ROLE OF TART CHERRY
IN THE PREVENTION OF HYPERTENSION AND
THE MODULATION OF INFLAMMATORY SIGNALING

by

Safiyah Mansoori

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Sciences

Fall 2022

ROLE OF TART CHERRY

IN THE PREVENTION OF HYPERTENSION AND

THE MODULATION OF INFLAMMATORY SIGNALING

by

Safiyah Mansoori

Approved: _____

Esther E. Biswas- Fiss, Ph.D., MB (ASCP)^{CM}
Chair of the Department of Medical and Molecular Sciences

Approved: _____

Kathleen Matt, Ph.D.
Dean of the College of Health Sciences

Approved: _____

Louis F. Rossi, Ph.D.
Vice Provost for Graduate and Professional Education and
Dean of the Graduate College

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Sheau Ching Chai, Ph.D., R.D.
Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Subhasis Biswas, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Esther Biswas- Fiss, Ph.D., MB (ASCP)^{CM}
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Sigrid Langhans, Ph.D.
Member of dissertation committee

ACKNOWLEDGMENTS

I am incredibly grateful to everyone who has supported me both personally and professionally throughout my Ph.D. experience. Pursuing this degree has been life changing and I am thankful for the opportunity to gain knowledge and contribute in a meaningful way.

I am thankful to my advisor Dr. Sheau Ching Chai for supporting me and giving me the opportunity to learn and grow inside and outside of the laboratory. You have provided me with valuable advice and taught me lessons that I will carry with me long after the Ph.D. Thank you for encouraging me to learn new research skills and confidently take on new challenges. I would also like to thank my committee members Dr. Esther Biswas, Dr. Subhasis Biswas, and Dr. Sigrid Langhans. Dr. Esther and Subhasis Biswas, thank you for providing invaluable guidance and support throughout my dissertation project. Thank you for allowing me to use your laboratory space for my cell culture work. I appreciate the time you took to look over my results and provide detailed feedback. I also want to thank Dr. Sigrid Langhans for providing valuable feedback, comments, and great ideas for improving my research project. I am grateful to all of my committee members for taking the time out to serve on my committee and for all of their support and advice every step of the project. I thank Dr. Richard Suminski for his expertise and help running and interpreting the regression analyses.

I am grateful to the past and present members of our laboratory, especially Bharathi Ramesh, Saiful Singar, Anita Dini, Breanna Nicholson, and Pengjun (Juno) Zhang for their support and friendship, and for making the Ph.D. more enjoyable. I would like to sincerely thank Jazzlyn Jones for teaching me how to work with cell

culture and perform western blots and to Albtool Alturkestani for her advice and help in optimizing my western blots. You two are incredible and your help has been invaluable to me. To my friend Iflah Laraib, thank you for your honest advice and unwavering support throughout the years. I will always remember the great times we had as Ph.D. students and I'm looking forward to new memories as graduates.

I dedicate this dissertation to my parents, Dr. Bashir and Samra Mansoori. My parents have always encouraged me to take on new challenges, to be confident and to be content with every situation in life. I am forever grateful to you for always believing in me and for being my biggest supporters. Your never ending love, encouragement, support, and prayers kept me going when things got tough. Thank you for teaching me to stay strong and trust God, and to never give up on my goals. I could not have come this far without you. To my sister Hafsah, thank you for all of your love and encouragement. I truly appreciate you showing up to support me in everything I do. I would also like to thank my brother and sister in law for all of their advice and support. To my friends, thank you for the love and for giving me something to look forward to on the weekends.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Advanced glycation end products (AGE)

Alzheimer's disease (AD)

American Heart Association (AHA)

Arachidonic acid (AA)

Block Food Frequency Questionnaire (FFQ)

Blood pressure (BP)

Body mass index (BMI)

Cardiovascular disease (CVD)

Chlorogenic acid (CHL)

Chronic systemic inflammation (CSI)

Coronary artery disease (CAD)

C-reactive protein (CRP)

Cyanidin-3-glucoside (C3G)

Cyanidin-3-rutinoside (C3R)

Diastolic blood pressure (DBP)

Dietary Approaches to Stop Hypertension (DASH)

Dietary Guidelines for Americans (DGA)

Dioxide (O2)

Extracellular-signal regulated kinases (ERK)

Fibroblast-like synoviocytes (FLS)

High fructose corn syrup (HFCS)

High-sensitivity c-reactive protein (hsCRP)

Histone acetyl transferase (HAT)

Human umbilical vein endothelial cells (HUVEC)

Hydrogen peroxide (H2O2)

Hydroxide (OH)

Inhibitor of the κB molecule (IκB)

Interferon-γ-inducible protein 10 (IP-10)

Interleukin 1 beta (IL-1β)

Interleukin 6 (IL-6)

Intestinal epithelial cell (IEC)

Ischemia-reperfusion (I/R)

Jun amino-terminal kinases (JNK)

Lipid hydroperoxide (LOOH)

Lipopolysaccharide (LPS)

Low-density lipoprotein cholesterol (LDL-C)

Macrophage inflammatory protein 2 (MIP-2)

Malondialdehyde (MDA)

Mean arterial pressure (MAP)

Messenger ribonucleic acid (mRNA)

Mitogen-activated protein kinase (MAPK)

National Health and Nutrition Examination Survey (NHANES)

Nicotinamide adenine dinucleotide phosphate (NADPH)

Nicotinamide adenine dinucleotide phosphate oxidase (NOX)

Nitric oxide (NO)

Nonsteroidal anti-inflammatory drug (NSAID)

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway

Osteoarthritis (OA)

Ovariectomized (Ovx)

Peroxisome proliferator-activated receptors (PPARs)

Physical Activity Scale for the Elderly (PASE)

Protocatechuic acid (PCA)

Reactive oxygen species (ROS)

Receptor for AGE (RAGE)

Secreted embryonic alkaline phosphatase (SEAP)

Superoxide dismutase (SOD)

Systolic blood pressure (SBP)

Tart cherries (TC)

Tart cherry (TC)

Thromboxane A2 (txa2)

Thymic stromal lymphopoietin (TSLP)

Tissue plasminogen activator (tPA)

Trolox equivalent antioxidant capacity (TEAC)

Tumor necrosis factor-alpha (TNF-α)

United States (US)

Upper respiratory tract symptoms (URTS)

Vanillic acid (VA)

Vascular smooth muscle cell (VSMC)

ABSTRACT

Hypertension increases the risk for vascular damage, atherosclerosis, and subsequent cardiovascular disease (CVD) related morbidity and mortalities. The development and progression of several chronic conditions, including hypertension, are influenced by inflammation. Chronic low-grade inflammation and hypertension, both prevalent in the aging population, can be modulated by dietary and lifestyle choices. Our laboratory has previously shown that 12 weeks of tart cherry (TC) juice consumption can reduce systolic BP and markers of inflammation and oxidative stress in older adults. There are several bioactive compounds in TC and evidence suggests these compounds in isolation can influence inflammatory signaling pathways that contribute to the pathogenesis of hypertension.

To first understand the impact of diet on BP, we conducted a cross-sectional study on 128 adults aged 65–80 years. Multiple linear regressions were conducted to examine the influence of major dietary factors on systolic and diastolic BP. We also wanted to understand the role of TC in reducing BP. To study this, human coronary artery endothelial cells (HCAEC) were exposed to 0-500μg/mL of TC extracts in the presence or absence of Angiotensin-II (Ang-II), which is known to increase BP and inflammation. Western blots were used to examine the effects of TC and/or Ang-II

on the protein expression of nitric oxide synthases and inflammatory molecules associated with the NF-κB signaling pathway.

Results of the cross-sectional study showed a significant association between intake of added sugar and systolic and diastolic BP in females. Whole fruit consumption was associated with a reduction in diastolic BP in both males and females. The regression model predicted that for every 0.71 cup increase in whole fruit consumption, there would be a 2.8 mmHg reduction in diastolic BP.

In the absence of Ang-II, TC exposure did not influence eNOS expression. Expression of iNOS was reduced by TC at all doses in the absence of Ang-II. Levels of p65 were significantly reduced at 62.5 and 125μg/mL compared to the control. Phosphorylated p65 was upregulated at the 62.5 μg/mL dose and ICAM-1 was similar between groups. In the presence of Ang-II, the 62.5 Ang and 125 Ang exposures resulted in a 0.75 fold and 0.71 fold reduction in iNOS respectively. Ang-II did not significantly affect NOS or inflammatory markers compared to the control. This could be due to the metabolism of Ang-II or loss of Ang-II type 1 receptor in cell culture.

Our findings support increased fruit consumption for the reduction of BP in older adults. Additionally, TC exposure can reduce the expression of iNOS which is known to contribute to the development of hypertension.

Chapter 1

INTRODUCTION

1.1 Hypertension

Blood pressure (BP) is the force of blood against arterial walls which can vary throughout the day under normal circumstances[1]. A hypertensive state causes damage to the heart and blood vessels resulting in cardiovascular disease (CVD)[2] and can contribute to life threatening diseases including myocardial infarction (MI) and stroke[1]. About half of the U.S. adult population has hypertension, but less than 25% of them have it under control[1].

Inflammation plays a role in the pathogenesis of hypertension, and although inflammation can be a useful response for injury repair or fighting off an infection, chronic inflammation can be detrimental[3]. Certain factors including overactivity of the renin-angiotensin-aldosterone system (RAAS), inflammation, and oxidative stress can contribute to increased BP[3]. These factors are thought to cause protein modifications resulting in them being identified as non-self and thus activating the immune system[3].

1.2 Endothelial Dysfunction

Inflammation can contribute to the development of endothelial dysfunction, which could be a mechanism for its role in the development of hypertension[3]. Blood vessels are lined with cells that can respond to changes in factors necessary for BP control. An important molecule for vascular tone regulation is nitric oxide (NO) produced by endothelial nitric oxide synthase[3]. Nitric oxide synthase (NOS) has three isoforms including endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS) that catalyze the reaction of L-arginine with molecular oxygen to produce NO and L-citrulline[4]. Problems with the L-arginine and NO pathway is linked to hypertension[4]. L-arginine is used as the substrate with reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) and molecular oxygen as co-substrates, flavin adenine dinucleotide, tetrahydrobiopterin (BH_4,) and flavin mononucleotide, as co-factors by the NOS isoforms[5]. The heme in the amino terminal oxygenase domain receives electrons from NADPH which bind to BH_4, L-arginine and molecular oxygen[5]. The electrons reduce and activate O_2 to oxidize L-arginine and L-citrulline and NO at the heme site.

The eNOS isoform has an important role in BP regulation[4]. NO, when released from endothelial cells, increases 3',5'-cyclic-guanosine monophosphate production and activation of its associated protein kinase under vascular smooth muscle cells (VSMC) leading to vasodilation[4]. Endothelial dysfunction can produce

a state of reduced NO bioavailability[4]. In endothelial dysfunction, there's an imbalance of vasoconstrictors and dilators which can reduce endothelium-dependent vasodilation[3]. Inflammation can play a role in reducing NOS activity and stimulating oxidative stress which also contributes to hypertension[3]. As a defense mechanism against pathogens, the body produces reactive oxygen species (ROS) until the tissue is repaired or the pathogen is deactivated[3]. A chronic inflammatory state can result in an overproduction of ROS leading to an imbalance between the breakdown and production of ROS, termed oxidative stress[3]. Oxidative stress contributes to the development of endothelial dysfunction by reducing NO bioavailability and by producing peroxynitrite which can oxidize 4-BH_4. BH_4 is a cofactor of eNOS, and without it there can be eNOS uncoupling which promotes the production of superoxide by eNOS instead of NO[3,6].

Under different disease conditions, eNOS can have varying phosphorylation levels at different sites[4]. The phosphorylation of Thr495 reduces eNOS function and the phosphorylation of Ser633, 1177, and 615 can activate eNOS[4]. The dysfunction and uncoupling of eNOS can be influenced by an L-arginine deficiency, the S-glutathionylation of eNOS, and the acetylation of eNOS[4].

It is important to understand the mechanisms regulating NO signaling under diseased conditions to improve therapeutic options[4]. It's also important to understand molecular mechanisms influencing eNOS dysfunction and changes in

NO bioavailability to help develop novel therapies for hypertension[4]. A deficiency in NO by superoxide anions can lead to endothelial dysfunction and high BP[4]. Many enzymatic systems can produce reactive oxygen species (ROS) including uncoupled eNOS, cyclooxygenase (COX), and NADPH oxidase (NOX)[4]. Additionally, ROS produced by one source can activate the production of ROS from other sources by influencing other enzyme systems[4]. NO produced by iNOS could contribute to hypertension[7]. This is because iNOS can react with superoxide to form peroxynitrite contributing to endothelial dysfunction[7]. Arginase activity can also be increased by iNOS, which competes with eNOS for L-arginine resulting in reduced bioavailability of NO, and eNOS uncoupling[7]. Inhibiting iNOS has been found to reduce hypertension, improve vascular function and reduce oxidative stress[7].

1.3 NF-κB Signaling Pathway

Activation of the NF-κB signaling pathway can also influence inflammation and oxidative stress by inducing the activation of cytokines and adhesion molecules to regulate the immune response[8,9]. In hypertension that is induced by angiotensin-II (Ang-II), immune T-cells express higher levels of NADPH oxidase components that contribute to ROS production[3]. There are five members of the NF-κB family of transcription factors including Rel A/p65, Rel B, c-Rel, p50 and p52that usually

occur in an inactive homo or heterodimer form in the cytoplasm[10]. There is ample evidence for the effect of Ang-II on inflammation and the NF-κB signaling pathway[9,11]. NF-κB is a transcription factor that can activate gene expression of cytokines and chemokines as well as inflammatory cells **(Fig. 1)**[12,13]. NF-κB can be activated through the canonical and noncanonical pathways. The canonical pathway can become activated in response to cytokine ligand receptor stimulation which causes the phosphorylation and subsequent degradation of IκBα, resulting in the freeing of dimers such as p50/p65 and p50/c-Rel and activation of NF-κB signaling **(Fig. 2)**. Activation of NF-κB by Ang-II can increase expression of cellular adhesion molecules and chemokines[9].

Cell adhesion molecules are located on the surface of cells which allows them to bind to extracellular matrix proteins or each other[14]. There are four major superfamilies of CAMs and intercellular adhesion molecules (ICAM) belong to the immunoglobulin superfamily[14]. Adhesion molecules such as ICAM-1 are important NF-κB target genes in endothelial cells that help inflammatory cells adhere to the vascular wall[15]. Endothelial cells express low levels of ICAM-1[16], but the expression can be upregulated by inflammatory stimuli.[14] Expression of ICAM-1 can also be a marker of aging[17]. The activity of ICAM-1 is required for Ang-II induced hypertension and blocking ICAM-1 inhibits inflammation, ROS production, and vascular remodeling and dysfunction[14]

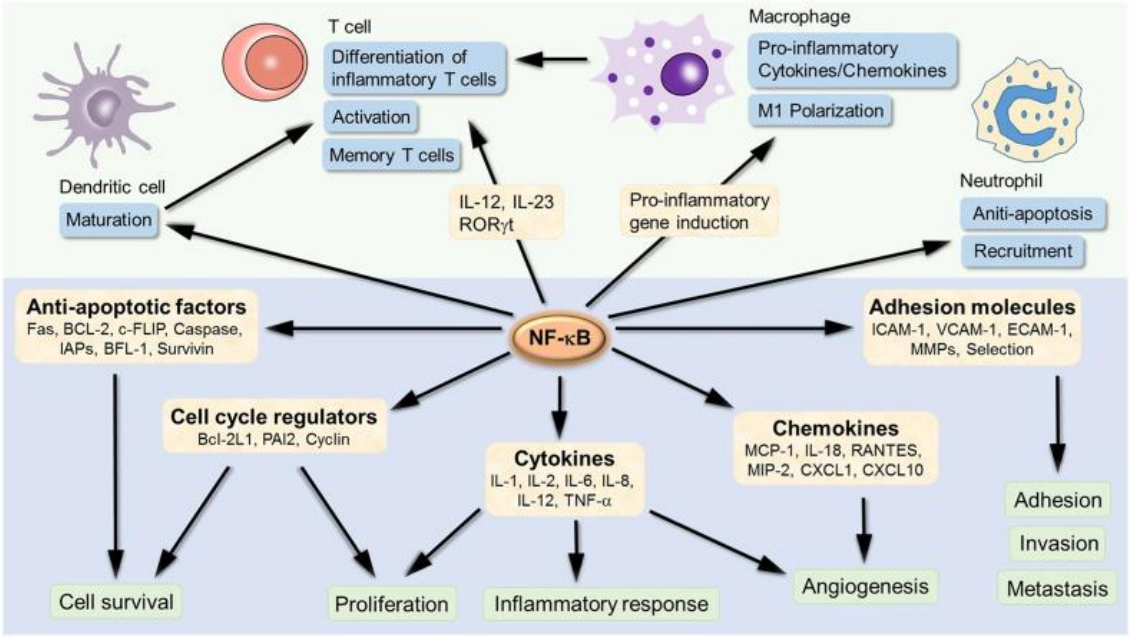


Figure 1. Impacts of NF-κB signaling in inflammation. Source: [13] When NF-κB is activated, it results in the transcription of genes related to inflammation resulting in increased chemokine, adhesion molecule, and cytokine production[13].

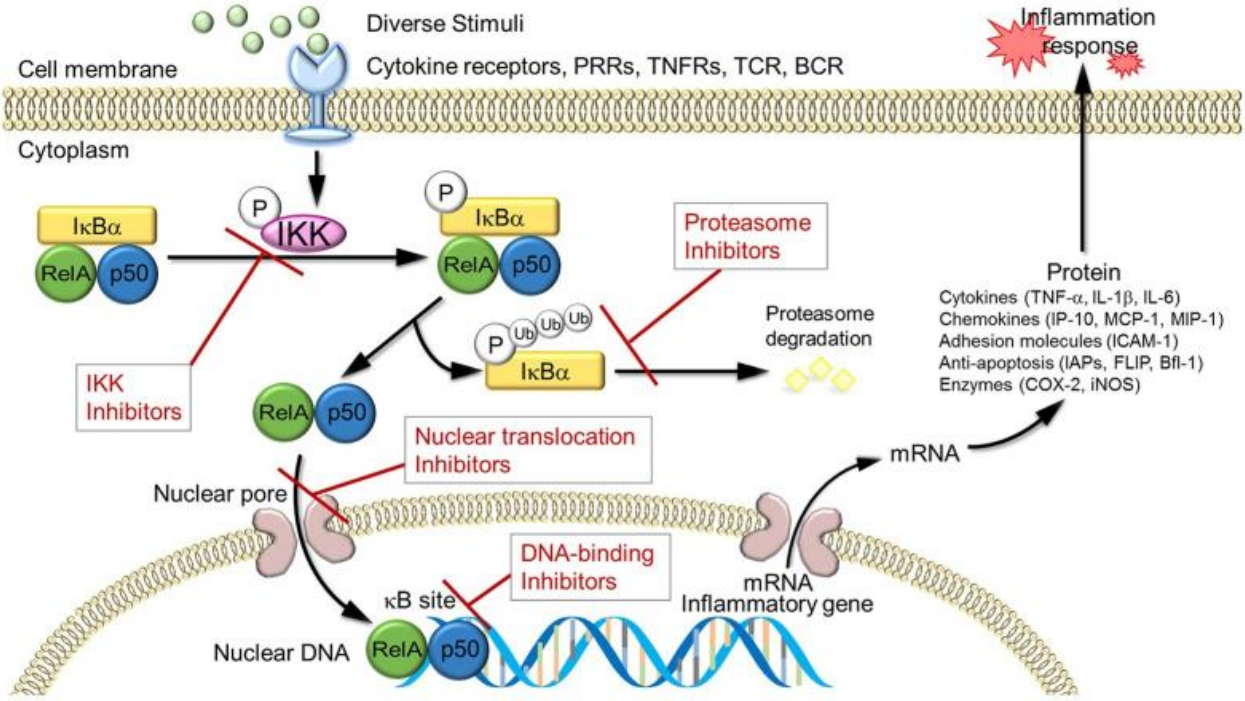


Figure 2. NF-κB signaling activation mechanism. Source: [13] In response to cytokine stimulation, NF-κB can become activated and produce inflammatory chemokines, cytokines, and enzymes[13].

1.4 Angiotensin-II Induced Hypertension

Ang-II is produced in vascular tissue and causes increases in BP through sodium and water retention and vasoconstriction[18]. Additionally, it can increase ROS production, endothelial dysfunction, NADPH oxidase activity, and inflammation[5,18]. There are two receptors used by Ang-II: the angiotensin type 1 and type 2 receptor[18]. The type 1 receptor plays a role in salt retention and blood pressure increases, however, Ang-II binding to the type 2 receptor promotes vasodilation[18].

Levels of Ang-II are usually elevated in patients with hypertension and contribute to high BP in mice infused with Ang-II[4]. Antagonists of Ang-II reduce BP in Ang-II dependent hypertension[4]. Ang-II can contribute to the uncoupling of eNOS[4]. An important mechanism contributing to the pathogenesis of hypertension is the uncoupling of eNOS[4]. Restoring eNOS coupling can be a good therapeutic strategy for the treatment of hypertension[4]. An Ang-II dependent initial activator to uncouple eNOS is NADPH oxidase, and it can be a good target to reduce hypertension[4].

Therapies for improving endothelial dysfunction and reducing eNOS uncoupling include drugs that interfere with the renin-angiotensin-aldosterone system (RAAS)[5].

1.5 Tart Cherry as a Therapeutic Agent for the Reduction of Blood Pressure

Dietary factors play an important role in the development of CVD[19]. Medication to reduce BP such as angiotensin-II type 1 receptor inhibitors, calcium channel antagonists, angiotensin converting enzyme inhibitors, and diuretics can help reduce BP, however, it may not address the underlying inflammation that contributes to the disease[3]. A healthy diet can be beneficial in reducing BP, CVD risk, and associated endothelial dysfunction and oxidative stress[2]. Drugs that affect the RAAS system have been found to elevate BH_4 levels, promote eNOS phosphorylation and expression and improve NO bioavailability, and thus may be useful in the prevention of endothelial dysfunction[5].

Plant based food contain many benefits including fiber and phytochemicals which may contribute to anti-hypertensive effects[19]. The polyphenols in plants are used in plant defense, to ward off bacterial pathogens and some of these polyphenols exert biological activity in humans[19]. Polyphenols are found in most fruit and plants and can be characterized into groups based on their chemical structures[19]. The amount and types of polyphenols can differ in plant products, and the potential benefits of polyphenols can be influenced by the overall dietary intake of polyphenol containing food[19].

Polyphenols may improve the bioavailability of NO through the activation of eNOS and iNOS[19]. An accumulation of ROS in the body due to normal cell

metabolism results in lipid, DNA, and protein damage[20]. Tart cherry (TC) consumption has been shown to reduce oxidative stress, inflammation and exert other beneficial effects on health in many studies[21]. Specifically, there is evidence that TC concentrate consumption is effective for BP lowering in humans,[21] however, the mechanism by which this occurs is not well understood. It is hypothesized that the effects of TC on BP occur through reductions in inflammation and oxidative stress, which subsequently reduces endothelial dysfunction[21]. Currently, a major obstacle in the field is that the effects of whole TC polyphenols on Ang-II induced endothelial dysfunction have not been studied in endothelial cells. Interventions for the prevention of age-related BP increases are important for reducing the burden of CVD[2]. TC is a fruit high in polyphenols. Chai et al. have previously seen beneficial impacts of this on inflammatory markers and DNA damage repair[21].

Several bioactive compounds are present in TC, and there is evidence suggesting that these compounds in isolation can influence inflammatory signaling pathways such as the NF-κB signaling pathway which can contribute to the pathogenesis of hypertension.

In this dissertation, I first study the influence of major dietary factors on systolic and diastolic BP in older adults. There is evidence that this may be due to the modulation of inflammation and oxidative stress that can contribute to hypertension. However, there are several gaps in our current knowledge of the

molecular mechanisms of BP reduction by TC. In this study, the dose-dependent effects of TC with and without Ang-II exposure on nitric oxide synthases, components of the NF-κB pathway, and intracellular adhesion molecule -1 in human coronary artery endothelial cells were determined.

Chapter 2

LITERATURE REVIEW EFFECTS OF TART CHERRY AND ITS METABOLITES ON AGING AND INFLAMMATORY CONDITIONS: EFFICACY AND POSSIBLE MECHANISMS

2.1 Introduction

Aging is an accumulation of molecular and cellular damage that increases the risk of disease and mortality[22]. Some factors that affect aging include genetics, environment, lifestyle, and dietary habits. Aging has far-reaching impacts including on the immune system and increases the number of pro-inflammatory markers in the blood[23]. The process of aging is associated, in part, with an increase in low-grade inflammation that makes the body more susceptible to age-associated diseases such as diabetes, hypertension, rheumatoid arthritis, and cancer[24]. The aging of hematopoietic stem cells and DNA damage responses can contribute to the manifestation of chronic inflammatory and autoimmune diseases in older adults[24].

Inflammation is a defense mechanism of the body against pathogens[25]. The process of inflammation is designed to repair damage and restore function[26]. Inflammation is usually associated with or a consequence of infections, allergens, toxic chemicals, obesity, a high-calorie diet, or radiation[25]. There are two types of

inflammation: acute and chronic. Acute inflammation is short-term and typically lasts a few minutes to a few days. Acute inflammation occurs in response to an inflammatory stimulus or injury and is important for tissue repair[27,28]. If the inflammatory stimulation persists, it is considered chronic inflammation, which contributes to chronic disease and tissue damage[27]. A chronic inflammatory state may be present in aged tissue, even if signs of infection are not present[29].

Chronic inflammatory diseases contribute to over half of deaths globally, including those from heart attacks, cancer, autoimmune diseases, strokes, and neurodegenerative diseases[30,31]. Common inflammatory cytokines found to be involved in the aging process include interleukin 6 (IL-6), interleukin 1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and high-sensitivity c-reactive protein (hsCRP)[28,30]. On a molecular level, the nuclear factor kappa B (NF- κB) signaling pathway seems to have the most impact on promoting inflammation in aging tissues by upregulating the expression of TNF-α, IL-2, and IL-6 pro-inflammatory genes[32]. Inflammation is typically treated with anti-inflammatory medication, such as nonsteroidal anti-inflammatory drug (NSAID), ibuprofen, or naproxen, which work by reducing the production and release of prostaglandins and inhibiting the activation of neutrophils[33]. However, the low-grade inflammation associated with aging can be modified with dietary changes[23].

In a previous study, we presented the inverse association between whole fruit intake and blood pressure[34]. Anthocyanin-rich fruits have been widely studied and regarded for their anti-inflammatory and antioxidant effects[35–37]. Belonging to the Rosaceae family, tart cherry (Prunus cerasus) is a drupe fruit with a higher acidity content than other types of cherries. As a part of the Amarelle variety, the Montmorency cherry is the most common type of tart cherry (TC) with red skin and lighter flesh compared to the Morello variety which has red-pigmented skin and dark flesh[38]. Balaton, another TC cultivar, is native to Hungary and is now grown in Michigan[39]. Many factors such as temperature, humidity, and stage of maturation affect the color, sugar, and phenol content of TC[40]. Total phenolic content decreases as the fruit ripens, whereas anthocyanin content, mainly cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside, increases[41]. After harvesting, the phenolic content of TC continues to increase during cold storage. TC is usually processed to frozen, powder, juice, or concentrate, and the chemical content of TC is altered as a result. For instance, almost half of the phenolic and anthocyanin content leaches out into the syrup. Among all processed products, frozen TC has the highest anthocyanin and phenolic contents[40]. On the other hand, TC juice concentrate has high phenolics with the highest oxygen radical absorbance capacity[42]. Although the phytochemical content of TC may be altered during processing stages, all processed

products show antioxidant properties to different extents. Concentrated TC juice seems to conserve the highest phytochemical content among other processed forms.

In this review, the effects of TC on inflammatory markers and risk factors that contribute to aging and inflammatory diseases are discussed. Effects of major TC compounds on inflammatory pathways, and the bioavailability of TC and its metabolites, as well as their anti-inflammatory effects, are also presented.

2.2 Effects of Tart Cherry on Inflammatory Conditions and Diseases

2.2.1 Cardiovascular Disease

CVD encompasses disorders of the heart and blood vessels and is the leading cause of mortality in the world[43]. Within the United States (US), one in four deaths is due to CVD, costing the US about $219 billion a year[44]. Atherosclerosis contributes to thrombotic complications associated with CVD and is an inflammatory process from initiation to progression[45].

Keane et al.[46] performed a randomized controlled trial in men with early hypertension to investigate the effects of TC concentrate consumption on vascular function. TC consumption was found to significantly reduce systolic blood pressure (SBP) at hours 1, 2, and 3 post-TC consumption, with a peak reduction of 7 ± 3 mmHg 2 h after consumption. The study determined that TC intake can acutely

reduce SBP in men with hypertension. This is important, given that high SBP and dietary factors are among the largest contributors to CVD[47].

Chai et al.[48] also found that TC could significantly reduce SBP. They conducted a randomized controlled trial in older adults aged 65-80 years that lasted 12 weeks and resulted in a significant reduction in SBP. This study differed from the one by Keane et al.[46] as more participants and both males and females were studied, a higher dose of TC concentrate (68 mL) was used, and the study was conducted for a longer period. SBP was significantly reduced in the TC group by a mean of 4.1 mmHg at the end of the 12-week study period, increasing by a mean of 5.4 mmHg in the control group. There were no changes in diastolic blood pressure (DBP) in either group. Biomarkers of oxidative stress and inflammation were also reduced from the TC intervention[21]. Levels of the DNA repair enzyme 8-oxoguanine glycosylase were significantly increased, and CRP was significantly reduced compared to the control. C-reactive protein (CRP) is an inflammatory marker used to predict CVD events including strokes, peripheral artery disease, heart attacks, and sudden cardiac death[49]. The researchers also found a reduction in low-density lipoprotein cholesterol (LDL-C) as compared to the control group. Data from these studies suggest that the daily incorporation of TC juice into the diet may be a reasonable intervention for the improvement of cardiovascular health in older adults[21,48].

Traustadottir et al.[50] performed a double-blind, placebo-controlled, clinical trial in older adults aged 61–75 years. This study included 12 healthy individuals (6 men and 6 women) who consumed a TC juice blend or a placebo (240 mL) twice daily for 14 days, separated by a 4-week washout period. Forearm ischemia-reperfusion (I/R) was performed at baseline and again after participants consumed the TC juice blend or the placebo intervention. The capacity to resist oxidative damage from I/R was measured as changes in levels of plasma F2-isoprostanes, which were reduced by TC consumption but unaffected by the placebo. The TC intervention also reduced the urinary excretion of the oxidized nucleic acids 8-hydroxy-2′-deoxyguanosine and 8-hydroxyguanosine. This data suggests that consumption of TC juice improves antioxidant defenses in older adults, as evidenced by an increased capacity to resist oxidative damage and reduced damage to nucleic acids.

Biro et al.[51] performed a cell-culture study to determine the effects of sour cherry extract on lipopolysaccharide (LPS)-induced endothelial inflammation. The sour cherry was extracted using ethanol and solid-phase extraction. They used human umbilical vein endothelial cells (HUVEC) and exposed them to 100 ng/ml of LPS and 50 ug/mL of anthocyanin Hungarian TC extract. TC was able to significantly reduce levels of the chemokine RANTES, also known as CCL5. RANTES is involved in several conditions, including atherosclerosis, cancer, and

autoimmune diseases[52]. TC reduced levels of GM-CSF, a growth factor and contributor to inflammation. In addition, levels of TNF-α, IL-6, and tissue plasminogen activator (tPA) were reduced by TC. Generally, tPA is known for its ability to dissolve blood clots, but it also can act as a cytokine in certain diseases[53]. The results suggest that TC was able to reduce inflammation in the HUVEC cells by suppressing cytokine and chemokine release. The impact of TC on thromboxane A2 (TxA2) and PGI2 of the arachidonic acid pathway was also studied. PGI2 is produced from the metabolism of arachidonic acid by COX-2 and PGI2 synthase[54]. LPS increased the levels of PGI2, and TC significantly reduced levels of PGI2, although TxA2 was not affected. Significant reductions in the messenger ribonucleic acid (mRNA) expression of enzymes involved in PGI2 synthesis were caused by the TC extract, through its effects on COX-1, COX-2, and PGI2 synthase. COX-2 is generally expressed in stimulated tissues and is associated with pro-inflammatory conditions[54]. The TC extract also affected oxidative stress and antioxidant levels by reducing the hydrogen peroxide (H_2O_2)-induced levels of reactive oxygen species (ROS) and increasing levels of glutathione peroxidase. The results of this study show that TC could reduce LPS-induced inflammation and may improve endothelial dysfunction[51].

2.2.2 Arthritis and Joint Diseases

Osteoarthritis (OA) typically involves an inflammation of synovium, synovitis, with increased levels of inflammatory cytokines in the serum and synovial fluid of patients with OA as compared to healthy individuals. Inflammation can occur in OA joints before cartilage degeneration can be observed. Joint deterioration may be a consequence of chronic low-grade inflammation in a large population of OA patients[55].

Kuehl et al.,[56] studied the effects of TC juice consumption on inflammatory biomarkers in participants with OA. This was a randomized, double-blind, placebo-controlled trial. Twenty female participants (aged 40–70 years) with OA were included in the study and consumed 21 oz of TC juice or a placebo for 21 days. They found that TC juice could significantly reduce serum CRP in women with OA.

Schumacher et al.,[57] also found a reduction in hsCRP from TC juice intake in OA patients. In this study, the sample size was larger than the study by Kuehl et al.[56] and contained 58 participants (44 males and 14 females; mean age 56.7 years) with mild to moderate OA. Participants consumed 16 oz of a TC-and-apple juice blend or a placebo every day for 6 weeks, with a one-week washout period before switching treatments. A total of 46 participants completed the entire study. In addition to hsCRP, the researchers found reductions in the WOMAC osteoarthritis index, a pain and function test, after TC intake.

When uric acid accumulates in the serum, joints, and tissues, it can cause gout, a type of inflammatory arthritis. Martin and Coles[58] conducted a 12-week, 2 × 2 crossover, randomized, placebo-controlled dietary intervention in overweight and obese participants to determine the effects of 100 % TC juice consumption on serum urate. Participants were randomly assigned to drink either a daily 240 mL (8 oz) of TC juice diluted 1:6, or a placebo drink for 4 weeks. After a 4-week washout period, the participants were switched to the opposite beverage for an additional 4-week. There were 26 participants in the study (18 women and 8 men) aged 30–52 years. Plasma urate was found to decrease significantly by about 19.2 % after the 4 weeks of TC juice consumption.

He et al.,[59] conducted a study to determine the anti-inflammatory and anti-oxidative effects of sour cherry anthocyanins on arthritis in male rats aged 6-7 weeks. The sour cherry anthocyanins were extracted using methanol and acidified water. Arthritis was induced in the rats through an intradermal injection with complete Freund's adjuvant. Anthocyanins, which were dissolved in water, were administered at 40, 20, and 10 mg/kg orally for 28 days. Levels of TNF-α and prostaglandin E2 (PGE2) were significantly reduced with anthocyanins at the 40 mg/kg dose. The total antioxidant capacity and superoxide dismutase (SOD) increased with the anthocyanins at all doses. The levels of malondialdehyde (MDA) were significantly reduced at 40 mg/kg.

2.2.3 Post-exercise Inflammation and Muscle Damage

Muscles are thought to become damaged during exercise as a result of mechanical and oxidative stress to the muscle fibers[60]. Oxidative stress can come from excess ROS and nitric oxide (NO) derivatives that exceed the antioxidant defense capacity of the body. Antioxidants have been a treatment of interest to reduce the muscle damage or stress associated with exercise[60]. Exercise-induced muscle damage is associated with an accumulation of inflammatory cells in muscle tissues. These cells can increase in muscles immediately after exercise, and the extent of accumulation can depend on how extensively the muscles are damaged[61].

Bowtell et al.,[60] conducted a study to determine the effect of TC juice on functional recovery from intensive knee extensor resistance exercise and in markers of inflammation and oxidative stress. Ten participants with a mean age of 27.8 years were included in this study. Participants consumed a total of 60 mL of TC concentrate or a fruit-concentrate placebo for seven days before, the day of, and two days after exercise. Participants completed intensive knee-training sessions and a two-week washout period. TC consumption led to significantly faster recovery and reduced protein carbonyl as compared to the placebo. Recovery of knee extensor maximum isometric strength was enhanced with TC juice consumption.

In a study by Bell et al.[62] the impact of TC concentrate on exercise-induced oxidative stress, inflammation, and muscle damage across three days of road-cycle racing was determined. Sixteen well-trained male cyclists (mean age 30 years ± 8 years) were included in the study. Participants consumed 60 mL of TC every day for a week. Participants performed a high intensity, simulated, stochastic road cycling trial for the last three days of the week. The researchers found that TC-attenuated lipid hydroperoxide (LOOH), a marker of oxidative stress, compared to the placebo group, and the greatest difference between the groups occurred on Day 3. Levels of IL-6 and hsCRP were significantly lower in the TC group as compared to the control group. This study demonstrated a useful application for TC consumption during repeated days of high intensity cycling and possibly other back-to-back days of sporting activities that cause increases in levels of inflammation and oxidative stress.

Exhaustively working out for a long time can upregulate chemotactic cytokine expression in the airways and result in inflammation of the respiratory mucosa. This is referred to as exercise-induced airway inflammation and can be triggered by many factors, including pollutants, oxidative stress, and hyperventilation[63]. Dimitriou et al.[63] conducted a study to determine the effects of a cherry juice blend on exercise-induced airway inflammation. This study was conducted with 20 marathon runners and included both males and females aged 14–40 years. Participants were asked to consume 472 mL of a TC-and-apple juice

blend or a placebo for five days prior to, the day of the marathon, and 48 h following the marathon (eight days total). The results indicated that serum CRP was significantly lower in the TC group compared to the placebo both 24 and 48 h after the marathon. In the placebo group, the incidence and severity of upper respiratory tract symptoms (URTS) were increased at 24 and 48 h following the marathon, compared to the pre-marathon levels. URTS were significantly higher in the placebo group at 24- and 48-h post-race as compared to the TC group. No URTS were reported in the TC group at 24- and 48-h post-marathon, as opposed to the placebo group, in which 50 % (5/10) of the runners developed URTS. TC may be a good treatment for exercise-induced pulmonary inflammation to protect or alleviate the URT from inflammatory symptoms.

2.2.4 Obesity

In the National Health and Nutrition Examination Survey, the prevalence of obesity among adults was 38.3 %, according to data collected from 2013 to 2016[47]. Additionally, according to 2015–2016 data, the prevalence was 20.6 % for individuals aged 12–19 years[47].

Obesity is caused by overeating, which results in calorie and/or nutrient overload, excess fat storage, weight gain, and disease development[28,64]. Calorie

overload can contribute to adipocyte dysfunction, which leads to an inflammatory response that can speed up triglyceride metabolism and increase the levels of free fatty acids in the blood. Over time, the presence of these free fatty acids can activate stress kinases that inhibit insulin signaling pathways, resulting in insulin resistance and β-cell damage[64,65]. Research has shown that the adipose tissue of obese mice have an overexpression of the proinflammatory cytokine TNF-α[66]. Further, adipose tissue plays a role in the regulation of glucose in the body, and the common complications that result from obesity include atherosclerosis, impaired glucose tolerance, and insulin resistance, which can result in type 2 diabetes mellitus (T2DM)[64].

Nemes et al.[64] conducted a study with mice to determine whether the Hungarian sour cherry variety VN1 could offset the effects of a high-fat diet in mice. The sour cherry was extracted using ethanol and filtration. The mice were divided into three groups: the first group, control (n = 11), received standard chow and tap water; a second group that consumed a high-fat diet that contained 5% sucrose (n = 12); and a third group that consumed a high-fat diet with sucrose and 60 mg/kg of TC extract daily in their drinking water (n = 12). After six weeks on these diets, the high-fat group, and the high-fat + TC group both showed a significant increase in levels of fasting blood glucose and a reduced glucose tolerance as compared to the control group. Between the two high-fat-diet groups, the addition of

TC was not able to reduce fasting blood glucose or improve glucose tolerance. The levels of IL-6 were significantly increased in the high-fat group as compared to the control, and there was no difference between the high-fat and TC-supplemented group and the control group. The levels of MCP-1 were significantly elevated in both the high-fat and high-fat + TC groups when compared to the control. In the high-fat mice, the levels of SOD were significantly reduced by the end of the experiment. TC increased the levels of SOD as compared to both the control and high-fat groups. The researchers concluded that TC anthocyanins (cyanidin-3-O-glucoside, cyanidin-3-O-glucosyl-rutinoside, and cyanidin-3-O-rutinoside) can reduce inflammatory cytokines in adipocytes and improve the antioxidant status in the body. TC may prevent the development of metabolic syndrome, and anthocyanin intake has the potential to enhance redox status and alleviate inflammation associated with obesity.

Martin et al.[67] conducted a 10-week randomized, placebo-controlled, crossover dietary intervention study with overweight and obese participants. The participants were randomly assigned to consume 240 mL of a placebo or 100 % TC juice every day for 4 weeks, followed by a washout period and consumption of the alternative beverage for 4 weeks. MCP-1 was significantly reduced by ~15.8 % from the TC intake and was slightly elevated from the placebo. Erythrocyte sedimentation

rate, a marker of chronic inflammation, was also reduced significantly by TC intake and increased from the placebo consumption.

TC may be able to alter adipose gene expression by changing the activity of tissue peroxisome proliferator-activated receptors (PPARs). In a study conducted by Seymour et al.,[68], the effect of anthocyanin-rich TC was tested on rats in a 90-day randomized, controlled animal trial. The rats that were fed a 1% (w/w) freeze-dried whole TC powder along with a high-fat diet had reduced hyperlipidemia and abdominal fat weight. Plasma IL-6 and TNF-α were reduced by 44 % and 40 %, respectively. The researchers also found that PPAR-α and PPAR-γ mRNA levels were reduced. Further, several NF-κB regulated transcripts, including IL-6 and TNF-α, were reduced in retroperitoneal fat in the TC group. The researchers observed that inflammation and expression of inflammation-related genes were reduced by dietary intake of anthocyanin-rich TC.

2.2.5 Cognitive Disorders

Cognitive decline and loss of neurons in the brain are characteristics of Alzheimer's disease (AD), and inflammation has been found in the postmortem brain tissue of AD patients[69]. The presence of inflammation has led to the hypothesis that enhanced microglial activation and other inflammatory factors made by the

brain can potentially damage neurons or axons[70]. In addition, inflammation may be detected or present early in the development of AD[70]. The brain uses acute inflammation as a defense against toxins or injury, but when there is too much inflammation or chronic inflammation, this can result in AD. This is thought to be due to the release of cytokines and activated microglial cells[69]. Aging is an important risk factor for the development of AD[70], and studies have shown that inflammation reduction can reduce the risk of developing AD [69,70].

Kim et al.[71] examined the protective effects of sour cherry phenolics in neuronal cells. The cherry phenolics were extracted using methanol. The study used neuronal PC 12 cells derived from rat pheochromocytoma under cell-damaging oxidative stress. The cells were preincubated with cherry phenolic extract and then treated with H_2O_2. The control group received no treatment with H_2O_2 or the cherry phenolic extract. The H_2O_2 caused a reduction in cell viability, but, with the cherry phenolics, the cell viability was improved in a dose-dependent manner. This may be due to the ability of TC phenolics to protect against oxidative stress in cells.

Carson[72] conducted a study to determine the effects of TC on neuropathic pain in non-diabetic peripheral polyneuropathies. This is a common condition that typically affects individuals aged 50 years and over and can develop from injury to nerve tissue, nerve compression, or autoimmune attacks[72]. The study included 12 patients with painful lower extremity peripheral neuropathy. The participants

described their pain as 9–10 out of 10 more than half of the time. All participants drank 8 oz of 100 % TC juice from concentrate every day for two weeks. At the end of the study, the participants rated their maximum peripheral neuropathy pain. Two patients reported no improvement, three patients reported a reduction of maximum pain to 5–8 (out of 10), five patients reported 2–4 (out of 10), and two patients reported 0–1 (out of 10). This indicates that more than half of the participants had a 50 % or greater reduction in maximum pain. It was concluded that a 2-week course of TC juice can significantly improve nondiabetic peripheral neuropathy pain in a majority of participants.

Chai et al.[73] measured the effect of TC concentrate on memory and cognitive performance in older adults aged 65–80 years. This was a 12-week parallel, randomized controlled trial. Thirty-four participants, with 17 in each group, completed the study. The TC juice included 68 mL of concentrate. After the intervention, participants in the TC group had higher contentment with memory scores, reduced movement time scores, and increased performance of the paired associates learning task as compared to the control group. The within-group analysis showed that visual sustained attention and spatial working memory improved after the 12-week consumption of TC juice as compared to baseline values. This study demonstrated that the daily intake of TC juice by older adults may help to improve subjective memory and cognitive abilities in older adults, as evidenced by increased

contentment with memory, improved visual sustained attention and spatial working memory, and reduced movement time and total errors made on learning new tasks.

2.2.6 Cancer

Inflammation may predispose an individual to develop cancer, as it plays an important role in tumor growth and progression[74]. Ogur et al.[75] investigated the anti-cancer effects of TC extract in mammary adenocarcinoma (MCF-7) and mouse mammary tumor cell (4T1) breast cancer cell lines in vitro. The TC extraction was performed using ethanol. The addition of encapsulated TC extract to the MCF-7 and 4T1 cell cultures significantly reduced cell proliferation. A concentration of 50 µg/ml encapsulated TC extract was found to inhibit cell growth by 37 % in the MCF-7 cells and 48 % in the 4T1 cells. A concentration of 100 µg/ml inhibited cell proliferation by over 50 % in both cell types[75]. Levels of asymmetric dimethylarginine were also significantly reduced in the cells after supplementation with TC. Asymmetric dimethylarginine is a NO synthase inhibitor and, thus, has the ability to reduce NO concentrations and potentially inhibit endothelial dysfunction[76].

Kang et al.[77] investigated the inhibitory effect of TC anthocyanins on tumor development in Apc^{Min} mice and reduction of proliferation in human colon cancer cell lines HT 29 and HCT 116. Rats were divided into five treatment groups, with 10

mice in each group and an equal number of males and females. The control group consumed a standard diet, the second group consumed the control diet in addition to 800 mg/l of anthocyanins from TC in their drinking water, the third group consumed the control diet in addition to 200 mg/l of cyanidin in their water, the fourth group had the control diet with 200 mg/l of sulindac (an NSAID) in their water, and the fifth group consumed a modified control diet with 200 g/kg of freeze-dried TC added. At the end of the 10-week intervention, the anthocyanin, cyanidin, and TC groups showed a reduction in the number and total volume of tumors in the cecum as compared to the control and sulindac groups. Treatment with anthocyanins or cyanidin resulted in a reduction in cell number in both the HCT 116 and HT 29 cells in a dose-dependent manner. Neither the anthocyanin nor the cyanidin treatment caused cytotoxicity, but cyanidin was more effective at inhibiting the growth of the cancer cell lines than the anthocyanins. The researchers concluded that TC anthocyanins and cyanidin may reduce the risk of colon cancer[75].

Anthocyanin-rich TC has been shown to reduce the risk of cancer through inhibition of cell proliferation or apoptosis. In an in-vitro study, Martin and Wooden[78] examined the effects of different concentrations of TC on apoptosis and cell proliferation in Apc^{Min} mice. MCF-7 cells were incubated with 0.03–30 % TC. The results indicated that mitogenesis and cell proliferation were decreased at concentrations greater than 10 %, likely due to necrosis. At TC concentrations less

than 10 %, cell proliferation was not different from that of the control group. Apoptosis was increased at concentrations of 3% and 10 % by 34%–44%, respectively. Apoptosis was significantly reduced, however, with the 1% TC juice treatment by 20 % as a result of banding of incubated cells to 1% TC juice, which is less dense than are the control cells. The results from this and many related studies suggest that consuming fruits rich in polyphenols, such as TC, might be beneficial for reducing the risk of cancer as well as other chronic diseases[78].

Combination therapy might be an effective treatment for diseases such as cancer. Pharmaceutical drugs are often associated with adverse effects on health. Sulindac, for instance, is an NSAID that has been shown to prevent the progression of adenomatous polyps in Apc^{Min} mice. Higher dosages of this drug, however, may increase the risk of gastrointestinal bleeding and ulceration. Bobe et al.[79] found that the application of a suboptimal dosage of sulindac in combination with dietary anthocyanin-rich Balaton TC extract may be more effective at inhibiting intestinal tumorigenesis in Apc^{Min} mice than the use of Sulindac alone. The TC extracts were prepared using methanol. A total of 141 mice, 4–5 weeks of age, were randomly placed in five groups with different dietary treatments. TC extract was added to the food of Groups 1–5 at concentrations of 0, 375, 750, 1500, or 3000 mg/ kg of diet, respectively. All diets contained 100 mg of sulindac. The other elements of the diet were the same in all groups. The mice were sacrificed after 19 weeks. The results

indicated that mice fed anthocyanin and sulindac had a 20 % smaller total tumor area and a 22 % lower tumor number in the small intestine. Statistically, however, there were no linear or quadratic associations between the anthocyanin dosages and tumor number or area. The data showed that the Apc^{Min} mice that consumed the lower two dosages of crude anthocyanin tended to have a lower number and burden of papillary tumors than did the ones who received the higher two dosages. The results of this study suggest that a combination diet of anthocyanin-rich TC extract with suboptimal dosages of sulindac could be a promising approach for colon cancer prevention in high-risk groups.

2.3 Mechanisms of Effectiveness

2.3.1 Components of Tart Cherry

Montmorency TC and its processed products are functional foods that are rich in phytochemicals, including isorhamnetin rutinoside, kaempferol, quercetin, catechin, epicatechin, procyanidins, and anthocyanins[39]. Anthocyanins are a type of flavonoid that gives pigmentation to plants and possesses antioxidative properties[39]. Cyanidin derivatives are the major anthocyanins of TC[39,80]. The analysis of Montmorency TC shows that the major anthocyanins in TC are cyanidin 3-glycosylrutinoside, followed by cyanidin 3-rutinoside and cyanidin sophoroside[39,80].

Kirakosyan et al.[39] found that 93 % of the total anthocyanins in Montmorency TC are cyanidins. The other anthocyanins in TC include peonidin 3-glucoside, cyanidin-3-glucoside, and isorhamnetin rutinoside[39,71]. The phenolic compounds in TC are hydroxycinnamates (such as chlorogenic acid and neochlorogenic acid), procyanidins, flavonol glycosides, and epicatechins[80]. The researchers Kirakosyan et al.[39] found melatonin (N-acetyl-5-methoxytryptamine) in frozen and individually quick-frozen powders of both Montmorency and Balaton TCs, with higher amounts in Montmorency. Dried TC and TC concentrates were not found to contain melatonin, which may be due to the instability of melatonin during processing and storage[39]. Montmorency TC skins have more total phenolics than do their pits or flesh[80].

Quercetin and kaempferol are flavonoids that have shown antioxidant, anti-inflammatory, cardioprotective, and anti-hypertensive effects in animal studies[81]. Sources of quercetin and kaempferol include tart cherries, blueberries, apples, onions, and chili peppers[39,81]. Cyanidins are common in berries such as acai berries, bilberries, and blackberries[82]. Research has shown that these compounds can individually affect inflammatory pathways. Note that some of the studies presented utilize pure polyphenolic compounds that were commercially purchased.

2.3.2 Modulation of Inflammatory Pathways

2.3.2.1 NF-κB pathway

NF-κB is a family of transcription factors that are involved with inflammation, proliferation, differentiation, and cell survival[83]. There are five subunits in the NF-κB family: p100/p52, p105/p50, RelA/p65, RelB, and c-Rel, which can form dimers to produce NF-κB transcription factors[83]. NF-κB transcription factors are usually inactive in unstimulated cells because they are bound to an inhibitor of the κB molecule (IκB). When IκB is degraded, NF-κB is freed[83]. Chronic systemic inflammation (CSI) is an underlying cause of the development and progression of atherosclerotic vascular lesions[84]. Quercetin reduces the formation of ROS by inhibiting or reducing the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (or NOX), xanthine oxidase, COX, and lipoxygenase[84]. IL-1β and TNF-α are two inflammatory mediators that are produced in response to pathogenic stimuli that affect the transcription of the NF-κB gene[84]. Chekalina et al.,[84] examined the effects of quercetin on the markers of CSI in stable coronary artery disease (CAD)[84]. The study included 85 patients (36 females and 49 males) aged 48–67 years. Patients in the experimental group consumed a daily dose of 120 mg of quercetin and received basic therapy (medication and lifestyle changes), while the control group received basic therapy

only. Cytokines, including IL-1β, TNF-α, and IL-10, were elevated in both groups of patients, but, after two months of the intervention, the quercetin group showed a reduction in levels of IL–1β. No significant changes in cytokine levels were noted in the control group, which received basic therapy. Expression of the IκBα gene was also significantly reduced in the patients' blood mononuclear cells after consuming the quercetin as compared to the response in the control group. These results show that quercetin in patients with stable CAD reduces the levels of IL-1β, TNF-α, and mRNA levels of IκBα[84]. This indicates a reduction in inflammatory cytokines by quercetin.

Ruiz et al.,[85] used mouse intestinal epithelial cell (IEC) line Mode-K to study the effects of quercetin and its enteric bacterial metabolites on proinflammatory gene expression. These cells were stimulated with TNF in the presence or absence of quercetin, taxifolin, alphitonin, and 3,4-dihydroxy-phenylacetic acid (bacterial metabolites of quercetin). Eight-week-old mice were fed 10 mg/kg of quercetin orally per day (n = 11), and the control group was fed its dissolvent, propylene glycol (n = 10). The mice were sacrificed after 10 weeks, and primary IEC cells were isolated from the ileum. These cells were treated with quercetin or its bacterial metabolites after stimulation with TNF. The results showed that quercetin significantly inhibited Akt phosphorylation and NF-κB–dependent interferon-γ-inducible protein 10 (IP-10) and macrophage inflammatory protein 2 (MIP-2)

expression. Quercetin also inhibited the recruitment of the NF-κB cofactor CBP/p300 to the IP-10 and MIP-2 gene promoters, which suggests that quercetin may target the TNF-induced transcriptional regulation of the chromatin. Quercetin was found to reduce total histone acetyl transferase (HAT) activity and block TNF-induced acetylation and/or phosphorylation of H3 at the IP-10 and MIP-2 gene promoters. It may be likely that the inhibitory effect of quercetin on the PI3 kinase/Akt signaling cascade may directly affect the NF-κB-dependent gene expression by modulating CBP/p300 recruitment and/or HAT activity at the chromatin. None of the bacterial metabolites inhibited TNF-induced IP-10, Akt phosphorylation, or MIP-2 protein production, which suggests that the bacterial transformation of quercetin may reduce its anti-inflammatory abilities. The results suggest that the mechanism by which quercetin may reduce TNF induced inflammation in IEC cells is by inhibiting cofactor recruitment at the chromatin of proinflammatory genes.

Sun and Li[86] studied the effect of cyanidin-3-glucoside (C3G) on fibroblast-like synoviocytes (FLS). When the researchers exposed the FLS cells to 10umol/l, 20umol/L, and 40umol/L of C3G for 12 h, followed by LPS for 12 h, they found that proinflammatory cytokines, such as TNFα, IL-1β, and IL-6, were all inhibited in a dose-dependent manner. The mRNA levels of these compounds were all significantly inhibited by C3G treatment in a dose-dependent manner, suggesting

possible anti-inflammatory effects of C3G in FLS cells. The activation of inflammatory pathways is necessary for the expression of pro-inflammatory cytokines, and C3G can inhibit LPS-induced proinflammatory cytokine production by affecting the NF-κB pathway. C3G treatment reduced the expression of p65 and the phosphorylation of IκBα. The protein levels of p-p65 and p-IκBα were also significantly reduced, indicating that C3G inhibited the NF-κB activation in FLS cells.

In another study, Jung et al.,[87] sought to determine the effects of C3G and cyanidin-3-rutinoside (C3R) against H_2O_2-induced oxidative stress and LPS-induced inflammation using RAW 264.7 murine macrophage cells. C3R was found to downregulate NF-κB expressions. LPS-induced IκBα degradation was significantly inhibited by both C3G and C3R as compared with control cells.

Allergic reactions involve a hypersensitivity response of the immune system[88]. Atopic dermatitis is a type of allergic reaction that presents as dry skin, hypersensitivity, thickening of the skin, and eczematous skin lesions. The researchers sought to determine the effects of C3R on allergic inflammation and the underlying mechanism, using the human mast cell line HMC-1[88]. These cells were treated with various concentrations of C3R (0.1-100 ug/mL) and stimulated with phorbol-12-myristate-13-acetate (PMA) and A23187 to induce allergic inflammation. The results indicated that C3R significantly reduced the secretion of

IL-1β, IL-6, and TNF-α and their mRNA levels. C3R inhibited the phosphorylation of NF-κB p65 in mast cells. Patients with allergic inflammatory diseases are sometimes treated with an inhibitor of caspase-1. This study found that the PMA/A23187 stimulation induced caspase-1 and was suppressed by C3R treatment. C3R also was found to reduce thymic stromal lymphopoietin (TSLP) and its mRNA levels as well as to reduce histamine release. TSLP is an epithelial-derived cytokine and is influenced by NF-κB activation. The researchers concluded that C3R may reduce allergic inflammation by affecting the NF-κB pathway and reducing inflammatory cytokine secretion and allergic mediators.

Kadioglu et al.[89] tested the anti-inflammatory activity of kaempferol in Jurkat leukemia cells. Molecular docking studies have shown that kaempferol shares comparable docking poses and binding energies with MG-132, a known NF-κB inhibitor. Kaempferol induced a dose-dependent inhibition of NF-κB activity in secreted embryonic alkaline phosphatase (SEAP)-driven NF-κB reporter cells with varying TNF-α concentrations with weaker inhibition as compared to MG-132. In addition, kaempferol (10 μM) inhibited NF-κB–DNA electromobility shift assay. TNF-α induction followed by treatment with kaempferol yielded inhibition of nuclear translocation of NF-κB p65. In-silico calculations were conducted on NF-κB pathway proteins, which indicated that kaempferol showed similar docking poses and comparable binding energies to MG-132. The binding energy implied that

kaempferol may intercalate into DNA and inhibit the binding of NF-κB to DNA. The results indicate that kaempferol affects the NF-κB pathway and may act as an anticancer compound.

Kim et al.[90] performed an animal and cell culture study to determine the effects of kaempferol on advanced glycation end products (AGE)-induced NF-κB activation. AGE is a common characteristic of aging and results from the reaction between carbohydrates and the free amino groups of proteins[90]. The results indicated that old rats (24 months old) had greater levels of AGE compared to young rats (6 months old). Short-term kaempferol supplementation for 10 days of 2 mg/kg or 4 mg/kg significantly reduced AGE in old mice as compared to un-supplemented old mice, and the receptor for AGE (RAGE) was reduced by a 4 mg/kg dose of kaempferol. Kaempferol supplementation reduced the DNA binding of NF-κB that increased with age and reduced the phosphorylation of IκBα and p65 as well as the translocation of the p50 and p65 subunits. The researchers also found that the expression of NF-κB-dependent genes and adhesion molecules MMP-9, MCP-1, RANTES, CAM-1, and ICAM-1 were all reduced by kaempferol intake. Similar results on AGE and RAGE expressions were determined when rat prostate endothelial cells (YPEN-1) were exposed to AGE and kaempferol. Kaempferol also significantly blocked total reactive species generation caused by AGE. The AGE-induced NF-κB activation was significantly blocked by the two NOX inhibitors,

APO and DPI, which suggests that kaempferol's attenuation of AGE-induced NF-κB activation may be through the inhibition of NOX-produced reactive species. These data suggest that kaempferol possesses anti-inflammatory and anti-oxidative properties, due to its effects on NF-κB and NOX and may have therapeutic potential for the treatment and prevention of age-related inflammation and disease[90]. **Figure 3** shows the molecular targets of TC compounds in the NF-κB inflammatory signaling pathway.

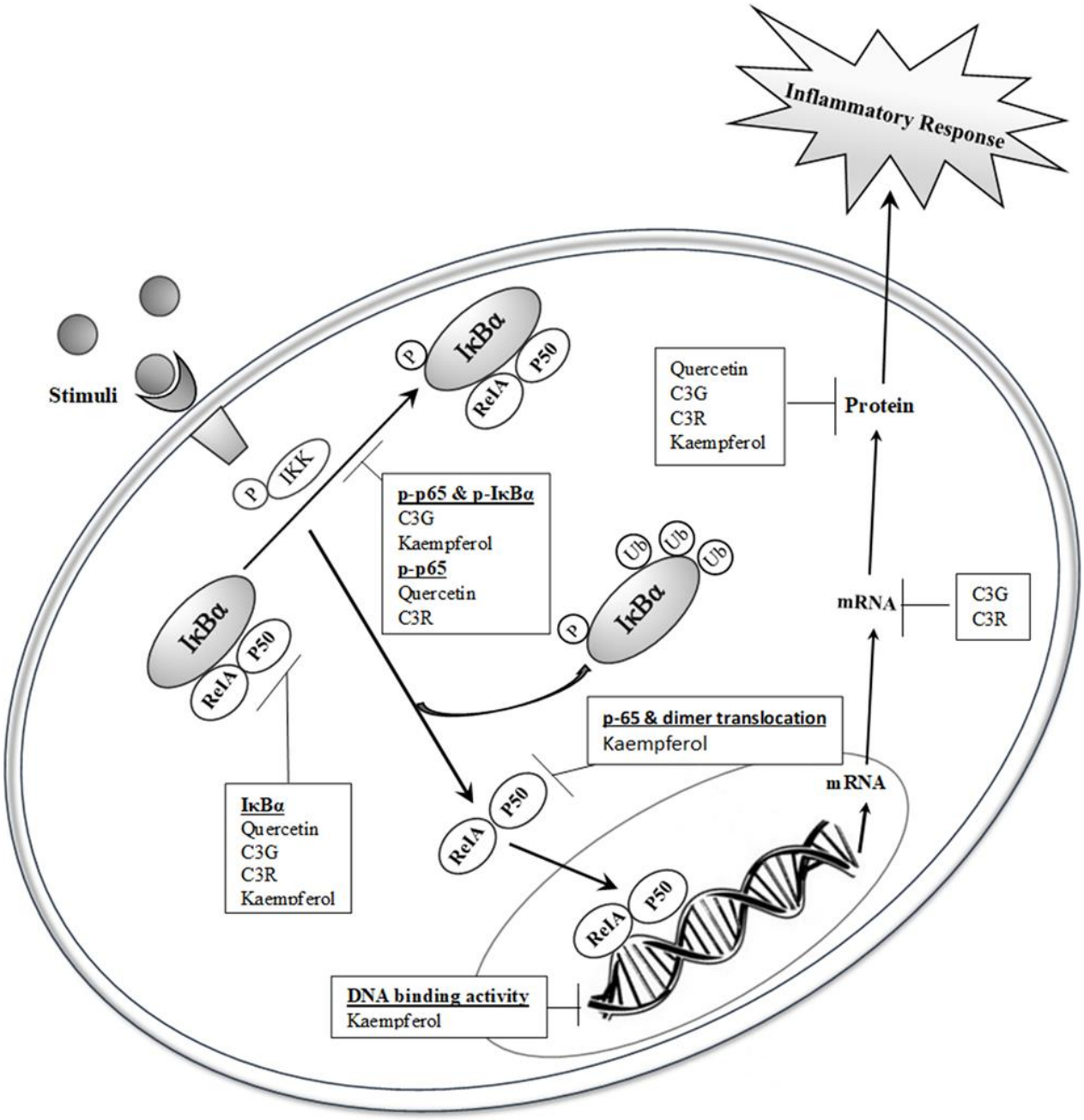


Figure 3. Influences of tart cherry components on the NF-κB pathway. The molecular targets of TC compounds in the NF-κB inflammatory signaling pathway. C3G, cyanidin 3-glucoside; C3R, cyanidin 3-rutinoside; IκBα, nuclear factor kappa light polypeptide gene enhancer in B-cells alpha.

2.3.2.2 MAPK pathway

Mitogen-activated protein kinase (MAPK) has three main families: p38/SAPKs (stress-activated protein kinases), extracellular-signal regulated kinases (ERK), and Jun amino-terminal kinases (JNK)[91]. The JNK family plays a role in inflammation and cytokine production[91]. When an inflammatory stimulus is experienced, macrophages and intracellular signaling pathways produce a signal for inflammatory mediators to become active[92]. The receptors activated in the inflammatory response, such as the TNF receptor family or the Toll receptors, trigger the MAPK and NF-κB pathways[92]. MAPK activation leads to the phosphorylation and activation of transcription factors in the cytoplasm or nucleus, which results in gene expression[92]. The p38 MAPK pathway can be stimulated by a variety of cytokines and can produce and activate inflammatory mediators to initiate leukocyte recruitment, thus playing a role in inflammation[92]. The activation of p38 MAPK also can generate inducible nitric oxide synthase (iNOS) and the production of an inflammatory response from macrophages[92].

Sun and Li[86] found that C3G could reduce LPS-induced pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in FLS. C3G treatment reduced the phosphorylation of ERK, p38, and JNK as well as the protein levels of p-ERK, p-p38, and p-JNK, indicating that C3G can inhibit MAPK activation, which may reduce the production of cytokines.

Jeon et al.,[88] found that C3R treatment could reduce PMA/A23187-induced secretion of IL-1β, IL-6, and TNF-α as well as their mRNA levels. C3R treatment also reduced the PMA/A23187-induced phosphorylation of JNK, ERK, and p38, indicating C3R's role in regulating the MAPK signaling pathway in mast cells[88]. **Figure 4** shows the molecular targets of TC compounds on the MAPK inflammatory signaling pathway.

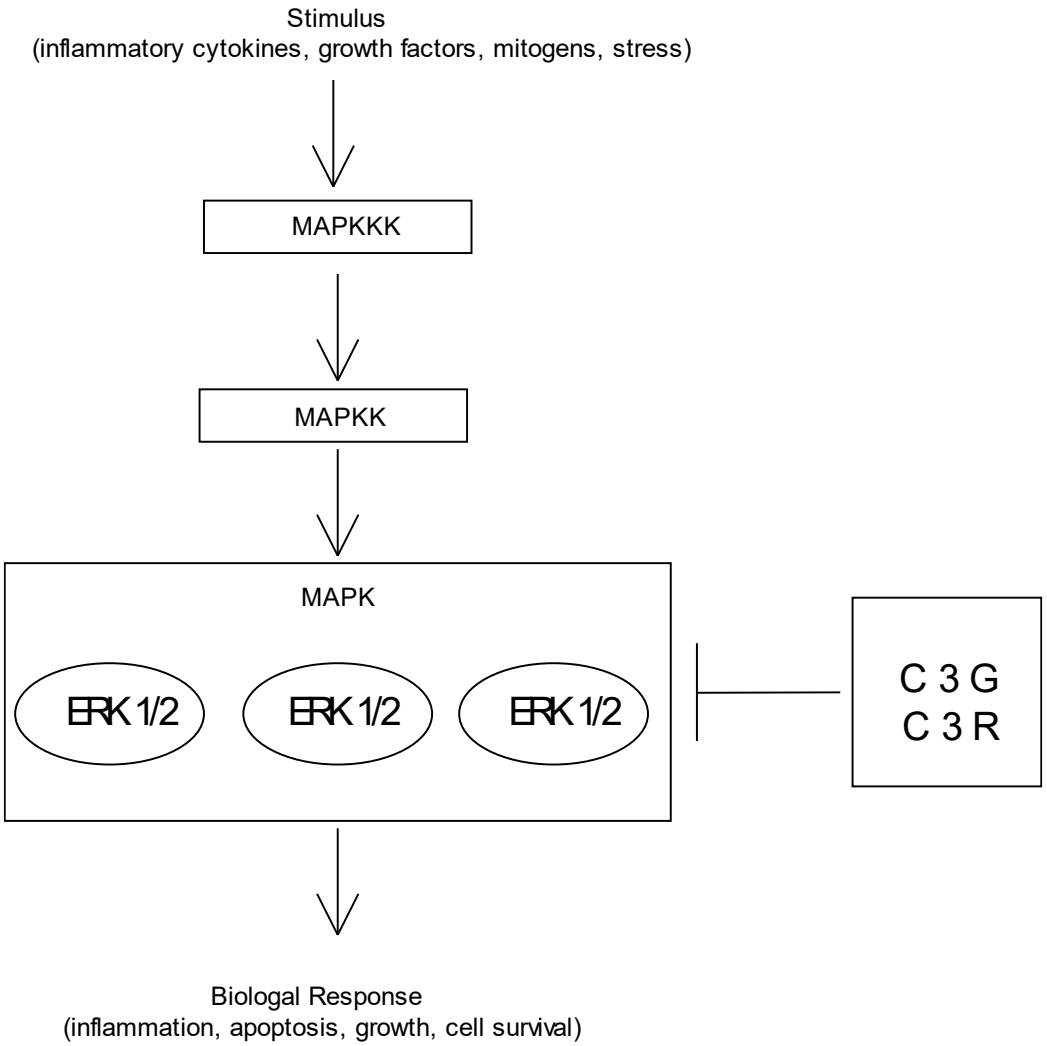


Figure 4. MAPK mechanism. Tart cherry compounds including cyanidin-3-glucoside and cyanidin-3-rutinoside can influence the MAPK pathway.

2.3.2.3 Arachidonic Acid Signaling Pathway

Arachidonic acid (AA) is a fatty acid, and its free form can be metabolized by four possible enzymatic pathways, including cyclooxygenase, lipoxygenase, cytochrome p450, and anandamide[93]. The COX pathway includes COX-1 and COX-2 as well as enzymes that help produce prostaglandins[93].

Jung et al.,[87] found that C3G and C3R significantly reduced H2O2-induced cytotoxicity in RAW 264.7 murine macrophage cells and ROS and DNA damage as compared to the control. These compounds also suppressed LPS-induced PGE2 production by over 50 % in a dose-dependent manner. Both compounds also inhibited COX-2 expression. The researchers found that C3R and C3G exhibited similar antioxidant and anti-inflammatory activities, suggesting that both anthocyanins are physiologically potent.

2.3.2.4 NADPH pathway

NOX genes produce transmembrane proteins that can cause oxygen to be reduced to superoxide[94]. NOX produces ROS, which can reduce the bioavailability of NO[94].

When ROS production exceeds the antioxidant defense system capacity, there is oxidative stress and damage to lipids, proteins, and DNA[87,95]. Examples of

ROS include dioxide (O2), H_2O_2, NO, and hydroxide (OH), which are constantly produced in aerobic cells[87,96]. Macrophages have an important role in the regulation of inflammation due to their production of NO, PGE2, and cytokines. Jung et al.,[87] found that C3G had a significantly higher nitrite-scavenging activity than did C3R at the same concentration. When RAW 264.7 murine macrophage cells were treated with anthocyanins, they suppressed the H_2O_2-induced cytotoxicity and DNA fragmentation. Both anthocyanins significantly reduced the production of NO and downregulated iNOS expression. The researchers concluded that the antioxidant effects of C3G and C3R may be related to the reduction of intracellular ROS production, inflammatory mediators, and DNA damage.

2.3.3 Polyphenol metabolites, bioavailability, and pharmacodynamics

Anthocyanins may contribute to the positive health effects of TC, but they are typically believed to have low bioavailability in the body, in ranges of 2% or less[97]. Processing and storage may reduce the biological activity and bioavailability of anthocyanins due to the formation of anthocyanin derivatives[39]. If parent compounds, in addition to metabolites and conjugated products of anthocyanins, are considered, however, the bioavailability may be greater[97]. The blood carries

nutrients to target organs, and, thus, the concentrations of anthocyanins and their metabolites in the plasma may be important for its physiological effects[97].

After food consumption, normal processes in the body can produce ROS, which contribute to reduced antioxidant capacity and the pathogenesis of chronic diseases[98]. Seymour et al.[98] found that TC antioxidant capacity in the plasma and urine can be detected even 12 h after consumption of 45–90 cherries. They conducted a crossover clinical trial study in which 12 healthy subjects were fed 45 or 90 individually quick-frozen TCs, and biological samples were collected over 12 h. At the 45-TC dose, there was a significant increase in mean trolox equivalent antioxidant capacity (TEAC) as compared to baseline detected at hours 1 and 12[98]. The TEAC for this dose reached its lowest point at hour 6, which corresponds to the elimination half-life of parent anthocyanins in plasma and with peak urinary excretion of anthocyanin metabolites. The consumption of 90 whole TCs resulted in significant increases in TEAC as compared to baseline at hours 1, 8, and 12[98]. This dose produced a positive AUC, indicating that the antioxidant capacity was significant throughout the study and counteracted ROS generation by food consumption. A delayed increase in antioxidant effects was observed 8–12 h after the consumption of both TC doses, indicating that active metabolites may have been produced by gut flora and reabsorbed into the systemic circulation.

Cyanidin and peonidin are major anthocyanin compounds in TC[39]. In a study by de Ferrars et al.[99], eight healthy male participants were fed a 500 mg bolus of 13C-labeled C3G and serum was collected and analyzed over a 48 -h period. A total of 17 13C-labeled compounds were detected in the serum, and the maximal concentrations of these compounds were seen 2–30 h post- consumption. Protocatechuic acid (PCA) and its metabolites, including vanillic acid (VA) and hippuric acid were the most abundant metabolites detected in the serum.

Keane et al.[100] investigated the time course of phenolic compounds in the body, following TC concentrate consumption, and determined the effect of phenolic compounds on vascular smooth muscle cell (VSMC) behavior. This double-blind, crossover study included 12 males with a mean age of 26 ± 3 years, who consumed 30 mL or 60 mL of TC concentrate. Blood was collected at baseline and 1, 2, 3, 4, and 8 h post-consumption. Levels of the phenolics chlorogenic acid (CHL), PCA, and VA were studied because these are the most abundant degradation products of the anthocyanins cyanidin and peonidin, which are detected in whole TC and concentrate[100]. The results indicated that both PCA and VA were most bioavailable in the plasma 1–2 h after Montmorency TC consumption. CHL was not detected in plasma post-TC consumption. When PCA and VA were separately applied to VSMC in vitro, there were no significant increases in migration behavior as compared to the control group. When PCA and VA were added together, however,

the migration of VSMC significantly increased as compared to the control group, although there were no effects on cell proliferation. The results suggest that PCA and VA may be beneficial for vascular remodeling.

PCA has been found to inhibit LPS-induced production of inflammatory markers in BV2 microglia cells, including TNF-α, IL-1β, IL-6, and PGE2, in a dose-dependent manner[101]. PCA also inhibited NF-κB p65 and IκBα phosphorylation and inhibited activation of the MAPKs p38, JNK, and ERK[101]. VA has been found to reduce LPS-induced inflammatory markers, such as TNF-α and IL-6, in mouse peritoneal macrophages[102]. It also was found to suppress COX-2 levels, PGE2, and NO production. VA reduced IκBα degradation and reduced Rel/p65 levels in the nucleus. Results of these studies suggest that PCA and VA are active TC metabolites that may contribute to their anti-inflammatory effects.

2.4 Conclusion

This review provides evidence that TC or its bioactive components can modulate several inflammatory cytokines and cellular and molecular targets in inflammatory signaling pathways, and thus may be considered an agent for the management of inflammatory diseases and associated symptoms. TC was shown to reduce SBP, hsCRP, TNF-α, IL-6 and other markers of inflammation and oxidative

stress, demonstrating promise for the management of inflammation associated with metabolic syndrome in adults. TC may also play a role in the reduction of pain associated with inflammatory diseases such as osteoarthritis and neuropathy, increases in antioxidative enzymes such as SOD, and improvements in cognitive functioning including improved memory and reduced movement time.

The effects of TC are clear in various diseases and models, including human, animal, and cell. These anti-inflammatory effects may be due to the bioactive compounds in TC, as studies have shown that the major compounds in TC affect inflammatory signaling pathways. Additionally, when TC is metabolized, the breakdown products also may affect these pathways. TC has potential as a therapeutic for inflammatory diseases and associated risk factors.

Based on the collective evidence of these studies, TC supplementation provides various health benefits when consumed over the course of a few weeks to a few months. Most of the studies included in this review provided participants TC concentrate or juice supplementation. Translation of this information to a dietary supplement in pill form would require further research into how this would affect the anthocyanin content and absorption in the body. Other considerations include long term impacts of daily supplementation, and the shelf-life of these supplements as the breakdown of anthocyanins can result from light exposure or moisture from storage.

Chapter 3

WHOLE FRUIT AND ADDED SUGAR INTAKE IS ASSOCIATED WITH BLOOD PRESSURE

3.1 Introduction

Hypertension affects one in three American adults and increases the risk for CVD, including heart disease, stroke, and heart failure, as well as non-cardiovascular conditions, including kidney disease and vision loss[1,103]. Although hypertension is common among adults of all ages, the prevalence of hypertension is significantly higher in adults aged 60 and over[104]. For years, a systolic BP of ≥140 mmHg and a diastolic BP of ≥90 mmHg were considered the threshold for hypertension[105]. New BP guidelines, however, classify hypertension as systolic BP ≥130 mmHg or diastolic BP ≥80 mmHg. This change was enacted due to evidence that shows a risk of CVD at lower BP values[106].

Common treatments for high BP include angiotensin-converting enzyme inhibitors, beta-blockers, calcium antagonists, and diuretics. The number of prescriptions for antihypertensive medications increased from 613.7 million in 2010 to 653 million in 2014, with antihypertensive medication costs exceeding $28 billion

in 2014[106,107]. It is projected that the total direct costs of hypertension will increase to about $220.9 billion by 2035[106,108]. Administration of antihypertensive agents for a few years to individuals with prehypertension may delay or prevent the transition to hypertension[109], however, lifestyle modifications are generally recommended by physicians as the first step towards BP management. In addition, taking hypertensive medications regularly may cause side effects, including a dry cough, dizziness, nausea, bradycardia, peripheral edema, and insomnia. These side effects, coupled with the fact that 84% of adults over the age of 57 are already taking at least one prescription medication per day, warrant the need for early dietary and lifestyle changes to lower BP[110].

Adherence to the Dietary Approaches to Stop Hypertension (DASH) eating plan is effective in the control or reduction of BP[111,112]. The DASH diet emphasizes the consumption of fruits, vegetables, low-fat foods, dairy, whole grains, and lean meat while limiting sodium intake to 2300 mg or less per day. The Mediterranean diet, which has been shown to reduce BP, is also popular among heart-healthy diets. The Mediterranean diet encourages the consumption of fish, unsaturated fats, whole grains, legumes, nuts, vegetables, and fruits[113]. These BP-lowering diets are proven strategies but require a long-term commitment and significant lifestyle changes, which may be difficult to maintain for some individuals. Some studies suggest that a small change in diet can have a significant impact on BP and cardiovascular health.

The impact of dietary factors on BP in older adults, however, is not clear. In this study, we sought to understand the dietary characteristics of older adults and to examine the association between dietary factors and BP in this population.

3.2 Materials and methods

3.2.1 Study Design and Participants

This cross-sectional study was conducted at the University of Delaware between 2015 and 2017. Males and females of diverse races and ethnicities who live in Newark, Delaware, and the surrounding areas were recruited. The eligibility criteria included males and females between the ages of 65 and 80 years who did not have a history of cancer, gastrointestinal disease, traumatic brain injury, stroke, diabetes, central nervous system disorders, Alzheimer's, dementia, or psychiatric illness. A total of 284 individuals were screened by phone, and 128 qualified individuals (57 males and 71 females) were invited to the Nurse Managed Primary Care Center for an in-person visit. During this visit, participants were asked to complete medical history, demographic, physical activity, and food frequency questionnaires. Anthropometrics and BP also were collected. BP data was available for 127 individuals (57 males and 70 females). This study was approved by the

University of Delaware Institutional Review Board. Informed consent was obtained from all participants prior to enrollment in the study.

3.2.2 Blood Pressure and Anthropometric Measurements

Participants were asked to sit quietly in a room for 5 minutes before BP was measured. A trained researcher measured the participants' arm to determine proper cuff size. Participants were asked to remain silent during BP readings. Two readings were taken on a digital BP monitor (HEM-907XL, Omron Healthcare, Inc., Lake Forest, IL, USA), and the average of the two was recorded. Mean arterial pressure (MAP) was derived from systolic and diastolic BP. MAP is defined as the average of arterial pressure during a single cardiac cycle and is calculated as follows: Diastolic BP + 1/3 pulse pressure, where the pulse pressure is the difference between the systolic and diastolic BP. Participants were asked to change into scrubs and to take off their shoes for the anthropometric measurements. Weight was measured using a digital scale and recorded in both pounds and kilograms. Height was measured in centimeters, using a stadiometer. Participants were asked to stand with their feet and back against the wall. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared.

3.2.3 Blood Pressure Classifications

Participants were considered to have (1) normal BP if their systolic BP was <120 mmHg and diastolic BP was <80 mmHg; (2) elevated BP if systolic BP was 120–129 mmHg and diastolic BP was <80 mmHg, and (3) high BP if systolic BP was ≥130 mmHg or diastolic BP was ≥80 mmHg[105]. If systolic and diastolic BP readings fell into different categories, the individual was considered to be a part of the higher BP category.

3.2.4 Diet, Physical Activity, and Demographic Questionnaires

The electronic form of the 2005 Nutrition Quest Block Food Frequency Questionnaire (FFQ) was used to assess the average daily intake of food, beverages, and supplements (Nutrition Quest, Berkeley, CA, USA). Approximately 110 food items were included, and each food item was accompanied by questions about the frequency of consumption and serving sizes. Frequency questions concerned intake year-round and accounted for seasonal food intake. This questionnaire was administered by a trained researcher. Measuring cups and spoons were provided as a reference for serving sizes. The Physical Activity Scale for the Elderly (PASE), designed to be used on individuals aged 65 and older, was used to determine physical activity in the participants[114]. The questionnaire contains items about the

individuals' leisure-time, household, and work-related activities performed over the past seven days. Various activities including reading, walking, dancing, gardening, and home repairs as well as volunteer or paid work. The demographic questionnaire was used to collect information about the participant's age, sex, education level, marital status, race/ethnicity, and income.

3.2.5 Statistical Analysis

All analyses were performed using the SPSS statistical software package, version 25.0 (IBM SPSS Inc., Chicago, IL, USA). Descriptive statistics are reported as means ± standard deviations for continuous variables, and percentages and frequencies, for categorical variables. Independent samples *t*-tests were used to compare continuous variables, and chi-square tests were used to compare categorical variable between males and females. Multiple linear regressions were used to determine the associations between dietary factors and BP in the overall population as well as split by gender. Model 1 (overall population) was adjusted for sex, age, income, total calorie intake, BMI, PASE scores, and BP medication use. Model 2 (split by sex) was adjusted for age, income, total calorie intake, BMI, PASE scores, and BP medication use. The independent variables in the models were normally distributed and did not have any outliers that were of concern. Effect sizes were

calculated as f^2 (effect size for independent variable) = squared semi partial (part) correlation coefficient for independent variable ÷ 1 − squared multiple correlation coefficient for the full model (R^2). The effect sizes were interpreted as follows: $f^2 <$ 0.02 small effect, 0.15 medium effect, and 0.35 large effect[115]. The significance level was set at $p < 0.05$.

3.3 Results

3.3.1 Participant Characteristics, Anthropometrics Measurements, and Demographics

Participant characteristics, including anthropometric and demographic data, are presented in **Table 1**. There were no significant differences between males and females in terms of age, BMI, anti-hypertensive medication use, education level, race, employment, or smoking status. The mean age was 70.8 ± 4.1 years and 70.6 ± 4.0 years for males and females, respectively. For BMI, the average participant was overweight, regardless of gender (29.1 ± 5.1 kg/m^2; $p = 0.85$). Of the participants, 32.8% held a graduate or professional degree, 86.7% were White, 73.4% were retired, 98.4% were not current smokers, and 69.5% were married. There were statistically significant differences between males and females in terms of height (173.8 ± 7.6 cm for males and 161.5 ± 5.4 cm for females, $p < 0.001$), weight (88.2

± 14.7 kg for males and 75.7 ± 15.7 kg for females, $p < 0.001$), income (45.6% of males and 22.5% of females earned $75,000 or more, $p = 0.025$), and marital status (91.2% of males and 52.1% of females were married, $p < 0.001$).

Variable	Total (*n* = 128)	Males (*n* = 57)	Females (*n* = 71)	*p*-value
	Mean ± SD			
Age (years)	70.7 ± 4.0	70.8 ± 4.1	70.6 ± 4.0	0.751
BMI (kg/m^2)	29.1 ± 5.1	29.2 ± 4.3	29.0 ± 5.7	0.848
Height (cm)	167.0 ± 8.9	173.8 ± 7.6	161.5 ± 5.4	0.000*
Weight (kg)	81.2 ± 16.4	88.2 ± 14.7	75.7 ± 15.7	0.000*
	n (%)			
Education level				0.587
High school/some college	41 (32)	21 (36.8)	20 (28.2)	
2-year degree	11 (8.6)	5 (8.8)	6 (8.5)	
4-year degree	34 (26.6)	12 (21.1)	22 (31.0)	
Graduate/professional degree	42 (32.8)	19 (33.3)	23 (32.4)	
Income				0.025*
Under $25,000	11 (8.6)	1 (1.8)	10 (14.1)	
$25,000–$49,999	20 (15.6)	8 (14.0)	12 (16.9)	
$50,000–$74,999	36 (28.1)	15 (26.3)	21 (29.6)	
$75,000–$99,999	9 (7.0)	4 (7.0)	5 (7.0)	
$100,000+	33 (25.8)	22 (38.6)	11 (15.5)	
Prefers not to say	19 (14.8)	7 (12.3)	12 (16.9)	
Race/Ethnicity				0.761
White	111 (86.7)	49 (86.0)	62 (87.3)	
Black or African American	7 (5.5)	3 (5.3)	4 (5.6)	
Asian	5 (3.9)	2 (3.5)	3 (4.2)	
Other	3 (2.3)	2 (3.5)	1 (1.4)	
Prefers not to say	2 (1.6)	1 (1.8)	1 (1.4)	
Marital status				0.000*
Single/never married	6 (4.7)	0 (0)	6 (8.5)	
Separated/divorced	25 (19.5)	3 (5.3)	22 (31.0)	
Married	89 (69.5)	52 (91.2)	37 (52.1)	
Widowed	7 (5.5)	1 (1.8)	6 (8.5)	

Living with someone	1 (0.8)	1 (1.8)	0 (0)	
Employment status				0.955
Retired	94 (73.4)	42 (73.7)	52 (73.2)	
Working	34 (26.6)	15 (26.3)	19 (26.8)	
Smoking Status				0.382
Current smoker	2 (1.6)	2 (3.5)	0 (0)	
Does not smoke	126 (98.4)	55 (96.5)	71 (100)	
Anti-hypertensive medication use				0.112
No medication	56 (44.1)	20 (35.1)	36 (51.4)	
1–3 medications	71 (55.9)	37 (64.9)	34 (48.6)	

Table 1. Characteristics of the study sample. Note: Values are mean ± SD for continuous variables and *n* (%) for categorical variables; income is reported in U.S. dollars ($). *$p < 0.05$. n=127 for anti-hypertensive medication use (M= 57, F= 70).

3.3.2 Blood Pressure, Physical Activity, and Dietary Characteristics

Table 2 shows that systolic BP (143.3 ± 17.1 mmHg for males and 130.6 ± 23.2 mmHg for females, $p = 0.001$), MAP (100.3 ±12.4 for males and 94.6 ± 16.2 mmHg for females, $p = 0.029$), and PASE scores (157.5 ± 69.0 for males and 118.9 ± 45.4 for females, $p < 0.001$) was statistically higher in males than in females. Diastolic BP was similar among male and female participants (78.9 ± 12.6 mmHg and 76.6 ± 14.1 respectively, $p = 0.34$). In terms of diet, daily calorie intake (1687.7 ± 707.5 kcals for males and 1510.6 ± 529.3 for females, $p = 0.12$), and most of the dietary intake was similar between sexes. Males, however, consumed more juice (0.4 ± 0.5 cups for males and 0.2 ± 0.3 cups for females, $p = 0.041$) and alcohol (1.3 ± 2.1 drink equivalents for males and 0.5 ± 1.1 drink equivalents for females, $p = 0.009$), whereas females consumed higher servings of vegetables (3.2 ± 1.9 servings for males and 4.4 ± 2.2 servings for females, $p = 0.001$).

Variable	Total (n = 127)	Males (n = 57)	Females (n = 70)	p-value
Systolic BP, mmHg	136.3 ± 21.6	143.3 ± 17.1	130.6 ± 23.2	0.001*
Diastolic BP, mmHg	77.6 ± 13.4	78.9 ± 12.6	76.6 ± 14.1	0.341
MAP, mmHg	97.2 ± 14.9	100.3 ± 12.4	94.6 ± 16.2	0.029*
Physical activity (PASE score)	136.1 ± 60.1	157.5 ± 69.0	118.9 ± 45.4	0.000*
Diet				
Total energy (kcal)	1589.5 ± 618.8	1687.7 ± 707.5	1510.6 ± 529.3	0.120
Protein (g)	64.1 ± 27.0	67.5 ± 32.0	61.4 ± 22.0	0.228
Carbohydrates (g)	181.4 ± 75.1	193.8 ± 87.8	171.5 ± 62.0	0.109
Fat (g)	66.9 ± 29.6	68.8 ± 31.4	65.4 ± 28.2	0.519
Saturated fat (g)	20.4 ± 10.0	21.2 ± 10.5	19.7 ± 9.7	0.422
Monounsaturated fat (g)	26.3 ± 11.4	26.7 ± 11.8	26.0 ± 11.1	0.741
Polyunsaturated fat (g)	15.1 ± 7.1	15.4 ± 7.7	15.0 ± 6.6	0.754
Trans fat (g)	1.7 ± 1.0	1.9 ± 1.1	1.6 ± 0.9	0.117
Dietary cholesterol (mg)	226.4 ± 126.8	248.1 ± 145.1	208.9 ± 107.9	0.093
Fiber (g)	17.9 ± 7.6	17.6 ± 8.6	18.1 ± 6.6	0.760
Sodium (mg)	2667.8 ± 1061.5	2747.1 ± 1176.5	2604.2 ± 963.2	0.461
Potassium (mg)	2691.4 ± 988.1	2702.9 ± 1119.9	2682.2 ± 876.2	0.910
Alcoholic intake, drink equivalents	0.9 ± 1.6	1.3 ± 2.1	0.5 ± 1.1	0.009*
Vegetables, serving	3.9 ± 2.1	3.2 ± 1.9	4.4 ± 2.2	0.001*
Total fruit (cup)	1.4 ± 0.8	1.4 ± 0.9	1.3 ± 0.7	0.416
Whole fruit (cup)	1.0 ± 0.7	1.0 ± 0.8	1.1 ± 0.7	0.788
Juices (cup)	0.3 ± 0.4	0.4 ± 0.5	0.2 ± 0.3	0.041*
Dairy (serving)	1.1 ± 0.8	1.2 ± 0.8	1.0 ± 0.9	0.428
Grains (serving)	3.5 ± 1.9	3.8 ± 1.9	3.2 ± 1.9	0.109
Fats (serving)	2.8 ± 1.5	2.7 ± 1.5	2.8 ± 1.5	0.722
Meat (serving)	2.0 ± 1.0	2.2 ± 1.2	1.9 ± 0.9	0.096
Added sugar (tsp)	9.1 ± 6.1	10.2 ± 7.5	8.3 ± 4.6	0.096
Fructose (g)	20.2 ± 11.1	21.7 ± 13.0	19.0 ± 9.2	0.200
Lactose (g)	9.2 ± 8.9	10.3 ± 9.0	8.3 ± 8.8	0.209
Maltose (g)	2.0 ± 1.0	2.0 ± 1.1	2.0 ± 0.9	0.976
Galactose (g)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.522
Sucrose (g)	26.6 ± 20.3	29.4 ± 25.8	24.3 ± 14.3	0.190
Glucose (g)	17.7 ± 9.5	19.0 ± 11.5	16.7 ± 7.4	0.192
Sweets and desserts (% daily kcals)	10.7 ± 8.3	11.6 ± 9.8	10.0 ± 6.8	0.290

Table 2. Blood pressure, physical activity, and dietary intake among males and females. Note: Values are reported as mean ± SD for continuous variables and n (%) for categorical variables. *$p < 0.05$.

3.3.3 Association Between Dietary Factors and Blood Pressure

The results of the regression analysis for the total sample and males and females separately are shown in **Table 3**. No significant associations were found between dietary factors and systolic BP when both males and females were included in the model. Whole fruit consumption, however, was associated with diastolic BP in both males and females ($\beta = -0.210$, $p = 0.040$; 95% CI = −7.7, −0.2). For every 0.71 cup increase in whole fruit consumption, the model predicted a 2.8 mmHg decrease in diastolic BP, when holding all other variable values in the model constant. When the model was split by sex, there was a significant association between intake of added sugar and systolic ($\beta = 0.721$, $p < 0.001$; 95% CI = 1.7, 5.6) and diastolic ($\beta = 0.514$, $p = 0.011$; 95% CI = 0.4, 2.8) BP in females after controlling for age, income, BMI, physical activity levels, daily calorie intake, and anti-hypertensive medication use. According to this model, a 2.3 teaspoon decrease in added sugar intake results in an 8.4 mmHg drop in systolic BP and a 3.7 mmHg drop in diastolic BP in females.

Table 3. Regression analysis of associations among food groups and systolic and diastolic blood pressure (BP).

Food Group	Total (n = 127)[a]				Males (n = 57)[b]				Females (n = 70)[b]			
	B	p-value	95% CI	Effect Size	B	p-value	95% CI	Effect Size	B	p-value	95% CI	Effect Size
Vegetables (serving)												
Systolic	−0.084	0.477	−3.196, 1.502	0.005	−0.183	0.368	−5.442, 2.056	0.019	−0.006	0.967	−3.360, 3.225	0.000
Diastolic	−0.031	0.792	−1.683, 1.287	0.001	−0.140	0.435	−3.369, 1.476	0.015	−0.113	0.486	−2.783, 1.341	0.009
Grain (serving)												
Systolic	−0.074	0.595	−3.889, 2.241	0.003	−0.259	0.262	−6.470, 1.806	0.030	−0.097	0.647	−6.252, 3.917	0.004
Diastolic	−0.048	0.732	−2.273, 1.601	0.001	0.055	0.787	−2.313, 3.034	0.002	−0.204	0.352	−4.677, 1.692	0.016
Meat (serving)												
Systolic	0.045	0.779	−5.698, 7.589	0.001	0.154	0.597	−6.337, 10.892	0.007	−0.066	0.750	−12.559, 9.103	0.002
Diastolic	−0.073	0.651	−5.160, 3.238	0.002	−0.227	0.376	−8.032, 3.099	0.019	−0.133	0.533	−8.908, 4.659	0.007
Dairy (serving)												
Systolic	0.076	0.488	−3.578, 7.448	0.004	0.100	0.586	−5.519, 9.639	0.007	−0.172	0.343	−14.358, 5.077	0.016
Diastolic	0.026	0.817	−3.076, 3.893	0.000	0.257	0.118	−1.027, 8.766	0.060	−0.295	0.116	−10.938, 1.235	0.045
Fat (serving)												
Systolic	0.134	0.293	−1.688, 5.552	0.010	0.067	0.754	−4.189, 5.742	0.002	−0.033	0.865	−6.428, 5.415	0.001
Diastolic	0.126	0.331	−1.160, 3.416	0.008	−0.098	0.602	−4.043, 2.373	0.006	0.000	0.998	−3.712, 3.705	0.000
Added sugar (tsp)												
Systolic	0.183	0.175	−0.292, 1.580	0.017	−0.035	0.883	−1.175, 1.014	0.000	0.721	0.000*	1.729, 5.562	0.259
Diastolic	0.201	0.143	−0.151, 1.031	0.020	0.267	0.210	−0.261, 1.153	0.038	0.514	0.011*	0.379, 2.780	0.124
Whole fruit (cup)												
Systolic	−0.113	0.262	−9.394, 2.581	0.011	−0.219	0.199	−12.241, 2.620	0.040	−0.087	0.509	−12.352, 6.199	0.008
Diastolic	−0.210	0.040*	−7.747, −0.178	0.039	−0.268	0.076	−9.125, 0.477	0.078	−0.194	0.157	−9.973, 1.645	0.037

Table 3. Regression analysis of associations among food groups and systolic and diastolic blood pressure (BP). [a]. Sex, age, income, total calorie intake, body mass index (BMI), Physical Activity Scale for the Elderly (PASE), and BP medication use were controlled for; [b]. Age, income, total calorie intake, BMI, PASE, and BP medication use were controlled for; B = standardized coefficient beta. * $p < 0.05$.

3.3.4 Blood Pressure Categories of Participants

Of the total sample (n = 127), 55.9% were taking between one and three anti-hypertensive medications. Among the BP medication users, 71.8% still had BP readings consistent with the high BP category, indicating that most anti-hypertensive medication users were still at high risk for CVD **(Table 4)**.

BP Category			
2017 AHA/ACC	**Total (*n* = 127)**	**Males (*n* = 57)**	**Females (*n* = 70)**
Normal	19 (15)	4 (7)	15 (21.4)
Elevated	9 (7)	4 (7)	5 (7.1)
High	99 (78)	49 (86)	50 (71.4)
Hypertension control among treated individuals[a]	**Total (*n* = 71)**	**Males (*n* = 37)**	**Females (*n* = 34)**
Normal	7 (9.9)	1 (2.7)	6 (17.6)
Elevated	13 (18.3)	2 (5.4)	11 (32.4)
High	51 (71.8)	34 (91.9)	17 (50)

Table 4. Participants categorized by blood pressure. Values are *n* (%); [a.] Blood pressure control for those being treated with anti-hypertensive medications. Participants were considered to have normal BP if their systolic BP was <120 mmHg and diastolic BP was <80 mmHg; elevated BP, if systolic BP was 120–129 mmHg and diastolic BP was <80 mmHg; and high BP, if systolic BP was ≥ 130 mmHg or diastolic BP was ≥80 mmHg.

3.3.5 Predicted Changes in Percentage of Population with High Blood Pressure

Our regression model predicted that decreasing added sugar intake results in an 8.4 mmHg drop in systolic BP and a 3.7 mmHg drop in diastolic BP in females, regardless of anti-hypertensive medication use. If females consume 2.3 teaspoons less added sugar, we predicted that 34.3% of females would have high BP readings, indicating a 12.9% drop in the percentage of females with hypertension readings and a 24.3% increase in the percentage of women with normal BP readings.

3.4 Discussion

We conducted a cross-sectional study to determine the associations between dietary factors and BP in older adults. Our analysis showed that 78% of the participants had hypertension, a percentage greater than the national prevalence of 71.8% in adults aged 60 and older[116]. This percentage also was above the Delaware hypertension prevalence rate of 61% in adults aged 65 and older[117]. The prevalence of hypertension in Delaware, however, is based on behavioral risk factor surveillance system data, which includes self-reported hypertension data; thus, actual values may be higher, as individuals with undiagnosed hypertension may not be accounted for.

Our regression model found a direct relationship between added sugar intake and both systolic and diastolic BP in females. The association between added sugar intake and BP remained significant even after controlling for typical factors that can affect BP, such as BMI, physical activity, total calorie intake, age, and anti-

hypertensive medication use. Consistent with our findings, other studies show a significant link between added sugar intake and hypertension[118]. In a meta-analysis, higher sugar intakes significantly increased systolic BP by 7.6 mmHg and diastolic BP by 6.1 mmHg[119]. In a study by Raben et al., the 10-week consumption of sucrose resulted in a 3.8 mmHg increase in systolic and 4.1 mmHg increase in diastolic BP[120].

In this study, most participants consumed about 10% or more of their daily calories from added sugar, with a mean intake of 9.1 teaspoons of added sugar per day and no significant difference in intake between males and females. The 2015 Dietary Guidelines for Americans (DGA) recommends that added sugar intake should be less than 10% of daily calories (200 calories for a 2000 calorie diet)[121]. Further, the American Heart Association (AHA) recommends restricting added sugar consumption to no more than half of one's daily discretionary calorie allowance, which is about 6 teaspoons (100 kcal) for females and 9 teaspoons (150 kcal) for males[122]. The DASH diet for heart health puts a more stringent limitation on added sugar intake, at three servings or less per week, equivalent to 9 teaspoons/week for an individual who follows a 1600-kcal diet. An analysis of the National Health and Nutrition Examination Survey (NHANES) 2013–2014 data revealed that only 42% of Americans aged 2 and over met the DGA recommendations[121]. Our findings showed that sugar intakes in this population were above the DGA, AHA, and DASH dietary guidelines.

A study that used NHANES data found that the main sources of added sugar in adults aged 50 and over were soda, desserts, and candy[123]. One 12-oz can of regular

soda contains about 39 g of sugar, equivalent to about 9.3 teaspoons of added sugar, which is above both the AHA and DASH guidelines. Our analysis suggests that reducing added sugar intake by 2.3 teaspoons, or about one-fourth of a can of soda, would significantly reduce both systolic and diastolic BP in females. This change in added sugar intake could potentially reduce the percentage of females with hypertension in our study from 47.1% to 21.4%.

Sucrose, glucose, and fructose were the main sources of dietary sugars in this population. Sucrose, or table sugar, is a disaccharide composed of equal parts glucose and fructose. In a study by Bunag et al.,[124] rats were given a sucrose solution instead of water to drink, and after 5 weeks, their systolic BP was elevated. This was thought to be due to overactivity of the sympathetic system in response to sucrose consumption. Glucose is a simple sugar that plays important roles in the body. It is also commonly found in syrups, candy, sports drinks, and desserts. Studies have shown that excess glucose may influence BP. A study conducted by Barbagallo et al.[125] demonstrated that excess glucose concentrations could significantly raise cytosolic free calcium concentrations in vascular smooth muscle cells in a dose and time dependent manner. Increases in vascular smooth muscle calcium concentrations have been associated with vasoconstriction and vascular resistance, which can increase BP[126]. Fructose is commonly consumed in the diet as high fructose corn syrup (HFCS). HFCS is produced by the isomerization of glucose to fructose, producing an inexpensive corn-based syrup that is sweeter than both sucrose and glucose. Fructose is a nonessential sugar that is found naturally in some foods, including fruit. It is also a

major constituent of many sugar-sweetened beverages and food items and comprises a large portion of dietary fructose[127].

Studies suggest that high fructose consumption has adverse effects on body composition and BP, but the mechanisms by which fructose stimulates hypertension are still unknown. One particular mechanism that may affect the reduction in urinary sodium excretion could be the impact of fructose on angiotensin II. Angiotensin II increases aldosterone production, which promotes sodium retention by the kidneys, leading to hypertension[128]. Farah et al.[129] observed the impacts of a high fructose diet in nocturnal mice. Mice consumed a high fructose diet for 8 weeks, and changes were seen only at night, a period of activity for mice. The researchers found that fructose increased nocturnal BP and plasma angiotensin II. In addition, responses to alpha-adrenergic blockades were augmented in fructose-fed mice, indicating an increase in sympathetic nerve activation. This increase in plasma angiotensin II in conjunction with sympathetic activation suggests that fructose activates a sympathetic pathway and may stimulate aldosterone production, causing sodium retention and a subsequent increase in BP. Another potential mechanism by which fructose could stimulate decreased urinary sodium excretion is through its interactions with salt absorption in the small intestines and the kidney tubules, through the fructose transporter Glut5. In another animal study, fructose-fed rats were found to have a reduction in urinary sodium excretion by the kidneys, which resulted in hypertension[130]. Urinary sodium excretion, however, did not decrease in mice that had a knockout of the Glut5

transporter, suggesting that Glut5 was the primary mechanism by which salt absorption was stimulated during a high fructose diet.

Collective evidence also suggests that diets high in added sugar promote body weight and fat gain, which can lead to metabolic syndrome, oxidative stress, and a dysregulation of lipid and carbohydrate metabolism. Research has shown that the main driving force of metabolic syndrome is insulin resistance, which is associated mainly with poorly patterned eating and the dramatic rise in obesity, diabetes, and CVD[131]. In previous years, metabolic syndrome was attributed to the overconsumption of fat in the Western diet. Recent studies, however, suggest that metabolic diseases can be largely attributed to the overconsumption of added sugars[131–134]. A meta-analysis by Te Morenga el al.[135] found that an increased intake of dietary sugars was significantly associated with increased body weight when adults consumed ad libitum diets. Another meta-analysis by Te Morenga et al.[119] reported that a high-sugar diet was associated with an increase in lipid profiles. These associations between sugar consumption and lipid concentrations occurred most consistently in studies that did not report significant weight changes. In the same study, they found that increased sugar consumption was significantly associated with BP, especially in trials lasting ≥8 weeks as evidenced by an increase in systolic and diastolic BP by 6.9 mmHg and 5.6 mmHg, respectively. Thus, diets high in added sugar promote changes in BP and lipid profiles and potentially increase CVD risk through the mechanisms of both body weight gain and metabolic syndrome.

It is interesting to note that the associations between added sugar intake and BP were significant in females but not in males. Studies show that a high fructose or sucrose diet can increase BP, with a greater increase generally occurring in male rodents[136,137]. However, Galipeau et al.[137] found that sex hormones play a role in response to a fructose diet in females. For instance, there were no significant differences between the female fructose-fed and control rats for BP after 9 weeks of 60% fructose consumption. In contrast, in the male fructose-fed rats, BP rose by the third week and continued to increase throughout the study compared to the male controls. In comparison, they looked at the effects of sex hormones on BP by comparing four groups of female rats: Control, fructose diet, ovariectomized (Ovx), and Ovx with fructose diet, and found that only the Ovx rats with fructose diets had a significant increase in BP. This suggested that female rats might have protection from fructose induced hypertension compared to male rats. However, when the female rats lose ovarian sex hormones through an Ovx, they also have increases in BP. Similarly, older women tend to have low levels of estrogen due to menopause, therefore this may explain why added sugar consumption was significantly associated with BP in females and why a reduction in added sugar has the potential to reduce BP levels in older women but not in men.

The present study also determined that increasing the consumption of whole fruit reduced diastolic BP in both males and females. In a 6-month dietary intervention study, educating participants to consume more fruits and vegetables led to a 1.4 ± 1.7 portion increase in fruit and vegetable intake and resulted in a mean 1.5 mmHg

reduction in diastolic BP and 4.0 mmHg reduction in systolic BP[138]. In addition, in a prospective cohort study, in which participants were followed up every 2 years over a span of 8 years, frequent fruit consumption (≥4 servings/ day) was associated with a 67% reduced incidence of hypertension in females and a 56% lower incidence in males as compared to the rates of infrequent consumers[139]. Clinical trial studies have shown that fruit such as grapes, tart cherries, and blueberries can reduce BP in adults[48,140,141].

Although the exact mechanisms of BP reduction by fruit are unknown, we do know that whole fruit contains fiber, vitamins, phytochemicals, and minerals that may contribute to their BP-lowering effects. In a study by Barone et al.,[140] grape polyphenol consumption for 30 days was found to reduce systolic BP in males with metabolic syndrome. The study also found a reduction in circulating inflammatory molecules and an improvement in brachial artery flow-mediated dilation response as compared to the placebo. The results of the study suggest that grape polyphenols may reduce BP by improving vascular endothelial function. The potassium content of fruit also may contribute to its BP-reducing properties[142]. In a meta-analysis by Whelton et al.,[143] potassium supplementation was associated with a 1.97 mmHg reduction in diastolic BP and a 3.11 mmHg reduction in systolic BP. We did not find any significant associations between the consumption of meat (defined as red meat, fish, poultry, beans, eggs, and other meats), vegetables, dairy, grains, or fat and BP in our sample. Other studies show mixed results in this regard. A cross-sectional study found an inverse association between consumption of low-fat dairy products and 24-hour

diastolic BP in older adults with hypertension[144]. Conversely, those who consumed seven or more servings of whole-fat dairy products per week had a 1.4 mmHg higher diastolic BP than did those who consumed less than one serving per week. In a study in which participants were followed up with every 2 years over the span of 8 years, there was no association between vegetable consumption and hypertension risk in middle-aged or older Korean adults[139]. In a prospective cohort study conducted with 28,926 females aged 45 and older, refined-grain intake was not associated with hypertension risk, although a high whole-grain intake was associated with a reduced risk of hypertension[145]. In an intervention study, high whole-grain (>80g/day) consumption for 6 weeks had no effect on BP[146].

This study has some limitations that need to be considered when interpreting our findings. A major limitation includes the small sample size of 128. In this study BP was measured twice at one visit. It has been suggested, however, that multiple readings over the course of two or more days result in more accurate BP determination. Additionally, it is important to note that most of the participants are White, and therefore the effects of added sugar and whole fruit consumption on BP might vary in other races due to genetic differences. This lack of diversity, in conjunction with the modest sample size, could limit the generalizability of these results. Due to the cross-sectional nature of this study, we could not assert causality. These findings are suggestive of potential BP reductions in women with reductions in added sugar and increases in solid fruit intake, however, clinical trial studies are necessary to confirm this. Therefore, although the findings regarding the effects of

added sugar consumption on BP in older women were novel and warrant further investigation, they should be considered highly preliminary. The strengths of the study include the use of a validated 110-item FFQ for dietary data collection.

3.5 Conclusions

Public health efforts to reduce hypertension prevalence among the elderly population should emphasize reducing added sugar consumption and increasing whole fruit consumption. Most of our participants consumed more added sugar than is seen in AHA and DASH recommendations. Further, considering that a majority of our sample was retired, an effort should be made to educate the older adult population about healthy eating and to make healthier foods affordable for individuals on a fixed income.

Chapter 4

THE IMPACT OF TART CHERRY AND ANGIOTENSIN-II ON INFLAMMATION IN HUMAN CORONARY ARTERY ENDOTHELIAL CELLS

4.1 Introduction

There is a strong relationship between hypertension and cardiovascular disease (CVD) risk[2,147]. Although about half of the U.S. adult population has hypertension, less than one-fourth of them have it under control[1]. Heart and blood vessel damage, common in a hypertensive state, can contribute to life threatening diseases including myocardial infarctions (MI) and strokes[1]. Risk factors for the pathogenesis of hypertension include aging, overactivity of the renin-angiotensin-aldosterone system (RAAS), inflammation, oxidative stress, and an unhealthy diet or lifestyl[3,19,147,148].

Consuming polyphenol-rich food or drinks has been shown to reduce blood pressure (BP) in adults [21,46]. Fruit, vegetables, and other plants contain various types and amounts of polyphenols that may provide health benefits[19]. Tart cherry (TC) consumption has been shown to reduce oxidative stress, inflammation, and other benefits[21,56,72,77]. This is thought to be due to the high amount of phytochemicals present in them[149]. In our laboratory, Chai and colleagues [21] have previously studied

the impact of 12- week TC juice consumption in older adults. Significant reductions in systolic BP, low-density lipoprotein (LDL) cholesterol, c-reactive protein, and an increase in plasma levels of the DNA repair enzyme 8-oxoguanine glycosylase were observed[21]. Increased systolic BP is common in adults aged 50 and above[150]. This is because aging is associated with increased BP due to changes in arterial collagen stiffness and reduced elasticity which can promote endothelial dysfunction[150,151].

Although there is evidence that TC consumption is effective in reducing BP in humans[21], the mechanism of these effects is not well understood. It is hypothesized that the effects of TC on BP occur through reductions in inflammation and oxidative stress, which subsequently reduces endothelial dysfunction[21]. The implication of TC in the modulation of endothelial function has been studied at the clinical level[46], but further studies are required to elucidate effects at the cellular level. Angiotensin-II (Ang-II) is a potent vasoconstrictor and its interaction with the Ang-II type 1 receptor (AT1R) can influence reactive species production contributing to the activation of the NF-κB signaling pathway and other inflammatory mechanisms[152].

In this study we investigated the dose-dependent effects of TC extracts on NOS and the modulation of the NF-κB signaling pathway in the presence or absence of Ang-II.

4.2 Methods

4.2.1 Tart Cherry Extraction

Freeze-dried TC powder was kindly provided by CherryPure Shoreline Fruit (Shoreline Fruit, LLC, Michigan, USA). Extraction was performed based on the methods by Iglesias-Carres et al.[153] with slight modifications. Ethanol was warmed to 55°C then diluted to 72% ethanol with 1% formic acid. Twenty-four milliliters of this solution were combined with 2g of TC powder. This was vortexed for 2 minutes then incubated at 55°C for 10 minutes and vortexed for 20 seconds. The TC and ethanol solution were then put on a shaking plate for 30 minutes at 450 rpm with protection from light. This was centrifuged at 9,500 xg for 10 minutes at 4°C. The supernatant was collected, and the procedure was repeated with the pellet. The two supernatants were combined and aliquoted into 1.5ml tubes. A SpeedVac was used to dry down the TC extracts and this was stored at -20°C until further use.

4.2.2 Total Phenol Assay

Dried TC extracts were weighted and diluted to 40,000µg/ml with phosphate buffered saline (PBS). Samples were filtered through a 0.22µm filter. The Folin-Ciocalteu method was used to determine the total phenol/ polyphenol content of the filtered TC extracts using the method by Ainsworth and Gillespie[154] with some

modifications. One hundred microliters of a sample, standard or blank was added to a new tube. Then 200µl of 10% Folin-Ciocalteu's phenol reagent was added and the tube was vortexed thoroughly. After the addition of 800µl of 700mM Na_2CO_3, the tubes were incubated at room temperature for 2 hours. Two hundred microliters of the sample, standard or blank was then transferred to a 96-well plate and the absorbance was measured at 765nm. Two standards curves were created using gallic acid: 3,000-10,000µM and 900-2,454µM. Results were blank corrected and expressed as gallic acid equivalents.

4.2.3 Total Anthocyanin Content

Total anthocyanin content was determined using the pH Differential method by Lee et al[155]. Tart cherry extracts were diluted to 40,000ug/ml using water. Samples were then filtered through a 0.22µm filter and diluted using a pH 1.0 potassium chloride buffer and a pH 4.5 sodium acetate buffer in separate tubes. Diluted samples were transferred to cuvettes and the absorbance was measured at 520nm and 700nm using a spectrophotometer. The anthocyanin pigment concentration in mg/L of cyanidin-3-glucoside equivalents was calculated using the equation:

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

Where A= ($A_{520nm} - A_{700nm}$)pH 1.0 – ($A_{520nm} - A_{700nm}$)pH4.5; MW is the molecular weight of cyanidin-3-glucoside: 449.2 g/mol; DF is the dilution factor; 10^3 is used to convert between g to mg; ε is 26,900 the molar extinction coefficient of cyanidin-3-glucoside in L/mol*cm; 1 is the pathlength in cm.

4.2.4 Cell Viability Assay

The Caymen Chem MTT Cell Proliferation Assay kit (Caymen Chem, Ann Arbor, Michigan, USA) was used to determine human coronary artery endothelial cell (HCAEC) viability in response to Ang-II and increasing doses of TC at 24 hours. HCAEC were seeded at 1.6 x 10^4 cells/well in two sterile 96-well plates. Then, 100nM of Ang-II or PBS was added to the cells. After a 30 minute incubation, 0, 25, 50, 100, 200, or 400ug/ml of TC was added to the appropriate wells. Cells were then incubated in a 5% CO_2 incubator at 37°C. After 24 hours the MTT reagent was added to each well. After a 3-4 hour incubation, the crystal dissolving solution was added and the absorbance at 570nm was measured. All samples and blanks were run in triplicate.

4.2.5 Cell Culture and Conditions

Primary HCAEC were purchased form American Type Cell Culture (ATCC, Manassas, VA, USA). The HCAEC were cultured in vascular cell basal media

supplemented with an endothelial cell growth kit containing 0.2% bovine brain extract (BBE), 2% fetal bovine serum, 10mM L-glutamine (ATCC, Manassas, VA, USA), and 0.1% Penicillin/Streptomycin. Cells were maintained in an incubator at 37°C with 5% CO_2 and media was replaced every other day. HCAEC were used between passages 2-4. Cells were counted using a hemocytometer. Cells were treated with various doses of TC (0, 62.5, 125, 250 and 500μg/mL) with or without 100nM of Ang-II then incubated for 24 hours in a 5% CO_2 incubator at 37°C and were harvested at ≥70% confluence. Cells were exposed as follows: Control (no TC, no Ang-II), Ang-II (no TC,100nM of Ang-II), 62.5 Ang (62.5 μg/ml TC, 100nM Ang-II), 125 Ang (125 μg/ml TC, 100nM Ang-II), 250 Ang (250 μg/ml TC, 100nM Ang-II), 500 Ang (500 μg/ml TC, 100nM Ang-II). After 24 hours cells harvested using trypsin and stored at -80°C until further use.

4.2.6 Western Blot

HCAEC were subject to RIPA lysis buffer containing an inhibitor cocktail and protein concentrations were determined using the Bradford assay with detection at 595 nm. Samples were mixed with 20% SDS and 10% cracking buffer and then incubated for 5 minutes at 95°C. A concentration of 20 μg/lane of total cellular protein was loaded and run on 10% Novex Tris-Glycine mini protein gels (Thermo Fisher Scientific) at 120V. A 10-250 kDa color- coded protein marker (Cell Signaling Technology (CST), Danvers, MA, USA) was used to determine molecular weight.

Proteins were transferred to 0.2μm nitrocellulose membranes using the Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, Hercules, CA) and blocked for 1 hour at room temperature in 1X TBST with 5% dry milk for eNOS, iNOS, p65, phosphorylated p65, and ICAM-1, in 1X TBST with 3% dry milk and 0.25% bovine serum albumin (Fisher Scientific) for β-actin.

Primary antibodies (CST, Danvers, MA, USA) were prepared using 1X TBST and 5% bovine serum albumin for eNOS (1:1,000, CST 32027), iNOS (1:1,000, CST 20609), p65 (1:1,000, CST 4764), phosphorylated p65 (1:1,000, CST 3033), and ICAM-1 (1:1,000, CST 67836). The primary antibody for β-actin was prepared using 1X TBST with 1% dry milk for (1:1,000, CST 8457). Incubation in primary antibody was performed overnight on a shaker at 4°C. Membranes were washed three times with 1X TBST for five minutes before incubation in the secondary antibody for 1 hour at room temperature. Secondary antibody was prepared using 1X TBST with 5% dry milk and HRP-linked anti-rabbit IgG (CST 7074) for eNOS, p65, phosphorylated p65, and ICAM-1 (1:2,000). The secondary antibody was prepared using 1X TBST with 1% dry milk for β-actin and iNOS (1:10,000).

Chemiluminesense visualization of protein bands was conducted using the Radiance Plus substrate (Azure Biosystems, Dublin, CA). The Invitrogen iBright FL-1500 was used to quantify bands for densitometry and values were normalized to levels of β-actin.

4.2.7 Statistical Analysis

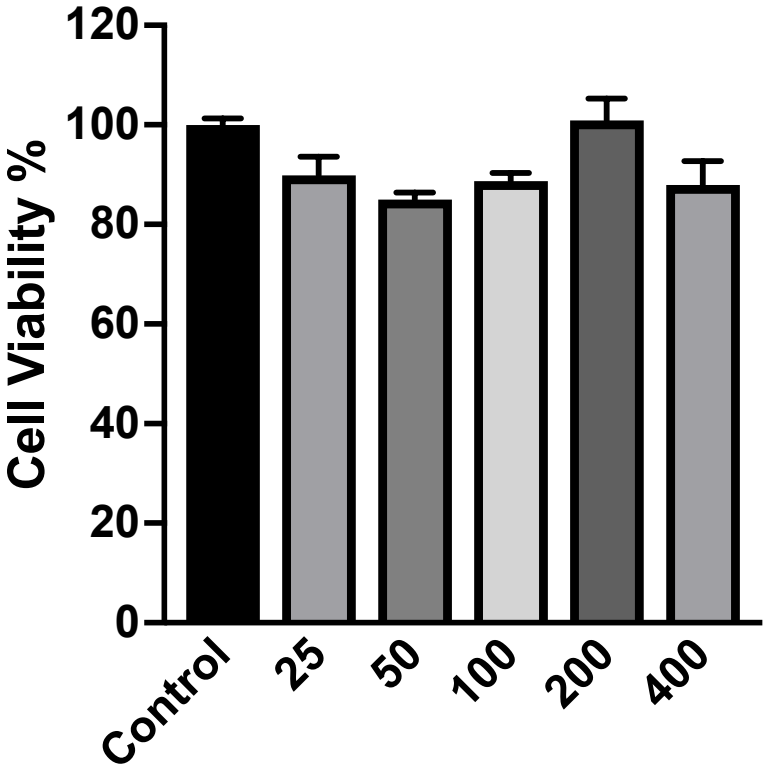
Data were analyzed using SPSS. The significance between-groups was determined for the F-C assay, pH differential assay, MTT assay, and Western blot using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test if results were normally distributed, otherwise the non-parametric Kruskal-Wallis test was used. Data are expressed as mean ± standard error mean (SEM). Values of $p<0.05$ are considered statistically significant.

4.3 Results

4.3.1 Polyphenols, Anthocyanins, and Cell Viability

Total polyphenols were 480.9mg GAE per g TC powder. The total monomeric anthocyanin content was 15.46 mg/L of cyanidin-3-glucoside equivalents. The viability of HCAEC after exposure to TC extracts for 24 hours was measured using the MTT assay. The TC extract concentrations ranged from 0 to 500ug/ml with the addition of Ang-II **(Fig. 5)**. There were no significant differences in percent cell viability with TC exposure at 24 hours. Additionally, Ang-II exposure did not significantly impact cell viability with or without TC exposure compared to the control.

TC (µg/ml)	-	25	50	100	200	400
Ang-II (100nM)	-	-	-	-	-	-



A.

TC (µg/ml)	-	-	25	50	100	200	400
Ang-II (100nM)	-	+	+	+	+	+	+

B.

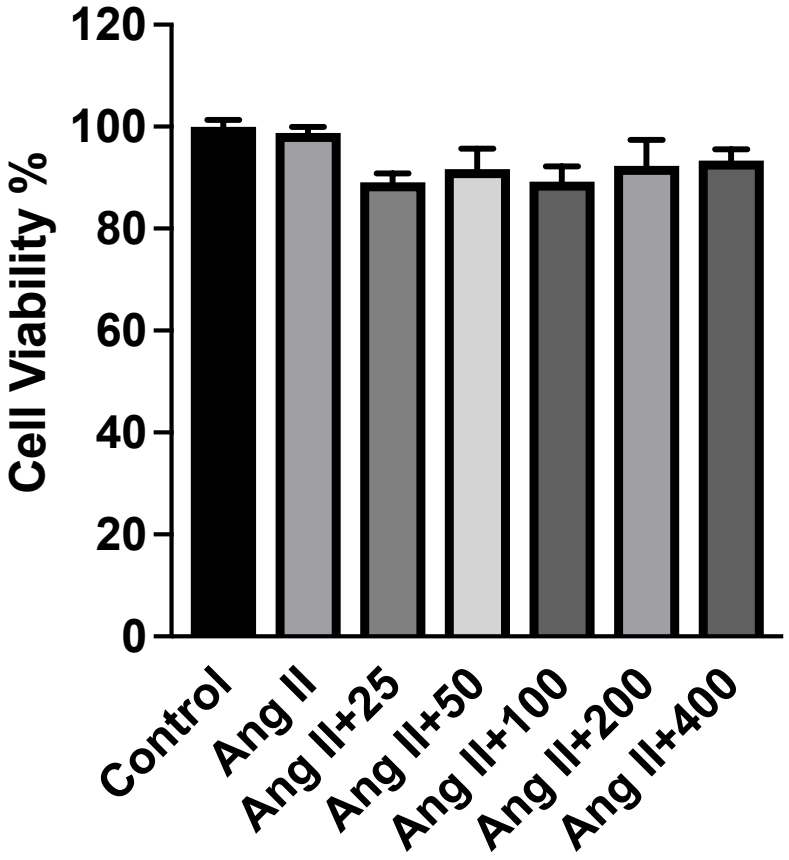
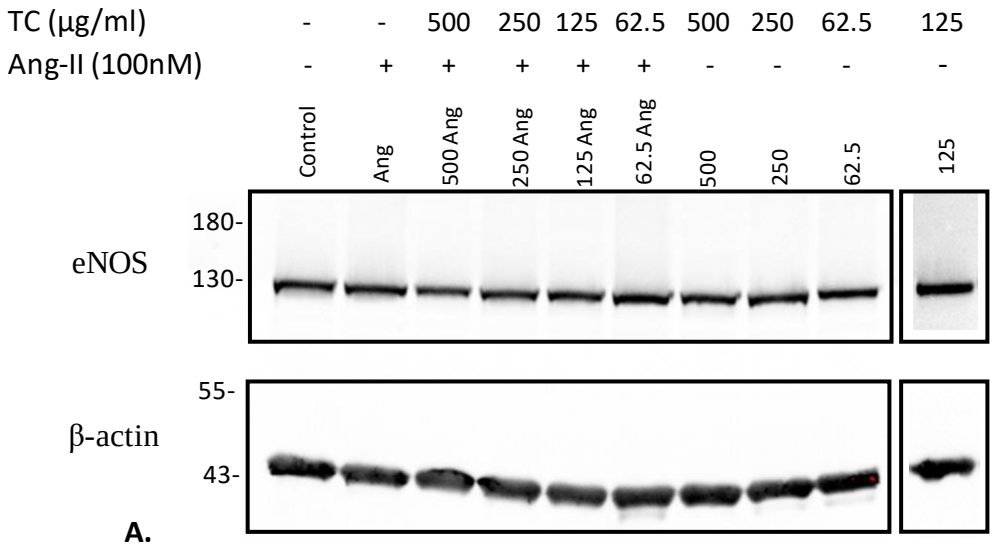


Figure 5. MTT HCAEC 24 hour viability in response to TC exposure in the presence of absence of Ang-II expressed as percent viability compared to control. Cells were exposed to levels of TC extracts between 0-400µg/mL with or without 100nM of Ang-II. Values are represented as mean ± SEM; (n=3). There were no significant differences between the groups. **A.** Percent viability of control and TC groups without Ang-II. **B.** Percent viability of control and TC groups with Ang-II.

4.3.2 Western Blots

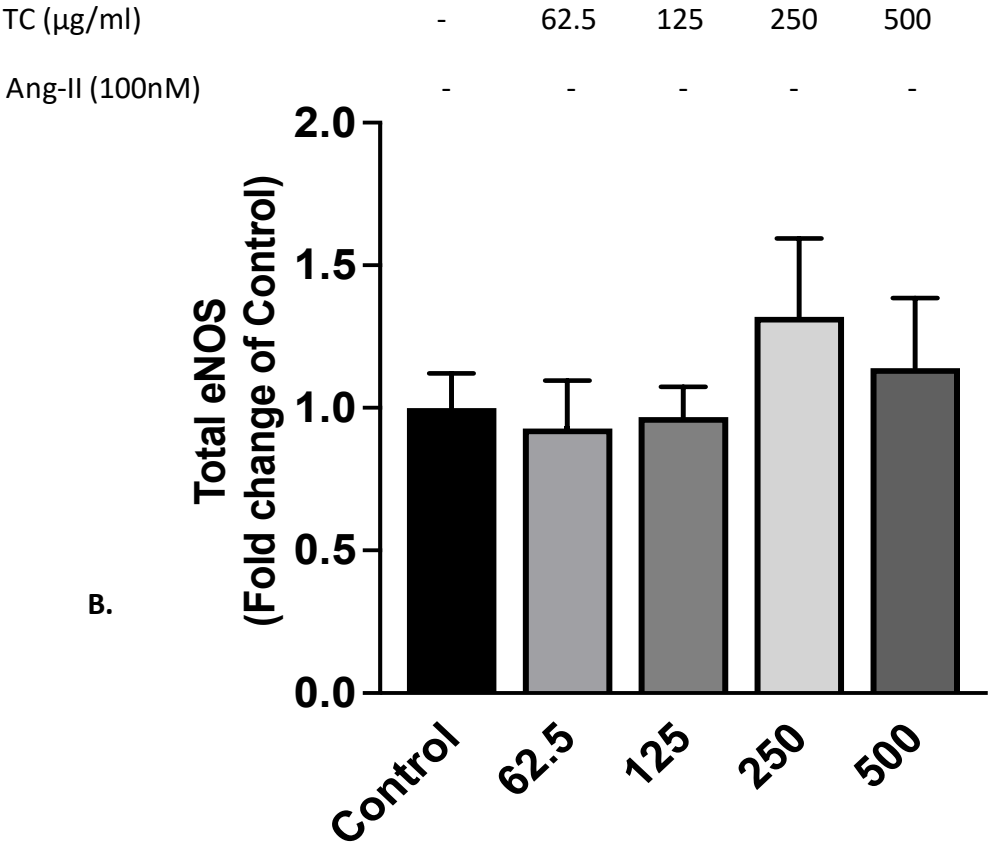
4.3.2.1 Endothelial Nitric Oxide Synthase

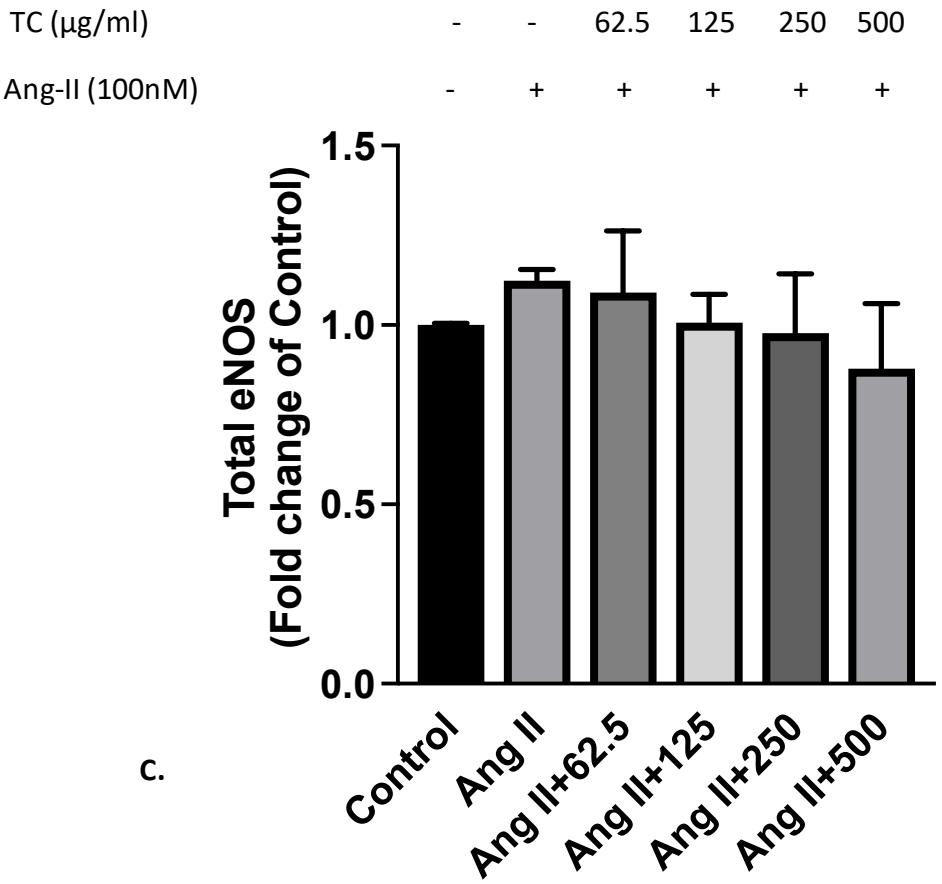
In this study, Ang-II exposure for 24 hours was not found to significantly affect eNOS protein expression levels in HCAEC total cell lysates. **Figure 6A** exhibits a representative western blot of eNOS samples. Exposure to various concentrations of TC extracts (0, 62.5, 125, 250, and 500 μg/ml) with Ang-II **(Fig. 6C)** or without Ang-II **(Fig. 6B)** for 24 hours showed no significant differences in eNOS levels between groups or compared to the control.


TC (µg/ml) - - 500 250 125 62.5 500 250 62.5 125
Ang-II (100nM) - + + + + + - - - -
Control
Ang
500 Ang
250 Ang
125 Ang
62.5 Ang
500
250
62.5
125
180-
130-
eNOS
55-
43-
β-actin

A.

B.


TC (µg/ml) - 62.5 125 250 500
Ang-II (100nM) - - - - -
Total eNOS (Fold change of Control)
2.0
1.5
1.0
0.5
0.0
Control
62.5
125
250
500

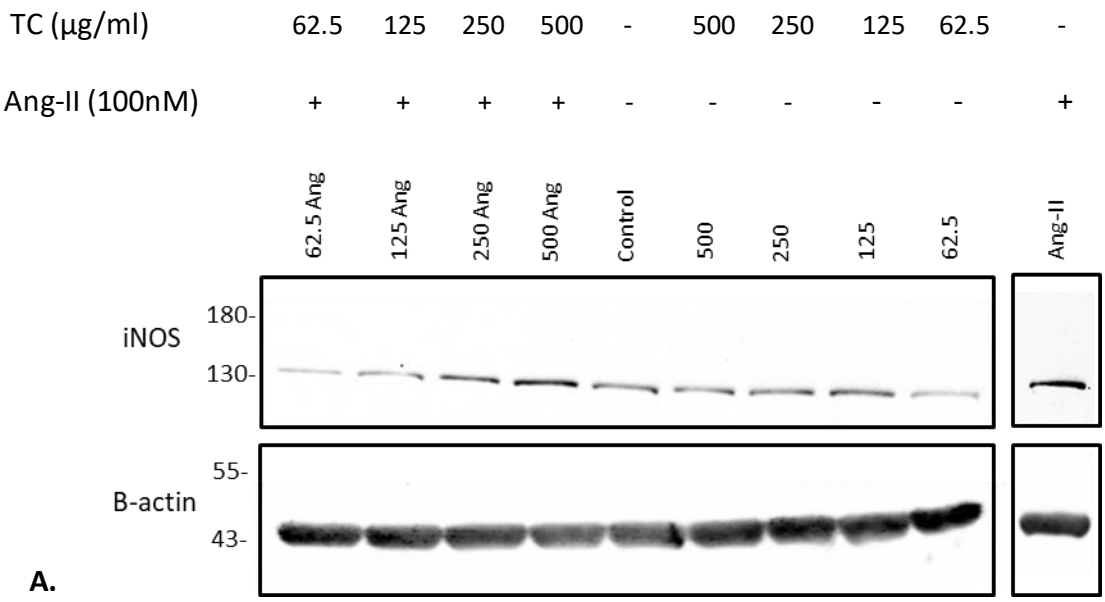


C.

Figure 6. Total eNOS expression in HCAEC with TC exposure in the presence or absence of Ang-II. Error bars represent the mean ± SEM; n=3. **A.** Western blot images for eNOS and β-actin; boxes represent images of different membranes. **B.** Fold change of total eNOS expression compared to the control in cells exposed to TC doses between 62.5µg/mL to 500µg/mL. **C.** Fold change of total eNOS expression compared to the control in cells exposed to TC doses between 62.5µg/mL to 500µg/mL with Ang-II. There were no significant differences between the groups.

4.3.2.2 Inducible Nitric Oxide Synthase

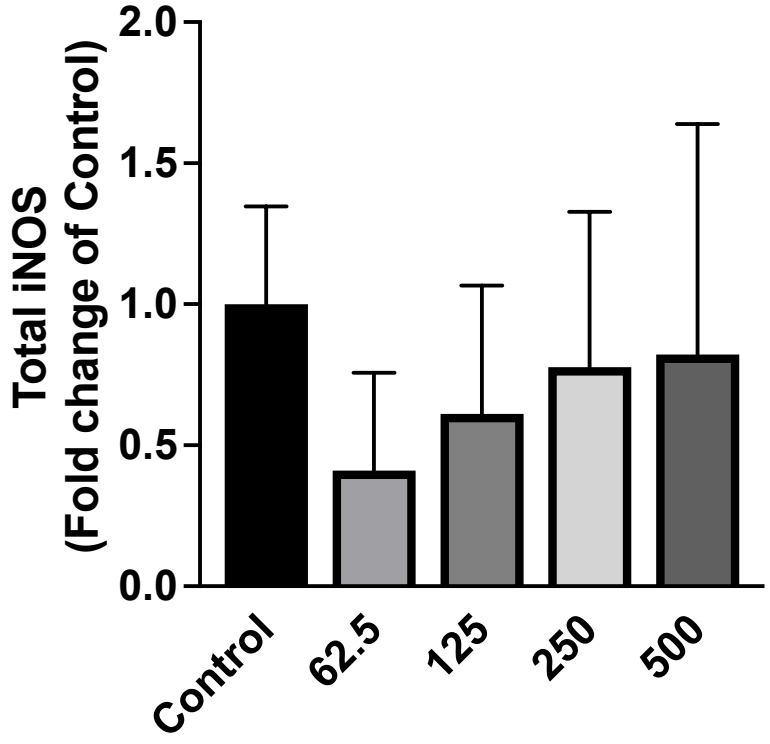
Figure 7A is a representative image of the western blot for all samples. There were no significant differences in iNOS protein expression in cells exposed to 62.5μg/mL – 500μg/mL TC compared to the control. In the absence of Ang-II, all of the TC exposures resulted in numerical reductions in iNOS below the control. A slight dose-dependent increase in iNOS was observed with increasing amounts of TC extract exposure in the absence of Ang-II **(Fig.7B)**. INOS expression was reduced at the 62.5 Ang *(p=0.016)* and 125 Ang *(p=0.050)* exposure levels compared to the 500 Ang **(Fig. 7C)**. Although this was not significantly different from the control, the data suggest a possibility for TC to reduce iNOS expression with 62.5μg/mL and 125μg/mL TC extracts in the presence **(Fig. 7C)** or absence of Ang-II **(Fig. 7B)**.

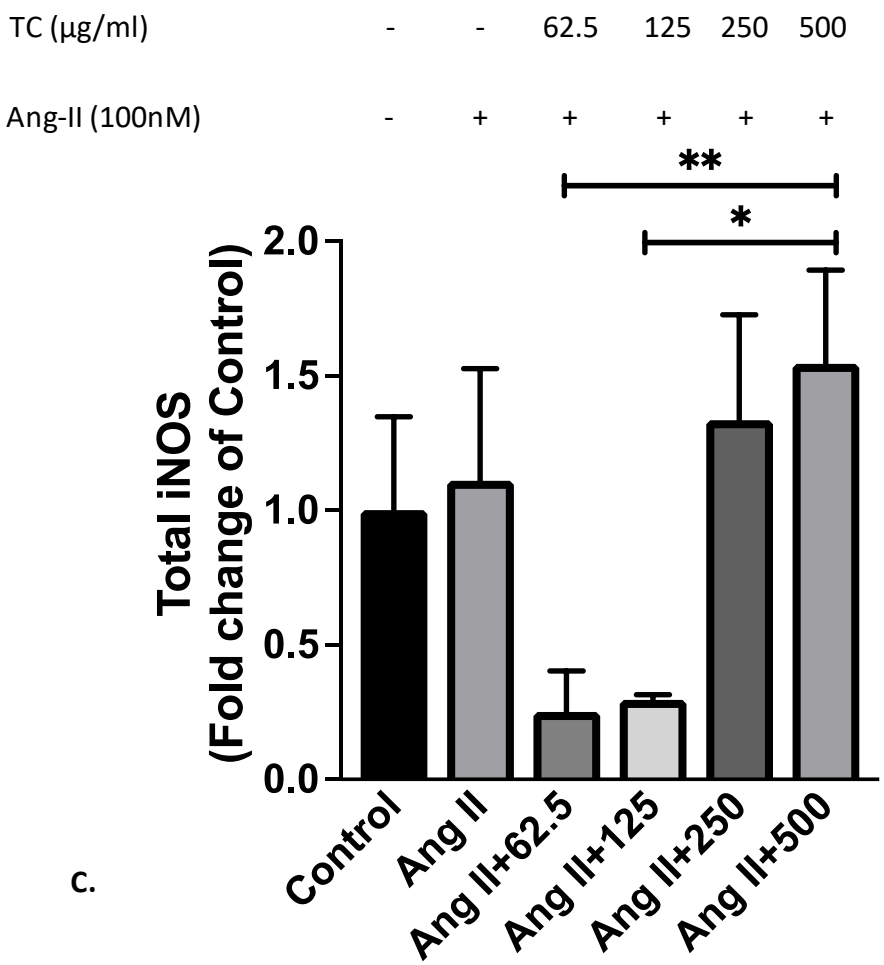

TC (µg/ml)
62.5
125
250
500
-
500
250
125
62.5
-
Ang-II (100nM)
+
+
+
+
-
-
-
-
-
+
62.5 Ang
125 Ang
250 Ang
500 Ang
Control
500
250
125
62.5
Ang-II
iNOS
180-
130-
B-actin
55-
43-

A.

TC (µg/ml)	-	62.5	125	250	500
Ang-II (100nM)	-	-	-	-	-

B.



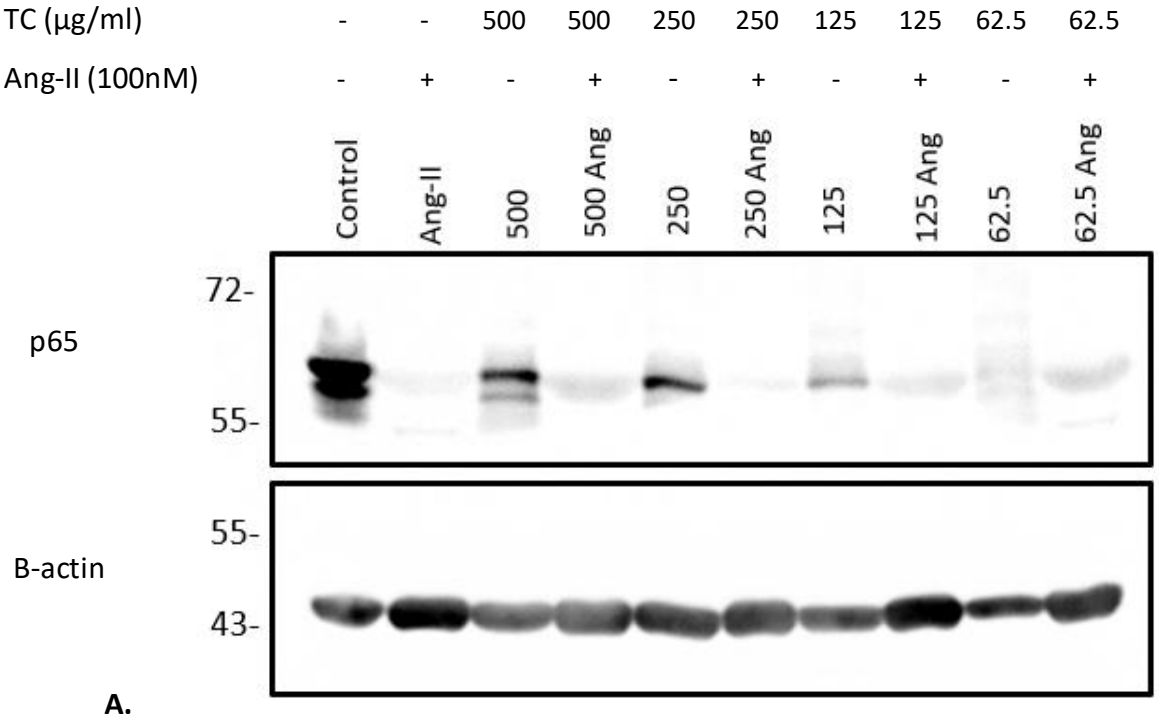


c.

Figure 7. Total iNOS expression in HCAEC with TC exposure in the presence or absence of Ang-II. Error bars represent the mean ± SEM; n=3. **A.** Western blot images for iNOS and β-actin; boxes represent images of different membranes. **B.** Fold change of total iNOS expression compared to the control using doses of TC between 62.5µg/mL to 500µg/mL . **C.** Fold change of total iNOS expression at TC doses of 62.5µg/mL to 500µg/mL with Ang-II exposure. Kruskal-Wallis test showed a significant difference at $p<0.05$ (*,**).

4.3.2.3 NF-κB p65

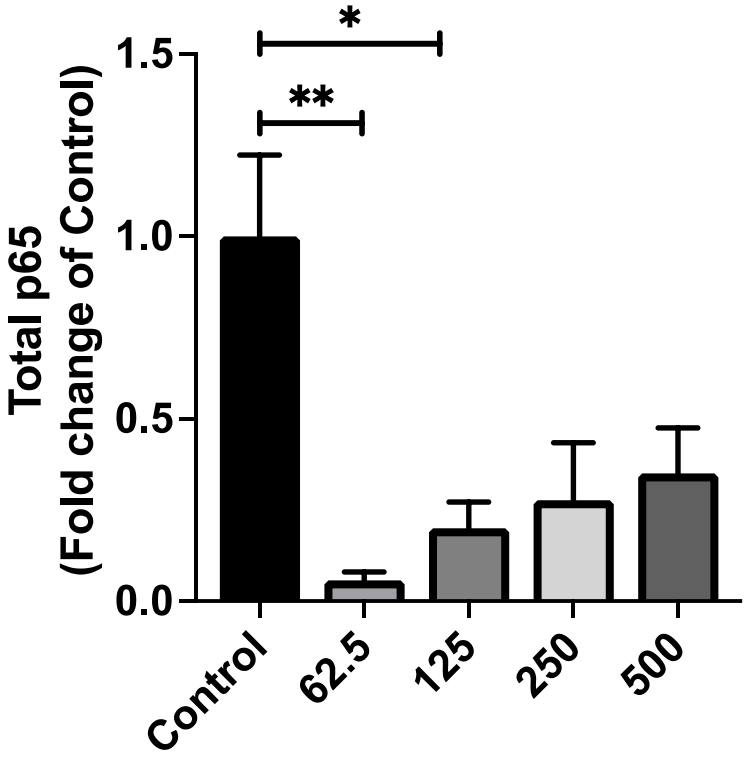
Figure 8A shows a representative image of the western blot for all samples. Levels of total p65 protein expression were higher in the control compared to the 62.5μg/ml *(p=0.003)* and the 125μg/ml exposures *(p=0.045)*. The cells exposed to the control conditions had the highest level of p65 expression compared to all other exposure conditions, although the expression was not significantly higher compared to the 250 and 500μg/mL TC exposures. Expression of p65 was increasing in a dose-dependent manner with TC extracts in the absence of Ang-II **(Fig. 8B)**. The Ang-II exposed cells had lower p65 compared to the control *(p=0.004)*. The 250 Ang and 500 Ang exposed cells had lower p65 expression than the control at levels of *p=0.008* and *p=0.017* respectively. The 62.5 Ang cells had a higher p65 expression than both the Ang-II *(p=0.029)* and the 250 Ang exposed cells *(p=0.05)* **(Fig. 8C)**.


TC (µg/ml) - - 500 500 250 250 125 125 62.5 62.5
Ang-II (100nM) - + - + - + - + - +
Control
Ang-II
500
500 Ang
250
250 Ang
125
125 Ang
62.5
62.5 Ang
p65
72-
55-
B-actin
55-
43-

A.

TC (µg/ml)	-	62.5	125	250	500
Ang-II (100nM)	-	-	-	-	-

B.



TC (µg/ml)	-	-	62.5	125	250	500
Ang-II (100nM)	-	+	+	+	+	+

C.

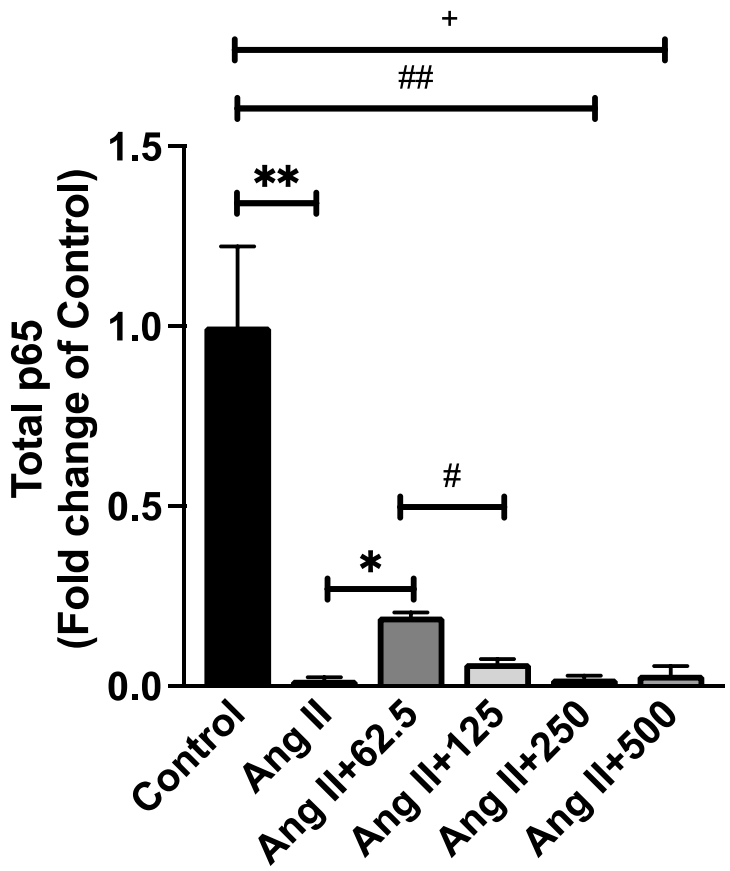
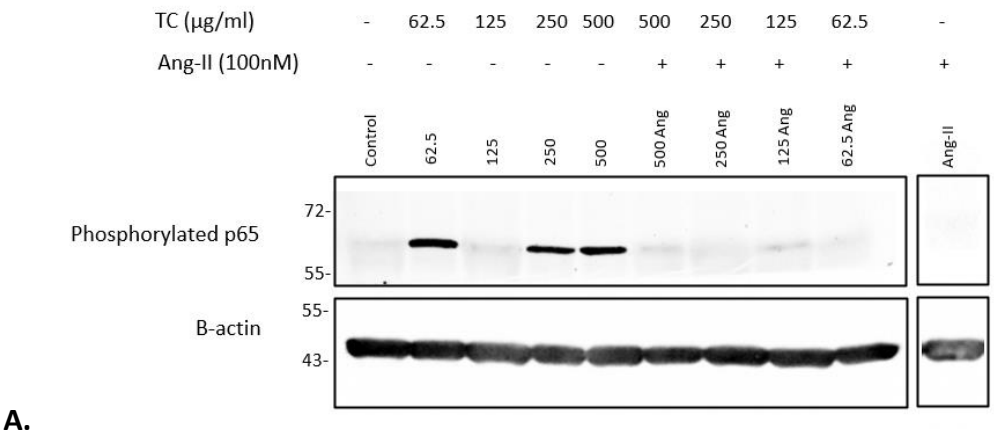


Figure 8. Total p65 expression in HCAEC with TC exposure in the presence or absence of Ang-II. Error bars represent the mean ± SEM; n=3. **A.** Representative western blot image for total p65 and β-actin for all samples. **B.** Fold change of total p65 expression compared to control at TC doses between 62.5µg/mL to 500µg/mL. **C.** Fold change of total p65 expression compared to the control with doses of TC between 62.5µg/mL to 500µg/mL and Ang-II exposure. Kruskal-Wallis test showed a significant difference at *$p<0.05$ (*, #, +), $p<0.01$(**, ##)*.

4.3.2.4 NF-κB Phosphorylated p65

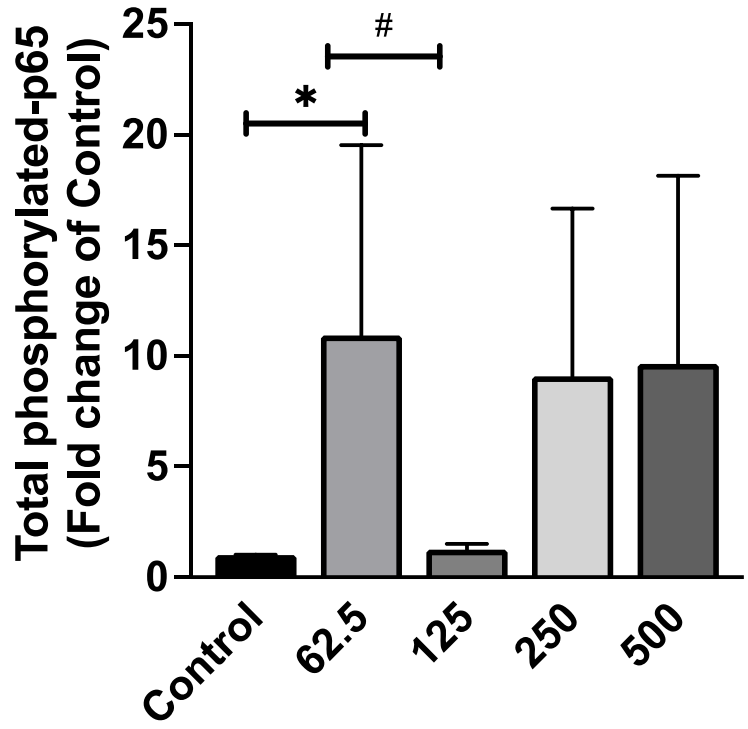
Figure 9A shows a representative image of the western blot for all samples. The levels of phosphorylated p65 protein expression were higher with the 62.5μg/mL TC exposure than the control *(p=0.018)*. This suggests that the 62.5μg/ml group had more NF-κB activation compared to the control **(Fig. 9B)**. Levels of phosphorylated p65 expression were increased with the 62.5 Ang exposure compared to the Ang-II exposure **(Fig. 9C)**.


TC (μg/ml) - 62.5 125 250 500 500 250 125 62.5 -
Ang-II (100nM) - - - - - + + + + +
Control
62.5
125
250
500
500 Ang
250 Ang
125 Ang
62.5 Ang
Ang-II
Phosphorylated p65
72-
55-
B-actin
55-
43-

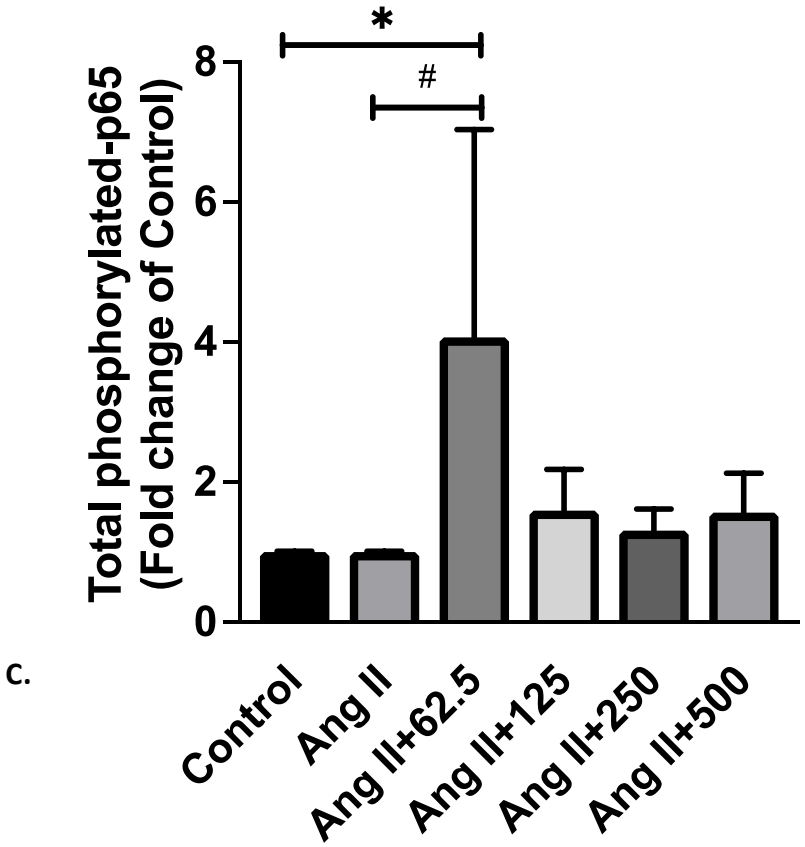
A.

TC (µg/ml)	-	62.5	125	250	500
Ang-II (100nM)	-	-	-	-	-

B.



TC (μg/ml)	-	-	62.5	125	250	500
Ang-II (100nM)	-	+	+	+	+	+

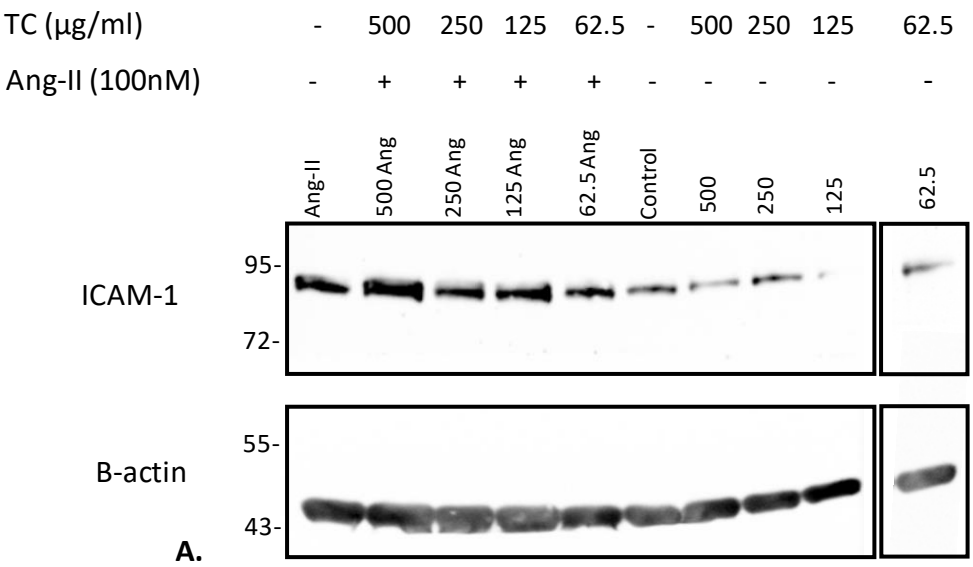


C.

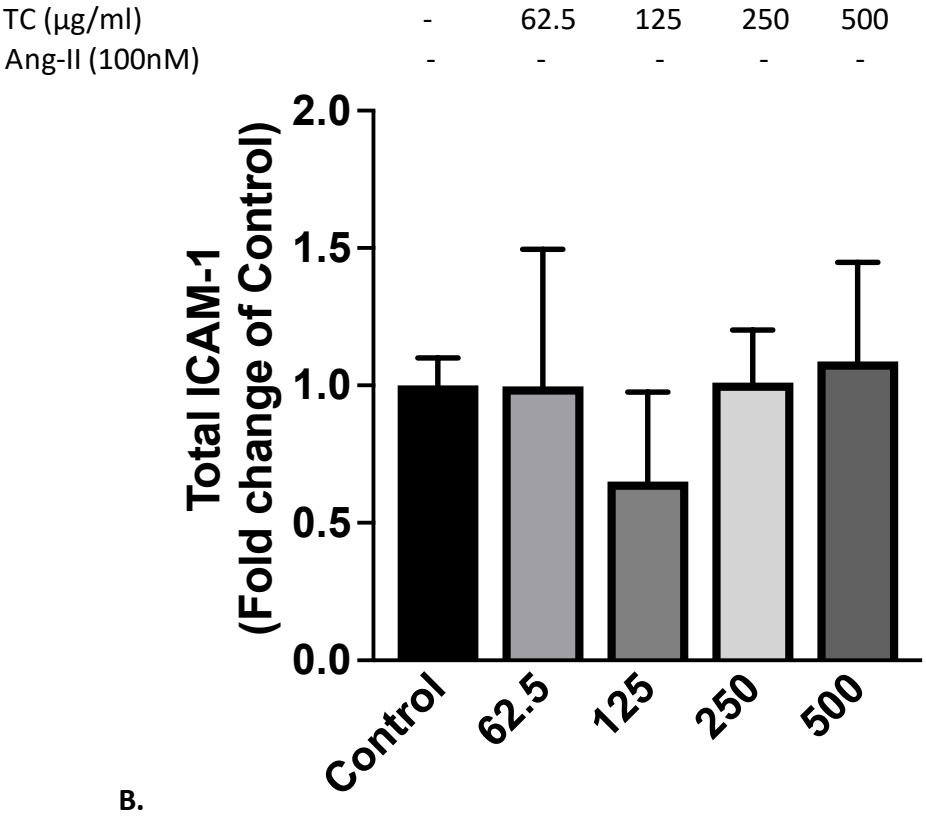
Figure 9. Total phospohorylated-p65 expression in HCAEC with TC exposure in the presence or absence of Ang-II. Error bars represent the mean ± SEM; n=3 (control and 62.5 Ang), n=2. **A.** Western blot images for phosphorylated-p65 and β-actin; boxes represent images of separate membranes. **B.** Fold change of total phosphorylated p65 expression compared to the control with TC doses between 62.5μg/mL to 500μg/mL. **C.** Fold change of total phosphorylated p65 expression compared to the control with TC doses between 62.5μg/mL to 500μg/mL and Ang-II exposure. Kruskal-Wallis test showed a significant difference at $p<0.05$(*, #).

4.3.2.5 Intercellular Adhesion Molecule-1

Figure 10A shows a representative image of the western blot for all samples. Levels of ICAM-1 protein expression were similar between the control and TC exposures in the absence of Ang-II. There was a slight reduction in ICAM-1 expression at the 125μg/mL exposure compared to the control, although this was not statistically significant **(Fig. 10B)**. The various TC doses did not significantly impact levels of ICAM-1 expression in the presence of Ang-II. Levels of ICAM-1 were similar between the control, Ang-II, and 62.5 Ang groups. The 250 Ang and 500 Ang groups had about two-fold higher ICAM-1 expression compared to the control, but this was not statically significant **(Fig. 10C)**.


TC (µg/ml)
- 500 250 125 62.5 - 500 250 125 62.5
Ang-II (100nM)
- + + + + - - - - -
Ang-II
500 Ang
250 Ang
125 Ang
62.5 Ang
Control
500
250
125
62.5
ICAM-1
95-
72-
B-actin
55-
43-

A.


TC (µg/ml) - 62.5 125 250 500
Ang-II (100nM) - - - - -
Total ICAM-1 (Fold change of Control)
2.0
1.5
1.0
0.5
0.0
Control
62.5
125
250
500

B.

TC (µg/ml)	-	-	62.5	125	250	500
Ang-II (100nM)	-	+	+	+	+	+

c.

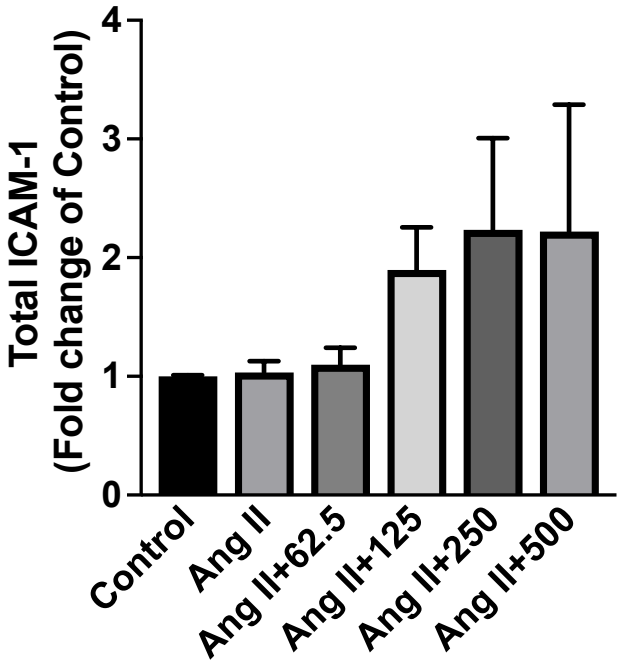


Figure 10. Total ICAM-1 expression in HCAEC with TC exposure in the presence or absence of Ang-II. Error bars represent the mean ± SEM; n=3 (control, 125 Ang, and 250 Ang), n=2. **A.** Western blot images for ICAM-1 and β-actin; boxes represent images of different membranes. **B.** Fold change of total ICAM-1 expression compared to the control with TC doses between 62.5µg/mL to 500µg/mL. **C.** Fold change of total ICAM-1 expression with doses of TC between 62.5µg/mL to 500µg/mL and Ang-II exposure. Kruskal-Wallis test showed no significant differences between groups.

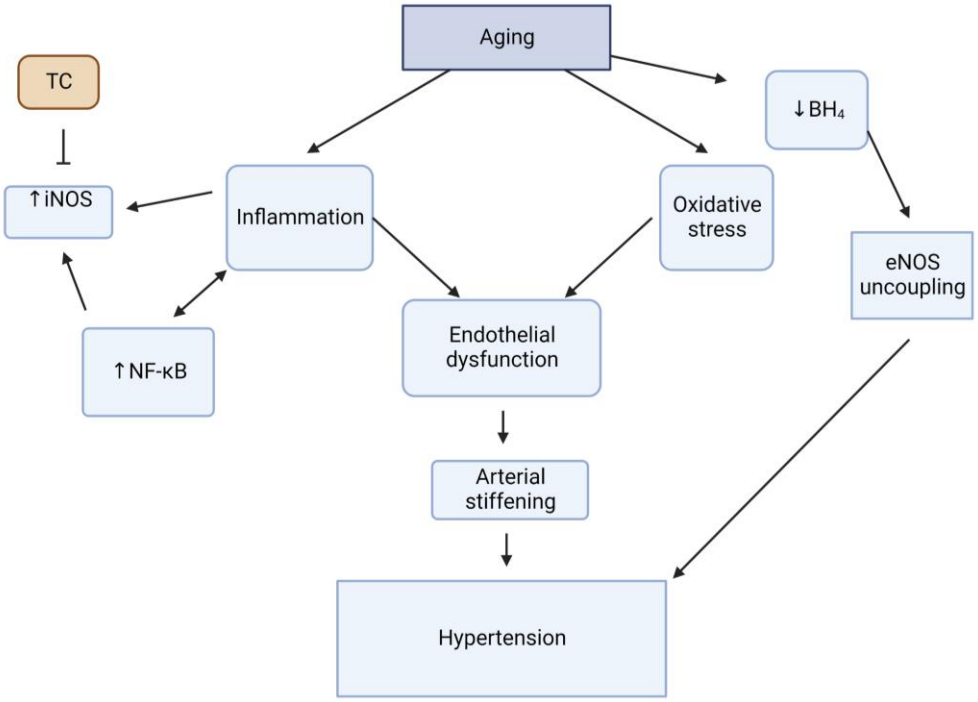


Figure 11. Potential effects of TC extracts on hypertension. Aging can contribute to hypertension by increasing inflammation and oxidative stress that leads to endothelial dysfunction and arterial stiffening. Increased inflammation and oxidative stress can contribute to increased iNOS, eNOS uncoupling, and NF-κB activation, leading to increased blood pressure. This image shows the potential mechanism of effects of TC extracts on hypertension. TC has the potential to reduce iNOS which may affect blood pressure.

4.4 Discussion

In this study, the impact of TC extracts on NOS and inflammatory markers in human coronary artery endothelial cells were investigated in the presence and absence of Ang-II. To our knowledge, this is the first study to examine the effects of whole TC extracts on NOS and components of the NF-κB pathway in human coronary artery endothelial cells (HCAEC).

Vascular endothelial cells line the blood vessels and produce molecules to regulate thrombosis, vasodilation, and vascular protection[156]. Oxidative stress and inflammation can influence endothelial cell function which can result in vascular endothelial dysfunction[157,158]. In the arteries, endothelial dysfunction encompasses changes in arterial phenotypes that can result in atherosclerosis, vasoconstriction, inflammation, and coagulation[151,156].

We examined HCAEC viability in response to Ang-II alone or in combination with 62.5- 400μg/mL of TC extracts for 24 hours. Percent cell viability was not affected by TC exposure with or without Ang-II, suggesting that these doses of TC are not toxic compared to the control. NO is a vasodilator that's produced by the endothelial cells to inhibit growth and inflammation, regulate vascular tone, and have an anticoagulant effect on platelets[159,160]. Endothelial dysfunction is a risk factor for CVD in which the bioavailability of NO can be affected[156,160]. Diminished NO is associated with increased oxidative stress in the vasculature,[161] and can be influenced by reductions in eNOS activity given its role in NO signaling and the regulation of

blood pressure and flow[158,160,162,163]. ENOS generally produces NO, however, it can become uncoupled by oxidative depletion of tetrahydrobiopterin (BH_4), and produce superoxide instead[161]. Ang-II can promote eNOS uncoupling and this may be dependent on its effects on the AT_1R[161]. Gallic acid, a compound in TC, has been found to reduce eNOS degradation in the aorta[158]. However, we did not observe any significant changes in eNOS protein expression due to Ang-II exposure with or without TC in this study.

The inflammatory roles of NO in the body are affected by iNOS, which is typically induced by cytokines or other factors[164]. Levels of iNOS protein expression were numerically lower at all TC concentrations in the absence of Ang-II, although this was not statistically different from the control. We did not observe any significant differences in iNOS expression between the Ang-II group and the control. Similarly, in a study by Shukitt-Hale et al.[165] when microglial cells were pre-treated with TC at doses of 0.0625, 0.125, 0.25, or 0.5mg/mL then exposed to lipopolysaccharide (LPS) overnight, iNOS expression was not affected by TC pre-treatment. However, we did notice that the expression of iNOS was reduced at the 62.5 Ang and 125 Ang doses compared to the 500 Ang dose and numerically reduced iNOS expression compared to the control. This could be due to increased cytotoxicity or reduced viability at higher TC doses and exposure times[165]. The results suggest that low concentrations of TC at the levels of 62.5 and 125µg/ml could potentially reduce iNOS in Ang-II induced states.

Endothelial cells and other tissues express the transcription factor NF-κB that influences the production of TNF-α, IL-6, and other inflammatory molecules[166]. The interaction of Ang-II with the Ang-II type 1 receptor (AT_1R) can influence reactive species production that contributes to the activation of the NF-κB signaling pathway[152]. Kim et al.[152] found that increases in Ang-II and the AT_1R during aging were accompanied by the generation of reactive species. They also found that increased Ang-II activated NF-κB signaling by phosphorylating p65[152]. We observed that p65 expression was lower in the Ang-II, 250 Ang, and 500 Ang groups compared to the control. The 62.5 Ang group also showed an increased amount of p65 compared to the Ang-II and 250 Ang groups. NF-κB is present in cells at low levels [166] and it was found that in unstimulated cells the basal levels of p65 are not zero[167]. Unphosphorylated p65 doesn't bind to DNA and thus is not transcriptionally active, however, it can become phosphorylated in response to a signal[166]. Although levels of p65 were significantly higher in the control group than all others, the levels of phosphorylated p65 were low in the control group, and especially lower than the 62.5 Ang group. One study demonstrated that expression of factors involved in NF-κB signaling is time-dependent, and expression can increase or decrease as time goes on[10]. This may explain the absence of increased NF-κB activation due to the long 24-hour Ang-II exposure in this study. Activation of NF-κB by Ang-II can increase expression of cellular adhesion molecules and chemokines[9].

Cell adhesion molecules are located on the surface of cells which allows them to bind to extracellular matrix proteins or to each other[14]. There are four major

superfamilies of CAMs and intercellular adhesion molecules (ICAM) belong to the immunoglobulin superfamily[14]. Adhesion molecules such as ICAM-1 are important NF-κB target genes in endothelial cells that help inflammatory cells adhere to the vascular wall[15]. Endothelial cells express low levels of ICAM-1[16], but the expression can be upregulated by inflammatory stimuli[14]. Expression of ICAM-1 can also be a marker of aging[17]. Activity of ICAM-1 is required for Ang-II induced hypertension and blocking ICAM-1 inhibits inflammation, ROS production, and vascular remodeling and dysfunction[14]. Levels of ICAM-1 expression were similar between groups in the absence of Ang-II. In the presence of Ang-II, ICAM-1 expression was similar to the control.

Previously, the effects of TC on endothelial cells such as HUVEC have been studied. However, endothelial cells from different vascular beds can have different mRNA and protein expression[168]. Furthermore, Lakota et al.[169] found that HCAEC were more susceptible to inflammation when exposed to serum amyloid A compared to human umbilical vein endothelial cells (HUVEC), supporting the use of HCAEC in inflammatory studies.

There is evidence of the anti-inflammatory and anti-oxidative effects of TC in human studies[21,48,73] In this study, in the absence of Ang-II the expression of eNOS numerically increased at TC doses of 250 and 500μg/mL, iNOS was numerically reduced at all doses, p65 expression was reduced at all doses, and ICAM-1 expression showed a numerical suppression at the 125μg/mL dose. Although some of these findings were not statistically significant, the data suggest a possible anti-

inflammatory role of TC extracts at 125μg/mL without Ang-II. However, these effects were not strongly observed in this study, and more studies are necessary to confirm the effects of TC on NOS and the activation of the NF-κB signaling pathway. One reason for this could be due to the long 24-hour exposure to TC. Shukitt-Hale et al[165] found that the optimal TC exposure time was 2 hours in HAPI rat microglial cells. Similarly, studies involving TC or blueberry extract exposure to cell culture have been limited to a few hours[165,170]. Additionally, TC extracts contain several compounds which could interact with each other or with Ang-II to produce compound combination effects[171]. More studies are needed to understand this relationship.

Ang-II can contribute to increased inflammation, the pathogenesis of hypertension, and the activation of the NF-κB pathway[9,11], which has made Ang-II signaling a potential therapeutic target[172]. We did not observe any significant effects of Ang-II treatment on eNOS, iNOS, phosphorylation of p65, or ICAM-1 protein expression compared to the control. In this study, HCAEC were exposed to Ang-II and TC for 24 hours. Various studies have determined the effects of Ang-II on cells for up to several minutes or a few hours[9,10]. Potential explanations for this observation include metabolism of Ang-II due to prolonged exposure or downregulation of AT_1R in cell culture[172]. In a study by Basu et al.,[172] when neuronal cells were exposed to 100nM of Ang-II for 24 hours, the Ang-II was rapidly metabolized and levels were near baseline 3 hours after administration. Furthermore, desensitization of AT_1R in cell culture could potentially explain the absence of significant inflammatory effects of Ang-II observed[173].

Chapter 5

CONCLUSIONS AND FUTURE DIRECTIONS

Aging can raise blood pressure (BP), increasing the risk for CVD, stroke, and diabetes[174]. In our laboratory, Chai et al.[21,48] have observed reduced systolic BP, low-density lipoprotein, total cholesterol, and c-reactive protein in older adults who consumed tart cherry (TC) juice for 12 weeks.

Additionally, we conducted a cross-sectional analysis to determine the relationship between major dietary factors and BP in older adults. It was found that 78% of participants had high BP[34]. We discovered that consuming added sugar was associated with increased systolic and diastolic BP in female participants. On the other hand, whole fruit consumption was associated with reduced diastolic BP in males and females[34]. The results of this study strengthened the assertion that fruit, such as TC, can influence BP in older adults.

TC contain a variety of compounds that may contribute to their health benefits. We conducted a thorough literature review and determined that TC have the potential to reduce systolic BP, hsCRP, TNF-α, IL-6 and other inflammatory markers associated with conditions such as cardiovascular disease, arthritis, cognitive disorders, and cancer[175].

Bioactive compounds in TC include quercetin, cyanidins, and kaempferol that have been shown to impact the NF-κB signaling pathway[84,87,89]. It was clear that TC could potentially reduce inflammation through the modulation of the NF-κB signaling pathway, which may contribute to the reductions in BP observed in the clinical trial.

Therefore, we studied the effects of TC extracts on NOS, components of the NF-κB pathway, and ICAM-1 in response to Ang-II in coronary artery endothelial cells. Levels of eNOS were not significantly influenced by TC with or without Ang-II exposure. Levels of iNOS were reduced by the 62.5 Ang and 125 Ang doses of TC and there was a numerical reduction in iNOS in the absence of Ang-II at all TC doses (62.5-500μg/mL). This suggests that TC exposure may potentially influence iNOS expression in HCAEC at low TC doses. Expression of p65 was reduced at the 62.5 and 125 μg/mL TC doses compared to the control with expression increasing with TC in a dose-dependent manner. The p65 expression was also reduced in the Ang-II, 250 Ang, and 500 Ang exposures compared to the control. Interestingly, the amount of phosphorylated p65 was much higher in the 62.5μg/mL TC exposed cells than in the control. The levels of ICAM-1 were not very different between the TC groups in the absence of Ang-II. However, when Ang-II was present, ICAM-1 expression numerically increased with TC in a dose-dependent manner. The results of this study suggest that TC may be able to reduce iNOS and levels of p65 in HCAEC.

Overall, the results from these studies support the idea that TC may play a role in reducing inflammation and oxidative stress. However, more studies are needed to better understand the effects of TC on NF-κB in the presence of Ang-II using

HCAEC. Supporting evidence herein can provide a foundation for future studies that wish to determine the role of TC consumption in preventing or delaying health conditions with an underlying inflammatory or oxidative stress cause.

REFERENCES

1. Centers for Disease Control and Prevention. High Blood Pressure Facts About Hypertension. https://www.cdc.gov/bloodpressure/facts.htm.

2. Fuchs FD, Whelton PK. High Blood Pressure and Cardiovascular Disease. *Hypertension*. 2020;75(2):285-292. doi:10.1161/HYPERTENSIONAHA.119.14240

3. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of Inflammation, Oxidative Stress, and Vascular Dysfunction in Hypertension. *Biomed Res Int*. 2014;2014:1-11. doi:10.1155/2014/406960

4. Li Q, Youn J-Y, Cai H. Mechanisms and consequences of endothelial nitric oxide synthase dysfunction in hypertension. *J Hypertens*. 2015;33(6):1128-1136. doi:10.1097/HJH.0000000000000587

5. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J*. 2012;33(7):829-837. doi:10.1093/eurheartj/ehr304

6. Yang Y-M, Huang A, Kaley G, Sun D. eNOS uncoupling and endothelial dysfunction in aged vessels. *Am J Physiol Circ Physiol*. 2009;297(5):H1829-H1836. doi:10.1152/ajpheart.00230.2009

7. Oliveira-Paula G, Lacchini R, Tanus-Santos J. Inducible Nitric Oxide Synthase as a Possible Target in Hypertension. *Curr Drug Targets*. 2014;15(2):164-174. doi:10.2174/1389450113146660227

8. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. *Mol Cancer*. 2013;12(1):86. doi:10.1186/1476-4598-12-86

9. Zhang L, Ma Y, Zhang J, Cheng J, Du J. A New Cellular Signaling Mechanism for Angiotensin II Activation of NF-κB. *Arterioscler Thromb Vasc Biol*. 2005;25(6):1148-1153. doi:10.1161/01.ATV.0000164624.00099.e7

10. Douillette A, Bibeau-Poirier A, Gravel S-P, et al. The Proinflammatory Actions of Angiotensin II Are Dependent on p65 Phosphorylation by the IκB Kinase Complex. *J Biol Chem*. 2006;281(19):13275-13284. doi:10.1074/jbc.M512815200

11. Wolf G, Wenzel U, Burns KD, Harris RC, Stahl RAK, Thaiss F. Angiotensin II activates nuclear transcription factor-κB through AT1 and AT2 receptors11See Editorial by Luft, p. 2272. *Kidney Int*. 2002;61(6):1986-1995. doi:10.1046/j.1523-1755.2002.00365.x

12. Tilstra JS, Clauson CL, Niedernhofer LJ, Robbins PD. NF-κB in Aging and Disease. *Aging Dis*. 2011;2(6):449-465. http://www.ncbi.nlm.nih.gov/pubmed/22396894.

13. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2(1):17023. doi:10.1038/sigtrans.2017.23

14. Lang P-P, Bai J, Zhang Y-L, et al. Blockade of intercellular adhesion molecule-1 prevents angiotensin II-induced hypertension and vascular dysfunction. *Lab Investig*. 2020;100(3):378-386. doi:10.1038/s41374-019-0320-z

15. Mussbacher M, Salzmann M, Brostjan C, et al. Cell Type-Specific Roles of NF-κB Linking Inflammation and Thrombosis. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.00085

16. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*. 2020;108(3):787-799. doi:10.1002/JLB.2MR0220-549R

17. Miguel-Hidalgo JJ, Nithuairisg S, Stockmeier C, Rajkowska G. Distribution of ICAM-1 immunoreactivity during aging in the human orbitofrontal cortex. *Brain Behav Immun*. 2007;21(1):100-111. doi:10.1016/j.bbi.2006.05.001

18. Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol Med*. 2010;2(7):247-257. doi:10.1002/emmm.201000080

19. Grosso G, Godos J, Currenti W, et al. The Effect of Dietary Polyphenols on Vascular Health and Hypertension: Current Evidence and Mechanisms of Action. *Nutrients*. 2022;14(3):545. doi:10.3390/nu14030545

20. Serino A, Salazar G. Protective Role of Polyphenols against Vascular Inflammation, Aging and Cardiovascular Disease. *Nutrients*. 2018;11(1):53. doi:10.3390/nu11010053

21. Chai S, Davis K, Zhang Z, Zha L, Kirschner K. Effects of Tart Cherry Juice on Biomarkers of Inflammation and Oxidative Stress in Older Adults. *Nutrients*. 2019;11(2):228. doi:10.3390/nu11020228

22. World Health Organization. Ageing and Health. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health. Published 2018.

23. Calder PC, Bosco N, Bourdet-Sicard R, et al. Health relevance of the modification of low grade inflammation in ageing (inflammageing) and the role of nutrition. *Ageing Res Rev*. 2017;40. doi:10.1016/j.arr.2017.09.001

24. Cavanagh MM, Weyand CM, Goronzy JJ. Chronic inflammation and aging: DNA damage tips the balance. *Curr Opin Immunol*. 2012;24(4):488-493. doi:10.1016/j.coi.2012.04.003

25. Hussain T, Tan B, Yin Y, Blachier F, Tossou MCB, Rahu N. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxid Med Cell Longev*. 2016;2016:1-9. doi:10.1155/2016/7432797

26. Arulselvan P, Fard MT, Tan WS, et al. Role of Antioxidants and Natural Products in Inflammation. *Oxid Med Cell Longev*. 2016;2016:1-15. doi:10.1155/2016/5276130

27. Germolec DR, Shipkowski KA, Frawley RP, Evans E. Markers of Inflammation. In: ; 2018:57-79. doi:10.1007/978-1-4939-8549-4_5

28. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444(7121):860-867. doi:10.1038/nature05485

29. Franceschi C, Campisi J. Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. *Journals Gerontol Ser A Biol Sci Med Sci*. 2014;69(Suppl 1):S4-S9. doi:10.1093/gerona/glu057

30. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med*. 2019;25(12):1822-1832. doi:10.1038/s41591-019-0675-0

31. Roth GA, Abate D, Abate KH, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392(10159):1736-1788. doi:10.1016/S0140-6736(18)32203-7

32. Chung HY, Kim DH, Lee EK, et al. Redefining Chronic Inflammation in Aging and Age-Related Diseases: Proposal of the Senoinflammation Concept. *Aging Dis*. 2019;10(2):367. doi:10.14336/AD.2018.0324

33. Abramson S, Korchak H, Ludewig R, et al. Modes of action of aspirin-like drugs. *Proc Natl Acad Sci*. 1985;82(21):7227-7231.

doi:10.1073/pnas.82.21.7227

34. Mansoori S, Kushner N, Suminski RR, Farquhar WB, Chai SC. Added Sugar Intake is Associated with Blood Pressure in Older Females. *Nutrients*. 2019;11(9):2060. doi:10.3390/nu11092060

35. Basu A, Du M, Leyva MJ, et al. Blueberries Decrease Cardiovascular Risk Factors in Obese Men and Women with Metabolic Syndrome. *J Nutr*. 2010;140(9):1582-1587. doi:10.3945/jn.110.124701

36. Medda R, Lyros O, Schmidt JL, et al. Anti inflammatory and anti angiogenic effect of black raspberry extract on human esophageal and intestinal microvascular endothelial cells. *Microvasc Res*. 2015;97:167-180. doi:10.1016/j.mvr.2014.10.008

37. Montrose DC, Horelik NA, Madigan JP, et al. Anti-inflammatory effects of freeze-dried black raspberry powder in ulcerative colitis. *Carcinogenesis*. 2011;32(3):343-350. doi:10.1093/carcin/bgq248

38. Papp N, Szilvássy B, Abrankó L, et al. Main quality attributes and antioxidants in Hungarian sour cherries: identification of genotypes with enhanced functional properties. *Int J Food Sci Technol*. 2010;45(2):395-402. doi:10.1111/j.1365-2621.2009.02168.x

39. Kirakosyan A, Seymour EM, Llanes DEU, Kaufman PB, Bolling SF. Chemical profile and antioxidant capacities of tart cherry products. *Food Chem*. 2009;115(1):20-25. doi:10.1016/j.foodchem.2008.11.042

40. Ferretti G, Bacchetti T, Belleggia A, Neri D. Cherry Antioxidants: From Farm to Table. *Molecules*. 2010;15(10):6993-7005. doi:10.3390/molecules15106993

41. Karaaslan M, Yılmaz FM, Karaaslan A, Vardin H. Synthesis and accumulation of anthocyanins in sour cherries during ripening in accordance with antioxidant capacity development and chalcone synthase expression. *Eur Food Res Technol*. 2016;242(2):189-198. doi:10.1007/s00217-015-2530-y

42. Ou B, Bosak KN, Brickner PR, Iezzoni DG, Seymour EM. Processed Tart Cherry Products-Comparative Phytochemical Content, in vitro Antioxidant Capacity and in vitro Anti-inflammatory Activity. *J Food Sci*. 2012;77(5):H105-H112. doi:10.1111/j.1750-3841.2012.02681.x

43. (WHO) WHO. Cardiovascular diseases (CVDs). http://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds). Published 2017. Accessed July 25, 2018.

44. Centers for Disease Control and Prevention. Heart Disease Facts. https://www.cdc.gov/heartdisease/facts.htm. Published 2020.

45. Libby P. Inflammatory Mechanisms: The Molecular Basis of Inflammation and Disease. *Nutr Rev*. 2007;65:S140-S146. doi:10.1111/j.1753-4887.2007.tb00352.x

46. Keane KM, George TW, Constantinou CL, Brown MA, Clifford T, Howatson G. Effects of Montmorency tart cherry (Prunus Cerasus L.) consumption on vascular function in men with early hypertension. *Am J Clin Nutr*. 2016;103(6):1531-1539. doi:10.3945/ajcn.115.123869

47. 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). doi:10.1161/CIR.0000000000000757

48. Chai SC, Davis K, Wright RS, Kuczmarski MF, Zhang Z. Impact of tart cherry juice on systolic blood pressure and low-density lipoprotein cholesterol in older adults: a randomized controlled trial. 2018;9(6). doi:10.1039/c8fo00468d

49. Ridker PM. Clinical Application of C-Reactive Protein for Cardiovascular Disease Detection and Prevention. *Circulation*. 2003;107(3):363-369. doi:10.1161/01.CIR.0000053730.47739.3C

50. Traustadóttir T, Davies SS, Stock AA, et al. Tart Cherry Juice Decreases Oxidative Stress in Healthy Older Men and Women. *J Nutr*. 2009;139(10):1896-1900. doi:10.3945/jn.109.111716

51. Biro A, Markovich A, Homoki JR, et al. Anthocyanin-Rich Sour Cherry Extract Attenuates the Lipopolysaccharide-Induced Endothelial Inflammatory Response. *Molecules*. 2019;24(19):3427. doi:10.3390/molecules24193427

52. Krensky AM, Ahn Y-T. Mechanisms of Disease: regulation of RANTES (CCL5) in renal disease. *Nat Clin Pract Nephrol*. 2007;3(3):164-170. doi:10.1038/ncpneph0418

53. Hu K, Yang J, Tanaka S, Gonias SL, Mars WM, Liu Y. Tissue-type Plasminogen Activator Acts as a Cytokine That Triggers Intracellular Signal Transduction and Induces Matrix Metalloproteinase-9 Gene Expression. *J Biol Chem*. 2006;281(4):2120-2127. doi:10.1074/jbc.M504988200

54. Dorris SL, Peebles RS. PGI 2 as a Regulator of Inflammatory Diseases. *Mediators Inflamm*. 2012;2012:1-9. doi:10.1155/2012/926968

55. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis*. 2013;5(2):77-94. doi:10.1177/1759720X12467868

56. Kuehl KS, Elliot DL, Sleigh AE, Smith JL. Efficacy of Tart Cherry Juice to Reduce Inflammation Biomarkers among Women with Inflammatory Osteoarthritis (OA). *J Food Stud*. 2012;1(1). doi:10.5296/jfs.v1i1.1927

57. Schumacher HR, Pullman-Mooar S, Gupta SR, Dinnella JE, Kim R, McHugh MP. Randomized double-blind crossover study of the efficacy of a tart cherry juice blend in treatment of osteoarthritis (OA) of the knee. *Osteoarthr Cartil*. 2013;21(8):1035-1041. doi:10.1016/j.joca.2013.05.009

58. Martin KR, Coles KM. Consumption of 100% Tart Cherry Juice Reduces Serum Urate in Overweight and Obese Adults. *Curr Dev Nutr*. 2019;3(5). doi:10.1093/cdn/nzz011

59. He Y, Zhou J, Wang Y, et al. Anti-inflammatory and anti-oxidative effects of cherries on Freund's adjuvant-induced arthritis in rats. *Scand J Rheumatol*. 2006;35(5):356-358. doi:10.1080/03009740600704155

60. BOWTELL JL, SUMNERS DP, DYER A, FOX P, MILEVA KN. Montmorency Cherry Juice Reduces Muscle Damage Caused by Intensive Strength Exercise. *Med Sci Sport Exerc*. 2011;43(8):1544-1551. doi:10.1249/MSS.0b013e31820e5adc

61. Peake JM, Neubauer O, Della Gatta PA, Nosaka K. Muscle damage and inflammation during recovery from exercise. *J Appl Physiol*. 2017;122(3):559-570. doi:10.1152/japplphysiol.00971.2016

62. Bell P, Walshe I, Davison G, Stevenson E, Howatson G. Montmorency Cherries Reduce the Oxidative Stress and Inflammatory Responses to Repeated Days High-Intensity Stochastic Cycling. *Nutrients*. 2014;6(2):829-843. doi:10.3390/nu6020829

63. Dimitriou L, Hill JA, Jehnali A, et al. Influence of a montmorency cherry juice blend on indices of exercise-induced stress and upper respiratory tract symptoms following marathon running—a pilot investigation. *J Int Soc Sports Nutr*. 2015;12(1). doi:10.1186/s12970-015-0085-8

64. Nemes, Homoki, Kiss, et al. Effect of Anthocyanin-Rich Tart Cherry Extract on Inflammatory Mediators and Adipokines Involved in Type 2 Diabetes in a High Fat Diet Induced Obesity Mouse Model. *Nutrients*. 2019;11(9):1966. doi:10.3390/nu11091966

65. Sears B, Perry M. The role of fatty acids in insulin resistance. *Lipids Health Dis*. 2015;14(1):121. doi:10.1186/s12944-015-0123-1

66. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose Expression of Tumor Necrosis Factor-α: Direct Role in Obesity-Linked Insulin Resistance. *Science (80-)*. 1993;259(5091):87-91. doi:10.1126/science.7678183

67. Martin KR, Burrell L, Bopp J. Authentic tart cherry juice reduces markers of inflammation in overweight and obese subjects: a randomized, crossover pilot study. *Food Funct*. 2018;9(10):5290-5300. doi:10.1039/C8FO01492B

68. Seymour EM, Lewis SK, Urcuyo-Llanes DE, et al. Regular Tart Cherry Intake Alters Abdominal Adiposity, Adipose Gene Transcription, and Inflammation in Obesity-Prone Rats Fed a High Fat Diet. *J Med Food*. 2009;12(5):935-942. doi:10.1089/jmf.2008.0270

69. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's Dement Transl Res Clin Interv*. 2018;4(1):575-590. doi:10.1016/j.trci.2018.06.014

70. Walker D, Lue L-F. Anti-inflammatory and Immune Therapy for Alzheimers Disease: Current Status and Future Directions. *Curr Neuropharmacol*. 2007;5(4):232-243. doi:10.2174/157015907782793667

71. Kim D-O, Heo HJ, Kim YJ, et al. Sweet and Sour Cherry Phenolics and Their Protective Effects on Neuronal Cells. *J Agric Food Chem*. 2005;53(26):9921-9927. doi:10.1021/jf0518599

72. Carson C. Tart Cherry Juice as a Treatment for Peripheral Neuropathy. 2015.

73. Chai SC, Jerusik J, Davis K, Wright RS, Zhang Z. Effect of Montmorency tart cherry juice on cognitive performance in older adults: a randomized controlled trial. *Food Funct*. 2019;10(7):4423-4431. doi:10.1039/C9FO00913B

74. Rakoff-Nahoum S. Why Cancer and Inflammation? *Yale J Biol Med*. 2006;79(3-4):123-130.

75. Ogur R, Istanbulluoglu H, Korkmaz A, Barla A, Tekbas OF, Oztas E. Report: investigation of anti-cancer effects of cherry in vitro. *Pak J Pharm Sci*. 2014;27(3):587-592.

76. Sibal L, C Agarwal S, D Home P, H Boger R. The Role of Asymmetric Dimethylarginine (ADMA) in Endothelial Dysfunction and Cardiovascular

Disease. *Curr Cardiol Rev*. 2010;6(2):82-90. doi:10.2174/157340310791162659

77. Kang S-Y, Seeram NP, Nair MG, Bourquin LD. Tart cherry anthocyanins inhibit tumor development in ApcMin mice and reduce proliferation of human colon cancer cells. *Cancer Lett*. 2003;194(1):13-19. doi:10.1016/S0304-3940(02)00583-9

78. Martin KR, Wooden A. Tart Cherry Juice Induces Differential Dose-Dependent Effects on Apoptosis, But Not Cellular Proliferation, in MCF-7 Human Breast Cancer Cells. *J Med Food*. 2012;15(11):945-954. doi:10.1089/jmf.2011.0336

79. Bobe G, Wang B, Seeram NP, Nair MG, Bourquin LD. Dietary Anthocyanin-Rich Tart Cherry Extract Inhibits Intestinal Tumorigenesis in APC Min Mice Fed Suboptimal Levels of Sulindac. *J Agric Food Chem*. 2006;54(25):9322-9328. doi:10.1021/jf0612169

80. CHAOVANALIKIT A, WROLSTAD RE. Anthocyanin and Polyphenolic Composition of Fresh and Processed Cherries. *J Food Sci*. 2004;69(1):FCT73-FCT83. doi:10.1111/j.1365-2621.2004.tb17859.x

81. Dabeek WM, Marra MV. Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular-Related Bioactivity in Humans. *Nutrients*. 2019;11(10):2288. doi:10.3390/nu11102288

82. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*. 2017;61(1):1361779. doi:10.1080/16546628.2017.1361779

83. Simmonds RE, Foxwell BM. Signalling, inflammation and arthritis: NF- B and its relevance to arthritis and inflammation. *Rheumatology*. 2008;47(5):584-590. doi:10.1093/rheumatology/kem298

84. Chekalina N, Burmak Y, Petrov Y, et al. Quercetin reduces the transcriptional activity of NF-kB in stable coronary artery disease. *Indian Heart J*. 2018;70(5):593-597. doi:10.1016/j.ihj.2018.04.006

85. Ruiz PA, Braune A, Hölzlwimmer G, Quintanilla-Fend L, Haller D. Quercetin Inhibits TNF-Induced NF-κB Transcription Factor Recruitment to Proinflammatory Gene Promoters in Murine Intestinal Epithelial Cells. *J Nutr*. 2007;137(5):1208-1215. doi:10.1093/jn/137.5.1208

86. Sun Y, Li L. Cyanidin-3-glucoside inhibits inflammatory activities in human

fibroblast-like synoviocytes and in mice with collagen-induced arthritis. *Clin Exp Pharmacol Physiol*. 2018;45(10):1038-1045. doi:10.1111/1440-1681.12970

87. Jung H, Kwak H-K, Hwang KT. Antioxidant and antiinflammatory activities of cyanidin-3-glucoside and cyanidin-3-rutinoside in hydrogen peroxide and lipopolysaccharide-treated RAW264.7 cells. *Food Sci Biotechnol*. 2014;23(6):2053-2062. doi:10.1007/s10068-014-0279-x

88. Jeon Y-D, Aye A, Song Y-J, Kim Y-H, Soh J-R, Jin J-S. Cyanidin 3-Rutinoside, an Anthocyanin Pigment of Schisandra chinensis Baill, Inhibits Allergic Inflammation. *J Med Food*. 2019;22(7):703-712. doi:10.1089/jmf.2018.4346

89. Kadioglu O, Nass J, Saeed MEM, Schuler B, Efferth T. Kaempferol Is an Anti-Inflammatory Compound with Activity towards NF-κB Pathway Proteins. *Anticancer Res*. 2015;35(5):2645-2650.

90. Kim JM, Lee EK, Kim DH, Yu BP, Chung HY. Kaempferol modulates pro-inflammatory NF-κB activation by suppressing advanced glycation endproducts-induced NADPH oxidase. *Age (Omaha)*. 2010;32(2):197-208. doi:10.1007/s11357-009-9124-1

91. Morrison DK. MAP Kinase Pathways. *Cold Spring Harb Perspect Biol*. 2012;4(11):a011254-a011254. doi:10.1101/cshperspect.a011254

92. Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. *Biochim Biophys Acta - Proteins Proteomics*. 2005;1754(1-2):253-262. doi:10.1016/j.bbapap.2005.08.017

93. Hanna VS, Hafez EAA. Synopsis of arachidonic acid metabolism: A review. *J Adv Res*. 2018;11:23-32. doi:10.1016/j.jare.2018.03.005

94. Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. *Cell Mol Immunol*. 2015;12(1):5-23. doi:10.1038/cmi.2014.89

95. Jacob RA, Burri BJ. Oxidative damage and defense. *Am J Clin Nutr*. 1996;63(6):985S-990S. doi:10.1093/ajcn/63.6.985

96. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Cell Mol Physiol*. 2000;279(6):L1005-L1028. doi:10.1152/ajplung.2000.279.6.L1005

97. Lila MA, Burton-Freeman B, Grace M, Kalt W. Unraveling Anthocyanin Bioavailability for Human Health. *Annu Rev Food Sci Technol*. 2016;7(1):375-393. doi:10.1146/annurev-food-041715-033346

98. Seymour EM, Warber SM, Kirakosyan A, et al. Anthocyanin pharmacokinetics and dose-dependent plasma antioxidant pharmacodynamics following whole tart cherry intake in healthy humans. *J Funct Foods*. 2014;11:509-516. doi:10.1016/j.jff.2014.08.007

99. de Ferrars RM, Czank C, Zhang Q, et al. The pharmacokinetics of anthocyanins and their metabolites in humans. *Br J Pharmacol*. 2014;171(13):3268-3282. doi:10.1111/bph.12676

100. Keane KM, Bell PG, Lodge JK, et al. Phytochemical uptake following human consumption of Montmorency tart cherry (L. Prunus cerasus) and influence of phenolic acids on vascular smooth muscle cells in vitro. *Eur J Nutr*. 2016;55(4):1695-1705. doi:10.1007/s00394-015-0988-9

101. Wang H, Wang H, Wang J, Wang Q, Ma Q, Chen Y. Protocatechuic Acid Inhibits Inflammatory Responses in LPS-Stimulated BV2 Microglia via NF-κB and MAPKs Signaling Pathways. *Neurochem Res*. 2015;40(8):1655-1660. doi:10.1007/s11064-015-1646-6

102. Kim M-C, Kim S-J, Kim D-S, et al. Vanillic acid inhibits inflammatory mediators by suppressing NF-κB in lipopolysaccharide-stimulated mouse peritoneal macrophages. *Immunopharmacol Immunotoxicol*. 2011;33(3):525-532. doi:10.3109/08923973.2010.547500

103. Go AS, Bauman MA, Coleman King SM, et al. An Effective Approach to High Blood Pressure Control. *J Am Coll Cardiol*. 2014;63(12):1230-1238. doi:10.1016/j.jacc.2013.11.007

104. CDC. Hypertension Prevalence and Control Among Adults. https://www.cdc.gov/nchs/products/databriefs/db289.htm. Published 2017. Accessed January 28, 2019.

105. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *J Am Coll Cardiol*. 2018;71(19):e127-e248. doi:10.1016/j.jacc.2017.11.006

106. 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). doi:10.1161/CIR.0000000000000558

107. Ritchey M, Tsipas S, Loustalot F, Wozniak G. Use of Pharmacy Sales Data to Assess Changes in Prescription- and Payment-Related Factors that Promote Adherence to Medications Commonly Used to Treat Hypertension, 2009 and 2014. *PLoS One*. 2016;11(7):e0159366. doi:10.1371/journal.pone.0159366

108. Khavjou O, Phelps D, Leib A. *Projections of Cardiovascular Disease Prevalence and Costs: 2015–2035*.; 2016. http://www.heart.org/idc/groups/heart-public/@wcm/@adv/documents/downloadable/ucm_491513.pdf. Accessed March 2, 2019.

109. Collier SR, Landram MJ. Treatment of prehypertension: lifestyle and/or medication. *Vasc Health Risk Manag*. 2012;8:613-619. doi:10.2147/VHRM.S29138

110. Qato DM, Alexander GC, Conti RM, Johnson M, Schumm P, Lindau ST. Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *JAMA*. 2008;300(24):2867-2878. doi:10.1001/jama.2008.892

111. Conlin PR. The dietary approaches to stop hypertension (DASH) clinical trial: implications for lifestyle modifications in the treatment of hypertensive patients. *Cardiol Rev*. 1999;7(5):284-288. http://www.ncbi.nlm.nih.gov/pubmed/11208239. Accessed December 28, 2018.

112. Sacks FM, Svetkey LP, Vollmer WM, et al. Effects on Blood Pressure of Reduced Dietary Sodium and the Dietary Approaches to Stop Hypertension (DASH) Diet. *N Engl J Med*. 2001;344(1):3-10. doi:10.1056/NEJM200101043440101

113. Widmer RJ, Flammer AJ, Lerman LO, Lerman A. The Mediterranean diet, its components, and cardiovascular disease. *Am J Med*. 2015;128(3):229-238. doi:10.1016/j.amjmed.2014.10.014

114. Washburn RA, McAuley E, Katula J, Mihalko SL, Boileau RA. The Physical Activity Scale for the Elderly (PASE). *J Clin Epidemiol*. 1999;52(7):643-651. doi:10.1016/S0895-4356(99)00049-9

115. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*.; 1988.

116. Dorans KS, Mills KT, Liu Y, He J. Trends in Prevalence and Control of Hypertension According to the 2017 American College of

Cardiology/American Heart Association (ACC/AHA) Guideline. *J Am Heart Assoc*. 2018;7(11). doi:10.1161/JAHA.118.008888

117. Hypertension BRFS Data Update - Delaware Health and Social Services - State of Delaware. https://www.dhss.delaware.gov/dhss/dph/dpc/hypertensionupdate.html. Accessed April 28, 2019.

118. Malik AH, Akram Y, Shetty S, Malik SS, Yanchou Njike V. Impact of Sugar-Sweetened Beverages on Blood Pressure. *Am J Cardiol*. 2014;113(9):1574-1580. doi:10.1016/j.amjcard.2014.01.437

119. Te Morenga LA, Howatson AJ, Jones RM, Mann J. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *Am J Clin Nutr*. 2014;100(1):65-79. doi:10.3945/ajcn.113.081521

120. Raben A, Vasilaras TH, Møller AC, Astrup A. Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. *Am J Clin Nutr*. 2002;76(4):721-729. doi:10.1093/ajcn/76.4.721

121. Bowman SA, Clemens JC, Martin CL, Anand J, Steinfeldt LC, Moshfegh AJ. *Highlights Added Sugars Intake of Americans: What We Eat in America, NHANES 2013-2014 What Percent of Americans Meet the DGA Added Sugars Recommendation? Figure 1. Estimated Percentage* of Americans Meeting the DGA 2015-2020 Added Sugars 1 Recommendation by Age, WWEIA, NHANES 2013-2014*.; 2017. www.ars.usda.gov/nea/bhnrc/fsrg. Accessed March 2, 2019.

122. American Heart Association. Added Sugars | American Heart Association. American Heart Association. http://www.heart.org/en/healthy-living/healthy-eating/eat-smart/sugar/added-sugars. Accessed October 14, 2018.

123. Drewnowski A, Rehm CD. Consumption of added sugars among US children and adults by food purchase location and food source. *Am J Clin Nutr*. 2014;100(3):901-907. doi:10.3945/ajcn.114.089458

124. Buñag RD, Tomita T, Sasaki S. Chronic sucrose ingestion induces mild hypertension and tachycardia in rats. *Hypertension*. 1983;5(2):218-225. doi:10.1161/01.HYP.5.2.218

125. Barbagallo M, Pang PK, Resnick LM, Shan J. Glucose-induced alterations of cytosolic free calcium in cultured rat tail artery vascular smooth muscle cells. Glucose-induced Alterations of Cytosolic Free Calcium in Cultured Rat Tail

Artery Vascular Smooth Muscle Cells. *J Clin Invest*. 1995;95(2):763. doi:10.1172/JCI117724

126. Ottolini M, Hong K, Sonkusare SK. Calcium signals that determine vascular resistance. *Wiley Interdiscip Rev Syst Biol Med*. March 2019:e1448. doi:10.1002/wsbm.1448

127. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr*. 2004;79(4):537-543. doi:10.1093/ajcn/79.4.537

128. Hussain M, Awan FR. Hypertension regulating angiotensin peptides in the pathobiology of cardiovascular disease. *Clin Exp Hypertens*. 2018;40(4):344-352. doi:10.1080/10641963.2017.1377218

129. Farah V, Elased KM, Chen Y, et al. Nocturnal hypertension in mice consuming a high fructose diet. *Auton Neurosci*. 2006;130(1-2):41-50. doi:10.1016/j.autneu.2006.05.006

130. Soleimani M. Dietary fructose, salt absorption and hypertension in metabolic syndrome: towards a new paradigm. *Acta Physiol*. 2011;201(1):55-62. doi:10.1111/j.1748-1716.2010.02167.x

131. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)*. 2005;2(1):5. doi:10.1186/1743-7075-2-5

132. Nakagawa T, Hu H, Zharikov S, et al. A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Physiol*. 2006;290(3):F625-F631. doi:10.1152/ajprenal.00140.2005

133. Stanhope KL. Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit Rev Clin Lab Sci*. 2016;53(1):52-67. doi:10.3109/10408363.2015.1084990

134. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest*. 2009;119(5):1322-1334. doi:10.1172/JCI37385

135. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ*. 2012;346(jan15 3):e7492-e7492. doi:10.1136/bmj.e7492

136. Hulman S, Falkner B. The Effect of Excess Dietary Sucrose on Growth, Blood

Pressure, and Metabolism in Developing Sprague-Dawley Rats. *Pediatr Res*. 1994;36(1):95-100. doi:10.1203/00006450-199407001-00017

137. Galipeau D, Verma S, McNeill JH. Female rats are protected against fructose-induced changes in metabolism and blood pressure. *Am J Physiol Circ Physiol*. 2002;283(6):H2478-H2484. doi:10.1152/ajpheart.00243.2002

138. John J, Ziebland S, Yudkin P, Roe L, Neil H. Effects of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. *Lancet*. 2002;359(9322):1969-1974. doi:10.1016/S0140-6736(02)98858-6

139. Kim J, Kim J. Association between Fruit and Vegetable Consumption and Risk of Hypertension in Middle-Aged and Older Korean Adults. *J Acad Nutr Diet*. 2018;118(8):1438-1449.e5. doi:10.1016/j.jand.2017.08.122

140. Barona J, Aristizabal JC, Blesso CN, Volek JS, Fernandez ML. Grape Polyphenols Reduce Blood Pressure and Increase Flow-Mediated Vasodilation in Men with Metabolic Syndrome. *J Nutr*. 2012;142(9):1626-1632. doi:10.3945/jn.112.162743

141. Johnson SA, Figueroa A, Navaei N, et al. Daily Blueberry Consumption Improves Blood Pressure and Arterial Stiffness in Postmenopausal Women with Pre- and Stage 1-Hypertension: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *J Acad Nutr Diet*. 2015;115(3):369-377. doi:10.1016/j.jand.2014.11.001

142. Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM. Dietary Approaches to Prevent and Treat Hypertension. *Hypertension*. 2006;47(2):296-308. doi:10.1161/01.HYP.0000202568.01167.B6

143. Whelton PK, He J, Cutler JA, et al. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. *JAMA*. 1997;277(20):1624-1632. http://www.ncbi.nlm.nih.gov/pubmed/9168293. Accessed May 24, 2019.

144. Lana A, Banegas JR, Guallar-Castillón P, Rodríguez-Artalejo F, Lopez-Garcia E. Association of Dairy Consumption and 24-Hour Blood Pressure in Older Adults with Hypertension. *Am J Med*. 2018;131(10):1238-1249. doi:10.1016/j.amjmed.2018.04.039

145. Wang L, Gaziano JM, Liu S, Manson JE, Buring JE, Sesso HD. Whole- and refined-grain intakes and the risk of hypertension in women. *Am J Clin Nutr*. 2007;86(2):472-479. doi:10.1093/ajcn/86.2.472

146. Ampatzoglou A, Atwal KK, Maidens CM, et al. Increased Whole Grain Consumption Does Not Affect Blood Biochemistry, Body Composition, or Gut Microbiology in Healthy, Low-Habitual Whole Grain Consumers. *J Nutr*. 2015;145(2):215-221. doi:10.3945/jn.114.202176

147. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol*. 2020;16(4):223-237. doi:10.1038/s41581-019-0244-2

148. Patrick DM, Van Beusecum JP, Kirabo A. The role of inflammation in hypertension: novel concepts. *Curr Opin Physiol*. 2021;19:92-98. doi:10.1016/j.cophys.2020.09.016

149. Mayta-Apaza AC, Marasini D, Carbonero F. Tart Cherries and health: Current knowledge and need for a better understanding of the fate of phytochemicals in the human gastrointestinal tract. *Crit Rev Food Sci Nutr*. September 2017:00-00. doi:10.1080/10408398.2017.1384918

150. Pinto E. Blood pressure and ageing. *Postgrad Med J*. 2007;83(976):109-114. doi:10.1136/pgmj.2006.048371

151. Castellon X, Bogdanova V. Chronic Inflammatory Diseases and Endothelial Dysfunction. *Aging Dis*. 2016;7(1):81. doi:10.14336/AD.2015.0803

152. Kim JM, Heo H-S, Ha YM, et al. Mechanism of Ang II involvement in activation of NF-κB through phosphorylation of p65 during aging. *Age (Omaha)*. 2012;34(1):11-25. doi:10.1007/s11357-011-9207-7

153. Iglesias-Carres L, Mas-Capdevila A, Bravo FI, Mulero M, Muguerza B, Arola-Arnal A. Optimization and characterization of Royal Dawn cherry (Prunus avium) phenolics extraction. *Sci Rep*. 2019;9(1):17626. doi:10.1038/s41598-019-54134-w

154. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat Protoc*. 2007;2(4):875-877. doi:10.1038/nprot.2007.102

155. Lee J, Durst RW, Wrolstad RE, et al. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J AOAC Int*. 2005;88(5):1269-1278. doi:10.1093/jaoac/88.5.1269

156. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. *Clin Sci*. 2011;120(9):357-375. doi:10.1042/CS20100476

157. Donato AJ, Black AD, Jablonski KL, Gano LB, Seals DR. Aging is associated with greater nuclear NFκB, reduced IκBα, and increased expression of proinflammatory cytokines in vascular endothelial cells of healthy humans. *Aging Cell*. 2008;7(6):805-812. doi:10.1111/j.1474-9726.2008.00438.x

158. Yan X, Zhang Q-Y, Zhang Y-L, Han X, Guo S-B, Li H-H. Gallic Acid Attenuates Angiotensin II-Induced Hypertension and Vascular Dysfunction by Inhibiting the Degradation of Endothelial Nitric Oxide Synthase. *Front Pharmacol*. 2020;11. doi:10.3389/fphar.2020.01121

159. Gallo G, Volpe M, Savoia C. Endothelial Dysfunction in Hypertension: Current Concepts and Clinical Implications. *Front Med*. 2022;8. doi:10.3389/fmed.2021.798958

160. Endemann DH. Endothelial Dysfunction. *J Am Soc Nephrol*. 2004;15(8):1983-1992. doi:10.1097/01.ASN.0000132474.50966.DA

161. Loot AE, Schreiber JG, Fisslthaler B, Fleming I. Angiotensin II impairs endothelial function via tyrosine phosphorylation of the endothelial nitric oxide synthase. *J Exp Med*. 2009;206(13):2889-2896. doi:10.1084/jem.20090449

162. Wood KC, Cortese-Krott MM, Kovacic JC, et al. Circulating Blood Endothelial Nitric Oxide Synthase Contributes to the Regulation of Systemic Blood Pressure and Nitrite Homeostasis. *Arterioscler Thromb Vasc Biol*. 2013;33(8):1861-1871. doi:10.1161/ATVBAHA.112.301068

163. Garcia V, Sessa WC. Endothelial NOS: perspective and recent developments. *Br J Pharmacol*. 2019;176(2):189-196. doi:10.1111/bph.14522

164. Zamora R, Vodovotz Y, Billiar TR. Inducible Nitric Oxide Synthase and Inflammatory Diseases. *Mol Med*. 2000;6(5):347-373. doi:10.1007/BF03401781

165. Shukitt-Hale B, Kelly ME, Bielinski DF, Fisher DR. Tart Cherry Extracts Reduce Inflammatory and Oxidative Stress Signaling in Microglial Cells. *Antioxidants (Basel, Switzerland)*. 2016;5(4). doi:10.3390/antiox5040033

166. Zhong H, May MJ, Jimi E, Ghosh S. The Phosphorylation Status of Nuclear NF-KB Determines Its Association with CBP/p300 or HDAC-1. *Mol Cell*. 2002;9(3):625-636. doi:10.1016/S1097-2765(02)00477-X

167. Zambrano S, Bianchi ME, Agresti A. High-Throughput Analysis of NF-κB Dynamics in Single Cells Reveals Basal Nuclear Localization of NF-κB and Spontaneous Activation of Oscillations. Mancini MA, ed. *PLoS One*.

2014;9(3):e90104. doi:10.1371/journal.pone.0090104

168. Lakota K, Mrak-Poljsak K, Rozman B, Sodin-Semrl S. Increased Responsiveness of Human Coronary Artery Endothelial Cells in Inflammation and Coagulation. *Mediators Inflamm*. 2009;2009:1-8. doi:10.1155/2009/146872

169. Lakota K, Mrak-Poljšak K, Rozman B, Kveder T, Tomšič M, Sodin-Semrl S. Serum Amyloid A Activation of Inflammatory and Adhesion Molecules in Human Coronary Artery and Umbilical Vein Endothelial Cells. *Eur J Inflamm*. 2007;5(2):73-81. doi:10.1177/1721727X0700500203

170. Najjar RS, Mu S, Feresin RG. Blueberry Polyphenols Increase Nitric Oxide and Attenuate Angiotensin II-Induced Oxidative Stress and Inflammatory Signaling in Human Aortic Endothelial Cells. *Antioxidants*. 2022;11(4):616. doi:10.3390/antiox11040616

171. Bulusu KC, Guha R, Mason DJ, et al. Modelling of compound combination effects and applications to efficacy and toxicity: state-of-the-art, challenges and perspectives. *Drug Discov Today*. 2016;21(2):225-238. doi:10.1016/j.drudis.2015.09.003

172. Basu U, Seravalli J, Madayiputhiya N, Adamec J, Case AJ, Zimmerman MC. Rapid metabolism of exogenous angiotensin II by catecholaminergic neuronal cells in culture media. *Physiol Rep*. 2015;3(2):e12287. doi:10.14814/phy2.12287

173. Outzen EM, Zaki M, Mehryar R, et al. Lipopolysaccharides, but not Angiotensin ll, lnduces Direct Pro-lnflammatory Effects in Cultured Mouse Arteries and Human Endothelial and Vascular Smooth Muscle Cells. *Basic Clin Pharmacol Toxicol*. 2017;120(4):335-347. doi:10.1111/bcpt.12697

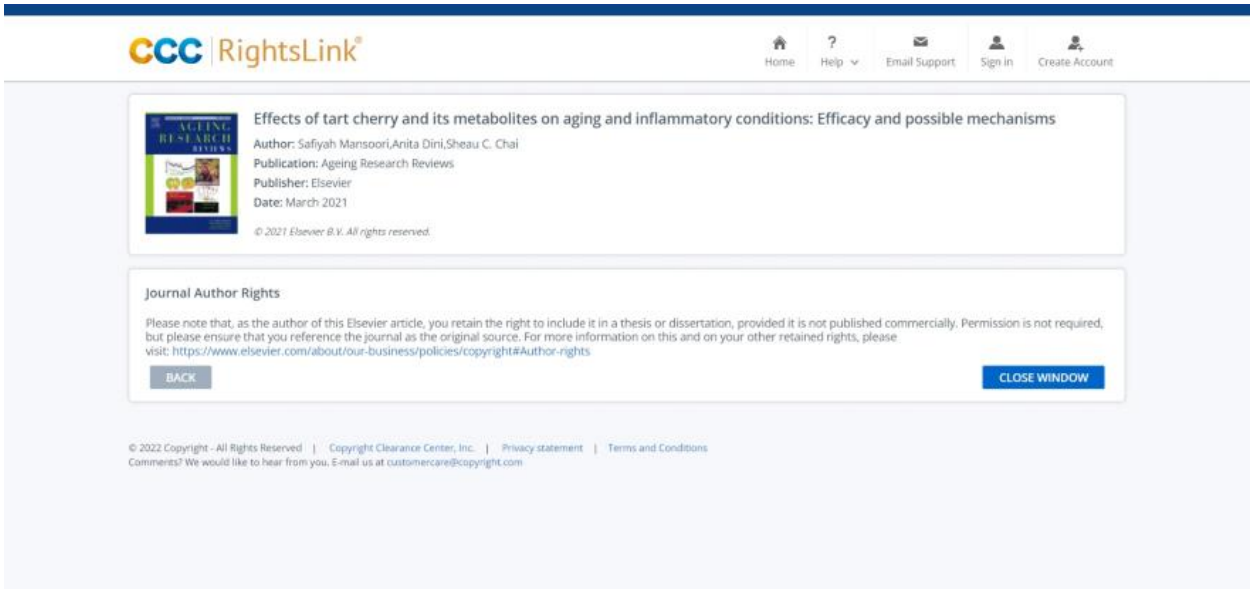
174. Gurven M, Blackwell AD, Rodríguez DE, Stieglitz J, Kaplan H. Does Blood Pressure Inevitably Rise With Age? *Hypertension*. 2012;60(1):25-33. doi:10.1161/HYPERTENSIONAHA.111.189100

175. Mansoori S, Dini A, Chai SC. Effects of tart cherry and its metabolites on aging and inflammatory conditions: Efficacy and possible mechanisms. *Ageing Res Rev*. 2021;66:101254. doi:10.1016/j.arr.2021.101254

176. Zimmerman MC, Lazartigues E, Sharma R V., Davisson RL. Hypertension Caused by Angiotensin II Infusion Involves Increased Superoxide Production in the Central Nervous System. *Circ Res*. 2004;95(2):210-216. doi:10.1161/01.RES.0000135483.12297.e4

Appendix A

PERMISSIONS AND RIGHTS



MDPI Open Access Information and Policy

All articles published by MDPI are made immediately available worldwide under an open access license. This means:

- everyone has free and unlimited access to the full-text of *all* articles published in MDPI journals;
- everyone is free to re-use the published material if proper accreditation/citation of the original publication is given;
- open access publication is supported by the authors' institutes or research funding agencies by payment of a comparatively low **Article Processing Charge (APC)** for accepted articles.

Permissions

No special permission is required to reuse all or part of article published by MDPI, including figures and tables. For articles published under an open access Creative Common CC BY license, any part of the article may be reused without permission provided that the original article is clearly cited. Reuse of an article does not imply endorsement by the authors or MDPI.

External Open Access Resources

Those who are new to the concept of open access might find the following websites or the *Open Access Explained!* video informative:

- **Wikipedia article on Open Access**
- **Open Access Network**

Meaning of Open Access

In accordance with major definitions of open access in scientific literature (namely the Budapest, Berlin, and Bethesda declarations), MDPI defines *open access* by the following conditions:

- peer-reviewed literature is freely available without subscription or price barriers,
- literature is immediately released in open access format (no embargo period), and
- published material can be re-used without obtaining permission as long as a correct citation to the original publication is given.

Open Access Explained!

Until 2008, most articles published by MDPI contained the note: "© *year* by MDPI (http://www.mdpi.org). Reproduction is permitted for noncommercial purposes". During 2008, MDPI journals started to publish articles under the **Creative Commons Attribution License** and are now using the latest version of the CC BY license, which grants authors the most extensive rights. All articles published by MDPI before and during 2008 should now be considered as having been released under the post-2008 Creative Commons Attribution License.

This means that all articles published in MDPI journals, including data, graphics, and supplements, can be linked from external sources, scanned by search engines, re-used by text mining applications or websites, blogs, *etc.* free of charge under the sole condition of proper accreditation of the source and original publisher. MDPI believes that open access publishing fosters the exchange of research results amongst scientists from different disciplines, thus facilitating interdisciplinary research. Open access publishing also provides access to research results to researchers worldwide, including those from developing countries, and to an interested general public. Although MDPI publishes all of its journals under the open access model, we believe that open access is an enriching part of the scholarly communication process that can and should co-exist with other forms of communication and publication, such as society-based publishing and conferencing activities.

Important Note: some articles (especially *Reviews*) may contain figures, tables or text taken from other publications, for which MDPI does not hold the copyright or the right to re-license the published material. Please note that you should inquire with the original copyright holder (usually the original publisher or authors), whether or not this material can be re-used.

Advantages of Open Access for Authors

The High Availability and Visibility of our open access articles is guaranteed through the free and unlimited accessibility of the publication over the Internet. Everyone can freely access and download the full text of all articles published with MDPI: readers of

Appendix B

IRB APPROVAL

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE:	December 6, 2016
TO:	Sheau Ching Chai, PhD, RD
FROM:	University of Delaware IRB
STUDY TITLE:	[681018-7] Effects of tart cherry juice on cognitive function and cardiovascular disease risk factors in older adults
SUBMISSION TYPE:	Continuing Review/Progress Report
ACTION:	Approved for Data Analysis Only
APPROVAL DATE:	December 6, 2016
EXPIRATION DATE:	December 16, 2017
REVIEW TYPE:	Expedited Review
REVIEW CATEGORY:	Expedited review category # *(8)*

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.

Appendix C

LIST OF PUBLICATIONS

Mansoori S, Dini A, Chai SC. Effects of Tart Cherry and Its Metabolites on Aging and Inflammatory Conditions: Efficacy and Possible Mechanisms (2021). *Ageing Research Reviews.*, 2021 Mar; 66:101254. Doi: 10.1016/j.arr.2021.101254.

Mansoori S, Kushner N, Suminski R, Farquhar WB, and Chai SC (2019). Added Sugar Intake is Associated with Blood Pressure in Older Females. *Nutrients.*, 2019 Sep; 11(9):2060. Doi: 10.3390/nu11092060.

Mansoori S and Chai SC. Impacts of Tart Cherry Extracts on Inflammation in Human Coronary Artery Endothelial Cells (2022). *Manuscript in Preparation.*

Published Abstracts and Poster Presentations:

Ramesh B, Isseks A, Martin J, **Mansoori S,** Browne A, Suminski R, Chai SC. Vitamin C and Copper Intake is Associated with Cognitive Function in Older Adults. Poster presented at the American Society for Nutrition Virtual Conference. *Current Developments in Nutrition. Nutrition.*, 2022 June; 6(1); Doi:10.1093/cdn/nzac051.080.

Mansoori S, Liberatore C, Ramirez A, Chai SC. Increased Sodium Consumption Is Associated with Abdominal Obesity in Older Adults. Poster presented at the American Society for Nutrition Virtual Conference. *Current Developments in Nutrition. Nutrition.*, 2021 June; 5(2); Doi: 10.1093/cdn/nzab055_040.

Chai SC, Cicalo C, Barish N, **Mansoori S**, Wright R, Callahan N. Effects of Freeze-dried Whole Grapes on Cognitive Performance in Postmenopausal Women. Poster presented at the American Society for Nutrition Virtual Conference. *Current Developments in Nutrition. Nutrition.*, 2020 May; 4(2); Doi: 10.1093/cdn/nzaa057_012.

Mansoori S, Suminski R, Kushner N, Cicalo C, Chai SC. Added Sugar and Solid Fruit Predict Blood Pressure in Older Adults. Presented at the American Society for Nutrition Conference, Baltimore, MD and College of Health Sciences Research Day, University of Delaware, Newark, DE USA. *Current Developments in Nutrition. Nutrition.* 2019 June; 3(1); Doi: 10.1093/cdn/nzz039.P18-030-19.

Li I, Katcher J, Liberatore C, Callahan N, **Mansoori S,** Barish N, Pacanowski C, Chai SC**.** The combined effect of Ashwagandha and B Vitamins on stress relief: Preliminary results from a pre- and post-intervention study. Poster presentation at the College of Health Sciences Research Day. University of Delaware. Newark, DE USA. 2019 February.

Chai SC, Kramer S, Brown N, McMahon J, **Mansoori S,** Zhang Z. Effects of Tart Cherry Juice on Biomarkers of Vascular Function. Poster presentation at American Society for Nutrition Conference. Boston, MA USA. *Current Developments in Nutrition. Biomarkers.* 2018 June. 2(11); Doi:10.1093/cdn/nzy029.