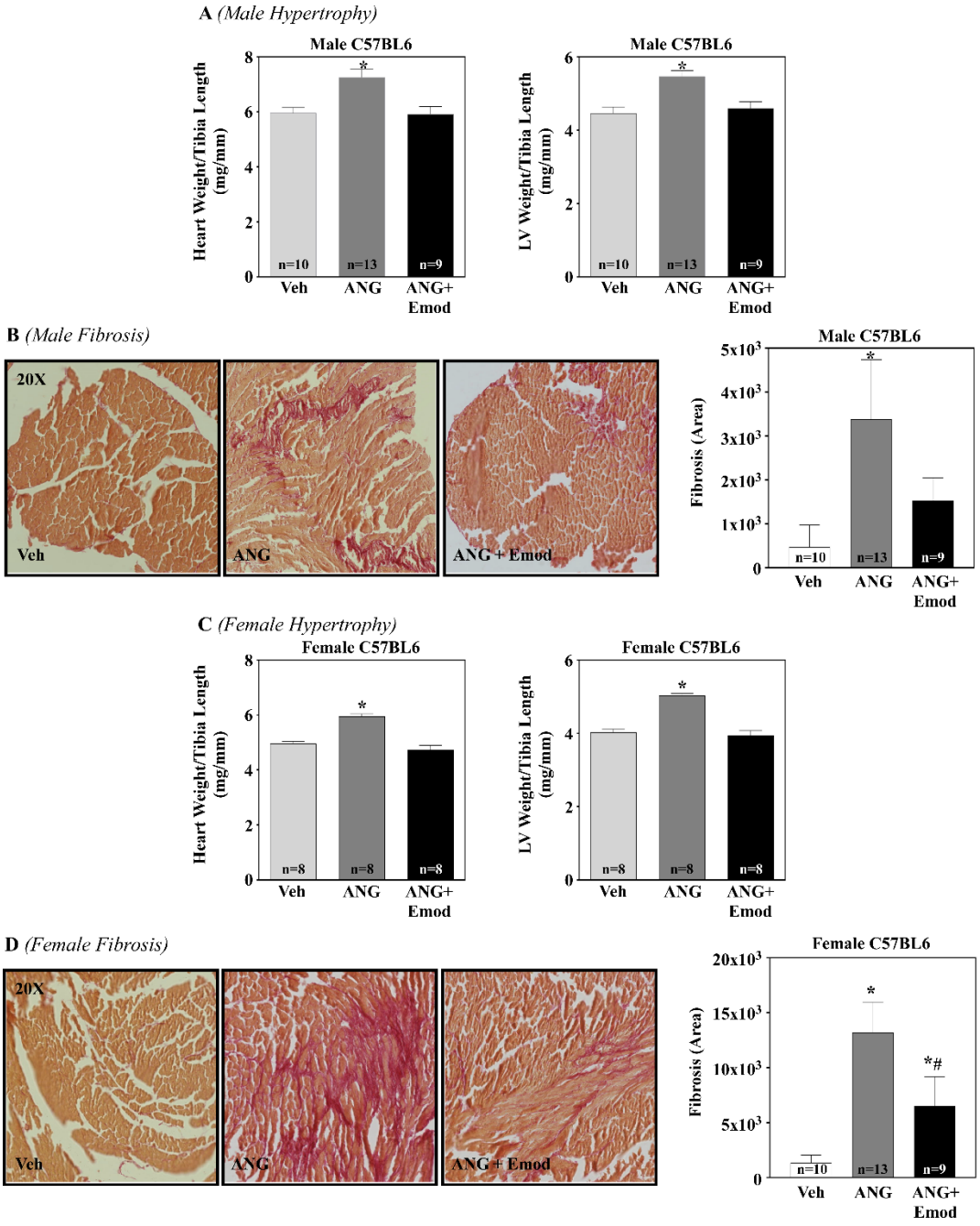
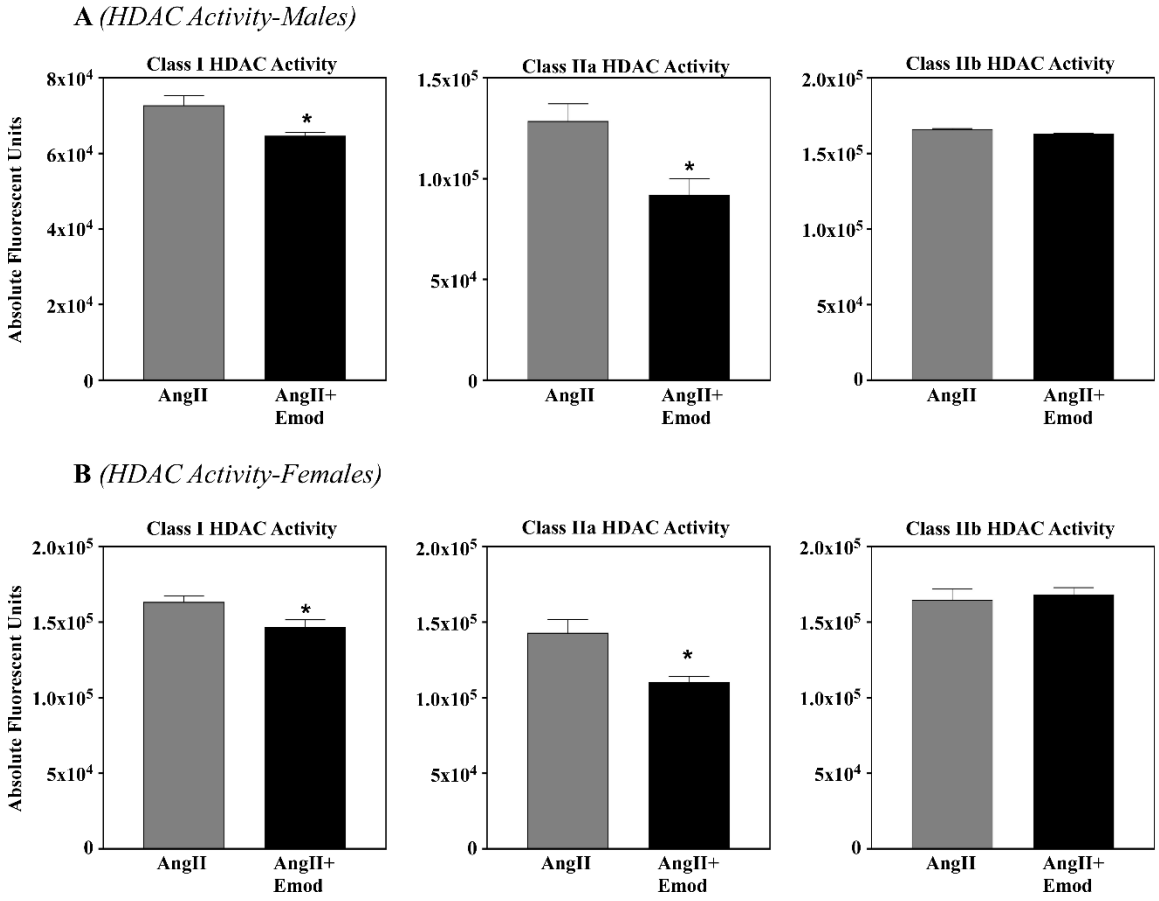



**Figure 6. Emodin attenuated angiotensin II-induced pathological hypertrophy and fibrosis in male and female mice.** C57BL/6 male and female mice were surgically implanted with a sham (Veh) or micro-osmotic pump containing angiotensin II (Ang; 1.5 μg/kg/min) and dosed with or without emodin (Emod, 30 mg/kg/day) for 14 days. 14

**Figure 6 cont.** days post Ang, whole hearts and left ventricles were dissected and assessed for hypertrophy and fibrosis. Whole heart weight to tibia length (HW/TL, mg/mm) and left ventricle weight to tibia length (LV/TL, mg/mm) of sham (Veh), Ang and Ang+Emod treated C57BL/6 male (A) and female (C) mice were examined at study end (14 days). Left ventriclular collagen of sham (Veh), Ang and Ang+Emod treated C57BL/6 mice was assessed via PicroSirius Red staining for male (B) and female (D) mice. ImageJ software was used to determine fibrotic area. GraphPad Prism was used to examine statistical significance. One-way ANOVA with Tukey's post-hoc analysis was used. Significance was set at $p<0.05$.




**Supplementary Figure 3. Emodin inhibited class I and IIa HDAC activity in angiotensin II-treated mice.** Classe I, IIa and IIb HDAC activity was examined in the left ventricles (LV) of AngII-treated male (A) and female (B) C57BL/6 mice treated with or without emodin (Emod) for 14 days. LV lysate was incubated with substrates selective of either class I, IIa or IIb HDACs for 2 hours prior to developer solution and fluorescence assessment via the BioTek Synergy plate reader (excitation/emission set at 360/460 nm). GraphPad Prism software was used to visualize data and determine significance ($p<0.05$). Student's t-test with Welch's Correction was used to determine significance.

**Table 1. Physiological Measures of Angiotensin II-infused Mice.**

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | **Vehicle** | **ANG II** | **ANG II + Emodin** | **Vehicle** | **ANG II** | **ANG II +Emodin** |
| Study Start BW (g) | 26.38 ± 2.44 | 26.40 ± 2.47 | 26.55 ± 1.75 | 19.08 ± 0.83 | 18.83 ± 1.05 | 18.90 ± 1.09 |
| Study End BW (g) | 26.89 ± 2.44 | 27.43 ± 2.39 | 24.07 ± 1.62 | 21.16 ± 0.87 | 21.25 ± 0.56 | 20.09 ± 1.45 |
| Systolic Blood Pressure (mmHg) | 124.95 ± 16.33 | 141.17 ± 15.80 * | 134.85 ± 21.11# | 123.98 ± 9.36 | 149.32 ± 14.77* | 137.59 ± 8.99# |

## Chapter 4: Emodin inhibited hypertension-induced cardiac hypertrophy and altered the gut microbiome.

### Abstract

Hypertension is a primary risk factor for the pathogenesis of cardiovascular disease, which is the leading cause of death around the world. One such cardiovascular disease that results from hypertension is heart failure, for which investigation of novel therapeutics is warranted. The gut microbiome has recently been implicated in heart failure. Emodin was shown to prevent pathological cardiac hypertrophy and attenuate fibrosis, two hallmarks of heart failure. Thus, we sought to associate the cardioprotective actions of Emodin with the microbiome in hypertensive mice and then to delineate the effects of Emodin on microbiota throughout the gastrointestinal tract and in feces of healthy mice. Emodin indeed blocked hypertension-induced cardiac hypertrophy and enriched microbial abundance of the cardioprotective bug, *Akkermansia.* Further, microbial diversity reduced before normalizing come day seven in mice supplemented with Emodin. Finally, Emodin enriched microbial abundance of the cardioprotective bugs, *Akkermansia, Roseburia* and *Allobaculum*. Our data suggest that Emodin was cardioprotective, in part, by enriching microbial abundance of bugs efficacious to the heart, such as *Akkermansia, Roseburia* and *Allobaculum*.

## Introduction

Hypertension is a major public health concern that contributes to the development of heart failure.[1] Hypertension impacts vascular resistance and loads the heart, which contributes to scar tissue formation (fibrosis) and heart enlargement (hypertrophy); this ultimately results in cardiac dysfunction and heart failure.[1]

According to the Global Burden of Disease study, poor quality diets have contributed to the rise in hypertension, heart failure and other cardiovascular disease components.[413] Indeed, diet is now considered the number one risk factor for preventable diseases in the United States and other developed nations.[413] Understanding how nutrients and other dietary components regulate cellular fate to promote health and longevity affords clinicians an opportunity to identify dietary strategies that can reduce and potentially reverse CVDs including hypertension and heart failure. This can be seen in the literature where increased intake of fruits and vegetables is linked to cardioprotection,[104,414] and in which studies identified fiber, vitamins, minerals and phytochemicals within these foods as capable of preventing and potentially treating pathological cardiac hypertrophy, fibrosis and dysfunction.[29,415,416]

Technological advancements have further impacted our understanding for diet in CVD health. This is evident in recent studies of the microbiome, which has emerged as a major player in CVD pathogenesis.[51,60,62] In fact, distinct microbial communities can be found in humans at risk of CVD [61] with dyslipidemia,[51] atrial fibrillation,[49] hypertension[62] and heart failure.[50] More importantly, angiotensin II-induced hypertension only increased pathological cardiac hypertrophy, fibrosis and dysfunction in conventionally raised mice

but not germ-free mice, suggesting that the microbiome is essential for hypertension-induced changes in the myocardium.[64] Consistent with this, fecal matter transplant (FMT) from hypertensive rats led to hypertension and dysbiosis in non-hypertensive rats.[65] Conversely, therapies that target and normalize the microbiome, similar to healthy controls, improve hypertension in rodents and humans.[417–419] As the diet plays a major role in regulating the microbiome, dietary components have the potential to regulate and/or maintain microbial homeostasis to prevent and/or ameliorate CVD and myocardial dysfunction.

Recent attention has been given to phytochemicals over the last decade in the prevention and treatment of CVD and pathological changes to the heart that results in heart failure.[385,420] Indeed, our group has published that an anthraquinone, emodin, found in rhubarb, buckthorn, knotweed, beans and cabbage can inhibit pathological cardiac remodeling.[357] Other groups have also highlighted the cardioprotective actions of emodin in the heart. For instance, Wu et al.[421] reported that emodin improved left ventricular function via increased phosphorylation of protein kinase B/glycogen synthase kinase-3 beta (pAkt/pGSK-3β) in diabetic rats with cardiomyopathy.[421] Emodin was also shown to attenuate activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome that led to decreased cardiomyocyte death in response to ischemia reperfusion (I/R) injury.[344] As mentioned above, we reported emodin inhibited pathological cardiomyocyte hypertrophy by attenuating Zn-dependent HDACs, while others have shown improvements via activation of $NAD^+$-dependent HDACs,[357,422] thus indicating the emodin can regulate the genome via epigenetic interactions. However, most of these studies focused on the actions of the parent compound, emodin, and

examined direct actions within cell culture or through intraperitoneal injections. Few studies have examined the impact for emodin on the microbiome,[423] nor have these studies elucidated emodin-microbiome interactions in a model of CVD.

As the microbiome has been implicated in modulating phytochemical efficacy,[424] it is imperative to elucidate the effects of emodin in the gut. Thus, the goal of this study was to examine the effects of emodin on the microbiome of hypertensive and healthy mice. Again, we observed that emodin attenuated pathological hypertrophy in hypertensive mice, with no changes in blood pressure. However, we also report several novel and interesting observations in this study: 1) hypertension decreased microbial diversity and altered the microbiome, increasing pathogenic bacteria (e.g. *Lachnospiraceae*); 2) emodin also decreased bacterial diversity in healthy mice by day 3 of ingestion, yet increased the expression of beneficial bacteria (e.g. Bacteroides thetaiotaomicron); 3) emodin normalized the microbiome in hypertensive animals similar to control mice and improved bacterial composition that favored leanness (e.g. *Roseburia*) and cardioprotection (e.g. *Akkermansia*); and 4) lastly, microbial composition was dependent on the route for emodin administration in which gavage dosed animals saw an increased in selective protective bacteria (e.g. *Roseburia*), while intraperitoneally dosed mice saw an increase in other protective bacterial species (e.g. Bacteroides thetaiotaomicron) suggesting that liver metabolism of emodin may impact microbial communities. While correlative, these findings suggest that the cardioprotective actions of emodin are driven, in part, through the regulation of the microbiome.

**Materials and Methods**

*Animal experiments:* All animal studies were performed in accordance with the University of Nevada Reno ICACUC. Female C57BL/6 mice were purchased from Jackson Labs and arrived at nine-weeks of age. Animals were and housed in the Office of Animal Resources at the University of Nevada, Reno. After one week of acclimation, mice (10-weeks of age) were randomly assigned to receive a subcutaneous sham operation (Sham) or a subcutaneous 14-day micro-osmotic pump (Alzet, model 1002) that contained angiotensin II (AngII, Bachem) which was administered at 1.5 μg/kg/min. Mice that were assigned to receive AngII were further randomly assigned to receive an intraperitoneal (i.p.) injection of vehicle control 1:1 solution of dimethyl sulfoxide and polyethylene glycol-300 (DMSO, Pharmco-AAPER; PEG-300, Acros Organics) or Emodin (SelleckChem, S2295) at 30 mg/kg/day dissolved in a 1:1 solution of DMSO:PEG-300. Sham further received vehicle control via IP injection. The 14-day experiment contained three different groups: non-hypertensive sham control (Sham); hypertension plus vehicle control (Hypert); and hypertension plus Emodin at 30 mg/kg/day (Hypert+Emodin). An n=8 mice/group were used in these studies. Mice were weighed and dosed with vehicle or Emodin via IP injection each day for 14 days. Mice were acclimated to a tail cuff blood pressure system (Coda High Throughput System, Kent Scientific) for 3-days prior to systolic blood pressure measurements at the study end. Cecum and hearts were dissected at the end of study. Whole heart and left ventricle weight was assessed. The tibia was dissected and measured. Heart weight (HW) and LV weight was normalized to tibia length as well as body weight. Cecum (n=3) was used for 16S rRNA sequencing.

Nine-week-old C57BL/6 mice were ordered from Jackson Labs and allowed to acclimate. Ten-week old healthy mice were randomly assigned to receive vehicle (1:1, DMSO:PEG-300) or Emodin at 30 mg/kg/day via oral gavage for seven days. Mice were weighed and dosed every day for seven days. Feces was collected every day for seven days. An n=4/treatment group was used for gavage studies. At study end, the gastrointestinal tract was dissected into individual components of the small and large intestine as well as the cecum. Tissues were immediately flash frozen and sent to Midwestern University for DNA extraction and 16S sequencing. Daily feces was also analyzed via 16S sequencing.

*Real-Time quantitative polymerase chain reaction (rt-qPCR):* RNA was isolated from the left ventricles of mice from the hypertension experiments via QIAzol (Qiagen) as previously described [357]. RNA concentration was determined via NanoDrop Spectrometry ND1000 and 500 ng RNA reverse transcribed to cDNA using the Verso cDNA Synthesis Kit (ThermoFisher Scientific). RT-qPCR was examined with Apex qPCR GREEN Master Mix (Genesee Scientific, 42–120) using primers for ANP (GCC GGT AGA AGA TGA GGT CAT, GCT TCC TCA GTC TGC TCA CTC') and BNP (5'-CGC TGG GAG GTC ACT CCT AT-3', GCT CTG GAG ACT GGC TAG GAC TT). Gene expression was examined using the BioRad CF96X real-time instrument.

*DNA extraction and 16S rRNA sequencing:*

*Statistical Analysis:* Statistical difference in gene expression and gross anatomy was determined via one-way ANOVA with Tukey's post-hoc analysis. Significance was set at $p<0.05$.

## Results

*Emodin inhibits pathological cardiac hypertrophy in response to hypertension.*

Angiotensin II (AngII) participates in the renin angiotensin system (RAS) and targets angiotensin receptors which subsequently activates vasoconstriction and signal transduction pathways in the heart.[7] As a result, AngII induces systemic hypertension and causes hallmarks of heart failure including cardiac hypertrophy and fibrosis.[7] To begin our studies, we tested the postulate that emodin would attenuate hypertension-induced pathological cardiac hypertrophy. Our data demonstrate that mice treated with AngII experienced an increase in systolic blood pressure, heart weight (HW) and left ventricular weight (LVW) compared to the sham, vehicle control group (Sham) (**Table 1**). Moreover, HW and LVW remained significantly increased when normalized to either tibia length or body weight (BW) (**Table 1**). Consistent with this increase in cardiac hypertrophy, we further demonstrate that hypertensive mice had increased gene expression of hypertrophy markers, the natriuretic peptides, ANP and BNP (**Supplemental Figure 1**). Notably, Emodin (Hypert + Emodin) attenuated HW and LVW, with and without normalization to tibia length and BW, when compared to hypertensive mice (**Table 1**). No changes were observed in BW or systolic blood pressure between emodin treated and hypertensive mice (**Table 1**). Lastly, Emodin inhibited ANP and BNP gene expression in hypertensive mice (**Supplemental Figure 1**). These data would suggest that Emodin is cardioprotective in this hypertension model.

*Hypertension increases pathogenic gut bacteria that is attenuated with emodin.*

The microbiome has recently been implicated in CVD development [64]. Moreover, it has been suggested that phytochemicals interact with the microbiome to protect the heart.[424] As we observed that Emodin attenuated hypertension-induced cardiac hypertrophy, we next sought to examine how this anthraquinone regulated microbes within the gut of hypertensive mice. Using 16S sequencing, we report microbial differences in the cecum of Hypert and Hypert + Emodin treated mice (**Figure 1A**). We also report that *Lachnospiraceae* was more abundant in hypertensive mice compared to healthy (Sham) mice and hypertensive mice treated with Emodin (Hypert + Emodin) (**Figure 1B**). Interestingly, we observed that *Akkermansia* was significantly increased in Emodin treated hypertensive mice compared to hypertensive mice (**Figure 1B & C**). This is interesting as *Akkermansia* has been associated with anti-obesity and anti-diabetes.[425,426] Combined, these data demonstrate that hypertension can negatively impact the microbiome by increasing pathogenic bacteria (e.g. *Lachnospiraceae*) and that Emodin can attenuate the expression of pathogenic bacteria (e.g. *Lachnospiraceae*) while increasing the expression of protective bacteria (e.g. *Akkermansia*).

*Emodin rapidly changes microbial diversity and bacterial composition throughout the gut.*

Data above demonstrate that hypertension impacts the microbiome and Emodin can normalize these pathogenic actions. As a next step, we examined how quickly Emodin could alter the microbiome in healthy mice. Mice were gavage dosed emodin (30 mg/kg.day) or placebo control and feces collected daily for seven days (**Figure 2A)**. 16S sequencing showed that emodin significantly reduced microbial diversity in the feces by

three days of emodin treatment (**Figure 2B**). Interestingly, these changes appeared to diminish by day 7, although still reduced (**Figure 2B**); this is in keeping with other findings that changes in the microbiome are dynamic and can return to homeostasis of the host.[59,427]

While fecal samples (**Figure 2B**) and the cecum above (**Figure 1**) suggest that Emodin alters the microbiome, it does not show if these changes are localized in the gut or take place throughout the digestive tract. As such, we investigated the effects of Emodin on microbial communities throughout the entire GI tract. We report that Emodin reduced taxonomical abundances in the duodenum, jejunum, ileum, cecum, cecum content, colon and feces after seven days of dosing (**Figure 3**). For example, *Lactobacillus* was enriched throughout the entire GI tract and in the feces of vehicle treated mice but not in Emodin treated mice (**Figure 3**). Further, Emodin reduced *Peptostreptococcaceae* in the duodenum, ileum, jejunum and in the colon (**Figure 3**). Not all of these changes were generalizable across the gut, where for instance, *Ruminococcaceae* was enriched in the colon of vehicle treated mice but not Emodin treated mice (**Figure 3**). These data demonstrate that Emodin can rapidly change the microbiome and that these anti-microbial actions can occur across the GI tract as well as within selective regions of the gut. While emodin did decrease the abundance of some microbes, enrichment was also observed. For example, *Allobaculum* was enriched in the jejunum and feces through day three of mice supplemented with Emodin. Most notably, Emodin increased the taxonomical abundance of *Akkermansia* in the colon and the feces (**Figure 3**); this also occurred in the cecum of hypertensive, emodin treated mice above. Additionally, *Roseburia* was enriched by emodin in the ileum, jejunum and colon

(**Figure 3**); similar to *Akkermansia, Roseburia* is also implicated in obesity and heart health.

**Discussion**

In this report, we showed that Emodin attenuated hypertension-induced cardiac hypertrophy, without impacting systolic blood pressure or body weight in female mice. This is consistent with a reduction in atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), which are two biomarkers of heart failure.[428] In addition, we report novel findings that: 1) emodin increased the abundance of cardioprotective bacteria (e.g. *Akkermansia*) in hypertensive mice; 2) emodin rapidly (within 3 days) decreased microbial diversity, while increasing the abundance of cardioprotective bacteria specifically in the colon (e.g. *Akkermansia*) as well as health-promoting bacteria (e.g. *Roseburia*) throughout the gut. These data show that microbial changes occur rapidly with dietary intervention and that emodin can improve bacterial abundance of known cardioprotective species in both healthy and hypertensive mice.

*Akkermansia* is a commensal bug that has gained much attention; this microbe is negatively correlated to obesity and type 2 diabetes.[429] In fact, probiotic supplementation of *Akkermansia* attenuated obesity and diabetes in rodents and humans.[425,426] As obesity and diabetes both increase the risk of developing CVDs,[1] *Akkermansia* is likely to protect the heart by ameliorating metabolic disease. Additionally, *Akkermansia* also acts as an anti-inflammatory microbe; inflammation is a common driver of CVD and heart dysfunction.[430,431] Consistent with this, *Akkermansia* supplementation reduced inflammation-induced damage and atherosclerosis in the hearts of Apolipoprotein E

deficient mice fed a Western Diet.[432] It should also be noted that rhubarb, a plant rich in emodin, also increased *Akkermansia* abundance and this was linked to inhibition of metabolic disease.[433] Here, we report that emodin enriched *Akkermansia*, independent of the route of administration (i.e. gavage vs. i.p.), and this increase was linked to attenuation of hypertension-induced cardiac hypertrophy. Combined, our data demonstrate that Emodin acts as a prebiotic of *Akkermansia*. Future studies that look at this probiotic in the cardioprotective actions in our hypertension model would be of interest as would in vitro analysis to determine if emodin directly improves *Akkermansia* growth.

Similar to *Akkermansia*, *Roseburia* is a microbe that has been linked to health promotion, and a diet rich in complex carbohydrates drives the increase in *Roseburia* abundance.[434] Of interest, *Roseburia* is a short chain fatty acid producing bacteria located in the intestine.[435] Short chain fatty acids like butyrate have been reported to inhibit histone deacetylase (HDAC) enzymes; butyrate also attenuates pathological cardiac remodeling and improves cardiac function via HDAC inhibition.[322,323] Specifically, orally supplemented sodium butyrate attenuated high fat diet-induced dysmetabolic symptoms including cardiac dysfunction, hypertrophy, fibrosis and apoptosis in mice.[323] Similar results were reported with streptozotocin-induced diabetic cardiomyopathy.[322] Both studies showed that butyrate inhibited HDAC activity in the heart.[322,323] *Roseburia* supplementation was also shown to decrease atherosclerotic lesions in germ-free mice, improve gut permeability and reduce inflammation compared to control counterparts.[436] Just as interesting, *Roseburia* inoculation increased histone H3 acetylation and gene expression in the gut.[436] However, the authors reported no change in epigenetic marks in

the aortas.[436] These data suggest that the butyrate-producing microbe *Roseburia* was able to regulate local but not systemic epigenetic marks. Consistent with these HDAC inhibitory actions, our previous work showed that Emodin reduced HDAC activity in the hearts of mice.[357] Moreover, in this report we showed that emodin increased *Roseburia* abundance, suggesting that emodin-mediated HDAC inhibition may, in part, be due to *Roseburia*-induced short chain fatty acids in addition to emodin inhibitory actions. It would be interesting to assess HDAC activity and pathological cardiac remodeling in germ-free mice treated with emodin, with and without *Roseburia* inoculation in hypertensive mice.

*Allobaculum* is a bacteria that was originally linked to leanness and a low-fat diet,[437,438] while increased *Allobaculum* is negatively linked to the pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α) and interleukin-1beta (IL-1β).[439] Similar to inflammation, diet-induced dyslipidemia and high cholesterol are negatively associated with *Allobaculum* enrichment.[440] Together, these actions suggest that *Allobaculum* is likely cardioprotective. Not surprisingly, *Allobaculum* abundance was positively correlated with spontaneously hypertensive rats treated with the angiotensin converting enzyme (ACE) inhibitor, Captopril.[441] Captopril would be expected to improve systolic blood pressure in spontaneously hypertensive rats, and as such it's unclear if *Allobaculum* helped improve hypertension or was a consequence of drug treatment.[441] In this report, we observed limited actions for Emodin on blood pressure regulation, and therefore it's unclear if *Allobaculum* enrichment plays a role in myocardial protection in our model.

Unlike the other bacteria discussed above, *Peptostreptococcaceae* was more abundant in mice treated with vehicle control than those gavaged Emodin. The role for *Peptostreptococcaceae* in human health remains unclear. For example, individuals with the autoimmune condition, primary immune thrombocytopenia, had lower levels of *Peptostreptococcaceae* compared to healthy controls.[442] Further, *Peptostreptococcaceae* correlated with circulating *N*-acyl-ethanolamines and 2-monoacyl-glycerols after omega fatty acid consumption.[443] These two reports would make it seem as if *Peptostreptococcaceae* may confer beneficial outcomes. However, others have shown that *Peptostreptococcaceae* is enriched with high fat diet.[444,445] In fact, *Peptostreptococcaceae* was reduced in ApoE knockout mice that consumed a high fat diet treated with the cholesterol-lowering agent, α-Cyclodextrin,[445] suggesting that lowering *Peptostreptococcaceae* is beneficial in this model. Here, we report that Emodin is cardioprotective and reduced *Peptostreptococcaceae* abundance, consistent with findings from the high fat diet studies. These mixed reports demonstrate that further studies on *Peptostreptococcaceae* in heart health are needed.

In conclusion, Emodin conferred cardioprotection in a model of hypertension and these cardioprotective benefits were positively linked with *Akkermansia, Rosuburia,* and *Allobaculum* abundance*;* and negatively associated with *Peptostreptococcaceaei enrichment*. Moreover, Emodin reduced microbiota diversity, which is consistent with reports on other phytochemicals that have been shown to have anti-microbial properties [328]. While our data suggest that the microbiome plays a role in the anti-hypertrophic actions of Emodin, this report is correlative and further research involving work with

germ-free animals, fecal matter transplant and in vitro assays are needed to define the bacteria important for emodin-mediated actions in the heart.

*TABLE 1. HYPERTENSION-INDUCED CHANGES IN HEART WEIGHT AND BODY COMPOSITION.*

| | Sham | Hypert | Hypert +Emodin |
|---|---|---|---|
| **Study End BW (g)** | 21.16 ± 0.87 | 21.25 ± 0.56 | 20.09 ± 1.45 |
| **Change in BW (g)** | 2.09 ± 0.14 | 2.43 ± 0.33 | 1.19 ± 0.25 |
| **Systolic Blood Pressure (mmHg)** | 123.98 ± 9.36 | 149.32 ± 14.77* | 137.59 ± 8.99* |
| **Heart Weight (mg)** | 83.00 ± 1.82 | 104.25 ± 3.20* | 79.40 ± 4.39 |
| **Left Ventricle Weight (mg)** | 68.00 ± 2.32 | 86.50 ± 2.33* | 67.00 ± 3.69 |
| **HW (mg)/TL (mm)** | 4.76 ± 0.06 | 6.06 ± 0.14* | 4.55 ± 0.23 |
| **LVW (mg)/TL (mm)** | 3.90 ± 0.13 | 5.03 ± 0.10* | 3.84 ± 0.20 |
| **HW (mg)/BW (g)** | 3.92 | 4.91* | 3.95 |
| **LVW (mg)/BW (g)** | 3.21 | 4.1* | 3.33 |

*BW, body weight; HW, heart weight; LVW, Left ventricle weight; Hypert, Hypertension*

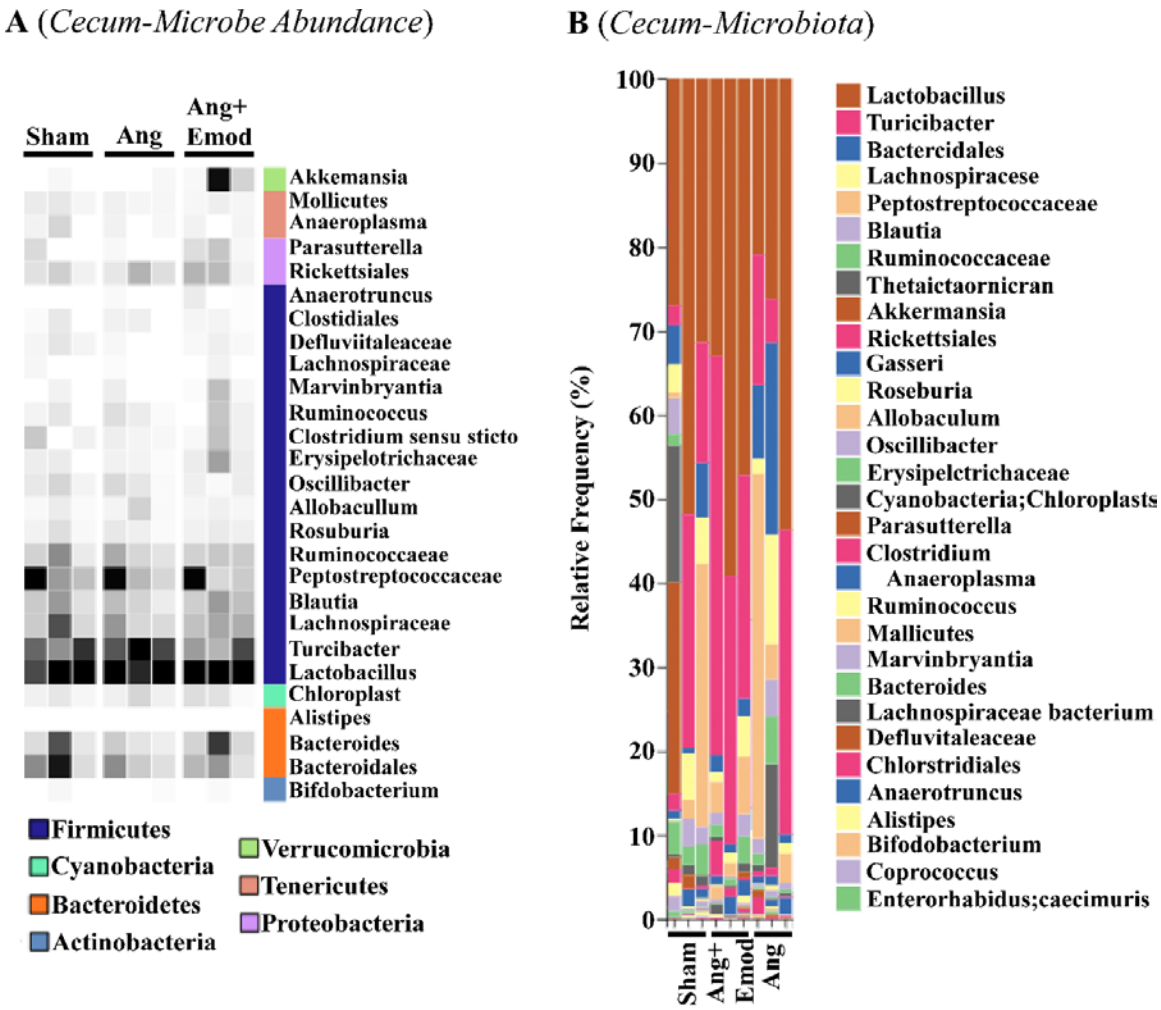



**Figure 1. Hypertension alters the cecal microbiome and this is restored by emodin treatment in mice.** Cecum was extracted from C57BL6/J female mice treated with vehicle, angiotensin ii (Ang) or Ang + emodin (Emod). 16S sequencing was performed and A) taxonomic abundance and B) microbiota profiles examined.

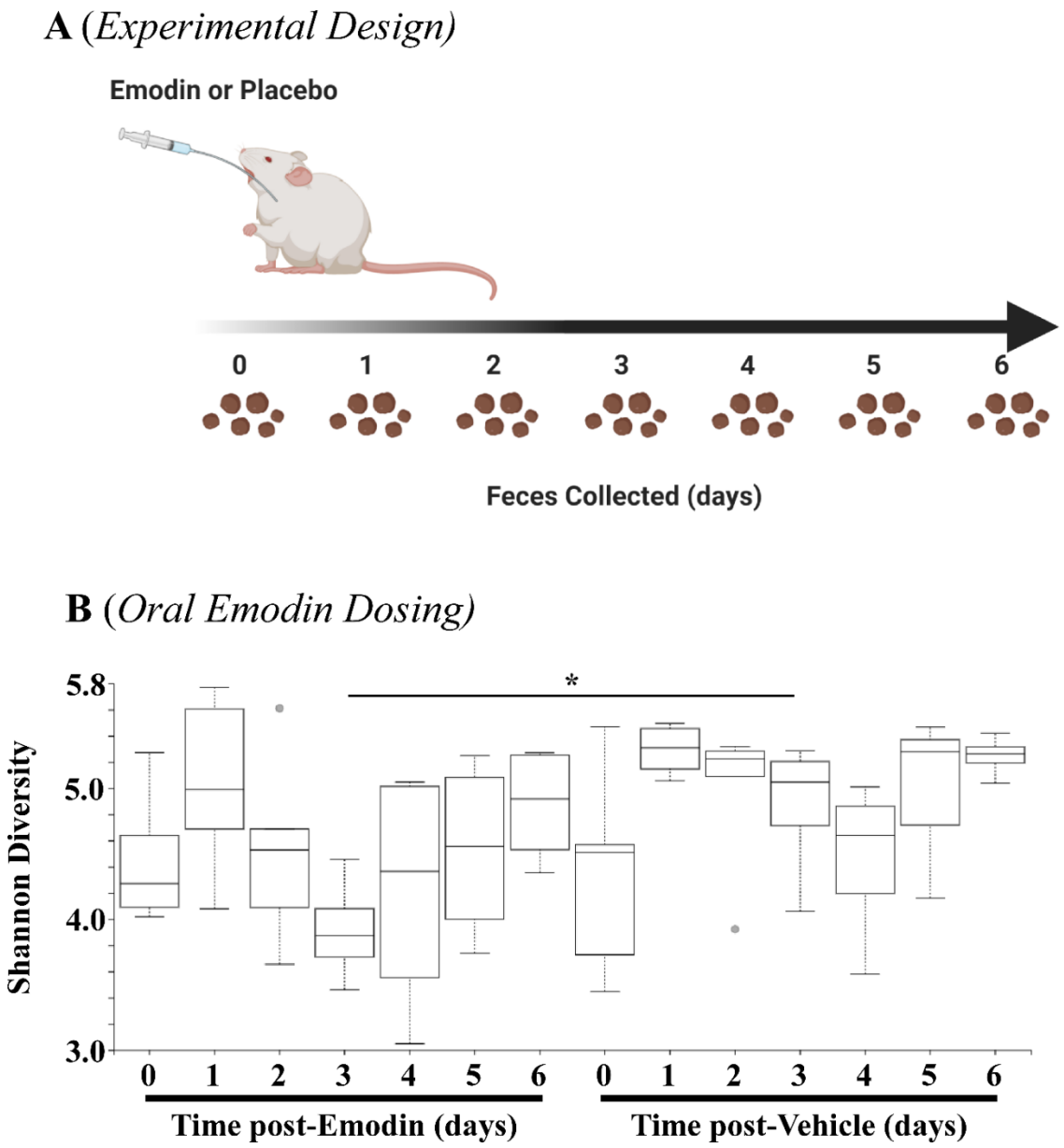



**Figure 2. Emodin rapidly changes microbial diversity in health C57BL6/J mice.** A) A model figure representing the study design. C57BL6/J mice were gavage dosed placebo or emodin for seven days. Each day, feces was collected and 16S sequencing used to examine microbial changes and diversity over time. B) Microbial diversity was determined overtime analyzed via Shannon Diversity.

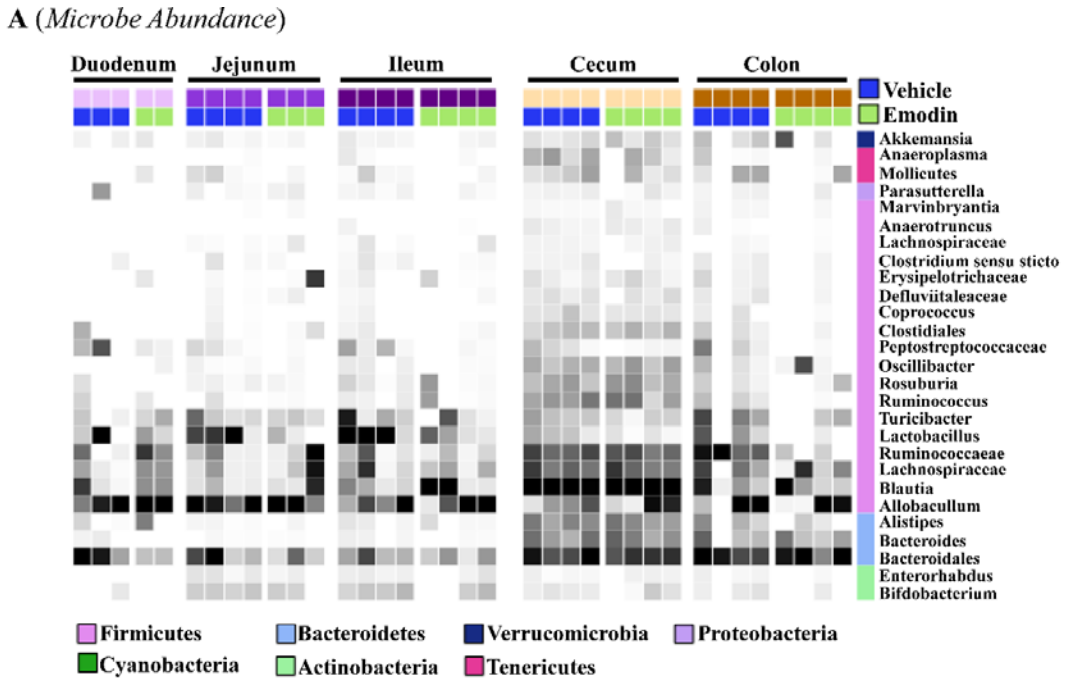
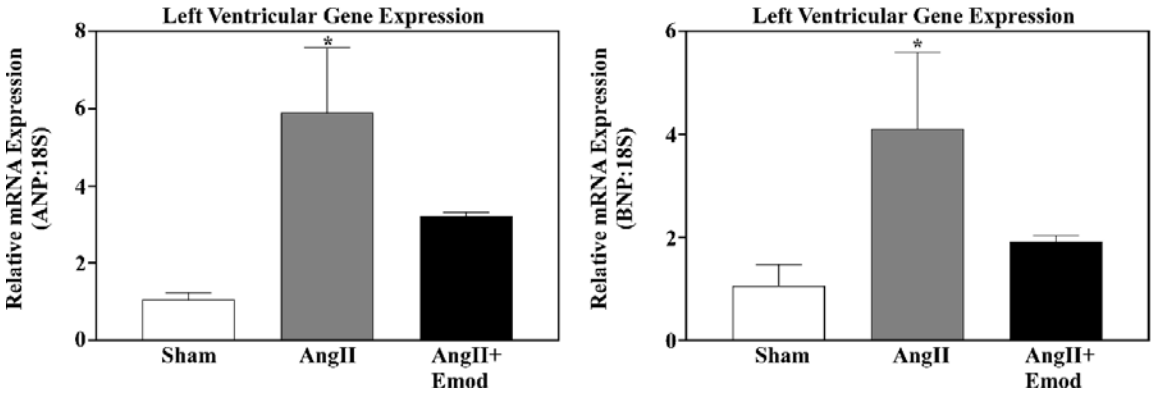



**Figure 3. Emodin alters microbial abundance throughout the GI tract.** A) Heat maps of phyla abundance within the duodenum, jejunum, ileum, cecum and colon of healthy C57BL/6 mice supplemented either vehicle control or Emodin.




**Supplemental Figure 1. Emodin attenuated angiotensin ii-induced natriuretic peptide expression in vivo.** C57BL6/J mice were treated with vehicle (Sham operated), angiotensin ii (AngII) or AngII+emodin (Emod) for two weeks. Hearts were removed and left ventricles (LV) dissected prior to RNA isolation. Quantitative PCR was used to examine ANP and BNP gene expression. One-way ANOVA with tukey's post-hoc was used to determine statistical significance ($p<0.05$).

# Chapter 5. Conclusions and Future Directions

## Conclusions

In conclusion, we found that phytochemicals similarly and differentially regulated pathways and signal transduction cascades and that these actions were associated with an attenuation in agonist-induced cardiomyocyte hypertrophy (**Chapter 2**). Indeed, phenylephrine (PE) as an $\alpha_1$-adrenergic agonist observably increased phosphorylation of the mitogen activated protein kinases (MAPKs), extracellular regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38, as well as protein kinase C/D (PKC/D). These actions of PE were associated with an increase in cardiomyocyte hypertrophy, which is a common hallmark of heart failure. In contrast, Apigenin, Baicalein, Berberine Hydrochloride (BHCl) and Emodin blocked PE-induced cardiomyocyte hypertrophy, but not via cytotoxicity. Moreover, the overlapping and divergent effects of Apigenin, Baicalein, BHCl and Emodin on the MAPKs and PKC/D highlight our hypothesis in that phytochemicals work through several mechanisms, both similar and different and at different magnitudes (e.g., Emodin reduced phosphorylated-PKD more than Apigenin). Interestingly, Emodin completely abolished PKD phosphorylation while Apigenin, Baicalein andBHCl only attenuated PKD phosphorylation.

Four phytochemicals, Apigenin, Baicalein, BHCl and Emodin, out of 18 were shown to be cardioprotective in **Chapter 2**. As our lab had previously found that these dietary compounds inhibited histone deacetylase (HDAC) activity, we next tested the postulate that Apigenin, Baicalein, BHCl and Emodin would attenuate HDAC activity in our cardiomyocyte experiments. Of the four, Emodin reduced HDAC activity the most. As HDAC inhibition is cardioprotective in experimental models of cardiovascular disease

(CVD), our next task sought to elucidate the effects of Emodin on HDAC activity in the heart and if an Emodin-rich plant, rhubarb, behaved similarly to Emodin (**Chapter 3**). Firstly, our data showed that Emodin and rhubarb inhibited HDAC activity similarly in a test tube, as both inhibited HDAC activity in a fast-on slow-off manner. Additionally, rhubarb blocked intracellular- and receptor-mediated cardiomyocyte hypertrophy in a comparable manner to Emodin. Of interest, Emodin reduced HDAC activity concomitant with increased histone acetylation. As these epigenetic modification data suggest differential gene expression, we next compared and contrasted the effects of Emodin to a well-known HDAC inhibitor, Trichostatin A (TSA), on gene expression in cardiomyocytes. Our data showed that Emodin and TSA affected similar pathways but also different. These data make sense as phytochemicals, per **Chapter 2**, indeed act in multi-faceted manners, including via HDAC inhibition. Finally, we found that the effects of Emodin in cardiomyocytes were reflected in hypertensive mice. Thus, we found Emodin to be cardioprotective both *in vitro* and *in vivo*.

Finally, we found it interesting that Emodin blocked hypertension-induced hypertrophy in mice despite lacking distribution to the heart. The microbiome has been implicated in heart failure. For example, germ-free mice infused with angiotensin II do not develop hypertension, hypertrophy nor fibrosis to the extent of their conventionally raised counterparts. Thus, we sought to characterize the effects of Emodin on hypertensive and healthy mice(**Chapter 4**). Our data linked the cardioprotective actions of Emodin in hypertensive mice with enrichment of *Akkermansia* (**Chapter 4**), a microbe that had previously been shown to be efficacious for diabetes and atherosclerosis. Intriguingly, healthy mice given Emodin via oral gavage also had enriched *Akkermansia*

which suggests Emodin increases microbial abundance of *Akkermansia* independent of administration route. Other notable microbes that were enriched in healthy mice supplemented with Emodin included *Roseburia* and *Allobaculum*.

**Future Directions**

In **Chapter 2**, our data showed that the phytochemical anthraquinone, Emodin completely abolished protein kinase D (PKD) phosphorylation while Apigenin, Baicalein and Berberine HCl only attenuated PKD phosphorylation to expression levels similar to those of vehicle treated neonatal rat ventricular myocytes (NRVMs). Indeed, Emodin has been shown to attenuate PKC expression and activity;[446] however, the actions of Emodin on PKC/D in the heart remain to be elucidated. In the heart, PKC drives PKD activity and nuclear translocation,[447] which has been shown to then phosphorylate nuclear-bound class II histone deacetylases (HDACs), resulting in HDAC nuclear export and de-repression of hypertrophic gene expression.[379] In contrast, PKD inhibition allowed class II HDACs to remain in the nucleus and prohibit gene expression.[379] This makes sense as HDACs downregulate gene expression. Thus, it would be interesting to test the importance of PKD in the anti-hypertrophic actions of Emodin in NRVMs.

We further found in **Chapter 4** that Emodin enriched the cardioprotective microbe, *Akkermansia*, despite the route of administration. Importantly, Emodin blocked angiotensin II-induced cardiac hypertrophy and fibrosis, as shown both phenotypically and via gene expression analysis (e.g., ANP, BNP, Col1 and CTFG). Thus, it would be interesting to test the importance of the microbiome for the cardioprotective actions of Emodin *in vivo*. This can be done via at least two methods: 1) running side-by-side

comparisons between conventionally raised and germ-free mice with heart disease and supplemented with or without Emodin and 2) taking fecal matter from mice treated with or without Emodin and performing transplants into mice with heart disease. As the microbiome has emerged a novel and important player in CVD and phytochemical-mediated health, elucidating the role of the microbiome further in the cardioprotective actions of Emodin is warranted.

## References


1. Virani SS, Alonso A, Benjamin EJ, et al. Heart disease and stroke statistics—2020 update: A report from the American Heart Association. *Circulation*. 2020;141(9):e139-e596. doi:10.1161/CIR.0000000000000757
2. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: Experimental findings and therapeutic strategies. *Pharmacol Ther*. 2010;128(1):191-227. doi:10.1016/j.pharmthera.2010.04.005
3. Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, Voipio-Pulkki LM. Apoptosis in human acute myocardial infarction. *Circulation*. 1997;95(2):320-323. doi:10.1161/01.CIR.95.2.320
4. Porrello ER, Mahmoud AI, Simpson E, et al. Transient regenerative potential of the neonatal mouse heart. *Science (80- )*. 2011;331(6020):1078-1080. doi:10.1126/science.1200708
5. Zebrowski DC, Jensen CH, Becker R, et al. Cardiac injury of the newborn mammalian heart accelerates cardiomyocyte terminal differentiation. *Sci Rep*. 2017;7(1):8362. doi:10.1038/s41598-017-08947-2
6. Xia P, Liu Y, Cheng Z. Signaling Pathways in Cardiac Myocyte Apoptosis. *Biomed Res Int*. 2016;2016:9583268. doi:10.1155/2016/9583268
7. Tham YK, Bernardo BC, Ooi JYY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. *Arch Toxicol*. 2015;89(9):1401-38. doi:10.1007/s00204-015-1477-x
8. Travers JG, Kamal FA, Robbins J, Yutzey KE, Blaxall BC. Cardiac fibrosis: The fibroblast awakens. *Circ Res*. 2016;118(6):1021-40. doi:10.1161/CIRCRESAHA.115.306565
9. Kai H, Mori T, Tokuda K, et al. Pressure overload-induced transient oxidative stress mediates perivascular inflammation and cardiac fibrosis through angiotensin II. *Hypertens Res*. 2006;29(9):711-8. doi:10.1291/hypres.29.711
10. De Barker JMT, Van Capelle FJL, Janse MJ, et al. Fractionated electrograms in dilated cardiomyopathy: Origin and relation to abnormal conduction. *J Am Coll Cardiol*. 1996;27(5):1071-8. doi:10.1016/0735-1097(95)00612-5
11. Ather S, Respress JL, Li N, Wehrens XHT. Alterations in ryanodine receptors and related proteins in heart failure. *Biochim Biophys Acta - Mol Basis Dis*. 2013;1832(12):2425-31. doi:10.1016/j.bbadis.2013.06.008
12. Asatryan. Cardiac Sodium Channel Dysfunction and Dilated Cardiomyopathy: A Contemporary Reappraisal of Pathophysiological Concepts. *J Clin Med*. 2019;8(7):1029. doi:10.3390/jcm8071029
13. Biasucci LM, Liuzzo G, Fantuzzi G, et al. Increasing levels of interleukin (IL)-1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation*. 1999;99(16):2079-2084. doi:10.1161/01.CIR.99.16.2079
14. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol*. 2018;15(9):505-522. doi:10.1038/s41569-018-0064-2

15. Riad A, Jäger S, Sobirey M, et al. Toll-Like Receptor-4 Modulates Survival by Induction of Left Ventricular Remodeling after Myocardial Infarction in Mice. *J Immunol*. 2008;180(10):6954-6961. doi:10.4049/jimmunol.180.10.6954
16. Satoh M, Shimoda Y, Maesawa C, et al. Activated toll-like receptor 4 in monocytes is associated with heart failure after acute myocardial infarction. *Int J Cardiol*. 2006;109(2):226-234. doi:10.1016/j.ijcard.2005.06.023
17. Kuusisto J, Kärjä V, Sipola P, et al. Low-grade inflammation and the phenotypic expression of myocardial fibrosis in hypertrophic cardiomyopathy. *Heart*. 2012;98(13):1007-1013. doi:10.1136/heartjnl-2011-300960
18. Fang L, Ellims AH, Beale AL, Taylor AJ, Murphy A, Dart AM. Systemic inflammation is associated with myocardial fibrosis, diastolic dysfunction, and cardiac hypertrophy in patients with hypertrophic cardiomyopathy. *Am J Transl Res*. 2017;9(11):5063-5073.
19. Fardet A, Boirie Y. Associations between food and beverage groups and major diet-related chronic diseases: An exhaustive review of pooled/meta-analyses and systematic reviews. *Nutr Rev*. 2014;72(12):741-762. doi:10.1111/nure.12153
20. Benjamin EJ, Virani SS, Callaway CW, et al. Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. *Circulation*. 2018;137(12):CIR.0000000000000558. doi:10.1161/CIR.0000000000000558
21. McEvoy CT, Temple N, Woodside J V. Vegetarian diets, low-meat diets and health: a review. *Public Health Nutr*. 2012;15(12):2287-2294. doi:10.1017/S1368980012000936
22. Ng TWK, Watts GF, Barrett PHR, Rye KA, Chan DC. Effect of weight loss on LDL and HDL kinetics in the metabolic syndrome: Associations with changes in plasma retinol-binding protein-4 and adiponectin levels. *Diabetes Care*. 2007;30(11):2945-50. doi:10.2337/dc07-0768
23. Chiu S, Williams PT, Krauss RM. Effects of a very high saturated fat diet on LDL particles in adults with atherogenic dyslipidemia: A randomized controlled trial. *PLoS One*. 2017;12(2). doi:10.1371/journal.pone.0170664
24. US Department Health and Human Services, Dietary Guidelines Advisory Committee. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. *Washingt USDA US Dep Heal Hum Serv*. 2015;53(9):1689-1699. doi:10.1017/CBO9781107415324.004
25. Health Canada. Food guide. *Gov Canada*. 2012:1-2. doi:http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php
26. Guidelines MD. Malaysian Dietary Guidelines. *Malaysian Diet Guidel*. 2015:2-3.
27. WHO | Diet, nutrition and the prevention of chronic diseases  Report of the joint WHO/FAO expert consultation. *WHO*. 2014.
28. NHMRC. Australian Dietary Guidelines: Recommended Daily Intakes. Eat for Health: Australian Dietary Guidelines. 2013.
29. Levitan EB, Lewis CE, Tinker LF, et al. Mediterranean and DASH diet scores and mortality in women with heart failure: The Women's Health Initiative. *Circ Heart Fail*. 2013;6(6):1116-1123. doi:10.1161/CIRCHEARTFAILURE.113.000495
30. Schwingshackl L, Boeing H, Stelmach-Mardas M, et al. Dietary Supplements and

Risk of Cause-Specific Death, Cardiovascular Disease, and Cancer: A Systematic Review and Meta-Analysis of Primary Prevention Trials. *Adv Nutr An Int Rev J*. 2017;8(1):27-39. doi:10.3945/an.116.013516

31. Boyes J, Bird A. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell*. 1991;64(6):1123-1134. doi:10.1016/0092-8674(91)90267-3
32. Serra RW, Fang M, Park SM, Hutchinson L, Green MR. A KRAS-directed transcriptional silencing pathway that mediates the CpG island methylator phenotype. *Elife*. 2014;2014(3):e02313. doi:10.7554/eLife.02313
33. Rountree MR, Bachman KE, Baylin SB. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat Genet*. 2000;25(3):269-277. doi:10.1038/77023
34. Nakatochi M, Ichihara S, Yamamoto K, et al. Epigenome-wide association of myocardial infarction with DNA methylation sites at loci related to cardiovascular disease. *Clin Epigenetics*. 2017;9(1):54. doi:10.1186/s13148-017-0353-3
35. Westerman K, Sebastiani P, Jacques P, Liu S, DeMeo D, Ordovás JM. DNA methylation modules associate with incident cardiovascular disease and cumulative risk factor exposure. *Clin Epigenetics*. 2018;11(1):142. doi:10.1101/471722
36. Gorenne I, Kumar S, Gray K, et al. Vascular smooth muscle cell sirtuin 1 protects against dna damage and inhibits atherosclerosis. *Circulation*. 2013;127(3):386-396. doi:10.1161/CIRCULATIONAHA.112.124404
37. Hsu CP, Zhai P, Yamamoto T, et al. Silent information regulator 1 protects the heart from ischemia/reperfusion. *Circulation*. 2010;122(21):2170-2182. doi:10.1161/CIRCULATIONAHA.110.958033
38. Gusterson RJ, Jazrawi E, Adcock IM, Latchman DS. The transcriptional co-activators CREB-binding protein (CBP) and p300 play a critical role in cardiac hypertrophy that is dependent on their histone acetyltransferase activity. *J Biol Chem*. 2003;278(9):6838-6847. doi:10.1074/jbc.M211762200
39. Antos CL, McKinsey TA, Dreitz M, et al. Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. *J Biol Chem*. 2003;278(31):28930-28937. doi:10.1074/jbc.M303113200
40. Kong Y, Tannous P, Lu G, et al. Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation*. 2006;113(22):2579-2588. doi:10.1161/CIRCULATIONAHA.106.625467
41. Williams SM, Golden-Mason L, Ferguson BS, et al. Class I HDACs regulate angiotensin II-dependent cardiac fibrosis via fibroblasts and circulating fibrocytes. *J Mol Cell Cardiol*. 2014;67:112-125. doi:10.1016/j.yjmcc.2013.12.013
42. Demos-Davies KM, Ferguson BS, Cavasin MA, et al. HDAC6 contributes to pathological responses of heart and skeletal muscle to chronic angiotensin-II signaling. *AJP Hear Circ Physiol*. 2014;307(2):H252-H258. doi:10.1152/ajpheart.00149.2014
43. Morales CR, Li DL, Pedrozo Z, et al. Inhibition of class I histone deacetylases blunts cardiac hypertrophy through TSC2-dependent mTOR repression. *Sci Signal*. 2016;9(422):ra34-ra34. doi:10.1126/scisignal.aad5736

44. Ferguson BS, Harrison BC, Jeong MY, et al. Signal-dependent repression of DUSP5 by class I HDACs controls nuclear ERK activity and cardiomyocyte hypertrophy. *Proc Natl Acad Sci U S A*. 2013;110(24):9806-9811. doi:10.1073/pnas.1301509110
45. Jeong MY, Lin YH, Wennersten SA, et al. Histone deacetylase activity governs diastolic dysfunction through a nongenomic mechanism. *Sci Transl Med*. 2018;10(427):eaao0144. doi:10.1126/scitranslmed.aao0144
46. Huttenhower C, Gevers D, Knight R, et al. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207-14. doi:10.1038/nature11234
47. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2015;14(1):20-32. doi:10.1038/nrmicro3552
48. Haghikia A, Li XS, Liman TG, et al. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arterioscler Thromb Vasc Biol*. 2018;38(9):2225-2235. doi:10.1161/ATVBAHA.118.311023
49. Zuo K, Li J, Wang P, et al. Duration of Persistent Atrial Fibrillation Is Associated with Alterations in Human Gut Microbiota and Metabolic Phenotypes. *mSystems*. 2019;4(6):e00422-19. doi:10.1128/msystems.00422-19
50. Hayashi T, Yamashita T, Watanabe H, et al. Gut microbiome and plasma microbiome-related metabolites in patients with decompensated and compensated heart failure. *Circ J*. 2019;83(1):182-192. doi:10.1253/circj.CJ-18-0468
51. Fu J, Bonder MJ, Cenit MC, et al. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. *Circ Res*. 2015;117(9):817-24. doi:10.1161/CIRCRESAHA.115.306807
52. Jones ML, Martoni CJ, Parent M, Prakash S. Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active Lactobacillus reuteri NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. *Br J Nutr*. 2012;107(10):1505-13. doi:10.1017/S0007114511004703
53. Yuille S, Reichardt N, Panda S, Dunbar H, Mulder IE. Human gut bacteria as potent class I histone deacetylase inhibitors in vitro through production of butyric acid and valeric acid. *PLoS One*. 2018;13(7):e0201073. doi:10.1371/journal.pone.0201073
54. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464(7285):59-65. doi:10.1038/nature08821
55. Koliada A, Syzenko G, Moseiko V, et al. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol*. 2017;17(1):120. doi:10.1186/s12866-017-1027-1
56. Odamaki T, Kato K, Sugahara H, et al. Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiol*. 2016;16:90. doi:10.1186/s12866-016-0708-5
57. Davenport ER, Mizrahi-Man O, Michelini K, Barreiro LB, Ober C, Gilad Y. Seasonal variation in human gut microbiome composition. *PLoS One*. 2014;9(3):e90731. doi:10.1371/journal.pone.0090731

58. Rashid MU, Zaura E, Buijs MJ, et al. Determining the long-term effect of antibiotic administration on the human normal intestinal microbiota using culture and pyrosequencing methods. *Clin Infect Dis*. 2015;60 Suppl 2:S77-84. doi:10.1093/cid/civ137
59. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559-63. doi:10.1038/nature12820
60. Jie Z, Xia H, Zhong SL, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun*. 2017;8(1):845. doi:10.1038/s41467-017-00900-1
61. Kelly TN, Bazzano LA, Ajami NJ, et al. Gut Microbiome Associates with Lifetime Cardiovascular Disease Risk Profile among Bogalusa Heart Study Participants. *Circ Res*. 2016;119(8):956-64. doi:10.1161/CIRCRESAHA.116.309219
62. Yan Q, Gu Y, Li X, et al. Alterations of the gut microbiome in hypertension. *Front Cell Infect Microbiol*. 2017;7:381.. doi:10.3389/fcimb.2017.00381
63. Kim S, Goel R, Kumar A, et al. Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. *Clin Sci*. 2018;132(6):701-718.. doi:10.1042/CS20180087
64. Karbach SH, Schönfelder T, Brandão I, et al. Gut Microbiota Promote Angiotensin II-Induced Arterial Hypertension and Vascular Dysfunction. *J Am Heart Assoc*. 2016;5(9):e003698. doi:10.1161/JAHA.116.003698
65. Adnan S, Nelson JW, Ajami NJ, et al. Alterations in the gut microbiota can elicit hypertension in rats. *Physiol Genomics*. 2017;49(2):96-104. doi:10.1152/physiolgenomics.00081.2016
66. Lampe JW, Chang JL. Interindividual differences in phytochemical metabolism and disposition. *Semin Cancer Biol*. 2007;17(5):347-353. doi:10.1016/j.semcancer.2007.05.003
67. Marín L, Miguélez EM, Villar CJ, Lombó F. Bioavailability of dietary polyphenols and gut microbiota metabolism: Antimicrobial properties. *Biomed Res Int*. 2015;2015:905215.. doi:10.1155/2015/905215
68. Gomes A, Oudot C, Macià A, et al. Berry-enriched diet in salt-sensitive hypertensive rats: Metabolic fate of (poly)phenols and the role of gut microbiota. *Nutrients*. 2019;11(11):2634. doi:10.3390/nu11112634
69. Vetrani C, Maukonen J, Bozzetto L, et al. Diets naturally rich in polyphenols and/or long-chain n-3 polyunsaturated fatty acids differently affect microbiota composition in high-cardiometabolic-risk individuals. *Acta Diabetol*. 2020;57(7):853-860. doi:10.1007/s00592-020-01494-9
70. Upadhyay S, Dixit M. Role of polyphenols and other phytochemicals on molecular signaling. *Oxid Med Cell Longev*. 2015;2015:1-15. doi:10.1155/2015/504253
71. Thakur VS, Deb G, Babcook MA, Gupta S. Plant phytochemicals as epigenetic modulators: role in cancer chemoprevention. *AAPS J*. 2014;16(1):151-163. doi:10.1208/s12248-013-9548-5
72. Pop RM, Popolo A, Trifa AP, Stanciu LA. Phytochemicals in cardiovascular and respiratory diseases: Evidence in oxidative stress and inflammation. *Oxid Med Cell Longev*. 2018;2018:1603872. doi:10.1155/2018/1603872

73. Scalbert A, Andres-Lacueva C, Arita M, et al. Databases on food phytochemicals and their health-promoting effects. *J Agric Food Chem*. 2011;59(9):4331-4348. doi:10.1021/jf200591d
74. Blekkenhorst L, Sim M, Bondonno C, et al. Cardiovascular Health Benefits of Specific Vegetable Types: A Narrative Review. *Nutrients*. 2018;10(5):595. doi:10.3390/nu10050595
75. Godoy LD, Lucas JE, Bender AJ, Romanick SS, Ferguson BS. Targeting the epigenome: Screening bioactive compounds that regulate histone deacetylase activity. *Mol Nutr Food Res*. 2016;61(4):10.1002/mnfr.201600744. doi:10.1002/mnfr.201600744
76. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. *Am J Clin Nutr*. 2004;79(5):727-747. doi:10.1093/ajcn/79.5.727
77. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15(10):7313-7352. doi:10.3390/molecules15107313
78. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angew Chemie - Int Ed*. 2011;50(3):586-621. doi:10.1002/anie.201000044
79. Cheynier V. Polyphenols in food are more complex then often thought. *Am J Clin Nutr*. 2005;81(1):223-229. doi:10.1093/ajcn/81.1.223S
80. Orzechowski A, Ostaszewski P, Jank M, Berwid SJ. Bioactive substances of plant origin in food--impact on genomics. *Reprod Nutr Dev*. 2002;42(5):461-477.
81. Vahid F, Zand H, Nosrat-Mirshekarlou E, Najafi R, Hekmatdoost A. The role dietary of bioactive compounds on the regulation of histone acetylases and deacetylases: A review. *Gene*. 2015;562(1):8-15. doi:10.1016/j.gene.2015.02.045
82. Nambiar VS, Sareen N, Daniel M, Gallego EB. Flavonoids and phenolic acids from pearl millet ( Pennisetum glaucum ) based foods and their functional implications. *Funct Foods Heal Dis*. 2012;2(7):251-264.
83. Bora-Tatar G, Dayangaç-Erden D, Demir AS, Dalkara S, Yelekçi K, Erdem-Yurter H. Molecular modifications on carboxylic acid derivatives as potent histone deacetylase inhibitors: Activity and docking studies. *Bioorganic Med Chem*. 2009;17(14):5219-5228. doi:10.1016/j.bmc.2009.05.042
84. Chrysant SG. The impact of coffee consumption on blood pressure, cardiovascular disease and diabetes mellitus. *Expert Rev Cardiovasc Ther*. 2017;15(3):151-156. doi:10.1080/14779072.2017.1287563
85. Harborne JB. *Fruit Phenolics*. Vol 31.; 1992. doi:10.1016/0031-9422(92)80092-S
86. Clifford MN. Chlorogenic acids and other cinnamates – nature, occurrence and dietary burden. *J Sci Food Agric*. 1999;79(3):362-372. doi:10.1002/(SICI)1097-0010(19990301)79:3<362::AID-JSFA256>3.0.CO;2-D
87. Brown OI, Allgar V, Wong KY-K. Coffee reduces the risk of death after acute myocardial infarction: A meta-analysis. *Coron Artery Dis*. 2016;27(7):566-572. doi:10.1097/MCA.0000000000000397
88. Lee SY, Ku HC, Kuo YH, et al. Caffeic acid ethanolamide prevents cardiac dysfunction through sirtuin dependent cardiac bioenergetics preservation. *J*

*Biomed Sci*. 2015;22(1):80. doi:10.1186/s12929-015-0188-1
89. Tan J, Ma Z, Han L, et al. Caffeic acid phenethyl ester possesses potent cardioprotective effects in a rabbit model of acute myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2005;289(5):H2265-71. doi:10.1152/ajpheart.01106.2004
90. Ren J, Zhang N, Liao H, et al. Caffeic acid phenethyl ester attenuates pathological cardiac hypertrophy by regulation of MEK/ERK signaling pathway in vivo and vitro. *Life Sci*. 2017;181:53-61. doi:10.1016/j.lfs.2017.04.016
91. Roy AJ, Stanely Mainzen Prince P. Preventive effects of p-coumaric acid on cardiac hypertrophy and alterations in electrocardiogram, lipids, and lipoproteins in experimentally induced myocardial infarcted rats. *Food Chem Toxicol*. 2013;60:348-354. doi:10.1016/j.fct.2013.04.052
92. Panda V, Laddha A, Nandave M, Srinath S. Dietary Phenolic Acids of Macrotyloma uniflorum (Horse Gram) Protect the Rat Heart Against Isoproterenol-Induced Myocardial Infarction. *Phyther Res*. 2016;30(7):1146-1155. doi:10.1002/ptr.5620
93. Wang F, Lu W, Zhang T, et al. Development of novel ferulic acid derivatives as potent histone deacetylase inhibitors. *Bioorganic Med Chem*. 2013;21(22):6973-6980. doi:10.1016/j.bmc.2013.09.021
94. Alam MA, Sernia C, Brown L. Ferulic acid improves cardiovascular and kidney structure and function in hypertensive rats. *J Cardiovasc Pharmacol*. 2013;61(3):240-249. doi:10.1097/FJC.0b013e31827cb600
95. Waldecker M, Kautenburger T, Daumann H, Busch C, Schrenk D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem*. 2008;19(9):587-593. doi:10.1016/j.jnutbio.2007.08.002
96. Tomás-Barberán FA, Clifford MN. Dietary hydroxybenzoic acid derivatives - Nature, occurrence and dietary burden. *J Sci Food Agric*. 2000;80(7):1024-1032. doi:10.1002/(SICI)1097-0010(20000515)80:7<1024::AID-JSFA567>3.0.CO;2-S
97. Cabrera C, Giménez R, López MC. Determination of tea components with antioxidant activity. *J Agric Food Chem*. 2003;51(15):4427-4435. doi:10.1021/jf0300801
98. Bøhn SK, Ward NC, Hodgson JM, Croft KD. Effects of tea and coffee on cardiovascular disease risk. *Food Funct*. 2012;3(6):575. doi:10.1039/c2fo10288a
99. Umadevi S, Gopi V, Elangovan V. Regulatory mechanism of gallic acid against advanced glycation end products induced cardiac remodeling in experimental rats. *Chem Biol Interact*. 2014;208(1):28-36. doi:10.1016/j.cbi.2013.11.013
100. Choi SY, Kee HJ, Jin L, et al. Inhibition of class IIa histone deacetylase activity by gallic acid, sulforaphane, TMP269, and panobinostat. *Biomed Pharmacother*. 2018;101:145-154. doi:10.1016/j.biopha.2018.02.071
101. Berkban T, Boonprom P, Bunbupha S, et al. Ellagic acid prevents L-NAME-induced hypertension via restoration of eNOS and p47phoxexpression in rats. *Nutrients*. 2015;7(7):5265-5280. doi:10.3390/nu7075222
102. Olgar Y, Ozturk N, Usta C, Puddu PE, Ozdemir S. Ellagic acid reduces l-type CA2+ current and contractility through modulation of NO-GC-CGMP pathways in

rat ventricular myocytes. *J Cardiovasc Pharmacol*. 2014;64(6):567-573. doi:10.1097/FJC.0000000000000153

103. Place RF, Noonan EJ, Giardina C. HDAC inhibition prevents NF-κB activation by suppressing proteasome activity: Down-regulation of proteasome subunit expression stabilizes IκBα. *Biochem Pharmacol*. 2005;70(3):394-406. doi:10.1016/j.bcp.2005.04.030
104. Wang X, Ouyang Y, Liu J, et al. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ*. 2014;349(July):g4490. doi:10.1136/bmj.g4490
105. Zhan J, Liu Y-J, Cai L-B, Xu F-R, Xie T, He Q-Q. Fruit and vegetable consumption and risk of cardiovascular disease: A meta-analysis of prospective cohort studies. *Crit Rev Food Sci Nutr*. 2017;57(8):1650-1663. doi:10.1080/10408398.2015.1008980
106. Kim Y, Je Y. Flavonoid intake and mortality from cardiovascular disease and all causes: A meta-analysis of prospective cohort studies. *Clin Nutr ESPEN*. 2017;20:68-77. doi:10.1016/j.clnesp.2017.03.004
107. Cheng IF, Breen K. On the ability of four flavonoids, baicilein, luteolin, naringenin, and quercetin, to suppress the fenton reaction of the iron-ATP complex. *BioMetals*. 2000;13(1):77-83. doi:10.1023/A:1009229429250
108. Mira L, Fernandez MT, Santos M, Rocha R, Florêncio MH, Jennings KR. Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. *Free Radic Res*. 2002;36(11):1199-1208. doi:10.1080/1071576021000016463
109. Sampson L, Rimm E, Hollman PCH, De Vries JHM, Katan MB. Flavonol and flavone intakes in US health professionals. *J Am Diet Assoc*. 2002;102(10):1414-1420. doi:10.1016/S0002-8223(02)90314-7
110. D'Andrea G. Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia*. 2015;106:256-271. doi:10.1016/j.fitote.2015.09.018
111. Knekt P, Kumpulainen J, Järvinen R, et al. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr*. 2002;76(3):560-568. doi:10.1093/ajcn/76.3.560
112. Punithavathi VR, Prince PSM. Combined effects of quercetin and α-tocopherol on lipids and glycoprotein components in isoproterenol induced myocardial infarcted Wistar rats. *Chem Biol Interact*. 2009;181(3):322-327. doi:10.1016/j.cbi.2009.07.002
113. Annapurna A, Reddy CS, Akondi RB, Rao SRC. Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. *J Pharm Pharmacol*. 2009;61(10):1365-1374. doi:10.1211/jpp/61.10.0014
114. Jalili T, Carlstrom J, Kim S, et al. Quercetin-supplemented diets lower blood pressure and attenuate cardiac hypertrophy in rats with aortic constriction. *J Cardiovasc Pharmacol*. 2006;47(4):531-541. doi:10.1097/01.fjc.0000211746.78454.50
115. Kumar M, Kasala ER, Bodduluru LN, Kumar V, Lahkar M. Molecular and biochemical evidence on the protective effects of quercetin in isoproterenol-

induced acute myocardial injury in rats. *J Biochem Mol Toxicol*. 2017;31(1):e21832. doi:10.1002/jbt.21832

116. Bartekova M, Radosinska J, Pancza D, Barancik M, Ravingerova T. Cardioprotective effects of quercetin against ischemia-reperfusion injury are age-dependent. *Physiol Res*. 2016;65 Suppl 1:S101-7.
117. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. *J Nutr*. 2007;137(11):2405-2411. doi:10.1093/jn/137.11.2405
118. Egert S, Bosy-Westphal A, Seiberl J, et al. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: A double-blinded, placebo-controlled cross-over study. *Br J Nutr*. 2009;102(7):1065-1074. doi:10.1017/S0007114509359127
119. Hung CH, Chan SH, Chu PM, Tsai KL. Quercetin is a potent anti-atherosclerotic compound by activation of SIRT1 signaling under oxLDL stimulation. *Mol Nutr Food Res*. 2015;59(10):1905-1917. doi:10.1002/mnfr.201500144
120. Bishayee K, Khuda-Bukhsh AR, Huh S-O. PLGA-Loaded Gold-Nanoparticles Precipitated with Quercetin Downregulate HDAC-Akt Activities Controlling Proliferation and Activate p53-ROS Crosstalk to Induce Apoptosis in Hepatocarcinoma Cells. *Mol Cells*. 2015;38(6):518-527. doi:10.14348/molcells.2015.2339
121. Vargas JE, Filippi-Chiela EC, Suhre T, Kipper FC, Bonatto D, Lenz G. Inhibition of HDAC increases the senescence induced by natural polyphenols in glioma cells. *Biochem Cell Biol*. 2014;92(4):297-304. doi:10.1139/bcb-2014-0022
122. Somerset SM, Johannot L. Dietary flavonoid sources in Australian adults. *Nutr Cancer*. 2008;60(4):442-449. doi:10.1080/01635580802143836
123. Suchal K, Malik S, Khan SI, et al. Molecular pathways involved in the amelioration of myocardial injury in diabetic rats by Kaempferol. *Int J Mol Sci*. 2017;18(5):1001. doi:10.3390/ijms18051001
124. Guo Z, Liao Z, Huang L, Liu D, Yin D, He M. Kaempferol protects cardiomyocytes against anoxia/reoxygenation injury via mitochondrial pathway mediated by SIRT1. *Eur J Pharmacol*. 2015;761:245-253. doi:10.1016/j.ejphar.2015.05.056
125. Kim DS, Ha KC, Kwon DY, et al. Kaempferol protects ischemia/reperfusion-induced cardiac damage through the regulation of endoplasmic reticulum stress. *Immunopharmacol Immunotoxicol*. 2008;30(2):257-270. doi:10.1080/08923970701812530
126. Suchal K, Malik S, Gamad N, et al. Kaempferol Attenuates Myocardial Ischemic Injury via Inhibition of MAPK Signaling Pathway in Experimental Model of Myocardial Ischemia-Reperfusion Injury. *Oxid Med Cell Longev*. 2016;2016:1-10. doi:10.1155/2016/7580731
127. Zhou M, Ren H, Han J, Wang W, Zheng Q, Wang D. Protective effects of kaempferol against myocardial ischemia/reperfusion injury in isolated rat heart via antioxidant activity and inhibition of glycogen synthase kinase-3β. *Oxid Med Cell Longev*. 2015;2015:1-8. doi:10.1155/2015/481405

128. Trivedi CM, Luo Y, Yin Z, et al. Hdac2 regulates the cardiac hypertrophic response by modulating Gsk3β activity. *Nat Med*. 2007;13(3):324-331. doi:10.1038/nm1552
129. Berger A, Venturelli S, Kallnischkies M, et al. Kaempferol, a new nutrition-derived pan-inhibitor of human histone deacetylases. *J Nutr Biochem*. 2013;24(6):977-985. doi:10.1016/j.jnutbio.2012.07.001
130. Dong Z, Yao K, Chen H, et al. Kaempferol targets RSK2 and MSK1 to Suppress UV radiation-induced skin cancer. *Cancer Prev Res*. 2014;7(9):958-967. doi:10.1158/1940-6207.CAPR-14-0126
131. Perkin AG. XXI. Myricetin. Part II. *J Chem Soc Trans*. 1902;81(0):203. doi:10.1039/ct9028100203
132. Semwal DK, Semwal RB, Combrinck S, Viljoen A. Myricetin: A dietary molecule with diverse biological activities. *Nutrients*. 2016;8(2):90. doi:10.3390/nu8020090
133. Hobbs CA, Swartz C, Maronpot R, et al. Genotoxicity evaluation of the flavonoid, myricitrin, and its aglycone, myricetin. *Food Chem Toxicol*. 2015;83:283-292. doi:10.1016/j.fct.2015.06.016
134. Qiu Y, Cong N, Liang M, Wang Y, Wang J. Systems Pharmacology Dissection of the Protective Effect of Myricetin Against Acute Ischemia/Reperfusion-Induced Myocardial Injury in Isolated Rat Heart. *Cardiovasc Toxicol*. 2017;17(3):277-286. doi:10.1007/s12012-016-9382-y
135. Tiwari R, Mohan M, Kasture S, Maxia A, Ballero M. Cardioprotective potential of myricetin in isoproterenol-induced myocardial infarction in wistar rats. *Phyther Res*. 2009;23(10):1361-1366. doi:10.1002/ptr.2688
136. Sun J, Sun G, Cui X, Meng X, Qin M, Sun X. Myricitrin Protects against Doxorubicin-Induced Cardiotoxicity by Counteracting Oxidative Stress and Inhibiting Mitochondrial Apoptosis via ERK/P53 Pathway. *Evidence-based Complement Altern Med*. 2016;2016:6093783. doi:10.1155/2016/6093783
137. Scarabelli TM, Mariotto S, Abdel-Azeim S, et al. Targeting STAT1 by myricetin and delphinidin provides efficient protection of the heart from ischemia/reperfusion-induced injury. *FEBS Lett*. 2009;583(3):531-541. doi:10.1016/j.febslet.2008.12.037
138. Zhang B, Chen Y, Shen Q, et al. Myricitrin Attenuates High Glucose-Induced Apoptosis through Activating Akt-Nrf2 Signaling in H9c2 Cardiomyocytes. *Molecules*. 2016;21(7):880. doi:10.3390/molecules21070880
139. Zhang B, Shen Q, Chen Y, et al. Myricitrin alleviates oxidative stress-induced inflammation and apoptosis and protects mice against diabetic cardiomyopathy. *Sci Rep*. 2017;7:44239. doi:10.1038/srep44239
140. Li LF, Lee CS, Lin CW, et al. Trichostatin A attenuates ventilationaugmented epithelial-mesenchymal transition in mice with bleomycin-induced acute lung injury by suppressing the Akt pathway. Lee JW, ed. *PLoS One*. 2017;12(2):e0172571. doi:10.1371/journal.pone.0172571
141. Martens S, Mithöfer A. Flavones and flavone synthases. *Phytochemistry*. 2005;66(20):2399-2407. doi:10.1016/j.phytochem.2005.07.013
142. Shukla S, Gupta S. Apigenin: A promising molecule for cancer prevention. *Pharm Res*. 2010;27(6):962-978. doi:10.1007/s11095-010-0089-7

143. Hu J, Li Z, Xu L, et al. Protective Effect of Apigenin on Ischemia/Reperfusion Injury of the Isolated Rat Heart. *Cardiovasc Toxicol*. 2015;15(3):241-249. doi:10.1007/s12012-014-9290-y
144. Zhu Y, Di S, Hu W, et al. A new flavonoid glycoside (APG) isolated from Clematis tangutica attenuates myocardial ischemia/reperfusion injury via activating PKCε signaling. *Biochim Biophys Acta - Mol Basis Dis*. 2017;1863(3):701-711. doi:10.1016/j.bbadis.2016.12.013
145. Yang X, Yang J, Hu J, Li X, Zhang X, Li Z. Apigenin attenuates myocardial ischemia/reperfusion injury via the inactivation of p38 mitogen-activated protein kinase. *Mol Med Rep*. 2015;12(5):6873-6878. doi:10.3892/mmr.2015.4293
146. Mahajan UB, Chandrayan G, Patil CR, et al. The protective effect of apigenin on myocardial injury in diabetic rats mediating activation of the PPAR-γ pathway. *Int J Mol Sci*. 2017;18(4). doi:10.3390/ijms18040756
147. Paredes-Gonzalez X, Fuentes F, Su Z-Y, Kong A-NT. Apigenin reactivates Nrf2 anti-oxidative stress signaling in mouse skin epidermal JB6 P + cells through epigenetics modifications. *AAPS J*. 2014;16(4):727-735. doi:10.1208/s12248-014-9613-8
148. Tseng T-HH, Chien M-HH, Lin W-LL, et al. Inhibition of MDA-MB-231 breast cancer cell proliferation and tumor growth by apigenin through induction of G2/M arrest and histone H3 acetylation-mediated p21 WAF1/CIP1 expression. *Environ Toxicol*. 2017;32(2):434-444. doi:10.1002/tox.22247
149. Lin Y, Shi R, Wang X, Shen H-M. Luteolin, a Flavonoid with Potential for Cancer Prevention and Therapy. *Curr Cancer Drug Targets*. 2008;8(7):634-646. doi:10.2174/156800908786241050
150. Harborne JB, Williams CA. Advances in flavonoid research since 1992. *Phytochemistry*. 2000;55(6):481-504. doi:10.1016/S0031-9422(00)00235-1
151. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P. Intake of flavonoids, carotenoids, Vitamins C and E, and risk of stroke in male smokers. *Stroke*. 2000;31(10):2301-2306. doi:10.1161/01.STR.31.10.2301
152. Keli SO, Hertog MGL, Feskens EJM, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: The Zutphen study. *Arch Intern Med*. 1996;156(6):637-642. doi:10.1001/archinte.156.6.637
153. Hu W, Xu T, Wu P, et al. Luteolin improves cardiac dysfunction in heart failure rats by regulating sarcoplasmic reticulum Ca2+-ATPase 2a. *Sci Rep*. 2017;7:41017. doi:10.1038/srep41017
154. Nai C, Xuan H, Zhang Y, et al. Luteolin Exerts Cardioprotective Effects through Improving Sarcoplasmic Reticulum Ca2+-ATPase Activity in Rats during Ischemia/Reperfusion in Vivo. *Evidence-based Complement Altern Med*. 2015;2015:365854. doi:10.1155/2015/365854
155. Wu X, Xu T, Li D, et al. ERK/PP1a/PLB/SERCA2a and JNK Pathways are involved in luteolin-mediated protection of rat hearts and cardiomyocytes following ischemia/reperfusion. Ai X, ed. *PLoS One*. 2013;8(12):e82957. doi:10.1371/journal.pone.0082957
156. Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phopholamban/SERCA2a regulatome. *Circ Res*. 2012;110(12):1646-1660.

doi:10.1161/CIRCRESAHA.111.259754
157. Kaikkonen L, Magga J, Ronkainen VP, et al. P38α regulates SERCA2a function. *J Mol Cell Cardiol*. 2014;67:86-93. doi:10.1016/j.yjmcc.2013.12.005
158. Blakeslee WW, Wysoczynski CL, Fritz KS, Nyborg JK, Churchill MEA, McKinsey TA. Class I HDAC inhibition stimulates cardiac protein SUMOylation through a post-translational mechanism. *Cell Signal*. 2014;26(12):2912-2920. doi:10.1016/j.cellsig.2014.09.005
159. Ganai SA, Farooq Z, Banday S, Altaf M. In silico approaches for investigating the binding propensity of apigenin and luteolin against class I HDAC isoforms. *Future Med Chem*. 2018;10(16):1925-1945. doi:10.4155/fmc-2018-0020
160. Fang F, Li D, Pan H, et al. Luteolin inhibits apoptosis and improves cardiomyocyte contractile function through the PI3K/Akt pathway in simulated ischemia/reperfusion. *Pharmacology*. 2011;88(3-4):149-158. doi:10.1159/000330068
161. Zhang X-W, Li W-F, Li W-W, et al. Protective effects of the aqueous extract of Scutellaria baicalensis against acrolein-induced oxidative stress in cultured human umbilical vein endothelial cells. *Pharm Biol*. 2011;49(3):256-261. doi:10.3109/13880209.2010.501803
162. Gasiorowski K, Lamer-Zarawska E, Leszek J, et al. Flavones from Root of Scutellaria Baicalensis Georgi: Drugs of the Future in Neurodegeneration? *CNS Neurol Disord - Drug Targets*. 2011;10(2):184-191. doi:10.2174/187152711794480384
163. Li-Weber M. New therapeutic aspects of flavones: The anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. *Cancer Treat Rev*. 2009;35(1):57-68. doi:10.1016/j.ctrv.2008.09.005
164. Kumar M, Kasala ER, Bodduluru LN, Dahiya V, Lahkar M. Baicalein protects isoproterenol induced myocardial ischemic injury in male Wistar rats by mitigating oxidative stress and inflammation. *Inflamm Res*. 2016;65(8):613-622. doi:10.1007/s00011-016-0944-z
165. Wang AW, Song L, Miao J, et al. Baicalein attenuates angiotensin II-induced cardiac remodeling via inhibition of AKT/mTOR, ERK1/2, NF-κB, and calcineurin signaling pathways in mice. *Am J Hypertens*. 2015;28(4):518-526. doi:10.1093/ajh/hpu194
166. Chen HM, Hsu JH, Liou SF, et al. Baicalein, an active component of Scutellaria baicalensis Georgi, prevents lysophosphatidylcholine-induced cardiac injury by reducing reactive oxygen species production, calcium overload and apoptosis via MAPK pathways. *BMC Complement Altern Med*. 2014;14(1):233. doi:10.1186/1472-6882-14-233
167. Zhang Y, Liao P, Zhu M, et al. Baicalin Attenuates Cardiac Dysfunction and Myocardial Remodeling in a Chronic Pressure-Overload Mice Model. *Cell Physiol Biochem*. 2017;41(3):849-864. doi:10.1159/000459708
168. Zhang S, Wang J, Pan J. Baicalin-loaded PEGylated lipid nanoparticles: characterization, pharmacokinetics, and protective effects on acute myocardial ischemia in rats. *Drug Deliv*. 2016;23(9):3696-3703. doi:10.1080/10717544.2016.1223218

169. Sun S-J, Wu X-P, Song H-L, Li G-Q. Baicalin ameliorates isoproterenol-induced acute myocardial infarction through iNOS, inflammation, oxidative stress and P38MAPK pathway in rat. *Int J Clin Exp Med*. 2015;8(12):22063-22072.
170. Chen Z, Wang Q. Activation of PPARγ by baicalin attenuates pulmonary hypertension in an infant rat model by suppressing HMGB1/RAGE signaling. *FEBS Open Bio*. 2017;7(4):477-484. doi:10.1002/2211-5463.12180
171. Huang X, He Y, Chen Y, et al. Baicalin attenuates bleomycin-induced pulmonary fibrosis via adenosine A2a receptor related TGF-β1-induced ERK1/2 signaling pathway. *BMC Pulm Med*. 2016;16(1):1-11. doi:10.1186/s12890-016-0294-1
172. Yan S, Wang Y, Liu P, et al. Baicalin attenuates hypoxia-induced pulmonary arterial hypertension to improve hypoxic cor pulmonale by reducing the activity of the p38 MAPK signaling pathway and MMP-9. *Evidence-based Complement Altern Med*. 2016;2016:2546402. doi:10.1155/2016/2546402
173. Liu X, Gu J, Fan Y, Shi H, Jiang M. Baicalin attenuates acute myocardial infarction of rats via mediating the mitogen-activated protein kinase pathway. *Biol Pharm Bull*. 2013;36(6):988-994.
174. Zong J, Zhang DP, Zhou H, et al. Baicalein protects against cardiac hypertrophy through blocking MEK-ERK1/2 signaling. *J Cell Biochem*. 2013;114(5):1058-1065. doi:10.1002/jcb.24445
175. Dong N, Xu B, Shi H, Tang X. Baicalein inhibits amadori-glycated albumin-induced mcp-1 expression in retinal ganglion cells via a microrna-124-dependent mechanism. *Investig Ophthalmol Vis Sci*. 2015;56(10):5844-5853. doi:10.1167/iovs.15-17444
176. Li L, Bao H, Wu J, et al. Baicalin is anti-inflammatory in cigarette smoke-induced inflammatory models in vivo and in vitro: A possible role for HDAC2 activity. *Int Immunopharmacol*. 2012;13(1):15-22. doi:10.1016/j.intimp.2012.03.001
177. Cherng CH, Lee KC, Chien CC, et al. Baicalin ameliorates neuropathic pain by suppressing HDAC1 expression in the spinal cord of spinal nerve ligation rats. *J Formos Med Assoc*. 2014;113(8):513-520. doi:10.1016/j.jfma.2013.04.007
178. Tsao R. Chemistry and biochemistry of dietary polyphenols. *Nutrients*. 2010;2(12):1231-1246. doi:10.3390/nu2121231
179. Vuong Q V., Golding JB, Nguyen M, Roach PD. Extraction and isolation of catechins from tea. *J Sep Sci*. 2010;33(21):3415-3428. doi:10.1002/jssc.201000438
180. Di Lorenzo A, Curti V, Tenore GC, Nabavi SM, Daglia M. Effects of Tea and Coffee Consumption on Cardiovascular Diseases and Relative Risk Factors: An Update. *Curr Pharm Des*. 2017;23(17):2474-2487. doi:10.2174/1381612823666170215145855
181. Wolfram S. Effects of green tea and egcg on cardiovascular and metabolic health. *J Am Coll Nutr*. 2007;26(4):373S-388S. doi:10.1080/07315724.2007.10719626
182. Chen T-S, Liou S-Y, Kuo C-H, et al. Green tea epigallocatechin gallate enhances cardiac function restoration through survival signaling expression in diabetes mellitus rats with autologous adipose tissue-derived stem cells. *J Appl Physiol*. 2017;123(5):1081-1091. doi:10.1152/japplphysiol.00471.2016
183. Liu J, Tang Y, Feng Z, Liu J, Liu J, Long J. (-)-Epigallocatechin-3-gallate attenuated myocardial mitochondrial dysfunction and autophagy in diabetic Goto-

Kakizaki rats. *Free Radic Res*. 2014;48(8):898-906. doi:10.3109/10715762.2014.920955

184. Yao YF, Liu X, Li WJ, et al. (−)-Epigallocatechin-3-gallate alleviates doxorubicin-induced cardiotoxicity in sarcoma 180 tumor-bearing mice. *Life Sci*. 2017;180:151-159. doi:10.1016/j.lfs.2016.12.004
185. Sun TL, Liu Z, Qi ZJ, Huang YP, Gao XQ, Zhang YY. (-)-Epigallocatechin-3-gallate (EGCG) attenuates arsenic-induced cardiotoxicity in rats. *Food Chem Toxicol*. 2016;93:102-110. doi:10.1016/j.fct.2016.05.004
186. Saeed NM, El-Naga RN, El-Bakly WM, Abdel-Rahman HM, Salah Eldin RA, El-Demerdash E. Epigallocatechin-3-gallate pretreatment attenuates doxorubicin-induced cardiotoxicity in rats: A mechanistic study. *Biochem Pharmacol*. 2015;95(3):145-155. doi:10.1016/j.bcp.2015.02.006
187. Othman AI, Elkomy MM, El-Missiry MA, Dardor M. Epigallocatechin-3-gallate prevents cardiac apoptosis by modulating the intrinsic apoptotic pathway in isoproterenol-induced myocardial infarction. *Eur J Pharmacol*. 2017;794:27-36. doi:10.1016/j.ejphar.2016.11.014
188. Xuan F, Jian J. Epigallocatechin gallate exerts protective effects against myocardial ischemia/reperfusion injury through the PI3K/Akt pathway-mediated inhibition of apoptosis and the restoration of the autophagic flux. *Int J Mol Med*. 2016;38(1):328-336. doi:10.3892/ijmm.2016.2615
189. Rice-evans C. Implications of the Mechanisms of Action of Tea Polyphenols as Antioxidants in vitro for Chemoprevention in Humans. *Proc Soc Exp Biol Med*. 1999;220(44377):262-266. doi:10.3181/00379727-220-44377
190. Higdon J V., Frei B. Tea Catechins and Polyphenols: Health Effects, Metabolism, and Antioxidant Functions. *Crit Rev Food Sci Nutr*. 2003;43(1):89-143. doi:10.1080/10408690390826464
191. Pan B, Quan J, Liu L, et al. Epigallocatechin gallate reverses cTnI-low expression-induced age-related heart diastolic dysfunction through histone acetylation modification. *J Cell Mol Med*. 2017;21(10):2481-2490. doi:10.1111/jcmm.13169
192. Liu J, Tang Y, Feng Z, et al. Acetylated FoxO1 mediates high-glucose induced autophagy in H9c2 cardiomyoblasts: Regulation by a polyphenol -(−)-epigallocatechin-3-gallate. *Metabolism*. 2014;63(10):1314-1323. doi:10.1016/j.metabol.2014.06.012
193. Trivedi CM, Min ML, Wang Q, Epstein JA. Transgenic overexpression of Hdac3 in the heart produces increased postnatal cardiac myocyte proliferation but does not induce hypertrophy. *J Biol Chem*. 2008;283(39):26484-26489. doi:10.1074/jbc.M803686200
194. Takamura T, Honda M, Sakai Y, et al. Gene expression profiles in peripheral blood mononuclear cells reflect the pathophysiology of type 2 diabetes. *Biochem Biophys Res Commun*. 2007;361(2):379-384. doi:10.1016/j.bbrc.2007.07.006
195. Ma J, Liew C-C. Gene profiling identifies secreted protein transcripts from peripheral blood cells in coronary artery disease. *J Mol Cell Cardiol*. 2003;35(8):993-998. doi:10.1016/S0022-2828(03)00179-2
196. Li H, Li Q, Liu Z, et al. The Versatile Effects of Dihydromyricetin in Health. *Evidence-based Complement Altern Med*. 2017;2017:1053617.

doi:10.1155/2017/1053617
197. Liu S, Ai Q, Feng K, Li Y, Liu X. The cardioprotective effect of dihydromyricetin prevents ischemia–reperfusion-induced apoptosis in vivo and in vitro via the PI3K/Akt and HIF-1α signaling pathways. *Apoptosis*. 2016;21(12):1366-1385. doi:10.1007/s10495-016-1306-6
198. Song Q, Liu L, Yu J, et al. Dihydromyricetin attenuated Ang II induced cardiac fibroblasts proliferation related to inhibitory of oxidative stress. *Eur J Pharmacol*. 2017;807:159-167. doi:10.1016/j.ejphar.2017.04.014
199. Meng G, Yang S, Chen Y, Yao W, Zhu H, Zhang W. Attenuating effects of dihydromyricetin on angiotensin II-induced rat cardiomyocyte hypertrophy related to antioxidative activity in a NO-dependent manner. *Pharm Biol*. 2015;53(6):904-912. doi:10.3109/13880209.2014.948635
200. Wu B, Lin J, Luo J, et al. Dihydromyricetin Protects against Diabetic Cardiomyopathy in Streptozotocin-Induced Diabetic Mice. *Biomed Res Int*. 2017;2017:1-13. doi:10.1155/2017/3764370
201. Zhou MQ, Shao L, Wu J, et al. Dihydromyricetin protects against lipopolysaccharide-induced cardiomyocyte injury through the toll-like receptor-4/nuclear factor-κB pathway. *Mol Med Rep*. 2017;16(6):8983-8988. doi:10.3892/mmr.2017.7742
202. Santos-Buelga C, Scalbert A. Proanthocyanidins and tannin-like compounds - Nature, occurrence, dietary intake and effects on nutrition and health. *J Sci Food Agric*. 2000;80(7):1094-1117. doi:10.1002/(SICI)1097-0010(20000515)80:7<1094::AID-JSFA569>3.0.CO;2-1
203. Rasmussen SE, Frederiksen H, Krogholm KS, Poulsen L. Dietary proanthocyanidins: Occurrence, dietary intake, bioavailability, and protection against cardiovascular disease. *Mol Nutr Food Res*. 2005;49(2):159-174. doi:10.1002/mnfr.200400082
204. Charradi K, Sebai H, Elkahoui S, Ben Hassine F, Limam F, Aouani E. Grape seed extract alleviates high-fat diet-induced obesity and heart dysfunction by preventing cardiac siderosis. *Cardiovasc Toxicol*. 2011;11(1):28-37. doi:10.1007/s12012-010-9101-z
205. Charradi K, Mahmoudi M, Elkahoui S, Limam F, Aouani E. Grape seed and skin extract mitigates heart and liver oxidative damage induced by a high-fat diet in the rat: gender dependency. *Can J Physiol Pharmacol*. 2013;91(12):1076-1085. doi:10.1139/cjpp-2013-0225
206. Feringa HHH, Laskey DA, Dickson JE, Coleman CI. The Effect of Grape Seed Extract on Cardiovascular Risk Markers: A Meta-Analysis of Randomized Controlled Trials. *J Am Diet Assoc*. 2011;111(8):1173-1181. doi:10.1016/j.jada.2011.05.015
207. Belcaro G, Ledda A, Hu S, Cesarone MR, Feragalli B, Dugall M. Grape seed procyanidins in pre- and mild hypertension: A registry study. *Evidence-based Complement Altern Med*. 2013;2013:1-5. doi:10.1155/2013/313142
208. Arora P, Ansari S, Nazish I. Study of antiobesity effects of ethanolic and water extracts of grapes seeds. *J Complement Integr Med*. 2011;8(1). doi:10.2202/1553-3840.1510

209. Mokni M, Hamlaoui-Guesmi S, Amri M, Marzouki L, Limam F, Aouani E. Grape seed and skin extract protects against acute chemotherapy toxicity induced by doxorubicin in rat heart. *Cardiovasc Toxicol*. 2012;12(2):158-165. doi:10.1007/s12012-012-9155-1
210. Boghdady NA El. Antioxidant and antiapoptotic effects of proanthocyanidin and ginkgo biloba extract against doxorubicin-induced cardiac injury in rats. *Cell Biochem Funct*. 2013;31(4):344-351. doi:10.1002/cbf.2907
211. Ammar ESM, Said SA, El-Damarawy SL, Suddek GM. Cardioprotective effect of grape-seed proanthocyanidins on doxorubicin-induced cardiac toxicity in rats. *Pharm Biol*. 2013;51(3):339-344. doi:10.3109/13880209.2012.729065
212. Yalçin E, Oruç E, Cavuşoğlu K, Yapar K. Protective role of grape seed extract against doxorubicin-induced cardiotoxicity and genotoxicity in albino mice. *J Med Food*. 2010;13(4):917-925. doi:10.1089/jmf.2009.0162
213. Li J, Liu H, Ramachandran S, et al. Grape Seed Proanthocyanidins Ameliorate Doxorubicin-Induced Cardiotoxicity. *Am J Chin Med*. 2010;38(03):569-584. doi:10.1142/S0192415X10008068
214. Sfaxi I, Charradi K, Limam F, El May MV, Aouani E. Grape seed and skin extract protects against arsenic trioxide induced oxidative stress in rat heart. *Can J Physiol Pharmacol*. 2016;94(2):168-176. doi:10.1139/cjpp-2015-0088
215. Badavi M, Mehrgerdi FZ, Sarkaki A, Naseri MKG, Dianat M. Effect of grape seed extract on lead induced hypertension and heart rate in rat. *Pakistan J Biol Sci*. 2008;11(6):882-887. doi:10.3923/pjbs.2008.882.887
216. Nazimabashir, Manoharan V, Miltonprabu S. Cadmium induced cardiac oxidative stress in rats and its attenuation by GSP through the activation of Nrf2 signaling pathway. *Chem Biol Interact*. 2015;242:179-193. doi:10.1016/j.cbi.2015.10.005
217. Zuo YM, Wang XH, Gao S, Zhang Y. Oligomerized grape seed proanthocyanidins ameliorates isoproterenol-induced cardiac remodeling in rats: Role of oxidative stress. *Phyther Res*. 2011;25(5):732-739. doi:10.1002/ptr.3331
218. Karthikeyan K, Sarala Bai BR, Niranjali Devaraj S. Efficacy of grape seed proanthocyanidins on serum and heart tissue lipids in rats subjected to isoproterenol-induced myocardial injury. *Vascul Pharmacol*. 2007;47(5-6):295-301. doi:10.1016/j.vph.2007.08.008
219. Nour MS, Sarhan NR, Mazroa SA, Gawish SA. Histological and immunohistochemical study of cardiac telocytes in a rat model of isoproterenol-induced myocardial infarction with a reference to the effect of grape seed extract. *Acta Histochem*. 2017;119(7):747-758. doi:10.1016/j.acthis.2017.09.007
220. Zhao G, Gao H, Qiu J, Lu W, Wei X. The molecular mechanism of protective effects of grape seed proanthocyanidin extract on reperfusion arrhythmias in rats in vivo. *Biol Pharm Bull*. 2010;33(5):759-767. doi:10.1248/bpb.33.759
221. Liang Y, Qiu J, Gao H-Q, Li B-Y. Protective effect of grape seed proanthocyanidins extracts on reperfusion arrhythmia in rabbits. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(3):223-230. doi:JST.JSTAGE/jnsv/55.223 [pii]
222. Shao ZH, Wojcik KR, Dossumbekova A, et al. Grape seed proanthocyanidins protect cardiomyocytes from ischemia and reperfusion injury via Akt-NOS signaling. *J Cell Biochem*. 2009;107(4):697-705. doi:10.1002/jcb.22170

223. Pataki T, Bak I, Kovacs P, Bagchi D, Das DK, Tosaki A. Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. *Am J Clin Nutr*. 2002;75(5):894-899. doi:10.1152/ajpheart.01243.2005
224. Guler A, Sahin MA, Yucel O, et al. Proanthocyanidin prevents myocardial ischemic injury in adult rats. *Int Med J Exp Clin Res*. 2011;17(11):326-331.
225. Heidker RM, Caiozzi GC, Ricketts ML. Grape seed procyanidins and cholestyramine differentially alter bile acid and cholesterol homeostatic gene expression in mouse intestine and liver. Avila MA, ed. *PLoS One*. 2016;11(4):e0154305. doi:10.1371/journal.pone.0154305
226. Heidker RM, Caiozzi GC, Ricketts ML. Dietary procyanidins selectively modulate intestinal farnesoid X receptor-regulated gene expression to alter enterohepatic bile acid recirculation: Elucidation of a novel mechanism to reduce triglyceridemia. *Mol Nutr Food Res*. 2016;60(4):727-736. doi:10.1002/mnfr.201500795
227. Downing LE, Heidker RM, Caiozzi GC, et al. A grape seed procyanidin extract ameliorates fructose-induced hypertriglyceridemia in rats via enhanced fecal bile acid and cholesterol excretion and inhibition of hepatic lipogenesis. Aguila MB, ed. *PLoS One*. 2015;10(10):e0140267. doi:10.1371/journal.pone.0140267
228. Downing LE, Ferguson BS, Rodriguez K, Ricketts ML. A grape seed procyanidin extract inhibits HDAC activity leading to increased Pparα phosphorylation and target-gene expression. *Mol Nutr Food Res*. 2017;61(2):1600347. doi:10.1002/mnfr.201600347
229. Chong J, Poutaraud A, Hugueney P. Metabolism and roles of stilbenes in plants. *Plant Sci*. 2009;177(3):143-155. doi:10.1016/j.plantsci.2009.05.012
230. Zordoky BNM, Robertson IM, Dyck JRB. Preclinical and clinical evidence for the role of resveratrol in the treatment of cardiovascular diseases. *Biochim Biophys Acta*. 2014;1852(6):http://dx.doi.org/10.1016/j.bbadis.2014.10.016. doi:10.1016/j.bbadis.2014.10.016
231. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. 1992;339(8808):1523-1526. doi:10.1016/0140-6736(92)91277-F
232. Sung MM, Dyck JRB. Therapeutic potential of resveratrol in heart failure. *Ann N Y Acad Sci*. 2015;1348(1):32-45. doi:10.1111/nyas.12839
233. Raj P, Lieben Louis X, Thandapilly SJ, Movahed A, Zieroth S, Netticadan T. Potential of resveratrol in the treatment of heart failure. *Life Sci*. 2014;95(2):63-71. doi:10.1016/j.lfs.2013.12.011
234. Raj P, Zieroth S, Netticadan T. An overview of the efficacy of resveratrol in the management of ischemic heart disease. *Ann N Y Acad Sci*. 2015;1348(1):55-67. doi:10.1111/nyas.12828
235. Chen YR, Yi FF, Li XY, et al. Resveratrol attenuates ventricular arrhythmias and improves the long-term survival in rats with myocardial infarction. *Cardiovasc Drugs Ther*. 2008;22(6):479-485. doi:10.1007/s10557-008-6141-8
236. Lin JF, Lin SM, Chih CL, et al. Resveratrol reduces infarct size and improves ventricular function after myocardial ischemia in rats. *Life Sci*. 2008;83(9-10):313-317. doi:10.1016/j.lfs.2008.06.016

237. Xuan W, Wu B, Chen C, et al. Resveratrol improves myocardial ischemia and ischemic heart failure in mice by antagonizing the detrimental effects of fractalkine. *Crit Care Med*. 2012;40(11):3026-3033. doi:10.1097/CCM.0b013e31825fd7da
238. Gu XS, Wang ZB, Ye Z, et al. Resveratrol, an activator of SIRT1, upregulates AMPK and improves cardiac function in heart failure. *Genet Mol Res*. 2014;13(1):323-335. doi:10.4238/2014.January.17.17
239. Kanamori H, Takemura G, Goto K, et al. Resveratrol reverses remodeling in hearts with large, old myocardial infarctions through enhanced autophagy-activating AMP kinase pathway. *Am J Pathol*. 2013;182(3):701-713. doi:10.1016/j.ajpath.2012.11.009
240. Juric D, Wojciechowski P, Das DK, Netticadan T. Prevention of concentric hypertrophy and diastolic impairment in aortic-banded rats treated with resveratrol. *Am J Physiol - Hear Circ Physiol*. 2007;292(5):H2138-H2143. doi:10.1152/ajpheart.00852.2006
241. Gupta PK, DiPette DJ, Supowit SC. Protective effect of resveratrol against pressure overload-induced heart failure. *Food Sci Nutr*. 2014;2(3):218-229. doi:10.1002/fsn3.92
242. Wojciechowski P, Juric D, Louis XL, et al. Resveratrol arrests and regresses the development of pressure overload- but not volume overload-induced cardiac hypertrophy in rats. *J Nutr*. 2010;140(5):962-968. doi:10.3945/jn.109.115006
243. Dong Q, Wu Z, Li X, et al. Resveratrol ameliorates cardiac dysfunction induced by pressure overload in rats via structural protection and modulation of Ca2+cycling proteins. *J Transl Med*. 2014;12(1):323. doi:10.1186/s12967-014-0323-x
244. Sung MM, Das SK, Levasseur J, et al. Resveratrol treatment of mice with pressure-overloadinduced heart failure improves diastolic function and cardiac energy metabolism. *Circ Hear Fail*. 2015;8(1):128-137. doi:10.1161/CIRCHEARTFAILURE.114.001677
245. Thandapilly SJ, Wojciechowski P, Behbahani J, et al. Resveratrol prevents the development of pathological cardiac hypertrophy and contractile dysfunction in the SHR without lowering blood pressure. *Am J Hypertens*. 2010;23(2):192-196. doi:10.1038/ajh.2009.228
246. Rimbaud S, Ruiz M, Piquereau J, et al. Resveratrol improves survival, hemodynamics and energetics in a rat model of hypertension leading to heart failure. de Windt LJ, ed. *PLoS One*. 2011;6(10):e26391. doi:10.1371/journal.pone.0026391
247. Yang DL, Zhang HG, Xu YL, et al. Resveratrol inhibits right ventricular hypertrophy induced by monocrotaline in rats. *Clin Exp Pharmacol Physiol*. 2010;37(2):150-155. doi:10.1111/j.1440-1681.2009.05231.x
248. Toklu HZ, Şehirli Ö, Erşahin M, et al. Resveratrol improves cardiovascular function and reduces oxidative organ damage in the renal, cardiovascular and cerebral tissues of two-kidney, one-clip hypertensive rats. *J Pharm Pharmacol*. 2010;62(12):1784-1793. doi:10.1111/j.2042-7158.2010.01197.x
249. Dolinsky VW, Chakrabarti S, Pereira TJ, et al. Resveratrol prevents hypertension and cardiac hypertrophy in hypertensive rats and mice. *Biochim Biophys Acta* -

*Mol Basis Dis*. 2013;1832(10):1723-1733. doi:10.1016/j.bbadis.2013.05.018
250. Biala A, Tauriainen E, Siltanen A, et al. Resveratrol induces mitochondrial biogenesis and ameliorates Ang II-induced cardiac remodeling in transgenic rats harboring human renin and angiotensinogen genes. *Blood Press*. 2010;19(3):196-205. doi:10.3109/08037051.2010.481808
251. Zaha VG, Young LH. AMP-activated protein kinase regulation and biological actions in the heart. *Circ Res*. 2012;111(6):800-814. doi:10.1161/CIRCRESAHA.111.255505
252. Cencioni C, Spallotta F, Mai A, et al. Sirtuin function in aging heart and vessels. *J Mol Cell Cardiol*. 2015;83:55-61. doi:10.1016/j.yjmcc.2014.12.023
253. Bagul PK, Deepthi N, Sultana R, Banerjee SK. Resveratrol ameliorates cardiac oxidative stress in diabetes through deacetylation of NFkB-p65 and histone 3. *J Nutr Biochem*. 2015;26(11):1298-1307. doi:10.1016/j.jnutbio.2015.06.006
254. Hirschey MD, Shimazu T, Jing E, et al. SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol Cell*. 2011;44(2):177-190. doi:10.1016/j.molcel.2011.07.019
255. Horton JL, Martin OJ, Lai L, et al. Mitochondrial protein hyperacetylation in the failing heart. *JCI Insight*. 2016;1(2). doi:10.1172/jci.insight.84897
256. Bo S, Togliatto G, Gambino R, et al. Impact of sirtuin-1 expression on H3K56 acetylation and oxidative stress: a double-blind randomized controlled trial with resveratrol supplementation. *Acta Diabetol*. 2018;55(4):331-340. doi:10.1007/s00592-017-1097-4
257. Venturelli S, Berger A, Böcker A, et al. Resveratrol as a Pan-HDAC Inhibitor Alters the Acetylation Status of Jistone Proteins in Human-Derived Hepatoblastoma Cells. Gaetano C, ed. *PLoS One*. 2013;8(8):e73097. doi:10.1371/journal.pone.0073097
258. Ammon H, Wahl M. Pharmacology of Curcuma longa. *Planta Med*. 1991;57(01):1-7. doi:10.1055/s-2006-960004
259. Alrawaiq NS, Abdullah A. A review of antioxidant polyphenol curcumin and its role in detoxification. *Int J PharmTech Res*. 2014;6(1):280-289.
260. Shen L, Ji HF. The pharmacology of curcumin: Is it the degradation products? *Trends Mol Med*. 2012;18(3):138-144. doi:10.1016/j.molmed.2012.01.004
261. Zeng C, Zhong P, Zhao Y, et al. Curcumin protects hearts from FFA-induced injury by activating Nrf2 and inactivating NF-κB both in vitro and in vivo. *J Mol Cell Cardiol*. 2015;79:1-12. doi:10.1016/j.yjmcc.2014.10.002
262. Aydin MS, Caliskan A, Kocarslan A, et al. Intraperitoneal curcumin decreased lung, renal and heart injury in abdominal aorta ischemia/reperfusion model in rat. *Int J Surg*. 2014;12(6):601-605. doi:10.1016/j.ijsu.2014.04.013
263. Imbaby S, Ewais M, Essawy S, Farag N. Cardioprotective effects of curcumin and nebivolol against doxorubicin-induced cardiac toxicity in rats. *Hum Exp Toxicol*. 2014;33(8):800-813. doi:10.1177/0960327114527628
264. Ciftci O, Turkmen NB, Taslıdere A. Curcumin protects heart tissue against irinotecan-induced damage in terms of cytokine level alterations, oxidative stress, and histological damage in rats. *Naunyn Schmiedebergs Arch Pharmacol*. 2018;391(8):783-791. doi:10.1007/s00210-018-1495-3

265. Lau WL, Khazaeli M, Savoj J, et al. Dietary tetrahydrocurcumin reduces renal fibrosis and cardiac hypertrophy in 5/6 nephrectomized rats. *Pharmacol Res Perspect*. 2018;6(2):e00385. doi:10.1002/prp2.385
266. Guo S, Meng XW, Yang XS, Liu XF, Ou-Yang CH, Liu C. Curcumin administration suppresses collagen synthesis in the hearts of rats with experimental diabetes. *Acta Pharmacol Sin*. 2018;39(2):195-204. doi:10.1038/aps.2017.92
267. Chen H, Yang X, Lu K, et al. Inhibition of high glucose-induced inflammation and fibrosis by a novel curcumin derivative prevents renal and heart injury in diabetic mice. *Toxicol Lett*. 2017;278:48-58. doi:10.1016/j.toxlet.2017.07.212
268. Li X, Cao T, Ma S, et al. Curcumin ameliorates cardiac dysfunction induced by mechanical trauma. *Eur J Pharmacol*. 2017;814:73-80. doi:10.1016/j.ejphar.2017.07.048
269. Wongcharoen W, Jai-Aue S, Phrommintikul A, et al. Effects of curcuminoids on frequency of acute myocardial infarction after coronary artery bypass grafting. *Am J Cardiol*. 2012;110(1):40-44. doi:10.1016/j.amjcard.2012.02.043
270. Sugawara J, Akazawa N, Miyaki A, et al. Effect of endurance exercise training and curcumin intake on central arterial hemodynamics in postmenopausal women: Pilot study. *Am J Hypertens*. 2012;25(6):651-656. doi:10.1038/ajh.2012.24
271. Akazawa N, Choi Y, Miyaki A, et al. Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. *Nutr Res*. 2012;32(10):795-799. doi:10.1016/j.nutres.2012.09.002
272. Panahi Y, Khalili N, Hosseini MS, Abbasinazari M, Sahebkar A. Lipid-modifying effects of adjunctive therapy with curcuminoids-piperine combination in patients with metabolic syndrome: Results of a randomized controlled trial. *Complement Ther Med*. 2014;22(5):851-857. doi:10.1016/j.ctim.2014.07.006
273. Mohammadi A, Sahebkar A, Iranshahi M, et al. Effects of supplementation with curcuminoids on dyslipidemia in obese patients: A randomized crossover trial. *Phyther Res*. 2013;27(3):374-379. doi:10.1002/ptr.4715
274. Chuengsamarn S, Rattanamongkolgul S, Phonrat B, Tungtrongchitr R, Jirawatnotai S. Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: A randomized controlled trial. *J Nutr Biochem*. 2014;25(2):144-50. doi:10.1016/j.jnutbio.2013.09.013
275. Balasubramanyam K, Varier RA, Altaf M, et al. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J Biol Chem*. 2004;279(49):51163-51171. doi:10.1074/jbc.M409024200
276. Neckers L, Trepel J, Lee S, et al. Curcumin is an Inhibitor of p300 Histone Acetylatransferase. *Med Chem (Los Angeles)*. 2006;2(2):169-174. doi:10.2174/157340606776056133
277. Yanazume T, Hasegawa K, Morimoto T, et al. Cardiac p300 is involved in myocyte growth with decompensated heart failure. *Mol Cell Biol*. 2003;23(10):3593-3606. doi:10.1128/MCB.23.10.3593
278. Morimoto T, Sunagawa Y, Kawamura T, et al. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. *J*

*Clin Invest*. 2008;118(3):868-878. doi:10.1172/JCI33160
279. Soflaei SS, Momtazi-Borojeni AA, Majeed M, Derosa G, Maffioli P, Sahebkar A. Curcumin: A Natural Pan-HDAC Inhibitor in Cancer | BenthamScience. Current Pharmaceutical Design. doi:10.2174/1381612823666171114165051
280. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. *Mol Pharm*. 2007;4(6):807-818. doi:10.1021/mp700113r
281. Prasad S, Tyagi AK, Aggarwal BB. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. *Cancer Res Treat*. 2014;46(1):2-18. doi:10.4143/crt.2014.46.1.2
282. Koleva II, van Beek TA, Soffers AEMF, Dusemund B, Rietjens IMCM. Alkaloids in the human food chain - natural occurrence and possible adverse effects. *Mol Nutr Food Res*. 2012;56(1):30-52. doi:10.1002/mnfr.201100165
283. Birdsall T, GS K. Therapeutic potential of an alkaloid found in several medicinal plants. *Altern Med Rev*. 1997;2(2):94-103.
284. Yin J, Gao Z, Liu D, Liu Z, Ye J. Berberine improves glucose metabolism through induction of glycolysis. *Am J Physiol Metab*. 2008;294(1):E148-E156. doi:10.1152/ajpendo.00211.2007
285. Wei W, Zhao H, Wang A, et al. A clinical study on the short-term effect of berberine in comparison to metformin on the metabolic characteristics of women with polycystic ovary syndrome. *Eur J Endocrinol*. 2012;166(1):99-105. doi:10.1530/EJE-11-0616
286. Shen N, Li X, Zhou T, et al. Shensong Yangxin Capsule prevents diabetic myocardial fibrosis by inhibiting TGF-β1/Smad signaling. *J Ethnopharmacol*. 2014;157:161-170. doi:10.1016/j.jep.2014.09.035
287. Gu HP, Lin S, Xu M, et al. Up-regulating relaxin expression by G-quadruplex interactive ligand to achieve antifibrotic action. *Endocrinology*. 2012;153(8):3692-3700. doi:10.1210/en.2012-1114
288. Soloff MS, Gal S, Hoare S, et al. Cloning, characterization, and expression of the rat relaxin gene. *Gene*. 2003;323(1-2):149-155. doi:10.1016/j.gene.2003.09.015
289. Xiao Z, Hao Y, Liu B, Qian L. Indirubin and Meisoindigo in the treatment of chronic myelogenous leukemia in China. *Leuk Lymphoma*. 2002;43(9):1763-1768. doi:10.1080/1042819021000006295
290. Wang Y, Hoi P, Chan J, Lee S. New Perspective on the Dual Functions of Indirubins in Cancer Therapy and Neuroprotection. *Anticancer Agents Med Chem*. 2014;14(9):1213-1219. doi:10.2174/1871520614666140825112924
291. Udumula MP, Medapi B, Dhar I, et al. The Small Molecule Indirubin-3'-Oxime Inhibits Protein Kinase R: Antiapoptotic and Antioxidant Effect in Rat Cardiac Myocytes. *Pharmacology*. 2015;97(1-2):25-30. doi:10.1159/000441727
292. González Arbeláez LF, Pérez Núñez IA, Mosca SM. Gsk-3 β inhibitors mimic the cardioprotection mediated by ischemic pre- and postconditioning in hypertensive rats. *Biomed Res Int*. 2013;2013:1-11. doi:10.1155/2013/317456
293. Yadav HN, Singh M, Sharma PL. Pharmacological inhibition of GSK-3β produces late phase of cardioprotection in hyperlipidemic rat: Possible involvement of HSP 72. *Mol Cell Biochem*. 2012;369(1-2):227-233. doi:10.1007/s11010-012-1386-8

294. Yadav HN, Singh M, Sharma PL. Modulation of the cardioprotective effect of ischemic preconditioning in hyperlipidaemic rat heart. *Eur J Pharmacol*. 2010;643(1):78-83. doi:10.1016/j.ejphar.2010.06.015
295. Yadav HN, Singh M, Sharma PL. Involvement of GSK-3β in attenuation of the cardioprotective effect of ischemic preconditioning in diabetic rat heart. *Mol Cell Biochem*. 2010;343(1-2):75-81. doi:10.1007/s11010-010-0500-z
296. Barillas R, Friehs I, Cao-Danh H, Martinez JF, del Nido PJ. Inhibition of Glycogen Synthase Kinase-3β Improves Tolerance to Ischemia in Hypertrophied Hearts. *Ann Thorac Surg*. 2007;84(1):126-133. doi:10.1016/j.athoracsur.2007.02.015
297. Jiang LL, Zhou SJ, Zhang XM, Chen HQ, Liu W. Sulforaphane suppresses in vitro and in vivo lung tumorigenesis through downregulation of HDAC activity. *Biomed Pharmacother*. 2016;78:74-80. doi:10.1016/j.biopha.2015.11.007
298. Myzak MC. Sulforaphane inhibits histone deacetylase in vivo and suppresses tumorigenesis in Apcmin mice. *FASEB J*. 2006;20(3):506-508. doi:10.1096/fj.05-4785fje
299. Clarke JD, Hsu A, Yu Z, Dashwood RH, Ho E. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. *Mol Nutr Food Res*. 2011;55(7):999-1009. doi:10.1002/mnfr.201000547
300. Myzak MC, Hardin K, Wang R, Dashwood RH, Ho E. Sulforaphane inhibits histone deacetylase activity in BPH-1, LnCaP and PC-3 prostate epithelial cells. *Carcinogenesis*. 2006;27(4):811-819. doi:10.1093/carcin/bgi265
301. Myzak MC, Tong P, Dashwood W-M, Dashwood RH, Ho E. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp Biol Med (Maywood)*. 2007;232(2):227-234. doi:232/2/227 [pii]
302. Dashwood RH, Ho E. Dietary histone deacetylase inhibitors: from cells to mice to man. *Semin Cancer Biol*. 2007;17(5):363-369. doi:10.1016/j.semcancer.2007.04.001
303. Singh P, Sharma R, McElhanon K, et al. Sulforaphane protects the heart from doxorubicin-induced toxicity. *Free Radic Biol Med*. 2015;86:90-101. doi:10.1016/j.freeradbiomed.2015.05.028
304. Li B, Kim DS, Yadav RK, Kim HR, Chae HJ. Sulforaphane prevents doxorubicin-induced oxidative stress and cell death in rat H9c2 cells. *Int J Mol Med*. 2015;36(1):53-64. doi:10.3892/ijmm.2015.2199
305. Li Z, Galli U, Becker LE, et al. Sulforaphane protects hearts from early injury after experimental transplantation. *Ann Transplant*. 2013;18(1):558-566. doi:10.12659/AOT.889342
306. Piao CS, Gao S, Lee GH, et al. Sulforaphane protects ischemic injury of hearts through antioxidant pathway and mitochondrial KATPchannels. *Pharmacol Res*. 2010;61(4):342-348. doi:10.1016/j.phrs.2009.11.009
307. Wu QQ, Zong J, Gao L, et al. Sulforaphane protects H9c2 cardiomyocytes from angiotensin II-induced hypertrophy. *Herz*. 2014;39(3):390-396. doi:10.1007/s00059-013-3849-4
308. Fernandes RO, Bonetto JHP, Baregzay B, et al. Modulation of apoptosis by sulforaphane is associated with PGC-1α stimulation and decreased oxidative stress

in cardiac myoblasts. *Mol Cell Biochem*. 2014;401(1-2):61-70. doi:10.1007/s11010-014-2292-z

309. Zhang Z, Wang S, Zhou S, et al. Sulforaphane prevents the development of cardiomyopathy in type 2 diabetic mice probably by reversing oxidative stress-induced inhibition of LKB1/AMPK pathway. *J Mol Cell Cardiol*. 2014;77:42-52. doi:10.1016/j.yjmcc.2014.09.022
310. Bai Y, Cui W, Xin Y, et al. Prevention by sulforaphane of diabetic cardiomyopathy is associated with up-regulation of Nrf2 expression and transcription activation. *J Mol Cell Cardiol*. 2013;57(1):82-95. doi:10.1016/j.yjmcc.2013.01.008
311. Ma T, Zhu D, Chen D, et al. Sulforaphane, a Natural Isothiocyanate Compound, Improves Cardiac Function and Remodeling by Inhibiting Oxidative Stress and Inflammation in a Rabbit Model of Chronic Heart Failure. *Med Sci Monit*. 2018;24:1473-1483. doi:10.12659/MSM.906123
312. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. *Biochem Soc Trans*. 2015;43(4):621-626. doi:10.1042/BST20150014
313. Kawai Y, Garduño L, Theodore M, Yang J, Arinze IJ. Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. *J Biol Chem*. 2011;286(9):7629-7640. doi:10.1074/jbc.M110.208173
314. Kee HJ, Kim GR, Kim IK, Jeong MH. Sulforaphane suppresses cardiac hypertrophy by inhibiting GATA4/GATA6 expression and MAPK signaling pathways. *Mol Nutr Food Res*. 2015;59(2):221-230. doi:10.1002/mnfr.201400279
315. Ho J-N, Yoon H-G, Park C-S, et al. Isothiocyanates Ameliorate the Symptom of Heart Dysfunction and Mortality in a Murine AIDS Model by Inhibiting Apoptosis in the Left Ventricle. *J Med Food*. 2012;15(9):781-787. doi:10.1089/jmf.2011.1906
316. Boyanapalli SSS, Li W, Fuentes F, et al. Epigenetic reactivation of RASSF1A by phenethyl isothiocyanate (PEITC) and promotion of apoptosis in LNCaP cells. *Pharmacol Res*. 2016;114:175-184. doi:10.1016/j.phrs.2016.10.021
317. Wang LG, Liu XM, Fang Y, et al. De-repression of the p21 promoter in prostate cancer cells by an isothiocyanate via inhibition of HDACs and c-Myc. *Int J Oncol*. 2008;33(2):375-380. doi:10.3892/ijo_00000018
318. Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. *J Nutr*. 2002;132(5):1012-1017. doi:10.1038/nrc3610
319. Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients*. 2015;7(4):2839-2849. doi:10.3390/nu7042839
320. Gao Z, Yin J, Zhang J, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes*. 2009;58(7):1509-1517. doi:10.2337/db08-1637
321. Aguilar EC, Leonel AJ, Teixeira LG, et al. Butyrate impairs atherogenesis by reducing plaque inflammation and vulnerability and decreasing NFκB activation.

*Nutr Metab Cardiovasc Dis*. 2014;24(6):606-613. doi:10.1016/j.numecd.2014.01.002

322. Chen Y, Du J, Zhao YT, et al. Histone deacetylase (HDAC) inhibition improves myocardial function and prevents cardiac remodeling in diabetic mice. *Cardiovasc Diabetol*. 2015;14(1):99. doi:10.1186/s12933-015-0262-8
323. Zhang L, Du J, Yano N, et al. Sodium Butyrate Protects -Against High Fat Diet-Induced Cardiac Dysfunction and Metabolic Disorders in Type II Diabetic Mice. *J Cell Biochem*. 2017;118(8):2395-2408. doi:10.1002/jcb.25902
324. Kang S-H, Seok YM, Song M -j., Lee H-A, Kurz T, Kim I. Histone Deacetylase Inhibition Attenuates Cardiac Hypertrophy and Fibrosis through Acetylation of Mineralocorticoid Receptor in Spontaneously Hypertensive Rats. *Mol Pharmacol*. 2015;87(5):782-791. doi:10.1124/mol.114.096974
325. Yoshida M, Ono H, Mori Y, Chuda Y, Mori M. Oxygenation of bisphenol A to quinones by polyphenol oxidase in vegetables. *J Agric Food Chem*. 2002;50(15):4377-4381. doi:10.1021/jf020206e
326. Wendlandt AE, Stahl SS. Quinone-catalyzed selective oxidation of organic molecules. *Angew Chemie - Int Ed*. 2015;54(49):14638-14658. doi:10.1002/anie.201505017
327. Tu Y, Sun W, Wan YG, et al. Dahuang Fuzi Decoction ameliorates tubular epithelial apoptosis and renal damage via inhibiting TGF-β1-JNK signaling pathway activation in vivo. *J Ethnopharmacol*. 2014;156:115-124. doi:10.1016/j.jep.2014.08.035
328. Dong X, Fu J, Yin X, et al. Emodin: A Review of its Pharmacology, Toxicity and Pharmacokinetics. *Phyther Res*. 2016;30(8):1207-1218. doi:10.1002/ptr.5631
329. Mueller SO, Schmitt M, Dekant W, et al. Occurrence of emodin, chrysophanol and physcion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. *Food Chem Toxicol*. 1999;37(5):481-491. doi:10.1016/S0278-6915(99)00027-7
330. Du Y, Ko KM. Effects of pharmacological preconditioning by emodin/oleanolic acid treatment and/or ischemic preconditioning on mitochondrial antioxidant components as well as the susceptibility to ischemia-reperfusion injury in rat hearts. *Mol Cell Biochem*. 2006;288(1-2):135-142. doi:10.1007/s11010-006-9129-3
331. Du Y, Ko KM. Effects of emodin treatment on mitochondrial ATP generation capacity and antioxidant components as well as susceptibility to ischemia-reperfusion injury in rat hearts: Single versus multiple doses and gender difference. *Life Sci*. 2005;77(22):2770-2782. doi:10.1016/j.lfs.2005.03.027
332. Wu Y, Tu X, Lin G, et al. Emodin-mediated protection from acute myocardial infarction via inhibition of inflammation and apoptosis in local ischemic myocardium. *Life Sci*. 2007;81(17-18):1332-1338. doi:10.1016/j.lfs.2007.08.040
333. Yen GC, Duh P Der, Chuang DY. Antioxidant activity of anthraquinones and anthrone. *Food Chem*. 2000;70(4):437-441. doi:10.1016/S0308-8146(00)00108-4
334. Cha TL, Chuang MJ, Tang SH, et al. Emodin modulates epigenetic modifications and suppresses bladder carcinoma cell growth. *Mol Carcinog*. 2015;54(3):167-177. doi:10.1002/mc.22084

335. Zhang H, Chen L, Bu HQ, et al. Effects of emodin on the demethylation of tumor-suppressor genes in pancreatic cancer PANC-1 cells. *Oncol Rep*. 2015;33(6):3015-3023. doi:10.3892/or.2015.3914
336. Pan FP, Zhou HK, Bu HQ, et al. Emodin enhances the demethylation by 5-Aza-CdR of pancreatic cancer cell tumor-suppressor genes P16, RASSF1A and ppENK. *Oncol Rep*. 2016;35(4):1941-1949. doi:10.3892/or.2016.4554
337. Iwanowycz S, Wang J, Altomare D, Hui Y, Fan D. Emodin bidirectionally modulates macrophage polarization and epigenetically regulates macrophage memory. *J Biol Chem*. 2016;291(22):11491-11503. doi:10.1074/jbc.M115.702092
338. Zhou D, Huang C, Lin Z, et al. Macrophage polarization and function with emphasis on the evolving roles of coordinated regulation of cellular signaling pathways. *Cell Signal*. 2014;26(2):192-197. doi:10.1016/j.cellsig.2013.11.004
339. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature*. 2013;496(7446):445-455. doi:10.1038/nature12034
340. Jin X, Liu MY, Zhang DF, et al. Natural products as a potential modulator of microglial polarization in neurodegenerative diseases. *Pharmacol Res*. 2019;145:104253. doi:10.1016/j.phrs.2019.104253
341. Li Y, Xu QQ, Shan CS, Shi YH, Wang Y, Zheng GQ. Combined use of emodin and ginsenoside Rb1 exerts synergistic neuroprotection in cerebral ischemia/reperfusion rats. *Front Pharmacol*. 2018;9(AUG):943. doi:10.3389/fphar.2018.00943
342. Ma Y, Xia X, Cheng J min, et al. Emodin Inhibits Inducible Nitric Oxide Synthase in a Rat Model of Craniocerebral Explosive Injury. *Neurochem Res*. 2014;39(9):1809-1816. doi:10.1007/s11064-014-1395-y
343. Liu T, Jin H, Sun QR, Xu JH, Hu HT. Neuroprotective effects of emodin in rat cortical neurons against β-amyloid-induced neurotoxicity. *Brain Res*. 2010;1347:149-160. doi:10.1016/j.brainres.2010.05.079
344. Ye B, Chen X, Dai S, et al. Emodin alleviates myocardial ischemia/reperfusion injury by inhibiting gasdermin D-mediated pyroptosis in cardiomyocytes. *Drug Des Devel Ther*. 2019;Volume 13:975-990. doi:10.2147/dddt.s195412
345. Swanson K V., Deng M, Ting JP-Y. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol*. 2019;19(8):477-489. doi:10.1038/s41577-019-0165-0
346. Pal T, Jana NR, Das PK. Spectrophotometric determination of magnesium(II) with emodin (1,3,8-trihydroxy-6-methylanthraquinone). *Analyst*. 1992;117(4):791-793. doi:10.1039/AN9921700791
347. Ooi JYY, Tuano NK, Rafehi H, et al. HDAC inhibition attenuates cardiac hypertrophy by acetylation and deacetylation of target genes. *Epigenetics*. 2015;10(5):418-430. doi:10.1080/15592294.2015.1024406
348. Kaneda R, Ueno S, Yamashita Y, et al. Genome-wide screening for target regions of histone deacetylases in cardiomyocytes. *Circ Res*. 2005;97(3):210-218. doi:10.1161/01.RES.0000176028.18423.07
349. Tan D, Wu J, Jing Z, Duan X, Cui Y, Liu S. Efficacy and safety of ginkgo injections in the treatment of angina pectoris caused by coronary heart disease in

China: a network Meta- analysis and systematic review. *J Tradit Chinese Med*. 2019;39(3):285-296.
350. García-Conesa MT, Chambers K, Combet E, et al. Meta-analysis of the effects of foods and derived products containing ellagitannins and anthocyanins on cardiometabolic biomarkers: Analysis of factors influencing variability of the individual responses. *Int J Mol Sci*. 2018;19(3):694. doi:10.3390/ijms19030694
351. Viguiliouk E, Glenn AJ, Nishi SK, et al. Associations between Dietary Pulses Alone or with Other Legumes and Cardiometabolic Disease Outcomes: An Umbrella Review and Updated Systematic Review and Meta-analysis of Prospective Cohort Studies. *Adv Nutr*. 2019;10(Suppl_4):S308-S319. doi:10.1093/advances/nmz113
352. Kwok CS, Gulati M, Michos ED, et al. Dietary components and risk of cardiovascular disease and all-cause mortality: a review of evidence from meta-analyses. *Eur J Prev Cardiol*. 2019;26(13):1415-1429. doi:10.1177/2047487319843667
353. Al-Shafei AIM, El-Gendy OAA. Regular consumption of green tea improves pulse pressure and induces regression of left ventricular hypertrophy in hypertensive patients. *Physiol Rep*. 2019;7(6). doi:10.14814/phy2.14030
354. Evans L, Ferguson B. Food Bioactive HDAC Inhibitors in the Epigenetic Regulation of Heart Failure. *Nutrients*. 2018;10(8):1120. doi:10.3390/nu10081120
355. Nienaber JJ, Tachibana H, Naga Prasad S V., et al. Inhibition of receptor-localized PI3K preserves cardiac β-adrenergic receptor function and ameliorates pressure overload heart failure. *J Clin Invest*. 2003;112(7):1067-79. doi:10.1172/JCI18213
356. Devereux RB, Wachtell K, Gerdts E, et al. Prognostic significance of left ventricular mass change during treatment of hypertension. *J Am Med Assoc*. 2004;292(19):2350-6. doi:10.1001/jama.292.19.2350
357. Evans LW, Bender A, Burnett L, et al. Emodin and emodin-rich rhubarb inhibits histone deacetylase (HDAC) activity and cardiac myocyte hypertrophy. *J Nutr Biochem*. 2020;79:108339. doi:10.1016/j.jnutbio.2019.108339
358. Breisch EA, White FC, Nimmo LE, McKirnan MD, Bloor CM. Exercise-induced cardiac hypertrophy: A correlation of blood flow and microvasculature. *J Appl Physiol*. 1986;60(4):1259-67. doi:10.1152/jappl.1986.60.4.1259
359. Lam CSP, Lyass A, Kraigher-Krainer E, et al. Cardiac dysfunction and noncardiac dysfunction as precursors of heart failure with reduced and preserved ejection fraction in the community. *Circulation*. 2011;124(1):24-30. doi:10.1161/CIRCULATIONAHA.110.979203
360. Dorn GW, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest*. 2005;115(3):527-37. doi:10.1172/JCI24178
361. Chiloeches A, Paterson HF, Marais R, Clerk A, Marshall CJ, Sugden PH. Regulation of Ras·GTP loading and Ras-Raf association in neonatal rat ventricular myocytes by G protein-coupled receptor agonists and phorbol ester: Activation of the extracellular signal-regulated kinase cascade by phorbol ester is mediated by Ras. *J Biol Chem*. 1999;274(28):19762-70. doi:10.1074/jbc.274.28.19762
362. Markou T, Cieslak D, Gaitanaki C, Lazou A. Differential roles of MAPKs and MSK1 signalling pathways in the regulation of c-Jun during phenylephrine-

induced cardiac myocyte hypertrophy. *Mol Cell Biochem*. 2009;322(1-2):103-12. doi:10.1007/s11010-008-9945-8
363. Filtz TM, Grubb DR, McLeod-Dryden TJ, Luo J, Woodcock EA. Gq-initiated cardiomyocyte hypertrophy is mediated by phospholipase Cβ1b. *FASEB J*. 2009;23(10):3564-70. doi:10.1096/fj.09-133983
364. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. *Lancet*. 2010;375(9709):132-140. doi:10.1016/S0140-6736(09)61717-7
365. Ross R. Atherosclerosis — An Inflammatory Disease. Epstein FH, ed. *N Engl J Med*. 1999;340(2):115-126. doi:10.1056/NEJM199901143400207
366. Hinglais N, Heudes D, Nicoletti A, et al. Colocalization of myocardial fibrosis and inflammatory cells in rats. *Lab Investig*. 1994;70(2):286-94.
367. Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, Lin A. Activation of NF-κB is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. *Proc Natl Acad Sci U S A*. 2001;98(12):6668-73. doi:10.1073/pnas.111155798
368. Zhao L, Cheng G, Jin R, et al. Deletion of Interleukin-6 Attenuates Pressure Overload-Induced Left Ventricular Hypertrophy and Dysfunction. *Circ Res*. 2016;118(12):1918-1929. doi:10.1161/CIRCRESAHA.116.308688
369. Kumar S, Wang G, Zheng N, et al. HIMF (Hypoxia-Induced Mitogenic Factor)-IL (Interleukin)-6 signaling mediates cardiomyocyte-fibroblast crosstalk to promote cardiac hypertrophy and fibrosis. *Hypertension*. 2019;73(5):1058-1070. doi:10.1161/HYPERTENSIONAHA.118.12267
370. Lai NC, Gao MH, Tang E, et al. Pressure overload-induced cardiac remodeling and dysfunction in the absence of interleukin 6 in mice. *Lab Investig*. 2012;92(11):1518-26. doi:10.1038/labinvest.2012.97
371. Fischer TA, Ludwig S, Flory E, et al. Activation of cardiac c-Jun NH2-terminal kinases and p38-mitogen-activated protein kinases with abrupt changes in hemodynamic load. *Hypertension*. 2001;37(5):1222-1228. doi:10.1161/01.HYP.37.5.1222
372. Burack WR, Sturgill TW. The activating dual phosphorylation of MAPK by MEK is nonprocessive. *Biochemistry*. 1997;36(20):5929-33. doi:10.1021/bi970535d
373. Ruppert C, Deiss K, Herrmann S, et al. Interference with ERKThr188 phosphorylation impairs pathological but not physiological cardiac hypertrophy. *Proc Natl Acad Sci*. 2013;110(18):7440-7445. doi:10.1073/pnas.1221999110
374. Slone S, Anthony SR, Wu X, et al. Activation of HuR downstream of p38 MAPK promotes cardiomyocyte hypertrophy. *Cell Signal*. 2016;28(11):1735-41. doi:10.1016/j.cellsig.2016.08.005
375. Zechner D, Thuerauf DJ, Hanford DS, McDonough PM, Glembotski CC. A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. *J Cell Biol*. 1997;139(1):115-27. doi:10.1083/jcb.139.1.115
376. Liao P, Georgakopoulos D, Kovacs A, et al. The in vivo role of p38 MAP kinases in cardiac remodeling and restrictive cardiomyopathy. *Proc Natl Acad Sci U S A*.

2001;98(21):12283-12288. doi:10.1073/pnas.211086598
377. Wang Y, Su B, Sah VP, Brown JH, Han J, Chien KR. Cardiac hypertrophy induced by Mitogen-activated Protein Kinase Kinase 7, a specific activator for c-Jun NH2-terminal kinase in ventricular muscle cells. *J Biol Chem*. 1998;273(10):5423-6. doi:10.1074/jbc.273.10.5423
378. Liu W, Zi M, Jin J, et al. Cardiac-specific deletion of Mkk4 reveals its role in pathological hypertrophic remodeling but not in physiological cardiac growth. *Circ Res*. 2009;104(7):905-14. doi:10.1161/CIRCRESAHA.108.188292
379. Monovich L, Vega RB, Meredith E, et al. A novel kinase inhibitor establishes a predominant role for protein kinase D as a cardiac class IIa histone deacetylase kinase. *FEBS Lett*. 2010;584(3):631-7. doi:10.1016/j.febslet.2009.12.014
380. Harrison BC, Kim M-S, van Rooij E, et al. Regulation of Cardiac Stress Signaling by Protein Kinase D1. *Mol Cell Biol*. 2006;26(10):3875-88. doi:10.1128/mcb.26.10.3875-3888.2006
381. Wu J, Nakashima S, Nakamura S, Matsuda H. Effects of Sanoshashinto on left ventricular hypertrophy and gut microbiota in spontaneously hypertensive rats. *J Nat Med*. 2020;74(2):482-486. doi:10.1007/s11418-020-01387-9
382. Reid BG, Stratton MS, Bowers S, et al. Discovery of novel small molecule inhibitors of cardiac hypertrophy using high throughput, high content imaging. *J Mol Cell Cardiol*. 2016;97:106-113. doi:10.1016/j.yjmcc.2016.04.015
383. Lim SS, Vos T, Flaxman AD, et al. 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*. 2012;380(9859):2224-2260. doi:10.1016/S0140-6736(12)61766-8
384. Butler T. Dietary management of heart failure: room for improvement? *Br J Nutr*. 2016;115(7):1202-1217. doi:10.1017/S000711451500553X
385. Islam MA, Alam F, Solayman M, Khalil MI, Kamal MA, Gan SH. Dietary Phytochemicals: Natural Swords Combating Inflammation and Oxidation-Mediated Degenerative Diseases. *Oxid Med Cell Longev*. 2016;2016:1-25. doi:10.1155/2016/5137431
386. Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. *J Nutr Biochem*. 2007;18(9):567-579. doi:10.1016/j.jnutbio.2006.10.007
387. Perez-Gregorio R, Simal-Gandara J. A Critical Review of Bioactive Food Components, and of their Functional Mechanisms, Biological Effects and Health Outcomes. *Curr Pharm Des*. 2017;23(19):2731-2741. doi:10.2174/1381612823666170317122913
388. Yang X-JJ, Seto E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene*. 2007;26(37):5310-5318. doi:10.1038/sj.onc.1210599
389. Li Y, Liu H, Ji X, Li J. Optimized separation of pharmacologically active anthraquinones in Rhubarb by capillary electrochromatography. In: *Electrophoresis*. Vol 21. ; 2000:3109-3115. doi:10.1002/1522-2683(20000901)21:15<3109::AID-ELPS3109>3.0.CO;2-Q
390. Lemon DD, Horn TR, Cavasin MA, et al. Cardiac HDAC6 Catalytic Activity is

Induced in Response to Chronic Hypertension. *J Mol Cell Cardiol*. 2012;51(1):41-50. doi:10.1016/j.yjmcc.2011.04.005.Cardiac
391. Heltweg B, Dequiedt F, Marshall BL, et al. Subtype selective substrates for histone deacetylases. *J Med Chem*. 2004;47(21):5235-5243. doi:10.1021/jm0497592
392. Palmer JN, Hartogensis WE, Patten M, Fortuin FD, Long CS. Interleukin-1β induces cardiac myocyte growth but inhibits cardiac fibroblast proliferation in culture. *J Clin Invest*. 1995;95(6):2555-2564. doi:10.1172/JCI117956
393. Deluca DS, Levin JZ, Sivachenko A, et al. RNA-SeQC: RNA-seq metrics for quality control and process optimization. *Bioinformatics*. 2012;28(11):1530-2. doi:10.1093/bioinformatics/bts196
394. Patro R, Mount SM, Kingsford C. Sailfish enables alignment-free isoform quantification from RNA-seq reads using lightweight algorithms. *Nat Biotechnol*. 2014;32(5):462-4. doi:10.1038/nbt.2862
395. Cunningham F, Amode MR, Barrell D, et al. Ensembl 2015. *Nucleic Acids Res*. 2015;43(D1):D662-669. doi:10.1093/nar/gku1010
396. Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2009;26(1):139-40. doi:10.1093/bioinformatics/btp616
397. Blakeslee WW, Lin YH, Stratton MS, et al. Class I HDACs control a JIP1-dependent pathway for kinesin-microtubule binding in cardiomyocytes. *J Mol Cell Cardiol*. 2017;112:74-82. doi:10.1016/j.yjmcc.2017.09.002
398. Yoshimura M, Yasue H, Okumura K, et al. Different secretion patterns of atrial natriuretic peptide and brain natriuretic peptide in patients with congestive heart failure. *Circulation*. 1993;87(2):464-469. doi:10.1161/01.CIR.87.2.464
399. Brown LA, Nunez DJR, Wilkins MR. Differential regulation of natriuretic peptide receptor messenger RNAs during the development of cardiac hypertrophy in the rat. *J Clin Invest*. 1993;92(6):2702-2712. doi:10.1172/JCI116887
400. Nakagawa O, Itoh H, Harada M, Komatsu Y, Yoshimasa T, Nakao K. Gene regulation of brain natriuretic peptide in cardiocyte hypertrophy by alpha1-adrenergic stimulation. *Clin Exp Pharmacol Physiol Suppl*. 1995;22(1):S183-5.
401. Ito K, Adcock IM. Histone Acetylation and Histone Deacetylation. *Mol Biotechnol*. 2003;20(1):099-106. doi:10.1385/mb:20:1:099
402. Gallo P, Latronico MVG, Gallo P, et al. Inhibition of class I histone deacetylase with an apicidin derivative prevents cardiac hypertrophy and failure. *Cardiovasc Res*. 2008;80(3):416-424. doi:10.1093/cvr/cvn215
403. Zhang X, Qin Q, Dai H, Cai S, Zhou C, Guan J. Emodin protects h9c2 cells from hypoxia-induced injury by up-regulating mir-138 expression. *Brazilian J Med Biol Res*. 2019;52(3):e7994. doi:10.1590/1414-431x20187994
404. Xu K, Zhou T, Huang Y, et al. Anthraquinone emodin inhibits tumor necrosis factor alpha-induced calcification of human aortic valve interstitial cells via the NF-κB pathway. *Front Pharmacol*. 2018;9(NOV):1328. doi:10.3389/fphar.2018.01328
405. Hall G, Hasday JD, Rogers TB. Regulating the regulator: NF-κB signaling in heart. *J Mol Cell Cardiol*. 2006;41(4):580-591. doi:10.1016/j.yjmcc.2006.07.006
406. Wang Y. Mitogen-activated protein kinases in heart development and diseases.

*Circulation*. 2007;116(12):1413-1423. doi:10.1161/CIRCULATIONAHA.106.679589
407. Lorenz K, Schmitt JP, Schmitteckert EM, Lohse MJ. A new type of ERK1/2 autophosphorylation causes cardiac hypertrophy. *Nat Med*. 2009;15(1):75-83. doi:10.1038/nm.1893
408. Liu W, Feng Q, Li Y, Ye L, Hu M, Liu Z. Coupling of UDP-glucuronosyltransferases and multidrug resistance-associated proteins is responsible for the intestinal disposition and poor bioavailability of emodin. *Toxicol Appl Pharmacol*. 2012;265(3):316-324. doi:10.1016/j.taap.2012.08.032
409. Wang S, Chen T, Chen R, Hu Y, Chen M, Wang Y. Emodin loaded solid lipid nanoparticles: Preparation, characterization and antitumor activity studies. *Int J Pharm*. 2012;430(1-2):238-246. doi:10.1016/j.ijpharm.2012.03.027
410. McKinsey TA. Therapeutic Potential for HDAC Inhibitors in the Heart. *Annu Rev Pharmacol Toxicol*. 2012;52(1):303-319. doi:10.1146/annurev-pharmtox-010611-134712
411. McKinsey TA, Olson EN. Toward transcriptional therapies for the failing heart: Chemical screens to modulate genes. *J Clin Invest*. 2005;115(3):538-546. doi:10.1172/JCI24144
412. Xie M, Kong Y, Tan W, et al. Histone deacetylase inhibition blunts ischemia/reperfusion injury by inducing cardiomyocyte autophagy. *Circulation*. 2014;129(10):1139-1151. doi:10.1161/CIRCULATIONAHA.113.002416
413. Afshin A, Sur PJ, Fay KA, et al. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2019;393(10184):1958-1972. doi:10.1016/S0140-6736(19)30041-8
414. Aune D, Giovannucci E, Boffetta P, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-A systematic review and dose-response meta-analysis of prospective studies. *Int J Epidemiol*. 2017;46(3):1029-1056. doi:10.1093/ije/dyw319
415. Pounis G, Costanzo S, Di Giuseppe R, et al. Consumption of healthy foods at different content of antioxidant vitamins and phytochemicals and metabolic risk factors for cardiovascular disease in men and women of the Moli-sani study. *Eur J Clin Nutr*. 2013;67(2):207-13. doi:10.1038/ejcn.2012.201
416. Tripp ML, Dahlberg CJ, Eliason S, et al. A Low-Glycemic, Mediterranean Diet and Lifestyle Modification Program with Targeted Nutraceuticals Reduces Body Weight, Improves Cardiometabolic Variables and Longevity Biomarkers in Overweight Subjects: A 13-Week Observational Trial. *J Med Food*. 2019;22(5):479-489. doi:10.1089/jmf.2018.0063
417. Yang T, Santisteban MM, Rodriguez V, et al. Gut Dysbiosis is Linked to Hypertension. *Hypertension*. 2015;65(6):1331-40. doi:10.1161/HYPERTENSIONAHA.115.05315
418. Galla S, Chakraborty S, Cheng X, et al. Disparate effects of antibiotics on hypertension. *Physiol Genomics*. 2018;50(10):837-845. doi:10.1152/physiolgenomics.00073.2018
419. Huang R, Wang K, Hu J. Effect of probiotics on depression: A systematic review and meta-analysis of randomized controlled trials. *Nutrients*. 2016;8(8):483.

doi:10.3390/nu8080483
420. Komici K, Conti V, Davinelli S, et al. Cardioprotective Effects of Dietary Phytochemicals on Oxidative Stress in Heart Failure by a Sex-Gender-Oriented Point of View. *Oxid Med Cell Longev*. 2020;2020:2176728. doi:10.1155/2020/2176728
421. Wu Z, Chen Q, Ke D, Li G, Deng W. Emodin protects against diabetic cardiomyopathy by regulating the AKT/GSK-3β Signaling pathway in the Rat Model. *Molecules*. 2014;19(9):14782-14793. doi:10.3390/molecules190914782
422. Gao J, Zhang K, Wang Y, et al. A machine learning-driven study indicates emodin improves cardiac hypertrophy by modulation of mitochondrial SIRT3 signaling. *Pharmacol Res*. 2020;155:104739. doi:10.1016/j.phrs.2020.104739
423. Zeng YQ, Dai Z, Lu F, et al. Emodin via colonic irrigation modulates gut microbiota and reduces uremic toxins in rats with chronic kidney disease. *Oncotarget*. 2016;7(14):17468-78. doi:10.18632/oncotarget.8160
424. Evans LW, Athukorala M, Martinez-Guryn K, Ferguson BS. The role of histone acetylation and the microbiome in phytochemical efficacy for cardiovascular diseases. *Int J Mol Sci*. 2020;21(11):4006. doi:10.3390/ijms21114006
425. Plovier H, Everard A, Druart C, et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med*. 2017;23(1):107-113. doi:10.1038/nm.4236
426. Depommier C, Everard A, Druart C, et al. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med*. 2019;25(7):1096-1103. doi:10.1038/s41591-019-0495-2
427. De Meij TGJ, Budding AE, De Groot EFJ, et al. Composition and stability of intestinal microbiota of healthy children within a Dutch population. *FASEB J*. 2016;30(4):1512-22. doi:10.1096/fj.15-278622
428. Koshman YE, Patel N, Chu M, et al. Regulation of connective tissue growth factor gene expression and fibrosis in human heart failure. *J Card Fail*. 2013;19(4):283-94. doi:10.1016/j.cardfail.2013.01.013
429. Dao MC, Everard A, Aron-Wisnewsky J, et al. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: Relationship with gut microbiome richness and ecology. *Gut*. 2016;65(3):426-36. doi:10.1136/gutjnl-2014-308778
430. Everard A, Belzer C, Geurts L, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A*. 2013;110(22):9066-9071. doi:10.1073/pnas.1219451110
431. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004;109(21 Suppl 1):II2-10. doi:10.1161/01.cir.0000129535.04194.38
432. Li J, Lin S, Vanhoutte PM, Woo CW, Xu A. Akkermansia muciniphila protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in Apoe-/- Mice. *Circulation*. 2016;133(24):2434-46. doi:10.1161/CIRCULATIONAHA.115.019645
433. Régnier M, Rastelli M, Morissette A, et al. Rhubarb Supplementation Prevents

Diet-Induced Obesity and Diabetes in Association with Increased Akkermansia muciniphila in Mice. *Nutrients*. 2020;12(10):2932. doi:10.3390/nu12102932
434. Tamanai-Shacoori Z, Smida I, Bousarghin L, et al. Roseburia spp.: A marker of health? *Future Microbiol*. 2017;12(2):157-170. doi:10.2217/fmb-2016-0130
435. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett*. 2009;294(1):1-8. doi:10.1111/j.1574-6968.2009.01514.x
436. Kasahara K, Krautkramer KA, Org E, et al. Interactions between Roseburia intestinalis and diet modulate atherogenesis in a murine model. *Nat Microbiol*. 2018;3(12):1461-1471. doi:10.1038/s41564-018-0272-x
437. Ravussin Y, Koren O, Spor A, et al. Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity*. 2012;20(4):738-47. doi:10.1038/oby.2011.111
438. Qiao Y, Sun J, Xie Z, Shi Y, Le G. Propensity to high-fat diet-induced obesity in mice is associated with the indigenous opportunistic bacteria on the interior of Peyer's patches. *J Clin Biochem Nutr*. 2014;55(2):120-8. doi:10.3164/jcbn.14-38
439. Sudun, Liu S, Xiao C, et al. Probiotic strains improve high-fat diet-induced hypercholesterolemia through modulating gut microbiota in ways different from atorvastatin. *Food Funct*. 2019;10(9):6098-6109. doi:10.1039/c9fo00444k
440. Martínez I, Perdicaro DJ, Brown AW, et al. Diet-induced alterations of host cholesterol metabolism are likely to affect the gut microbiota composition in hamsters. *Appl Environ Microbiol*. 2013;79(2):516-24. doi:10.1128/AEM.03046-12
441. Yang T, Aquino V, Lobaton GO, et al. Sustained captopril-induced reduction in blood pressure is associated with alterations in Gut-Brain axis in the spontaneously hypertensive rat. *J Am Heart Assoc*. 2019;8(4):e010721. doi:10.1161/JAHA.118.010721
442. Liu C, Cheng L, Ji L, et al. Intestinal microbiota dysbiosis play a role in pathogenesis of patients with primary immune thrombocytopenia. *Thromb Res*. 2020;190:11-19. doi:10.1016/j.thromres.2020.03.012
443. Castonguay-Paradis S, Lacroix S, Rochefort G, et al. Dietary fatty acid intake and gut microbiota determine circulating endocannabinoidome signaling beyond the effect of body fat. *Sci Rep*. 2020;10(1):15975. doi:10.1038/s41598-020-72861-3
444. Liu D, Huang J, Luo Y, et al. Fuzhuan Brick Tea Attenuates High-Fat Diet-Induced Obesity and Associated Metabolic Disorders by Shaping Gut Microbiota. *J Agric Food Chem*. 2019;67(49):13589-13604. doi:10.1021/acs.jafc.9b05833
445. Sakurai T, Sakurai A, Chen Y, et al. Dietary α-cyclodextrin reduces atherosclerosis and modifies gut flora in apolipoprotein E-deficient mice. *Mol Nutr Food Res*. 2017;61(8):10.1002/mnfr.201600804. doi:10.1002/mnfr.201600804
446. Kim DSHL, Koonchanok NM, Geahlen RL, Ashendel CL, Chang CJ. Structure activity relationship study of emodin derivatives based on the protein-tyrosine kinase and protein kinase C inhibitory activities and cytotoxicity. *Nat Prod Lett*. 1997;10(3):173-180. doi:10.1080/10575639708041191
447. Iwata M, Maturana A, Hoshijima M, et al. PKCε-PKD1 signaling complex at Z-discs plays a pivotal role in the cardiac hypertrophy induced by G-protein coupling

receptor agonists. *Biochem Biophys Res Commun*. 2005;327(4):1105-1113. doi:10.1016/j.bbrc.2004.12.128