

**TART CHERRY JUICE CONSUMPTION REDUCES BLOOD PRESSURE IN
OLDER ADULTS**

by

Kristina Davis

A thesis submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Master of Science in Human Nutrition

Spring 2016

© 2016 Kristina Davis
All Rights Reserved

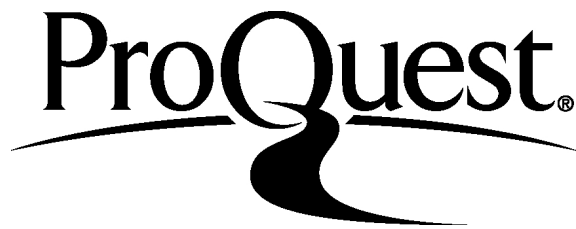
ProQuest Number: 10156535

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10156535

Published by ProQuest LLC (2016). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

**TART CHERRY JUICE CONSUMPTION REDUCES BLOOD PRESSURE IN
OLDER ADULTS**

by

Kristina Davis

Approved: _____
Sheau Ching Chai, Ph.D., R.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____
P. Michael Peterson, Ed.D.
Chair of the Department of Behavioral Health and Nutrition

Approved: _____
Kathleen S. Matt, Ph.D.
Dean of the College of Health Sciences

Approved: _____
Ann L. Ardis, Ph.D.
Senior Vice Provost for Graduate and Professional Education

ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor Dr. Sheau Ching Chai, for always pushing me to excel both academically and professionally. I am very grateful for the knowledge and wisdom she has shared with me throughout every step of this process. I would also like to thank my committee members, Dr. Marie Kuczmarski and Dr. Regina Sims Wright for their continuous help and support. I greatly appreciate the help of Dr. Barry Bodt and Dr. Ryan Pohlig, for all of their input and statistical expertise. I would also like to thank the undergraduate volunteers I had the pleasure of working with who made the research process flow flawlessly. And finally, I would like to thank my family and friends, whose endless encouragement and inspiration brought me to where I am today.

TABLE OF CONTENTS

LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii

Chapter

1	INTRODUCTION	1
2	LITERATURE REVIEW	3
2.1	Definition and Prevalence of Cardiovascular Disease in the U.S.	3
2.2	Atherosclerosis	3
2.3	Cardiovascular Disease Risk Factors	5
2.3.1	Hypertension.....	5
2.3.2	Dyslipidemia.....	7
2.3.3	Atherogenic Risk Ratio	8
2.4	Medications Used for the Treatment of Cardiovascular Disease	9
2.5	Diet and Cardiovascular Disease.....	9
2.6	Antioxidant Content of Tart Cherries.....	10
2.7	Health Benefits of Tart Cherries	12
2.7.1	Cardioprotective Effects of Tart Cherry Consumption	12
3	SPECIFIC AIMS	18
4	METHODS.....	19
4.1	Subject Recruitment, Inclusion and Exclusion Criteria	19
4.2	Study Design	20
4.3	Procedures	20
4.3.1	Phone Screening	20
4.3.2	Study Visits	20
4.3.3	Baseline (Visit 2) and Final (Visit 5)	21
4.3.4	Juice Pick-Up (Visits 3 and 4).....	22

4.4	Description of Instruments	22
4.5	Statistical Analysis	24
5	RESULTS.....	27
5.1	Demographic Information and Baseline Characteristics	27
5.2	Lipid Profile and Atherogenic Risk Ratios	27
5.3	Blood Pressure.....	28
5.4	Anthropometric Measurements, Dietary Intake, and Physical Activity..	28
6	DISCUSSION.....	29
7	CONCLUSION	34
	REFERENCES	35
Appendix		
A	TABLES AND FIGURES.....	43
B	IRB APPROVAL LETTER	48
C	STUDY VISIT FORMS	50
C.1	Demographic Questionnaire	51
C.2	Medical History Questionnaire.....	52
C.3	Montreal Cognitive Assessment.....	53
C.4	Physical Activity Screening for the Elderly (PASE).....	54
C.5	Food Frequency Questionnaire.....	55
C.6	Juice Instructions	58
C.7	Juice Calendar	59
C.8	Three-Day Food Record Instructions	60

LIST OF TABLES

Table A.1. Participant characteristics at baseline.	45
Table A.2. Results of 2x2 ANOVA comparing change in biochemical parameters from baseline to final in tart cherry and placebo groups.	46
Table A.3. Results of 2x2 ANOVA comparing change in dietary and physical activity variables from baseline to final in tart cherry and placebo groups.	47

LIST OF FIGURES

Figure A.1. Flow of study participants.	44
Figure A.2. Overview of study visit procedures.....	44

ABSTRACT

Cardiovascular disease (CVD) continues to be the leading cause of death in the United States. It was estimated that 80% of people who died of CVD were age 65 or older. Hypertension and dyslipidemia are major risk factors for CVD. Common treatments for high blood pressure (BP) and dyslipidemia include medications, but there is question as to whether natural sources may be adequate to improve CVD risk factors. Studies have suggested age-associated related cardiovascular conditions may be ameliorated by the antioxidants in tart cherry. Hence, a randomized intervention trial was conducted evaluating whether participants consuming 16 fluid ounces (fl. oz.) of tart cherry juice daily would reduce CVD risk factors including total cholesterol, low-density protein cholesterol, high-density lipoprotein cholesterol, triglycerides, atherogenic risk ratios, and systolic and diastolic BP when compared to a placebo group consuming 16 fl. oz. of placebo daily for 12 weeks. Participants consisted of 37 generally healthy older adults between the ages of 65-80 years. Participants were randomly assigned to the tart cherry group or placebo group and were blinded to their group assignment. Overnight fasting blood samples and BP were collected at baseline and final. Dietary intake and physical activity were also assessed. The baseline characteristics of participants in both groups were similar, with the exception of physical activity, which was significantly greater in the tart cherry group. Results of 2x2 ANOVA show a significant difference between groups in change in systolic BP from baseline to final. In the tart cherry group, systolic BP decreased by 5.7mmHg (4.0%) and in the placebo group systolic BP increased by 5.4mmHg (p

value 0.0188). There were no significant changes in any of the other variables. The present finding also indicated that an additional 236 kilocalorie intake daily from juice did not significantly increase body weight. In conclusion, daily incorporation of tart cherry juice into diet reduced systolic BP in older adults and could be a plausible intervention for improved cardiovascular health in older adults.

Chapter 1

INTRODUCTION

The risk of cardiovascular disease (CVD) increases as adults age and is the leading cause of death in adults over the age of 65.¹ The two most common types of CVD include coronary heart disease and stroke, and the process of atherosclerosis plays a large role in the development of these types of CVD.²⁻⁴ Risk factors for the development of atherosclerosis and CVD can be classified as modifiable, including obesity, dyslipidemia, hypertension, physical inactivity, and smoking, or non-modifiable, including age, sex and family history.³ Treatment and prevention of CVD focuses around the modifiable risk factors.

Consumption of a healthful diet high in fruit and vegetables has been associated with a decreased prevalence of cardiovascular disease.⁵⁻⁷ In addition, consumption of fruits containing various vitamins and polyphenolic compounds may also decrease CVD prevalence. Tart cherries are high in polyphenolic compounds that have antioxidant and anti-inflammatory properties.⁸ Health benefits associated with the consumption of tart cherries may include decreased muscle damage following strenuous exercise, improved sleep and circulating melatonin concentrations, reduction of colonic tumor development, and a decrease in CVD risk factors.⁹⁻²¹ Various in vitro, animal and human studies have investigated the effects of tart cherries on numerous measures of cardiovascular health and disease prevalence.¹⁵⁻²¹ Results are fairly consistent in suggesting the cardioprotective effects associated with tart cherry consumption. However, it is not clear as to how long a tart cherry juice

intervention must be to obtain cardiovascular health benefits. Therefore, further research needs to be completed investigating different supplementation periods in an older adult population between the ages of 65-80.

Chapter 2

LITERATURE REVIEW

2.1 Definition and Prevalence of Cardiovascular Disease in the U.S.

According to the World Heart Federation, CVD can be defined as a “broad term for a range of diseases affecting the heart and blood vessels.”²² Specifically, the most common types of CVD include coronary heart disease (CHD) and stroke.¹ In the year 2013, it was estimated that over 800 thousand people died from CVD, making CVD the leading cause of death in the U.S. In addition, this disease costs the U.S. billions of dollars every year. It is projected that the total cost of all types of CVD in 2015 will cost 656 billion dollars and this figure is expected to nearly double, reaching 1208 billion by the year 2030.¹

The risk for developing CVD increases with age, and adults at and above the age of 65 make up over 80% of deaths attributable to CVD. In addition, it is estimated that approximately 68.5% of adults over the age of 60 and 85.3% of adults over the age of 80 currently have CVD.¹ Since the number of adults at or above the age of 65 is rapidly increasing and projected to exceed 82 million by the year 2040, CVD can be considered a major health threat.²³

2.2 Atherosclerosis

The process of atherosclerosis, or the hardening of the arteries, is thought to play a large role in the development of CVD, specifically CHD and stroke.²⁻⁴

Atherosclerosis occurs in the innermost arterial wall, called the intima. The intima is

comprised of smooth muscle cells lined by endothelial cells.² Under normal vascular conditions, monocytes circulate in the blood stream and respond to injury on the arterial wall. In the presence of low-density lipoprotein (LDL), the monocytes will slip under blood vessel cells and engulf the LDL molecule, becoming foam cells. The foam cells will develop into thin layers lining the arterial walls, forming fatty streaks. Over time, these fatty streaks will continue to build up and will eventually harden and calcify into atherosclerotic plaque.³ This plaque will be covered by a thin fibrous coating and embedded smooth muscle cells. As the plaque builds up, blood flow will be blocked, leading to a higher BP. As BP increases, it is possible for the fibrous capping on the plaque to rupture, releasing dangerous thrombus into the blood stream.² In addition, as the atherosclerosis process continues and plaque builds up, the arterial lumen will eventually be blocked, leading to ischemic conditions and cardiovascular event, such as CHD or stroke.

It is known that inflammation may also contribute to the atherosclerosis process.²⁴ The majority of cells present at the site of plaque rupture are macrophages, T cells, and mast cells, all of which are inflammatory mediators. The macrophages contain metalloproteinases, which break down collagen within the fibrous cap, leaving it more prone to rupture. In addition, the activation of smooth muscle cells present in the fibrous cap will secrete factors that will attract additional monocytes, therefore increasing the inflammatory response and promoting a local pro-coagulant effect, mainly due to the action of interleukin-6 (IL-6). Furthermore, C-reactive protein (CRP), which is increased in the presence of IL-6, may have an affect on nitric oxide (NO). CRP may reduce the production and bioavailability of NO, leading to a reduction in NO at the site of atherosclerosis. NO is a peptide that inhibits platelet

adherence and aggregation and suppresses vasoconstriction. A reduction in NO will further contribute to the inflammatory and atherogenic processes.²⁴

2.3 Cardiovascular Disease Risk Factors

Risk factors for atherosclerosis and CVD include, but are not limited to, family history, age, sex, obesity, dyslipidemia, hypertension, diabetes, physical inactivity, and cigarette smoking. A poor diet, or one that is low in fruits, vegetables, whole grains, and high in fat and processed meats has also been associated with CVD.⁵ Having one or more of the above risk factors increases one's risk for developing atherosclerosis, and subsequently, CVD.³ Risk factors can be classified as modifiable or non-modifiable. Non-modifiable risk factors include family history, age, sex and diabetes. Therefore, modifiable risk factors include obesity, dyslipidemia, hypertension, physical inactivity, and cigarette smoking.³

2.3.1 Hypertension

Hypertension, or high BP, is the biggest risk factor for stroke, and BP regulation plays a large role in the prevention of heart attacks. An untreated BP below 120/80mmHg has been recommended for cardiovascular health.¹ According to data from the National Health and Nutrition Examination Survey (NHANES), prevalence of hypertension increases as adults age, and 62% of males and 67.8% of females between the ages of 65-74 have hypertension, while 76.4% of males and 79.9% of females over the age of 74 have hypertension.¹ Out of all the older adults with hypertension, 81.7% are taking medication for it, yet only 54.1% of individuals have successfully controlled their BP. Prevalence of hypertension in this population can be very life altering. Hypertension has been shown to shorten overall life expectancy, as

well as life expectancy without CVD. A variety of factors can contribute to prevalence and severity of hypertension, such as genetic factors, age, ethnicity, physical activity and dietary factors.¹

The Framingham Heart Study monitored borderline isolated systolic hypertension (systolic BP=140-159mmHg; diastolic BP<90mmHg) and examined that prevalence of isolated systolic hypertension increased in both sexes from ages 30-70.²⁵ Throughout the course of the observation period, over 80% of individuals with isolated systolic hypertension progressed to full blown hypertension (systolic BP>140mmHg; diastolic BP>90mmHg). It is important to note that based on current recommendations, this definition of borderline isolated systolic hypertension would be classified as full hypertension. When compared to normotensive individuals, those with borderline isolated systolic hypertension were 1.5 times more likely to have CVD and 1.6 times more likely to die from CVD complications over a long-term observation period. In another observational study, men over the age of 60 who had systolic BP measurements ≥ 130 mmHg or diastolic BP measurements ≥ 81 mmHg were 2.2 times more likely to have CVD compared to men with systolic BP measurements <116mmHg or diastolic BP measurements <44mmHg.²⁶

Several age-related changes are thought to play a role in the development of hypertension in the older adult population.²⁷ These changes include an altered regulation of vascular tone, leading to an increase in vascular stiffness. These changes in vascular tone include the build-up of collagen and alterations in elastin content, including decreased elastin and increases in both elastin fractures and calcification. These changes lead to an increase in vascular stiffness, which can be measured by pulse wave velocity. This measure is consistently increased in adults over the age of

50 when compared to younger adults. Consequently, both of these changes also increase the presence and development of atherosclerosis.²⁷ Another difference that may be seen with age is the increase in the thickness of the intima. This increase has been associated with an increased risk of myocardial infarction and stroke following a 6-year follow up in subjects over the age of 65.²⁸

2.3.2 Dyslipidemia

Dyslipidemia has also been related to prevalence and severity of atherosclerosis and CVD.¹ Dyslipidemia can be defined as altered blood lipid levels, including high levels of total cholesterol (TC), LDL, and triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL). The American Heart Association defines an ideal lipid profile as follows: TC<200mg/dL, LDL<140mg/dL, HDL>40mg/dL and TG <150mg/dL.^{1,29} In regards to TC, 55.9% of adults in the U.S. have TC levels that are not ideal, meaning levels are high (>240mg/dL) or borderline-high (between 200-240mg/dL).¹ In addition, 31.7%, 19.9%, and 25.1%, of individuals do not have LDL, HDL, and TG levels, respectively, within the recommendations.¹ Recently, the number of individuals with unfavorable lipid levels has been declining, but this decline is thought to be related to an increase in use of cholesterol-lowering medications rather than changes in diet and lifestyle.^{1,30} The American Heart Association proposes that older adults may have suboptimal lipid levels due to continued exposure to cholesterol from the diet as well as changes in pathophysiological disease mechanisms, leading to alterations in cholesterol synthesis and metabolism.²⁷

The Framingham Heart Study compared lipid levels at an initial observation period and again following an 8-year follow up. Results were consistent in that there

was an inverse association with HDL and prevalence of CVD and a direct relationship with TC and prevalence of CVD in men and women over the age of 49. Individuals with the highest levels of HDL (80th percentile) had two times less risk of developing CHD when compared to those with the lowest levels of HDL (20th percentile).³¹ This risk was also confirmed in an additional observational study of individuals over the age of 71. Results showed a consistent relationship of HDL with CHD, where individuals with low HDL (<35mg/dL) were 2.5 times more likely to die from CHD than individuals with high HDL ($\geq$ 60mg/dL). There was also a significant association seen with TC and CHD, as individuals with high levels of TC (240mg/dL) were 1.8 times more likely to die from CHD than individuals with low levels of TC (160-199mg/dL), but this relationship was only observed in women.³²

2.3.3 Atherogenic Risk Ratio

While the entire lipid profile can be viewed as a marker of cardiovascular health, recent evidence is suggesting that the use of atherogenic risk ratios may be more valid in determining cardiovascular health and CVD risk.³³ Atherogenic risk ratios can be defined as TC/HDL and LDL/HDL. When comparing healthy individuals and those who have suffered from a heart attack, the TC/HDL ratio rather than absolute values of TC and HDL has been shown to have a higher predictive capability in regards to CHD. The LDL/HDL ratio has also been shown to be significant, as well as similar to the TC/HDL ratio.

An optimal TC/HDL ratio can be defined as <4.5 for men and <4.0 for women. A TC/HDL ratio above 5.0 for men and 4.5 for women is considered to be at risk for CVD. An optimal LDL/HDL ratio can be defined as <3.0 for men and <2.5 for

women. An LDL/HDL ratio above 3.5 for men and above 3.0 for women is considered to be at risk.³³

2.4 Medications Used for the Treatment of Cardiovascular Disease

Currently, many individuals rely on medication for the treatment or management of risk factors for CVD and atherosclerosis, such as dyslipidemia and hypertension.³⁴ Common medications include ACE inhibitors, beta-blockers, calcium antagonists, and diuretics. ACE inhibitors may reduce the risk of stroke, CHD, and major cardiovascular events by 20-30%. Furthermore, calcium-antagonists may reduce the risk of stroke by up to 30-40%, but they do not decrease the risk of CHD. Diuretics and beta-blockers may reduce risk of stroke by up to 38%, and they may reduce the risk of CHD by up to 16%.³⁴

While these medications have been shown to be effective in the treatment and prevention of CVD, taking these medications regularly may cause negative side effects. Possible side effects include dry cough, dizziness, bradycardia, peripheral edema and insomnia. These side effects paired 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 a natural remedy to the problem of CVD.³⁵

2.5 Diet and Cardiovascular Disease

Recently, many research studies have focused on different diets when it comes to the prevention and treatment of CVD.^{5,36,37} Diets associated with a high intake of fruits, vegetables, legumes, whole grains and fish have been shown to be inversely associated with the prevalence of CVD.⁵⁻⁷ Epidemiological studies also support the existence of an inverse association between a fruit and vegetable-rich diet and CVD,

in part, due to their antioxidant content. According to NHANES data, the cumulative mortality from stroke and CVD decreased as fruit and vegetable consumption increased. Those who consumed the lowest amount of fruits and vegetables had a mortality risk of 14.5%, and those who had the highest consumption rate had a mortality risk of just 6.1%.³⁸ This difference may be due to certain nutrients found in fruits and vegetables, such as potassium, antioxidants, folic acid, and fiber.^{38,39} It has been shown that the risk of CHD decreases by 7% for each standard portion of fruit consumed per day.³⁹ The decreased risk for CVD associated with fruit consumption may be attributed to the polyphenolic compounds found in many fruits.⁴⁰ Research has shown that polyphenolic compounds may act as antioxidants and interfere with the development of CVD, leading to an inverse relationship between plasma antioxidants and CVD.⁴¹

2.6 Antioxidant Content of Tart Cherries

Tart cherries are a rich source of polyphenolic compounds, especially proanthocyanins, anthocyanins, and flavonols, all of which are strong antioxidants.⁸ Tart cherries are a particularly rich source of anthocyanins, specifically cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside, cyanidin-3-glucoside, and their aglycone, cyanidin.⁴² The anthocyanin compounds found in the tart cherry are responsible for the dark red color of the skin, which is where the majority of the anthocyanin compounds are contained.⁴³ Common flavonoids in tart cherries include isorhamnetin rutinoside, kaempferol, quercetin and melatonin, an antioxidant involved in the regulation of sleep and wake cycles.⁴⁴ This specific antioxidant detoxifies the hydroxyl radical, the peroxynitrite anion, singlet oxygen and NO.⁴⁵

Compared to Bing cherries, which are sweeter and more commonly consumed in the U.S., Montmorency tart cherries have higher total antioxidant activity, as well as a greater content of total phenolic compounds. Bing cherries contain 1.85 milligrams/Gallic Acid Equivalents/gram fresh weight (mg/GAE/g/fw) of phenolic compounds and Montmorency tart cherries contain 4.07 mg/GAE/g fw.⁴³ Total antioxidant activity can be measured via oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP). According to those measures, Bing sweet cherries have an ORAC value of 14.94 μmoles Trolox Equivalents (TE)/g/fw, and a FRAP value of 15.90 μmoles TE/g/fw. Tart cherries have an ORAC value of 27.57 μmoles TE/g/fw and a FRAP value more than two times greater than Bing cherries, at 37.56 μmoles TE/g/fw. The level of antioxidant activity seen in tart cherries is similar to other fruits commonly known as being high in antioxidants such as blueberries, strawberries and pomegranates.⁴⁶ In addition, tart cherries have higher amounts of antioxidants per portion size than red wine, dark chocolate, and orange juice, placing them as number 14 out of the top 50 highest antioxidant containing foods.⁸

An analysis of Montmorency tart cherry's polyphenolic content was conducted by Ou et al.⁴⁶ They compared various processing methods, including tart cherry juice concentrate, as well as dried, frozen and canned tart cherries, as it is unknown how processing may affect the polyphenolic and antioxidant properties of the fruit. The polyphenolic content of the frozen cherries, 4.18 mg/GAE/g/fw, was similar to the value gathered by Chaovanalikit et. al, as mentioned above.^{43,46} However, in nearly all measures of antioxidant activity, including ORAC and several other measures including hydroxyl, peroxy, and super oxide radical absorbance capacities, tart cherry

juice concentrate had higher values than the other processed cherries, with the exception of super oxide radical absorbance capacity, which had a value that was slightly less than that of dried cherries. According to this analysis, one gram of tart cherry juice concentrate contains approximately 264.9mg/GAE of phenolic compounds, which is also higher than the other processed tart cherries.⁴⁶

2.7 Health Benefits of Tart Cherries

The health benefits associated with consumption of tart cherries have been studied extensively in various populations.⁹⁻¹⁴ Due to the melatonin content of tart cherries, research has been investigating the effect of tart cherry juice on sleep and melatonin levels. Drinking tart cherry juice before bed has been shown to significantly improve symptoms related to insomnia, including reduced sleep disturbances and increased urinary melatonin.^{12,13} Tart cherry consumption has also been studied in regards to exercise.⁹⁻¹¹ Compared to placebo, athletes consuming tart cherry juice had significantly less muscle damage, quicker recovery time, and less pain during extreme bouts of exercise. Finally, tart cherry consumption may significantly reduce colonic tumor development in rats susceptible to colon cancer.¹⁴

2.7.1 Cardioprotective Effects of Tart Cherry Consumption

Aside from the health benefits mentioned above, tart cherry consumption may be related to improvements in cardiovascular health and many research studies have looked at the relationship of tart cherry juice consumption and variables related to CVD¹⁵⁻²¹. This beneficial effect may be due to the consumption of polyphenolic compounds found in tart cherries and their anti-inflammatory and antioxidant capabilities.⁴⁷

When compared to ibuprofen and the non-steroidal anti-inflammatory drug (NSAID) naproxen, the aglycone cyanidin from tart cherries showed comparable anti-inflammatory activities, when comparing cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) inhibition activities. COX eventually forms a precursor for prostaglandins, one of the messenger molecules involved in inflammation. Ibuprofen had COX-I and COX-II inhibition activities of 47.5% and 39.8%, naproxene had inhibition activities of 54.3% and 39.8%, and cyanidin had inhibition activities of 38.7 and 46.8, respectively.⁴⁷ Ou et al. found tart cherry juice concentrate had higher inhibitory actions than other types of processed tart cherries, but failed to find any significant inhibitory effects of any type of tart cherry on COX-II.⁴⁶ Numerous studies have investigated the anti-inflammatory and antioxidant effects of tart cherries in relation to health.

A further possible mechanism associated with intake of whole grains and fiber-containing foods in decreasing serum cholesterol is the binding of bile acids.¹⁵ Once these compounds bind the bile acids, the bile is excreted, which stimulates the conversion of liver and plasma cholesterol to additional bile acids, therefore lowering cholesterol. This mechanism has also been observed in relation to cherries, and analysis showed that cherries can bind bile acids up to 5%, which is a value similar to the binding capacities of dark leafy vegetables and oat cereals.¹⁵ Another study showed that a cherry extract containing 20 μ M/GAE of phenolic compounds completely inhibited LDL oxidation.¹⁶ Both of these studies used sweet cherries, and as it has been shown that tart cherries contain higher levels of polyphenolic compounds, it can be expected that tart cherries may lead to similar, if not even more significant results.⁴³ These actions of polyphenolic compounds found in tart cherries

may have a positive effect on cholesterol levels, and furthermore, the development of CVD.

Keane et al. completed a study looking at the in vitro effects of tart cherry and phenolic compounds on vascular smooth muscle cells.¹⁷ The proper functioning of vascular smooth muscle cells (VSMCs) is essential to avoid build up of atherosclerotic plaques, as VSMCs are responsible for normal, healthy vasculature tone. Authors hypothesized that Montmorency tart cherries may contain vasoactive compounds that could modulate VSMCs in vitro. VSMCs came from 12 healthy non-smoking males. Participants had blood samples taken upon arrival as well as 1, 2, 3, 4, and 8 hours post tart cherry juice consumption. Participants were consuming either 30 or 60ml of a Montmorency tart cherry juice that provided the equivalent of approximately 90 or 180 tart cherries, respectively. Authors were also looking at presence of protocatechuic acid (PCA), vanillic acid (VA), and chlorogenic acid (CHL), which are all major metabolites of anthocyanins. Results showed that one-hour following consumption of both doses of tart cherry juice, PCA levels were significantly higher. In regards to VA, there were no significant increases following the lower dose but there were significant increases seen following the higher dose at 1, 5, and 8 hours following baseline. There were no significant effects on CHL following either dose. In terms of VSMCs, there was a significant increase in cell migration following baseline, but no difference in cell proliferation. VSMC migration is protective and increases stability, therefore reducing risk of atherosclerotic plaque rupture. This study provides support for the cardioprotective effects of tart cherry juice consumption, but it may not be directly related to the individual metabolites mentioned above.¹⁷

In an animal study, Seymour et al. hypothesized that an intake of a diet supplemented with tart-cherry powder would modify indices of metabolic syndrome as well as the action of peroxisome-proliferator activated receptor (PPAR).¹⁸ PPAR is a transcription factor in control of certain genes that modify blood lipoprotein metabolism and tissue lipolysis, therefore altering blood lipids and fat metabolism and improving insulin resistance. Male Dahl-Salt Sensitive rats were fed a standard diet supplemented with 1% of a tart cherry powder by weight, or a control diet, for 90 days. The tart cherry powder consisted of freeze-dried Montmorency cherries. Following consumption of the tart cherry powder, there was a significant decrease in TC, TG, and glucose. There was also, expectedly, a significant increase in plasma antioxidant status.¹⁸

Seymour et. al duplicated the methods described above with obesity-prone (Zucker) rats consuming a higher-fat diet.¹⁹ These rats are more prone to obesity, hyperlipidemia, insulin resistance and systemic inflammation. Rats were randomized to consume a tart cherry powder as 1% of their total diet by weight or a control diet for 90 days. After 90 days, fasting glucose, TC and TG concentrations were significantly reduced. Plasma IL-6 and TNF- α were also significantly reduced, by 44% and 40%, respectively. In accordance with the previous results, PPAR- α mRNA was significantly increased in the tart cherry group, which supports a possible mechanism to the change in hyperlipidemia. Results reported in this study are particularly relevant due to America's high prevalence of obesity and CVD.¹⁹

The prevalence of CVD has also been thought to be related to oxidative stress. Traustidottir et al. investigated this relationship in older adults.²⁰ Oxidative stress is considered an "imbalance between the rate of formation and the rate of clearance of

reactive oxygen and nitrogen species (RONS)” and may play a key role in the development of atherosclerosis.²⁰ Consumption of antioxidants, such as those in tart cherries, may decrease oxidative stress. Authors hypothesized that consumption of a tart cherry juice may decrease oxidative stress following an acute stress. Participants were 12 healthy older adults between the ages of 61-75. At baseline, ischemic reperfusion (IR) occurred and participants were randomized to consume 240ml of a tart cherry or placebo juice twice daily for 14 days. IR was repeated once again at the final visit. After a 14-day washout period participants completed the opposite arm of the study. Results showed that as expected, after all four trials, isoprostanes, a marker of acute oxidative damage, increased, but there were no differences before the IR trial. Participants consuming the tart cherry juice had a reduced response to the IR trial, as well as significant decreases in 8-OHdG and 8-oxo-G, long-lived markers of oxidative damage, compared to the placebo trial. Results from this study suggest that tart cherry juice consumption may reduce markers of oxidative stress, but only following an IR trial.²⁰

Lynn et al. conducted a 6-week open label trial where 47 healthy participants between the ages of 30-50 consumed 30ml of a Montmorency tart cherry concentrate or a lemonade placebo beverage.²¹ The placebo beverage was chosen because the energy and macronutrient content was similar to the tart cherry juice, while being devoid of plant compounds, antioxidants and vitamins. Participants consumed the tart cherry concentrate or placebo beverage once daily for six weeks. The primary outcome variable was pulse wave velocity (PWV) and the secondary outcome variable was change in CRP. Change in BP, TC, HDL and body weight was also assessed. Measurements were taken twice, at baseline and again at final. Throughout the six-

week intervention period, there was one dropout, due to dislike of the taste of the cherry juice. There were no dropouts related to any adverse side effects. Results showed no significant effects of the tart cherry juice in changes of PWV, CRP, BP, TC, HDL or body weight. While there were no significant changes, it needs to be noted that this intervention was only 6 weeks and there may need to be a longer intervention period to notice significant differences.²¹

Chapter 3

SPECIFIC AIMS

Aim 1: Determine the difference at baseline and post-intervention in serum TC, LDL, HDL and TG concentrations between the tart cherry group consuming 16 fl. oz. of tart cherry juice daily and the placebo group consuming 16 fl. oz. of placebo juice daily for 12 weeks.

Aim 2: Determine the difference at baseline and post-intervention in atherogenic risk ratios between the tart cherry group consuming 16 fl. oz. of tart cherry juice daily and the placebo group consuming 16 fl. oz. of placebo juice daily for 12 weeks. The atherogenic risk ratios were defined as TC/HDL and LDL/HDL.

Aim 3: Determine the difference at baseline and post-intervention in systolic BP and diastolic BP in the tart cherry group consuming 16 fl. oz. of tart cherry juice daily and the placebo group consuming 16 fl. oz. of placebo juice daily for 12 weeks.

Chapter 4

METHODS

4.1 Subject Recruitment, Inclusion and Exclusion Criteria

Men and women of diverse race and ethnicities who live in Newark, Delaware and the surrounding areas were recruited and screened. Recruiting materials were placed in 55+ communities, nursing homes, event halls, churches, and public bulletin boards. Advertisements were also placed in the Delaware News Journal.

Inclusion criteria included men and women between the ages of 65-80 who consumed ≤ 5 servings of fruits and vegetables per day. Individuals taking any medications that may influence brain function or have had any prior diagnosis or history of central nervous system or psychiatric disorders, traumatic brain injury, stroke, impaired cognitive function, or any uncontrolled disease or disorder such as diabetes, gastrointestinal disease, cancer or chronic disease were excluded. Additionally, heavy smokers (>20 cigarettes/day) or individuals allergic to tart cherry were excluded from this study.

A flow chart of participants is presented in Figure 1. A total of 378 individuals displayed interest in the study and 284 were screened over the phone. Following the initial phone screening, 129 potential participants were invited to the study site to complete a screening visit. Based on inclusion and exclusion criteria, a total of 37 participants were enrolled in the study and randomized to intervention. There is final data for 34 participants, and reasons for drop out included juice intolerability, loss of

interest, and an unrelated medical condition. Juice compliance for the 12-week intervention was 94.2%.

4.2 Study Design

The present study was a 12-week, randomized, placebo controlled trial. Thirty-seven (37) eligible participants were randomly assigned to consume either 16 fl. oz. tart cherry juice or placebo juice daily for 12 weeks. Participants were blinded to which group they were in until the study was closed. Both tart cherry and placebo juices were isocaloric with similar color, sugar content and flavor. Placebo juice was devoid of tart cherry and its polyphenols. All juice was prepared specifically according to the Cherry Marketing Institute directions and properly stored until consumption. All procedures and protocol were approved by the Institutional Review Board at the University of Delaware.

4.3 Procedures

4.3.1 Phone Screening

Upon initial interest, participants completed a pre-phone screening. Information obtained from the phone screening included name, sex, age, date of birth, contact information and presence of any chronic conditions outlined in the exclusion criteria. Following the phone screening individuals were given more information about the study and qualified participants were scheduled for a screening visit.

4.3.2 Study Visits

All visits took place at the University of Delaware's Nurse Managed Health Center at the STAR campus. All procedures are outlined in Figure 2. Upon arrival for

the screening visit, participants read and signed informed consent. Following informed consent, anthropometric measurements, including weight, height, and hip and waist circumferences, and BP were taken. Participants then filled out questionnaires regarding demographic, medical history and physical activity information as well as a food frequency questionnaire (FFQ) while aided by research personnel. The Montreal Cognitive Assessment (MoCA) was administered to assess cognitive status (Appendix C.3). Finally, participants were provided with instructions to complete a three-day food record and physical activity questionnaire prior to the next study visit if they were deemed eligible for the study. At this visit participants received \$20 compensation.

Following the screening visit, eligibility was assessed and participants were notified of their eligibility and the remaining study visits were scheduled.

4.3.3 Baseline (Visit 2) and Final (Visit 5)

Eligible participants were scheduled for visits 2, 3, 4 and 5. At visits 2 and 5, participants completed all baseline and final measurements. These visits were completed following a 10-12 hour fast and blood samples were collected upon arrival. Anthropometric measurements, including weight and hip and waist circumferences, and BP were taken. Participants reviewed their completed three-day food record and physical activity questionnaire with research personnel for completion and accuracy.

Participants were given their assigned juice and instructions regarding consumption and storage. Participants were instructed to consume 16 fl. oz. of the juice per day, 8 fl. oz. in the morning and 8 fl. oz. in the evening (6-8 hours later) for the entirety of the 12-week intervention (Appendix C.6). At this time participants were provided with four bottles of juice, with one bottle being enough for one week.

They were instructed to keep the one bottle they are drinking in the fridge, and the remainder in the freezer. At each relevant study visit, participants were provided with a calendar (Appendix C.7). This calendar has designated spaces for each day and each serving of juice. Participants were instructed to mark the space for each serving of juice consumed.

Participants received \$20 compensation for the completion of Visit 2, and \$60 compensation for the completion of Visit 5.

4.3.4 Juice Pick-Up (Visits 3 and 4)

At weeks four and eight, participants returned to the study center for visits 3 and 4, where they picked up a refill of juice. At this time participants also returned any unused juice and the calendar they had been filling out. They again received enough for four weeks. At each of these visits, the participants received \$10 compensation.

4.4 Description of Instruments

Blood Draw

Fasting venous blood was collected at baseline and final visits following a 10-12 hour fast. Blood samples were collected in the appropriate vacutainers and centrifuged within one hour of collection. Serum and plasma were separated by centrifuging at 3500rpm for 15 minutes at 4°C using a Beckman Allegra 6KR centrifuge.

Lipid Profile

Serum was analyzed for TC, HDL, LDL and TG using a Genesys 10S UV-Vis Spectrophotometer. All reagents and standards were from Pointe Scientific.

Blood Pressure

An Omron Digital Blood Pressure monitor HEM-907XL Intelli Sense sphygmomanometer was used for measuring BP. Two successive measurements were taken after the participant had sat in a quiet room with their feet placed on the ground for five minutes. Results were recorded as the average of the two measurements.

Anthropometrics

A stadiometer was used to measure height. Participants stood barefoot with their back against the wall and their head in the Frankfort horizontal plane. Height was measured in centimeters, as well as feet and inches. Weight was measured in kilograms and pounds with minimal clothing on a digital scale. Hip and waist circumference were measured in centimeters using a non-stretchable measuring tape. Hip circumference was measured as the participant was standing, with the researcher standing at eye level at the widest point of the hips. Waist circumference was measured according to NHANES protocol.⁴⁸

Medical History Questionnaire

At visit 1 (screening), participants filled out a medical history questionnaire (example shown in Appendix C.2). This questionnaire covers family history, as well as the participant's medical history and regular medications. This questionnaire is used to rule out any participants with any chronic or acute disease that fall under exclusion criteria.

Physical Activity Questionnaire

The questionnaire used to assess physical activity was the Physical Activity Scale for the Elderly (PASE) (example shown in Appendix C.4). The PASE gathers information about the participant's physical activity over the past seven days, including walking, light, moderate and vigorous physical activity, housework, and

physical activity completed at volunteer activities or paid work. This questionnaire has been proven to be reliable and valid for the assessment of physical activity in community-dwelling older adults.⁴⁹

Food Frequency Questionnaire

The Nutrition Quest Block Food Frequency Questionnaire (FFQ) was used to assess participant's average dietary intake (sample questions shown in Appendix C.5).

⁵⁰ The purpose of this questionnaire is to quantify daily consumption of fruits and vegetables for each participant. This questionnaire has been proven to be both reliable and valid when compared to nutrient intakes as measured by four 2-day diet records from middle aged and older adults.⁵¹

Food Diary and Nutrition Data System for Research

The week prior to the baseline and final visits, participants completed a three-day food record, where they wrote down everything they eat and drank for two weekdays and one weekend day prior to the scheduled visit (Appendix C.8). Participants were given instructions regarding estimation of portion sizes and directions on how to record various foods or recipes. Participants reviewed their completed food diary with research personnel for completion and accuracy. Diet information was analyzed using the Nutrition Data System for Research (NDSR) (University of Minnesota, 2014). This database was developed specifically for the analyses of 24-hour recalls, food diaries, menus and recipes.⁵²

4.5 Statistical Analysis

All statistical analysis was performed using a program from the Statistical Analysis System, John's Manufacturing Program (JMP) 2016. Descriptive statistics were used to describe all data in the form of means, standard errors and frequencies.

Comparisons for baseline sociodemographic characteristics, including sex and age, were performed. Additionally, comparisons of anthropometric, biochemical and dietary variables, namely weight, BMI, TC, LDL, HDL, TG, systolic BP and diastolic BP, atherogenic risk ratios, PASE score, and selected nutrient intakes, were performed. A p value <0.05 was used to denote statistical significance.

Aim 1: Determine the difference at baseline and post-intervention in serum TC, LDL, HDL and TG concentrations between the tart cherry group consuming 16 fl. oz. of tart cherry juice daily and the placebo group consuming 16 fl. oz. of placebo juice daily for 12 weeks.

To assess Aim 1, means and standard errors were calculated at baseline and post-intervention. A 2x2 ANOVA was used to compare the differences in serum TC, LDL, HDL and TG concentrations between groups.

Aim 2: Determine the difference at baseline and post-intervention in atherogenic risk ratios between the tart cherry group consuming 16 fl. oz. of tart cherry juice daily and the placebo group consuming 16 fl. oz. of placebo juice daily for 12 weeks. The atherogenic risk ratios were defined as TC/HDL and LDL/HDL.

To assess Aim 2, means and standard errors were calculated at baseline and post-intervention. A 2x2 ANOVA was used to compare the differences in atherogenic risk ratios between groups.

Aim 3: Determine the difference at baseline and post-intervention in systolic BP and diastolic BP in the tart cherry group consuming 16 fl. oz. of tart cherry juice daily and the placebo group consuming 16 fl. oz. of placebo juice daily for 12 weeks.

To assess Aim 3, means and standard errors were calculated at baseline and post-intervention. A 2x2 ANOVA was used to compare the differences in systolic BP and diastolic BP between groups.

Participants with missing baseline or final values for any variable were excluded from the 2x2 ANOVA for that particular variable.

Chapter 5

RESULTS

5.1 Demographic Information and Baseline Characteristics

Demographic characteristics of the population are presented in Table 1. Mean age was 70 ± 0.8 , and 69.5 ± 1.0 , in the tart cherry and placebo groups respectively and was not significantly different. The majority of the sample had at least a 4-year college degree, was white and married. In the tart cherry group, half of the participants were working while half were retired and in the placebo group, the majority of participants were retired.

There were no significant differences at baseline in any variables other than PASE (Tables 2 and 3). PASE was significantly higher in the tart cherry group than in the placebo group at baseline (222.5 ± 7.7 ; 135.8 ± 7.5 ; p value 0.0052).

5.2 Lipid Profile and Atherogenic Risk Ratios

There were no significant changes in TC, LDL, HDL, or TG when comparing baseline and final values in the tart cherry and placebo groups (Table 2). Although not significant, TC increased by 1.9% and 10.8% in the tart cherry and placebo groups respectively, and LDL decreased by 2.5% in the tart cherry group and increased by 14.4% in the placebo group. HDL increased by 1.6% and 9.7%, and TG increased by 6.0% and 1.8% from baseline to final in the tart cherry and placebo groups, respectively. There no significant changes in either TC/HDL or LDL/HDL from baseline to final in either group.

5.3 Blood Pressure

Between the groups, there were significant changes in systolic BP following 12 weeks of tart cherry juice consumption (p value 0.0188; f value 6.1215) (Table 2). In the tart cherry group, systolic BP decreased by 4.0% and in the placebo group systolic BP increased by 4.0%. Diastolic BP remained relatively unchanged and there were no significant differences.

5.4 Anthropometric Measurements, Dietary Intake, and Physical Activity

Weight and BMI at baseline and final were not significantly different between groups. Due to the potential effects of dietary intake and physical activity on cardiovascular health, measurements of dietary intake and physical activity were collected and assessed. Intake of dietary components including total energy, protein, carbohydrates, fat, cholesterol and fiber were not significantly different between the two groups. There were no significant differences when comparing change between baseline and final PASE scores in either group.

Chapter 6

DISCUSSION

Various studies have reported the cardiovascular health benefits of tart cherry juice consumption in animals and humans.¹⁵⁻²¹ It has been proposed the mechanism behind tart cherry's health benefits is related to the bioactive compounds present in tart cherries, including various polyphenolic compounds and vitamins that act as antioxidants. However, it is important to continue research in this area due to the staggering impact CVD has in the aging population and to also investigate different intervention time periods and mechanisms.

The present study, examining a 12-week tart cherry juice intervention, did report improvements in cardiovascular health via a 4.0% reduction of systolic BP. To date, the present study is the first to note a significant change in systolic BP following an intervention of tart cherry juice. Diebolt et al. reported a significant reduction in systolic BP following a short-term oral administration of polyphenolic compounds in rats.⁵³ Changes in BP could be related to observed changes in vascular reactivity of the thoracic aorta leading to enhanced endothelium relaxation, therefore leading to reduced BP. When adults consumed ~100g per day of a drink containing various berries, systolic BP and diastolic BP were significantly reduced following eight weeks.⁵⁴ This reduction could be attributable to action of polyphenolic compounds as determined by bioavailability analysis. Tart cherries are rich sources of polyphenolic compounds, and thus it can be reasoned that the 4.0% reduction in systolic BP seen in the present study was due to the presence of polyphenolic compounds in the tart

cherry juice. Future studies should focus on the polyphenolic compounds present in tart cherry juice specifically. Bioavailability analysis of the tart cherry juice provided in the study should be completed to quantify the total amount of polyphenolic compounds contained in the juice.

The present study failed to find any significant changes in TC, LDL, HDL, TG, or either of the atherogenic risk ratios using 2X2 ANOVA. It is plausible that more participants need to complete the analysis to detect changes in these parameters. A total of three participants withdrew from the intervention, therefore reducing the power to detect changes. However, Seymour et al found significant reductions in both TC and TG after rats were fed a standard diet supplemented with 1% tart cherry powder for 90 days.¹⁸ Furthermore, there were significant increases in peroxisome proliferator activated receptor- α (PPAR- α) transcript level, PPAR- α target acyl co-enzyme A oxidase (ACO) mRNA, and ACO activity. PPAR- α target ACO mRNA is a marker of PPAR- α activation, and PPAR- α action reduces TC and TG. PPAR- α also regulates ACO activity, which decreases fat storage and increases fat oxidation. Therefore, the mechanism behind the reduction in TC and TG could partly be explained by the action of ACO. This mechanism also suggests that a longer intervention period may lead to changes in lipid profile.

The goal population for this study was generally healthy older adults. While the population was free from chronic diseases, the population was overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$), and hypertensive (Systolic BP $> 140 \text{ mmHg}$; Diastolic BP $> 90 \text{ mmHg}$). Levels of TC, LDL, HDL and TG, as well as the atherogenic risk ratios, did fall within the recommendations based on cardiovascular health. Due to the inverse relationship of BP to CVD prevalence and outcomes, results from the present study are

encouraging. The tart cherry group had a reduction in systolic BP from 141.4mmHg to 135.7mmHg. This reduction of 5.7mmHg resulted in a systolic BP of 135.7mmHg, which is no longer considered hypertensive. As reported in the Framingham Heart study, those with hypertension were 1.5 times more likely to have CVD and 1.6 times more likely to die from CVD complications when compared to normotensive individuals.²⁵ Therefore, this shift from a BP in the hypertension range to the prehypertension range is important in improving overall cardiovascular health, which in turn improves longevity and quality of life.

Lynn et al. conducted an open-label, randomized placebo controlled study investigating the effect of a tart cherry juice on cardiovascular health.²¹ This study failed to find any significant differences in any measure of cardiovascular health following tart cherry juice intervention, but this intervention period was only six weeks. Due to the significant decrease in systolic BP found in the present study, it is likely that a longer intervention period is necessary for cardiovascular health, compared to just six weeks. It is also important to note that the intervention used by Lynn et al. was an open-label trial, meaning participants knew whether they were consuming tart cherry juice or placebo juice. This is not an ideal study design compared to the single-blind study design used in the present study.

Despite the addition of 236 kcal per day to normal dietary intake, there was no significant change in weight. According to NHANES data, adults are consuming approximately 209 kcal per day from fruit drinks.⁵⁵ Therefore, it is plausible that the participants in the present study replaced their average daily fruit drink with the tart cherry or placebo juice. Therefore, there was no overall increase in energy intake and no significant increase in weight.

The purpose of the present analysis was to compare changes before and after the intervention between the tart cherry and placebo groups. This comparison was accomplished by running 2x2 ANOVA. However, with this analysis, means across groups and time points cannot be compared individually. In order to complete these comparisons, repeated measures ANOVA would have to be run.

A limitation of the present study includes differences in physical activity at baseline between groups. The placebo group had significantly less physical activity at baseline compared to the tart cherry group. As it was not directly associated with any variables of interest, it was inappropriate to treat physical activity as a covariate. It was expected that physical activity would increase from baseline to final, as most baseline visits were completed during colder months and the majority of final visits were completed during spring or summer. However not significant, there was a decrease in physical activity levels seen from baseline to final in both groups. An additional limitation is the possibility of under or over reporting in dietary intake. Participants may be conscious of recording their intake for three days and therefore may stray from their usual diet. However, all food records were reviewed with participants for completion and accuracy. Finally, the majority of the research population was white, married and earned $\geq \$75,000$ per year. The results may not be generalizable to populations that do not share those characteristics. Finally, exclusion of missing data may limit the power of the study.

Strengths include the randomized control trial study design. Participants were blinded to which group they were in and juices were identical in color and taste. The inclusion of the atherogenic risk ratios is also a strength. The atherogenic risk ratios encompass a wider span of cardiovascular health and may be more sensitive indicators

of cardiovascular health rather than the individual cholesterol levels. Finally, another strength of the present study includes the analysis of possible confounding variables including dietary intake. It is important to recognize that dietary intake varies between season, and the three-day food records were able to take that into consideration.

Future studies in this area could consider different study designs. To avoid the possibility of significant differences between groups at baseline, a crossover method could be used. In addition, a longer time period and a consumption exceeding 16 fl. oz. per day should be contemplated. It is possible that 16 fl. oz. daily for 12 weeks was not long enough for the beneficial components present in tart cherry juice to accumulate and reach their full action.

Chapter 7

CONCLUSION

In conclusion, the present study found a significant difference in systolic BP in older adults consuming 16 fl. oz. of tart cherry juice per day when compared to a placebo group. This reduction in systolic BP moved participants from hypertensive to prehypertension, therefore improving overall cardiovascular health, as well as longevity and quality of life. This difference suggests the consumption of a tart cherry juice, which is high in polyphenolic compounds, may be beneficial for cardiovascular health in older adults. Results are encouraging, as the astounding prevalence of CVD in the aging population is increasing medication use, and therefore health-expenditure related to CVD. A natural solution to the pressing issue of CVD is necessary. It is important to continue studying consumption of tart cherry juice over longer time periods to determine if there may be a more significant effect on cardiovascular health including beneficial changes in lipid profiles, atherogenic risk ratios and BP. Future research should also investigate the effects of an intervention of tart cherry juice on vascular function and possible mechanisms of how tart cherry juice reduces systolic BP specifically.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics-2016 update: A report from the american heart association. *Circulation*. 2015.
2. Habauzit V, Morand C. Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: An update for clinicians. *Therapeutic advances in chronic disease*. 2011;2040622311430006.
3. Nelms, M. Sucher, K. Lacey, K. Long Roth, S. *Nutritional therapy & pathophysiology*. Second ed. Wadsworth Cengage Learning; 2011.
4. What is atherosclerosis? National Heart, Lung and Blood Institute Web site. <http://www.nhlbi.nih.gov/health/health-topics/topics/atherosclerosis>. Updated 2014. Accessed 1/18, 2016.
5. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willett WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. *Am J Clin Nutr*. 2000;72(4):912-921.
6. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. *J Nutr*. 2012;142(7):1304-1313.

7. Oyebode O, Gordon-Dseagu V, Walker A, Mindell JS. Fruit and vegetable consumption and all-cause, cancer and CVD mortality: Analysis of health survey for england data. *J Epidemiol Community Health*. 2014;68(9):856-862.
8. Halvorsen BL, Carlsen MH, Phillips KM, et al. Content of redox-active compounds (ie, antioxidants) in foods consumed in the united states. *Am J Clin Nutr*. 2006;84(1):95-135.
9. Keuhl K, Perrier E. Efficacy of tart cherry juice in reducing muscle pain during running. *Journal of the International Society of Sports Nutrition*. 2010;7:17.
10. Howatson G, McHugh M, Hill J, et al. Influence of tart cherry juice on indices of recovery following marathon running. *Scand J Med Sci Sports*. 2010;20(6):843-852.
11. Connolly DA, McHugh MP, Padilla-Zakour OI, Carlson L, Sayers SP. Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *Br J Sports Med*. 2006;40(8):679-83; discussion 683.
12. Pigeon WR, Carr M, Gorman C, Perlis ML. Effects of a tart cherry juice beverage on the sleep of older adults with insomnia: A pilot study. *Journal of medicinal food*. 2010;13(3):579-583.
13. Howatson G, Bell PG, Tallent J, Middleton B, McHugh MP, Ellis J. Effect of tart cherry juice (*prunus cerasus*) on melatonin levels and enhanced sleep quality. *Eur J Nutr*. 2012;51(8):909-916.

14. Kang S, Seeram NP, Nair MG, Bourquin LD. Tart cherry anthocyanins inhibit tumor development in apc min mice and reduce proliferation of human colon cancer cells. *Cancer Lett.* 2003;194(1):13-19.
15. Kahlon T, Smith G. In vitro binding of bile acids by blueberries (< i> vaccinium spp.), plums (< i> prunus spp.), prunes (< i> prunus spp.), strawberries (< i> fragaria X ananassa), cherries (< i> malpighia punicifolia), cranberries (< i> vaccinium macrocarpon) and apples (< i> malus sylvestris). *Food Chem.* 2007;100(3):1182-1187.
16. Heinonen IM, Meyer AS, Frankel EN. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J Agric Food Chem.* 1998;46(10):4107-4112.
17. 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.* 2015:1-11.
18. Seymour EM, Singer AA, Kirakosyan A, Urcuyo-Llanes DE, Kaufman PB, Bolling SF. Altered hyperlipidemia, hepatic steatosis, and hepatic peroxisome proliferator-activated receptors in rats with intake of tart cherry. *Journal of medicinal food.* 2008;11(2):252-259.
19. Seymour E, 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. *Journal of medicinal food.* 2009;12(5):935-942.
20. Traustadottir 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.

21. Lynn A, Mathew S, Moore CT, et al. Effect of a tart cherry juice supplement on arterial stiffness and inflammation in healthy adults: A randomised controlled trial. *Plant foods for human nutrition*. 2014;69(2):122-127.
22. Cardiovascular disease. World Heart Foundation Web site. <http://www.world-heart-federation.org>. Updated 2015. Accessed January 12, 2015.
23. 2014 national population projections: Summary tables. US Census Bureau Web site. <http://www.census.gov/population/projections/data/national/2014/summarytables.html>. Updated 2014. Accessed January 5, 2015.
24. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004;109(21 Suppl 1):II2-10.
25. Sagie A, Larson MG, Levy D. The natural history of borderline isolated systolic hypertension. *N Engl J Med*. 1993;329(26):1912-1917.
26. Sesso HD, Stampfer MJ, Rosner B, et al. Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in men. *Hypertension*. 2000;36(5):801-807.
27. Lakatta EG, Levy D. Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises: Part I: Aging arteries: A "set up" for vascular disease. *Circulation*. 2003;107(1):139-146.

28. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson Jr SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med*. 1999;340(1):14-22.
29. Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease: A scientific statement from the american heart association. *Circulation*. 2011;123(20):2292-2333.
30. Ford ES, Capewell S. Trends in total and low-density lipoprotein cholesterol among US adults: Contributions of changes in dietary fat intake and use of cholesterol-lowering medications. . 2013.
31. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels: The framingham study. *JAMA*. 1986;256(20):2835-2838.
32. Corti M, Guralnik JM, Salive ME, et al. HDL cholesterol predicts coronary heart disease mortality in older persons. *JAMA*. 1995;274(7):539-544.
33. Millan J, Pinto X, Munoz A, et al. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag*. 2009;5:757-765.
34. Trialists' Collaboration, Blood Pressure Lowering Treatment. Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: Results of prospectively designed overviews of randomised trials. *Lancet*. 2000;356(9246):1955-1964.

35. 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.
36. Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a mediterranean diet. *N Engl J Med*. 2013;368(14):1279-1290.
37. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to mediterranean diet and health status: Meta-analysis. *BMJ*. 2008;337:a1344.
38. Bazzano LA, He J, Ogden LG, et al. Fruit and vegetable intake and risk of cardiovascular disease in US adults: The first national health and nutrition examination survey epidemiologic follow-up study. *Am J Clin Nutr*. 2002;76(1):93-99.
39. Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable consumption and risk of coronary heart disease: A meta-analysis of cohort studies. *J Nutr*. 2006;136(10):2588-2593.
40. Stipanuk M, Caudill M. *Biochemical, physiological, and molecular aspects of human nutrition*. Third ed. Elsevier; 2013.
41. Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. *World journal of cardiology*. 2014;6(6):462.
42. 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.

43. Chaovanalikit A, Wrolstad R. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *J Food Sci.* 2004;69(1):FCT67-FCT72.
44. Kirakosyan A, Seymour E, Llanes DEU, Kaufman PB, Bolling SF. Chemical profile and antioxidant capacities of tart cherry products. *Food Chem.* 2009;115(1):20-25.
45. Burkhardt S, Tan DX, Manchester LC, Hardeland R, Reiter RJ. Detection and quantification of the antioxidant melatonin in montmorency and balaton tart cherries (*prunus cerasus*). *J Agric Food Chem.* 2001;49(10):4898-4902.
46. 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.
47. Seeram N, Momin R, Nair M, Bourquin L. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine.* 2001;8(5):362-369.
48. Consultation WE. Waist circumference and waist-hip ratio. . 2011.
49. Washburn RA, Smith KW, Jette AM, Janney CA. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol.* 1993;46(2):153-162.
50. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol.* 1986;124(3):453-469.

51. Mares-Perlman JA, Klein BE, Klein R, Ritter LL, Fisher MR, Freudenheim JL. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. *J Nutr*. 1993;123(3):489-501.
52. University of minnesota: NDSR. University of Minnesota Nutrition Coordinating Center Web site. <http://www.ncc.umn.edu/products/ndsr.html>. Updated 2014. Accessed 10/25, 2015.
53. Diebolt M, Bucher B, Andriantsitohaina R. Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. *Hypertension*. 2001;38(2):159-165.
54. Erlund I, Koli R, Alfthan G, et al. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. *Am J Clin Nutr*. 2008;87(2):323-331.
55. Han E, Powell LM. Consumption patterns of sugar-sweetened beverages in the united states. *Journal of the Academy of Nutrition and Dietetics*. 2013;113(1):43-53.

Appendix A

TABLES AND FIGURES

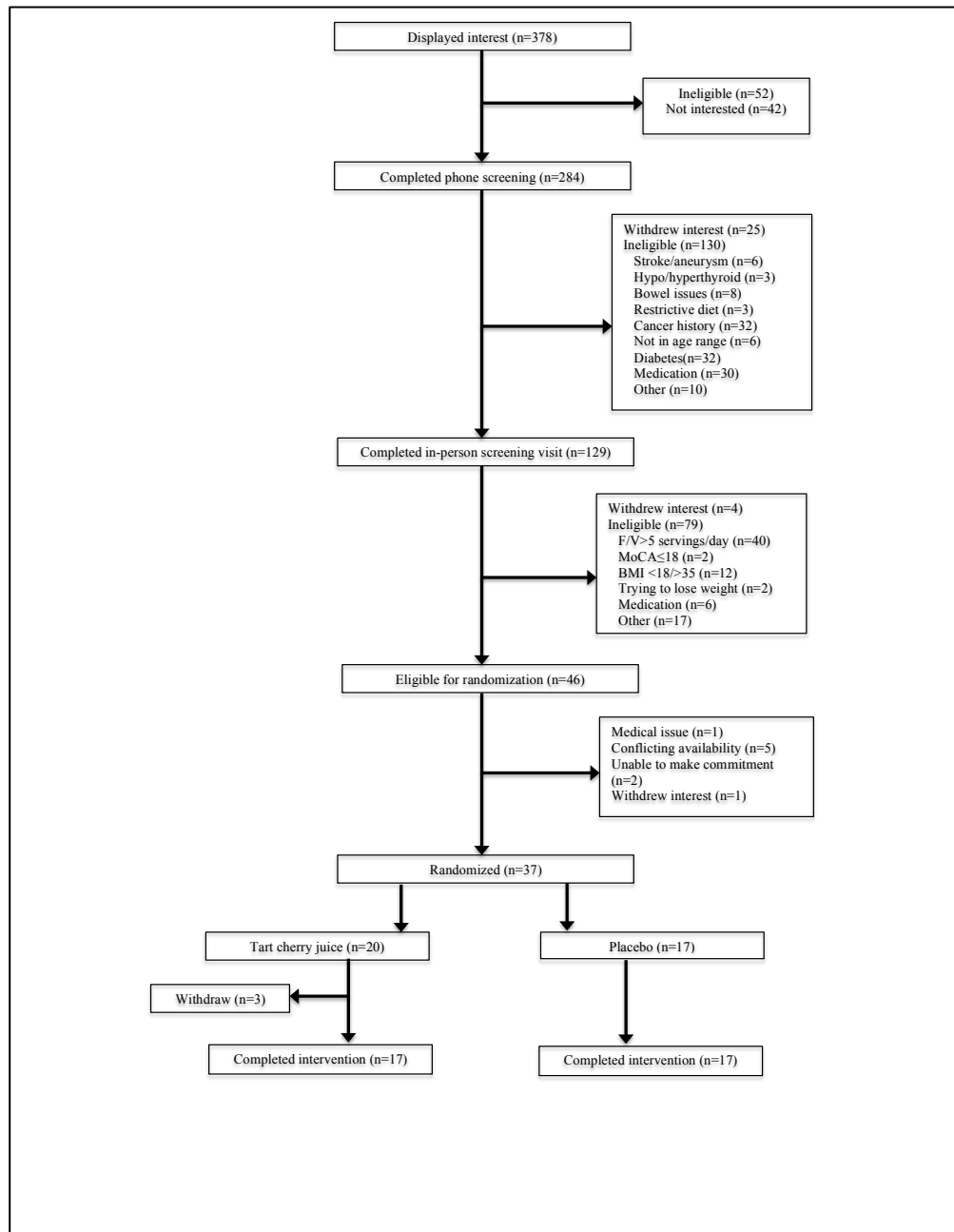


Figure A.1. Flow of study participants.

	Screening	Baseline Week 0	PU#1 & 2 Weeks 4 & 8	Final Week 12
Informed consent	✓			
Anthropometric measurements	✓	✓		✓
Demographic Questionnaire	✓			
Medical History Questionnaire	✓			
MoCA	✓			
PASE	✓	✓		✓
FFQ	✓			
Blood draw		✓		✓
Receive juice		✓	✓	
Return juice			✓	✓
Review 3-day record		✓		✓

Figure A.2. Overview of study visit procedures.

Table A.1. Participant characteristics at baseline.

	Tart Cherry Group n=20	Placebo Group n=17
<i>Sex, female</i>	12	8
<i>Age, yrs</i>	70±0.8	69.5±1.0
<i>Height, cm</i>	165.5±1.5	169.6±1.9
<i>Weight, lb</i>	172.2±0.5	173.1±0.5
<i>BMI</i>	28.5±0.1	27.3±0.1
<i>Education Level</i>		
Completed high school	5 (25%)	5 (29%)
2-year college degree	4 (20%)	1 (6%)
4-year college degree	7 (35%)	6 (36%)
Higher education	4 (20%)	5 (29%)
<i>Income</i>		
Under 25,000	3 (15%)	0 (0%)
25,000-49,999	0 (0%)	1 (6%)
50,000-74,999	8 (40%)	2 (12%)
75,000-99,999	0 (0%)	3 (18%)
Over 100,000	5 (25%)	8 (46%)
Prefer not to say	4 (20%)	3 (18%)
<i>Race</i>		
Asian	2 (10%)	0 (0%)
Black or African American	1 (5%)	2 (12%)
White	16 (80%)	15 (88%)
Prefer not to say	1 (5%)	0 (0%)
<i>Marital Status</i>		
Single never married	2 (10%)	1 (6%)
Separated/Divorced	4 (20%)	3 (18%)
Married	14 (70%)	13 (76%)
<i>Employment Status</i>		
Retired	10 (50%)	15 (88%)
Working	10 (50%)	2 (12%)

Values for age, height, weight and BMI are reported as means± standard error of the mean. All other values are frequencies.

Table A.2. Results of 2x2 ANOVA comparing change in biochemical parameters from baseline to final in tart cherry and placebo groups.

	Tart Cherry Group		Placebo Group		F Value	P value
	Pre	Post	Pre	Post		
<i>Anthropometric Parameters</i>						
Weight, lb	172.2±0.5	172.7±0.5	173.1±0.5	174.2±0.5	0.2313	0.6338
BMI, kg/m ²	28.5±0.1	28.5±0.1	27.3±0.1	27.5±0.1	0.1772	0.6766
<i>Biochemical Parameters</i>						
TC, mg/dL	150.4±4.5	153.2±4.5	156.1±4.5	172.9±4.5	2.3838	0.1324
LDL, mg/dL	64.7±4.8	63.1±4.8	72.8±4.7	83.3±4.7	1.6431	0.2103
HDL, mg/dL	62.6±1.9	63.6±1.9	58.9±2.0	64.6±2.0	1.4899	0.2314
TG, mg/dL	124.7±7.3	132.2±7.2	120.9±6.8	123.1±6.8	0.1464	0.7047
<i>Atherogenic RR</i>						
TC/HDL	2.7±0.1	2.6±0.1	2.8±0.1	2.8±0.1	0.0182	0.8936
LDL/HDL	1.2±0.1	1.1±0.1	1.3±0.1	1.4±0.1	0.2369	0.6301
<i>Blood Pressure</i>						
Systolic	141.4±2.3	135.7±2.3	133.4±2.3	138.8±2.3	6.1215	0.0188*
Diastolic	79.7±1.4	78.8±1.4	78.1±1.4	77.8±1.4	0.0382	0.8463

Values are means±standard error of the mean. BMI, body mass index. TC, total cholesterol. LDL, low-density lipoprotein. HDL, high-density lipoprotein. TG, triglycerides.

P and F value represent the comparison of the difference from pre to post in the tart cherry group compared to the difference from pre to post in the placebo group.

*P values <0.05 indicates a statistically significant difference from baseline to final between groups.

Table A.3. Results of 2x2 ANOVA comparing change in dietary and physical activity variables from baseline to final in tart cherry and placebo groups.

	Tart Cherry Group		Placebo Group		F Val.	P Val.
	Pre	Post	Pre	Post		
<i>Dietary Intake</i>						
Energy, kcal	1709.4±95.1	1716.9±95.1	2008.7±92.3	1879.5±92.3	0.5325	0.4710
Protein, g	75.5±5.2	74.1±5.2	86.3±5.1	75.1±5.1	0.9222	0.3443
% total energy	17.6	17.3	17.2	16.0		
Carbohydrate, g	195.9±10.0	181.8±10.0	234.1±9.7	221.3±9.7	0.0050	0.9439
% total energy	45.8	42.4	48.4	47.1		
Fat, g	67.4±5.6	75.7±5.6	75.0±5.5	76.2±5.5	0.4058	0.5288
% total energy	35.5	40.0	33.6	36.5		
Cholesterol, mg	203.0±38.2	294.5±38.2	262.0±37.1	333.9±37.1	0.0669	0.7976
Fiber, g	20.7±1.0	16.5±1.0	21.4±1.0	20.0±1.0	2.0276	0.1645
<i>Physical Activity</i>						
PASE, score	222.5±7.7 ^a	190.6±7.7 ^a	135.8±7.5	126.3±7.5	2.5635	0.1195

Values are means ±standard error of the mean. PASE, physical activity scale for the elderly.

P and F value represent the comparison of the difference from pre to post in the tart cherry group compared to the difference from pre to post in the placebo group.

Values with same superscript are significantly different at baseline with $p < 0.05$.

Appendix B

IRB APPROVAL LETTER



RESEARCH OFFICE

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

DATE: December 2, 2015

TO: Sheau Ching Chai, PhD, RD
FROM: University of Delaware IRB

STUDY TITLE: [681018-6] Effects of tart cherry juice on cognitive function and cardiovascular disease risk factors in older adults

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED

APPROVAL DATE: December 2, 2015

EXPIRATION DATE: December 16, 2016

REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # (8)

* Closed to New Enrollment

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
STUDY VISIT FORMS

C.1 Demographic Questionnaire

Subject ID: _____	Study #: _____
Interviewer: _____	Date: _____
Session #: _____	

Demographic Questionnaire

Gender: ☐ Male ☐ Female **Age:** _____

Highest level of education:

☐ Less than high school	☐ High school or equivalent
☐ Some college	☐ 2-year college degree (Associates)
☐ 4-year college degree (BS, BA)	☐ Master's degree
☐ Doctoral degree	☐ Professional degree (MD, JD, etc)

Annual household income:

☐ Under \$25,000	☐ \$25,000 - \$49,999	☐ \$50,000 - \$74,999
☐ \$75,000 - \$99,999	☐ Over \$100,000	☐ Prefer not to say

Ethnicity and race:

Choose one:

☐ Hispanic or Latino	☐ Not Hispanic or Latino	☐ Prefer not to say
---	---	--

Choose one or more:

☐ American Indian/Alaska Native	☐ Asian	☐ Native Hawaiian or Other Pacific Islander
☐ Black or African American	☐ White	☐ Prefer not to say

Current marital status:

☐ Single never married	☐ Married
☐ Separated/Divorced	☐ Widowed ☐ Living with another

C.2 Medical History Questionnaire

Subject ID: _____
 Interviewer: _____
 Session #: _____

Study #: _____
 Date: _____

Medical History Questionnaire

FAMILY & PERSONAL HEALTH HISTORY

Has any person, related by blood, had any of the following:

	Yes	No	Relationship
High blood pressure			
Stroke			
Heart attack before age 55			
Blood or clotting disorder			
Cholesterol or blood fat disorder			
Glaucoma			
Cancer (type):			
Alcohol/drug problems			
Psychiatric illness			
Suicide			

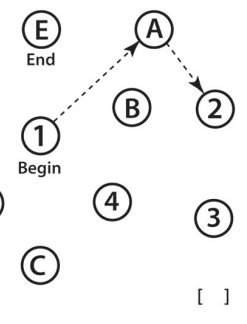
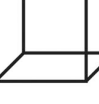
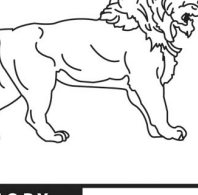
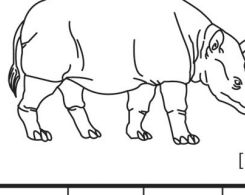
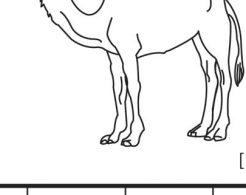
HEIGHT _____ WEIGHT _____

Have you ever had or have you now: (Please check at right of each item and if yes, indicate year of first occurrence)

	Yes	No	Year
High blood pressure			
Rheumatic fever			
Heart trouble			
Pain or pressure in chest			
Shortness of breath			
Asthma			
Pneumonia			
Chronic cough			
Head or neck radiation treatments			
Tumor or cancer (specify)			
Malaria			
Thyroid trouble			
Diabetes			
Serious skin disease			
Mononucleosis			

	Yes	No	Year
Hay fever			
Allergy injection therapy			
Arthritis			
Concussion			
Frequent or severe headache			
Dizziness or fainting spells			
Severe head injury			
Paralysis			
Disabling depression			
Excessive worry or anxiety			
Ulcer (duodenal or stomach)			
Intestinal trouble			
Pilonidal cyst			
Frequent vomiting			
Gallbladder trouble or gallstones			

C.3 Montreal Cognitive Assessment

MONTREAL COGNITIVE ASSESSMENT (MOCA) Version 7.1 Original Version				NAME : Education : Sex :	Date of birth : DATE :																		
VISUOSPATIAL / EXECUTIVE   Copy cube		Draw CLOCK (Ten past eleven) (3 points)			POINTS ____/5																		
NAMING   		____/3																					
MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.		<table border="1" style="width: 100%; border-collapse: collapse; text-align: center;"> <tr> <th></th> <th>FACE</th> <th>VELVET</th> <th>CHURCH</th> <th>DAISY</th> <th>RED</th> </tr> <tr> <td>1st trial</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>2nd trial</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>				FACE	VELVET	CHURCH	DAISY	RED	1st trial						2nd trial						No points
	FACE	VELVET	CHURCH	DAISY	RED																		
1st trial																							
2nd trial																							
ATTENTION Read list of digits (1 digit/ sec.).		Subject has to repeat them in the forward order [] 2 1 8 5 4 Subject has to repeat them in the backward order [] 7 4 2			____/2																		
Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors		[] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B			____/1																		
Serial 7 subtraction starting at 100 [] 93 [] 86 [] 79 [] 72 [] 65		4 or 5 correct subtractions: 3 pts , 2 or 3 correct: 2 pts , 1 correct: 1 pt , 0 correct: 0 pt			____/3																		
LANGUAGE Repeat : I only know that John is the one to help today. [] The cat always hid under the couch when dogs were in the room. []		____/2																					
Fluency / Name maximum number of words in one minute that begin with the letter F [] _____ (N ≥ 11 words)		____/1																					
ABSTRACTION Similarity between e.g. banana - orange = fruit [] train - bicycle [] watch - ruler		____/2																					
DELAYED RECALL Has to recall words WITH NO CUE		<table border="1" style="width: 100%; border-collapse: collapse; text-align: center;"> <tr> <th>FACE</th> <th>VELVET</th> <th>CHURCH</th> <th>DAISY</th> <th>RED</th> </tr> <tr> <td>[]</td> <td>[]</td> <td>[]</td> <td>[]</td> <td>[]</td> </tr> </table>			FACE	VELVET	CHURCH	DAISY	RED	[]	[]	[]	[]	[]	Points for UNCUEd recall only	____/5							
FACE	VELVET	CHURCH	DAISY	RED																			
[]	[]	[]	[]	[]																			
Optional Category cue Multiple choice cue		____/5																					
ORIENTATION [] Date [] Month [] Year [] Day [] Place [] City		____/6																					
© Z.Nasreddine MD Administered by: _____		www.mocatest.org Normal ≥ 26 / 30		TOTAL ____/30 Add 1 point if ≤ 12 yr edu																			

C.4 Physical Activity Screening for the Elderly (PASE)

4. Over the past 7 days, how often did you engage in moderate sport and recreational activities such as doubles tennis, ballroom dancing, hunting, ice skating, golf without a cart, softball or other similar activities?

[0.] NEVER [1.] SELDOM [2.] SOMETIMES [3.] OFTEN
 ↓ (1-2 DAYS) (3-4 DAYS) (5-7 DAYS)
 GO TO Q.#5 ↓ ↓ ↓

4a.	What were these activities?
4b.	On average, how many hours per day did you engage in these moderate sport and recreational activities?
[1.] LESS THAN 1 HOUR	[2.] 1 BUT LESS THAN 2 HOURS
[3.] 2-4 HOURS	[4.] MORE THAN 4 HOURS

5. Over the past 7 days, how often did you engage in strenuous sport and recreational activities such as jogging, swimming, cycling, singles tennis, aerobic dance, skiing (downhill or cross-country) or other similar activities?

[0.] NEVER [1.] SELDOM [2.] SOMETIMES [3.] OFTEN
 ↓ (1-2 DAYS) (3-4 DAYS) (5-7 DAYS)
 GO TO Q.#6 ↓ ↓ ↓

5a.	What were these activities?
5b.	On average, how many hours per day did you engage in these strenuous sport and recreational activities?
[1.] LESS THAN 1 HOUR	[2.] 1 BUT LESS THAN 2 HOURS
[3.] 2-4 HOURS	[4.] MORE THAN 4 HOURS

6. Over the past 7 days, how often did you do any exercises specifically to increase muscle strength and endurance, such as lifting weights or pushups, etc.?

[0.] NEVER [1.] SELDOM [2.] SOMETIMES [3.] OFTEN
 ↓ (1-2 DAYS) (3-4 DAYS) (5-7 DAYS)
 GO TO Q.#7 ↓ ↓ ↓

6a.	What were these activities?
6b.	On average, how many hours per day did you engage in exercises to increase muscle strength and endurance?
[1.] LESS THAN 1 HOUR	[2.] 1 BUT LESS THAN 2 HOURS
[3.] 2-4 HOURS	[4.] MORE THAN 4 HOURS

C.5 Food Frequency Questionnaire

nutritionquest.com

NutritionQuest

WELCOME

ABOUT YOU

BREAKFAST

FRUIT

VEGGIES/PASTA

MEATS

BREADS/SPREADS

SNACKS/DESSERTS

DRINKS

VITAMINS

LIFESTYLE

YOUR RESULTS

Stop Survey

BACK

NEXT

How often do you eat the following foods, all year round? Estimate your average for the whole year.

Oranges or tangerines

✓ How often do you eat oranges or tangerines?

More Info

Never

A few times per year

Once per month

2-3 times per month

Once per week

2 times per week

3-4 times per week

5-6 times per week

Every day

✓ How many do you eat, on the days you eat them?

1/2

One

Two

All the rest of the questions are about how often you eat the foods all year round.

How often do you eat the following vegetables **ALL YEAR ROUND**, including fresh, frozen, canned or in stir-fry, at home or in a restaurant?

Broccoli

✓ How often do you eat broccoli?

[More Info](#)

New

A few times per year

Once per month

2-3 times per month

Once per week

2 times per week

3-4 times per week

5-6 times per week

Every day

✓ How much broccoli do you eat, on the days you eat it?



WELCOME

ABOUT YOU

BREAKFAST

FRUIT

VEGGIES/PASTA

MEATS

BREADS/SPREADS

SNACKS/DESSERTS

DRINKS

VITAMINS

LIFESTYLE

YOUR RESULTS

Stop Survey

NutritionQuest

nutritionquest.com

How often do you eat the following foods, all year round? Estimate your average for the whole year.

Oranges or tangerines

✓

How often do you eat oranges or tangerines?

More Info

Never

A few times per year

Once per month

2-3 times per month

Once per week

2 times per week

3-4 times per week

5-6 times per week

Every day

✓

How many do you eat, on the days you eat them?

1/2

One

Two

57

C.6 Juice Instructions

Juice Instructions

The juice we are providing you with is concentrated. When we say you will drink 2 cups a day of the juice, this includes the juice concentrate and water. Instructions are below. Please be sure that you are drinking 2 cups a day, and do not share with anyone else. We are giving you 4 bottles. One bottle is enough for 7 days. Store the extra bottles in the freezer, and take out one at a time to defrost in the fridge the day before you need it. If you have any leftover juice when you come to pick up the refill, please bring the leftovers with you. Also, please try to keep your diet and physical activity constant throughout this 12-week period. If you have any questions call us at 831-7218.

Starting today: _____

You will drink **2 cups** a day of this juice.

- One cup in the **morning**
- **6-8 hours** later, you will drink another cup

To make the mixed juice:

1. Shake the bottle of juice concentrate.
2. Using the measuring cup we provided you with, mix:
 - **4 ounces** of the juice concentrate
 - **4 ounces** of water
3. Drink and enjoy!

You have enough juice for about 4 weeks. You will come to pick up refills on:

_____ & _____

Thank you for participating in our study!

C.7 Juice Calendar

October 2015

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
				1 AM [] PM []	2 AM [] PM []	
4 Defrost bottle AM [] PM []	5 AM [] PM []	6 AM [] PM []	7 AM [] PM []	8 AM [] PM []	9 AM [] PM []	10 AM [] PM []
11 Defrost bottle AM [] PM []	12 AM [] PM []	13 AM [] PM []	14 AM [] PM []	15 AM [] PM []	16 AM [] PM []	17 AM [] PM []
18 AM [] PM []	19 Pick Up Juice  AM [] PM []	20 AM [] PM []	21 AM [] PM []	22 AM [] PM []	23 AM [] PM []	24 AM [] PM []
25 AM [] PM []	26 AM [] PM []	27 AM [] PM []	28 AM [] PM []	29 AM [] PM []	30 AM [] PM []	31 AM [] PM []

C.8 Three-Day Food Record Instructions

Diet Record Instructions

1. The week prior to your visit, choose 3 days to record everything you eat and drink. Choose two weekdays and one weekend day.
2. On the first day you have chosen, starting when you wake up write down everything you eat and drink that day. It may be best to carry around the small diary with you so you can record the food as soon as you have eaten it.
3. Start by writing down the date and time the food was consumed.
4. Include as much detail as possible.
 - a. Important details to include:
 - i. Brand (McDonald's, Sargento, Quaker, etc.)
 - ii. How prepared (baked or fried?)
 - iii. Fat-free, low-fat, sugar-free, reduced-sodium, low-sodium, etc.
5. If you added toppings to your food, include that too.
 - a. Example: Adding Parmesan cheese to spaghetti, adding mayo to a sandwich.
6. It is also important to include the amount consumed. Refer to the next page for some helpful hints and examples. Common kitchen measurements include:
 - a. Teaspoons and tablespoons
 - b. Cups
 - c. Ounces
7. Lastly, during the 3-days chosen, you may consume a homemade recipe. If possible, include the recipe with the diary and how much the recipe makes. For example, everyone has a different way to make chili. It would be helpful to know the specific recipe you use to make your chili. That way, we get the best idea of what you consumed.