ABSTRACT

Studies have demonstrated that anthocyanins can function as antioxidants, reduce inflammation, and improve dyslipidemia. Tart cherries are anthocyanin-rich, making them particularly attractive as a functional food to improve cardiovascular disease (CVD) risk. There have been few published studies to date examining the impact of tart cherries on biomarkers of dyslipidemia and inflammation, particularly in overweight and obese individuals at high risk for these conditions. This study evaluated the effect of consuming 100% tart cherry juice daily on blood lipids including total cholesterol, low-density lipoprotein cholesterol (LDL-C), calculated very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), and the CVD risk ratios, as well as the inflammatory biomarkers interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), monocyte chemotactic protein-1 (MCP-1), and erythrocyte sedimentation rate (ESR) following a 4-week period. Based on the high anthocyanin content of tart cherries, it was hypothesized that the lipid and inflammatory profiles would be significantly improved following the intervention. A total of 26 men and women completed this 4-week randomized, single-blind, placebo-controlled, crossover study. Participants were randomized to drink either 8 ounces of placebo beverage or tart cherry juice daily for 4 weeks. Following a 4-week washout period, the alternate beverage was consumed. Ultimately, this investigation demonstrated no statistically significant alterations in any of the lipid or
inflammatory biomarkers when analyzed across time and between interventions (p > 0.05). As expected, glucose and insulin parameters remained stable over the duration of the study, as well as self-reported physical activity level, total calorie consumption, and macronutrient intake. However, trans-fat was reported to be significantly higher during the cherry arm of the study as compared to the placebo arm (p < 0.05), potentially confounding other results. Although the results of this study were equivocal, it is feasible that a higher dose, longer treatment duration, or more susceptible target population may be required to elicit significant effects. Consequently, further investigation is necessary to clarify this research.
DEDICATION

For my parents.

Who taught me to pursue excellence, take the road less traveled, and always do what I am afraid to do. Without your love and support, I am not sure who or where I would be today.
ACKNOWLEDGMENTS

There are many people to whom I am grateful for enabling me to reach this milestone in my academic career. First, I would like to thank my mentor, Dr. Keith Martin, for his guidance in conducting this clinical research study and in writing my thesis, as well as for patiently facilitating my understanding of the associated laboratory procedures. I found his enthusiasm and passion for the research process to be both fun and motivational.

I would also like to thank my additional committee members, Dr. Tinna Traustadottir and Dr. Sonia Vega-López for the insight that they provided throughout this process. Dr. Traustadottir was extremely supportive and donated an immense amount of time aiding my statistical analysis, and Dr. Vega-López provided invaluable input and alternative ways of looking at collected data. I chose my committee based on the premise that by surrounding myself with the best, I would be inspired to elevate my nutritional knowledge to a higher level. I reaped the benefits of this decision and am indebted to my outstanding committee.

For their help with blood and laboratory analysis, a thank you goes out to our phlebotomist, Ginger Hook, and my classmate, Lisa Norman, who dedicated many hours to this research while also making it enjoyable.

I would like to extend a special thanks to a few people that, without their support and encouragement, I would not be where I am today. To Dr. Kathy Frier, who promoted my personal growth and development over the past 2 years, to Dr. Carol Johnston, who encouraged me to follow my dreams and apply to the
program earlier than I believed possible, and to my family, who have provided financial and emotional support and have never stopped believing in me.
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Chapter 1

Introduction

Cardiovascular disease (CVD), obesity, and diabetes are chronic conditions associated with low-grade inflammation and oxidative damage to cells and their constituents (1). Diet has the potential to mediate these effects. Several epidemiological studies have demonstrated a protective effect by fruits and vegetables against the development of chronic diseases, and it has been suggested that plant compounds called polyphenols are largely responsible for this effect. (2-4). As major antioxidants in our diet, polyphenols function to protect cells against oxidative damage by up-regulating the expression of certain antioxidant and detoxifying enzymes (5) and by scavenging free radicals and chelating metals (6). Studies also show that polyphenols may protect against CVD by inhibiting the oxidation of low-density lipoprotein (LDL) associated with the development of atherosclerosis, improving endothelial function by inducing vasorelaxation, inhibiting platelet aggregation, and functioning as an anti-thrombotic agent (1). Polyphenols have also been shown to exert anti-inflammatory effects (7).

Anthocyanins are one of the major classes of polyphenols that exhibit a wide range of biological activities. They are water-soluble pigments that give fruit and vegetables their red, purple, and blue colors, and primary sources include blueberries, raspberries, black currants, purple grapes, strawberries, and cherries (6). Because of their high anthocyanin content, many studies on anthocyanin activity have been done using tart cherries as a source. Such studies have
demonstrated that anthocyanins within this fruit have antioxidant activity greater than alpha-tocopherol (8, 9) and display activity comparable to the commercial antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (6)(9). A study conducted by Saric et al. (10) in mice showed that the consumption of tart cherry juice exerted an antioxidant effect through increased activity of superoxide dismutase (SOD) and glutathione peroxidase (Gpx). An improved antioxidant effect has also been shown in humans in a study completed by Traustadottir et al. (11) where tart cherry juice increased the capacity of older adults to resist oxidative damage following an acute oxidative challenge.

There are limited studies to support that anthocyanins from cherries may protect against dyslipidemia. In a study conducted by Seymour et al. (12), Dahl-SS rats fed a tart cherry-enriched diet exhibited significantly reduced total cholesterol and triglycerides compared to the control diet (75 compared to 87 mg/dL total cholesterol, and 28 compared to 34 mg/dL triglycerides, respectively). In a more recent study by Seymour et al. (13), Zucker fatty rats fed tart cherry powder demonstrated a similar reduction in lipid values when compared to those fed a control diet (185 compared to 207 mg/dL total cholesterol, and 284 compared to 241 mg/dL triglycerides, respectively). Although several studies have shown cherry consumption to have a beneficial effect on lipid levels in rats, a study completed by Kelley et al. (14) in humans failed to duplicate these results. In this study, total cholesterol, LDL-cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured
in healthy men and women and were not affected by the consumption of Bing sweet cherries.

While relatively few studies have examined the effect of a cherry intervention on serum lipid levels in both human and animal models, a larger number of studies have been conducted with the purpose of measuring alterations in inflammatory markers. Obesity greatly contributes to the inflammatory process, as an overabundance of white adipose tissue (WAT) increases the production and secretion of several inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (15). C-reactive protein (CRP) is an inflammatory marker that is a predictive factor for coronary heart disease, and its levels are directly related to IL-6 production. In the study by Kelley et al. (14) an inverse relationship was demonstrated between the human consumption of Bing sweet cherries for a 28-day period and serum CRP. In the study by Seymour et al. (13) Zucker rats experienced a 14% increase in lean body mass and an 18% decrease in fat mass after consuming cherry powder for 12 weeks, and also demonstrated a significant decrease in retroperitoneal (abdominal) and plasma IL-6 and TNF-α expression. Additional studies have shown cherries to modulate inflammatory pathways by inhibiting cyclooxygenase (COX) 1 and 2 enzymes (16). Non-steroidal anti-inflammatory drugs such as aspirin function by irreversibly inhibiting the activity of the COX enzymes (7), and one in vitro study showed anthocyanins to have greater anti-inflammatory activity than aspirin (9).
There have been many studies evaluating and substantiating the role of tart cherries as a potent antioxidant source. However, studies exploring the ability of tart cherries to lower serum lipid values and markers of inflammation have reported conflicting outcomes. There is some evidence that tart cherries have lipid-lowering capacity, but results derived from such studies have generally used rodent models and may therefore be less relevant to humans. There have been some promising studies conducted on the ability of cherries to lower markers of inflammation, but additional well-controlled research studies in humans are necessary. It would be beneficial to recruit participants possessing elevated baseline plasma lipid values and inflammatory markers since this study population is more prone to develop CVD and may be more likely to respond to a dietary intervention. Overweight or obese individuals have a greater potential to exhibit dyslipidemia and/or inflammation. Very few studies demonstrating the effects of tart cherry supplementation on lipid values and inflammatory markers within an overweight or obese population have been published.

**Purpose**

The purpose of this randomized, crossover, single blinded, placebo-controlled study was to examine the effects of tart cherry juice consumption on serum lipids (total cholesterol, triglycerides, LDL-C, calculated VLDL-C, HDL-C) and inflammatory markers (ESR, MCP-1, IL-6, TNF-α, hsCRP, and IL-10) in overweight and obese subjects from the greater Phoenix region.
Hypotheses and Aims

- **Hypothesis:**
  
  Daily consumption of 100% tart cherry juice will improve biomarkers of dyslipidemia and inflammation without disrupting glucose homeostasis.

- **Specific Aim 1:**
  
  To determine whether daily tart cherry consumption will improve biomarkers of dyslipidemia (total cholesterol, triglycerides, LDL-C, calculated VLDL-C, HDL-C, and calculated CVD risk ratios) without altering glucose homeostasis in overweight and obese individuals.

- **Specific Aim 2:**
  
  To determine whether daily tart cherry consumption will affect biomarkers of inflammation (ESR, hsCRP, TNF-alpha, IL-6, MCP-1, IL-10).

Definition of Terms

**Overweight:** Overweight is defined as having a BMI between 25.0 kg/m$^2$ and 30.0 kg/m$^2$.

**Obese:** Obese is defined as having a BMI greater than or equal to 30.0 kg/m$^2$.

Delimitations and Limitations

Participants in this study were overweight and obese men and women of at least 18 years of age from the Phoenix area. They were non-smokers, had never been diagnosed with type 1 or 2 diabetes, had no unresolved infections, diseases, or inflammatory conditions, and were not pregnant or lactating. They were weight stable for 6 months prior to enrolling in the study, were not planning to lose
weight over the 3 month study duration, and had a body fat of at least 20% for men and 25% for women at the study baseline. The body fat parameter was implemented to ensure that a BMI of greater than 25.0 kg/m$^2$ primarily reflected higher adiposity rather than lean body mass. The inclusion criteria were established to include a population most likely to be at risk of dyslipidemia and inflammation. Thus, results cannot be generalized to individuals who do not fit these criteria or to the American population as a whole.

Because participants will only be consuming tart cherry juice for a total of 28 days, it is possible that this may not be enough time to elicit changes in serum lipids and inflammatory markers. The compliance of the participants is another factor that must be considered in the study. It is expected that participants will avoid other high anthocyanin containing fruits and fruit juices, and that diet and exercise will remain consistent throughout the duration of the study. This will be evaluated by having participants submit one 24-hr dietary record per week and a physical activity questionnaire at the completion of each 4-week study arm. It will be assumed that the dietary records will reflect typical daily intake.
Chronic Disease

The increasing prevalence of chronic disease is becoming a global epidemic and, according to a report published by the World Health Organization (WHO) in 2005, kills approximately 17 million people annually (17, 17, 17). In the United States alone, chronic conditions such as CVD, cancer, and diabetes are responsible for 7 out of every 10 deaths, and in 2005 it was estimated that nearly 1 out of every 2 adults suffered from one or more forms of chronic illness (18). CVD is an umbrella term referring to all diseases that affect the cardiovascular system, but frequently refers to those associated with atherosclerosis and the narrowing of the arteries, such as coronary heart disease (CHD). Of all chronic diseases, CVD has the highest mortality rate and is the leading cause of death in the United States, accounting for 34.3 percent of all deaths in 2006 (19). Although CVD (including heart disease and stroke) has consistently remained the leading cause of death in the United States since 1919 (20), the prevalence of diabetes is escalating at an alarming rate. Between 1958 and 2008, the number of people with diabetes has increased more than 10-fold from 1.6 million to 18.8 million, and in 2007 nearly 8 percent of adults over 20 years of age had been diagnosed with the disease (21). Based on current trends, the CDC estimates that by 2050 the number of people with diabetes will double or even triple (22). Diabetes and CVD share many of the same risk factors, a cluster of symptoms which have been
termed the metabolic syndrome. The National Cholesterol Education Program’s Adult Treatment Panel III report (NCEP ATP III) included abdominal obesity, atherogenic dyslipidemia (identified by low serum concentration of HDL cholesterol and high triglycerides), hypertension, insulin resistance/glucose intolerance, thrombosis, and inflammation (clinically recognized by elevated CRP levels) as the 6 major components of the metabolic syndrome (23). Obesity, defined as a BMI ≥ 30 kg/m², is a risk factor for both CVD and metabolic syndrome. Similar to trends seen in diabetes, the prevalence of obesity in the United States has drastically increased over the past several decades, more than doubling since 1980 (24). The most recent reports published by the CDC estimate that 33.8% of American adults age 20 and older are obese (25).

**Inflammation**

Inflammation is a normal biological response that occurs when tissues become injured or infected, functioning to protect the body from foreign pathogens and particles, to remove dead and damaged cells, and to initiate the healing process (5). The affected tissues produce inflammatory cytokines and chemokines, which in turn function as part of a signaling pathway to orchestrate the inflammatory response by regulating target cells and other mediators of inflammation. Inflammation must occur in order for infections to be resolved and for wounds to heal, and can be categorized as either acute or chronic. Acute inflammation occurs when body tissues are first exposed to an injury, irritant, or infection, and progresses to chronic inflammation if the stimulant is not removed.
Chronic inflammation is associated with the simultaneous repair and destruction of inflamed tissues, as well as an alteration in the types of cells involved in the inflammatory response. Some of the primary cytokines and chemokines involved in inflammation include tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and monocyte chemotactic protein-1 (MCP-1). TNF-α and IL-1β are potent cytokines and key mediators of inflammation and both induce fever and IL-6 synthesis (IL-6 is also secreted by adipocytes). IL-6 in turn acts along with TNF-α to stimulate the liver to produce acute phase proteins (27), which participate in the inflammatory response through modulation of the immune system. CRP is one such acute phase protein, and is particularly significant in that it is both a risk factor and a prognostic marker for CHD and its progression (28). MCP-1 is a chemokine that is responsible for activating certain types of cells, such as monocytes, and provoking their migration to inflamed tissues (27). Once in the inflamed regions monocytes can differentiate into phagocytic macrophages, which act immunologically to clear debris in the cell and defend against foreign bodies (29). Macrophages contribute to the cyclical nature of chronic inflammation by producing IL-1β, TNF-α, and additional MCP-1, and also play a significant role in the progression of atherosclerosis through plaque formation (27, 30). IL-10 is an anti-inflammatory cytokine that has inhibitory effects on the production of pro-inflammatory cytokines (27), and is therefore very important in ameliorating chronic inflammation. Chronic inflammation is problematic in that it
tends to be systemic, generating additional inflammatory sites in other regions of the body, and plays a primary role in the etiology of many chronic diseases (26).

**Obesity and Inflammation**

CVD, diabetes, and obesity are interrelated in that they are each associated with chronic low-grade inflammation. Obesity refers to an accumulation of adipose tissue and is a major contributor to inflammation. Recent studies have exposed the biologically active nature of adipocytes and their ability to modulate the inflammatory response by functioning as an endocrine organ that secretes adipokines, chemokines, and cytokines (29). In a review article by Gustafson (30), mammalian adipose tissue is characterized as white adipose tissue (WAT) or brown adipose tissue (BAT). These two forms are similar metabolically but differ in that WAT primarily stores energy, while BAT dispels energy as heat. WAT plays an active role in the pathophysiological processes leading to the development of inflammatory diseases, and is composed of both subcutaneous and visceral adipose tissue. Subcutaneous adipose tissue stores most of the body’s energy reserves and resides in the upper and lower body, while visceral adipose tissue surrounds and supplies the internal organs with energy.

Adipose tissue is composed of mature adipocytes and a group of smaller cells that include pre-adipocytes, fibroblasts, and macrophages among many others (29). The adipocyte is a multifunctional cell, engaging in lipid synthesis, lipid storage, and the secretion of both pro-inflammatory and anti-inflammatory factors (30). Under normal circumstances, adipose tissue primarily secretes anti-
inflammatory molecules, but as lipid accumulates and cells hypertrophy, the adipocytes, preadipocytes, and macrophages within the adipose tissue can secrete a variety of inflammatory cytokines and hormones such as CRP, resistin, inducible nitric oxide synthase (iNOS), IL-6, and MCP-1 (31-34). Enlarged adipocytes also secrete the hormone leptin, which is pro-inflammatory and decreases the secretion of adiponectin, a hormone with anti-inflammatory properties (29).

Visceral adipose tissue has a higher rate of lipolysis (fatty acid turnover), an increased infiltration of inflammation-inducing macrophages, and a higher expression of IL-6, MCP-1, and additional inflammatory related markers than subcutaneous adipose tissue (35, 36, 36). With increasing adiposity, monocytes move from the blood to adipose tissue where they differentiate into inflammatory macrophages and are activated to express inflammatory cytokines such as MCP-1, IL-6, and TNF-α (30). There is evidence to suggest that macrophages may be attracted to high levels of necrotic tissue induced by hypoxia in the expanding adipocytes(29). Visceral adipose tissue contains a higher concentration of infiltrated macrophages ((36), and macrophages may be a primary source of circulating inflammatory molecules associated with obesity (29), providing one possible explanation for intra-abdominal adiposity (as measured by WC) being a component of the metabolic syndrome and an independent risk factor for CVD and diabetes.
Figure 1. The cycle of inflammation between macrophages and adipocytes in obese visceral adipose tissue increases chronic disease risk. Adapted from Hirai, S. et al., 2010. (37).

**Obesity, Inflammation, and the Vasculature**

The inflammation associated with obesity also exerts a negative effect on the vasculature within the body, leading to an increased risk of atherosclerosis and CVD. Epicardial adipose tissue (EAT) is the deposition of visceral fat around the heart and coronary arteries that is associated with intima-media thickness and the inflammatory response that contributes to disease etiology (38). Similar to visceral adiposity, EAT is subject to macrophage infiltration and is also a source of bioactive molecules including resistin, TNF-α, and IL-6 (38, 39), all of which contribute to chronic inflammation and adverse affects on cardiac function. Patients with coronary artery disease have a higher level of macrophage
infiltration into EAT (39). Perivascular adipose tissue (PAT) refers to the
deposition of fat around the blood vessels, which may contribute to vascular
stiffness observed in the obese (40). Like EAT, PAT is metabolically active and
releases a variety of inflammatory cytokines and factors that act to attenuate
vascular relaxation and health (41).

A large accumulation of PAT may be associated with the secretion of an
unfavorable profile of relaxation factors, atherogenic cytokines, and smooth
muscle growth factors that result in adverse function and morphological
alterations of blood vessels (40). The inflammatory cytokine TNF-α plays a
primary role in deterring the healthy relaxation of blood vessels by inhibiting
endothelial nitric oxide (NO) vasodilation, which leads to subsequent arteriole
constriction (42). The increased deposition of PAT is also associated with a
reduction in the adipokine adiponectin, which modulates the inflammatory
pathway in a variety of ways and has a major impact on endothelial function.
Adiponectin opposes the negative effects of TNF-α on the blood vessel by
decreasing inflammation and adhesion molecule expression, macrophage
recruitment and TNF-α production, formation of atherosclerotic foam cells
(produced by lipid accumulation in macrophages), and reducing platelet
aggregation and smooth muscle cell proliferation (43-45). In addition, as a
regulator of endothelial NO production, adiponectin helps directly determine
blood vessel tone (46). The obesity-associated reduction of adiponectin clearly
has a detrimental effect on the cardiovascular system and greatly contributes to vascular complications.

**Cholesterol and Lipoproteins**

Cholesterol is a type of lipid that has multiple functions in the body and is characterized by a four-ring core structure. It exists only in animal tissues and is an essential component of cell membranes, and a precursor for bile acids, steroid sex hormones, and vitamin D (47). Although it is required physiologically, cholesterol can be synthesized endogenously in the liver and is therefore not an essential dietary component. Dietary intake, however, can affect cholesterol synthesis, as saturated fatty acids have been demonstrated to enhance its endogenous production. However, contrary to what might be expected, dietary consumption of cholesterol has a very minimal impact on serum cholesterol concentrations (47).

Lipoproteins are compounds comprised of lipids and proteins that function to transport hydrophobic lipids through the blood to tissues (47). The structure of lipoproteins confers stability in the aqueous environment of the blood, consisting of a hydrophilic outer shell in which the polar components of proteins and phospholipids are situated, and an inner surface lined with the hydrophobic portions of proteins and phospholipids. The outer layer of lipoproteins also contains apolipoproteins, which confer specificity to the lipoproteins, permitting recognition by their corresponding cellular receptors (47). Being insoluble in water, triglycerides and cholesterol esters reside in the core of the lipoprotein
where they are shielded from contact with the blood. When dietary fats such as triglycerides, phospholipids, and cholesterols are ingested, they are emulsified by bile salts and incorporated into water-soluble micelles, which enter enterocytes during absorption. Upon reaching the enterocyte, the lipids are released from micelles and are incorporated into lipoproteins, which travel through the lymphatic system and into the bloodstream. The four major classes of lipoproteins include chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), and each is categorized based on size and the lipid and protein composition that determines their density (23). The higher the ratio of protein to lipid, the greater the density of the lipoprotein. Chylomicrons have the lowest density of the lipoproteins, being composed of 88% TG, 7% phospholipids (PL), 4% cholesterol, and only 1% protein. Chylomicrons transport dietary lipids from the enterocyte through the lymph system, the blood, and then to the liver (47). When in the bloodstream, lipoprotein lipase (LPL) hydrolyzes and releases TG from the chylomicron to the adipose tissue, and the chylomicron and its remaining contents (referred to as a chylomicron remnant) then return to the liver.

VLDLs are composed of 54% TG, 22% cholesterol, 16% PL, and 8% protein (48) and transport TG from the liver to body tissues and adipose tissue. TG are also transferred from VLDL to HDL in exchange for cholesterol through the action of cholesterol esterase transport protein (CETP) (47). As VLDL progressively loses its TG and fatty acids, cholesterol becomes more concentrated
and the lipoprotein temporarily transitions to an intermediate density lipoprotein (IDL) and then ultimately to LDL. When the level of cholesterol in the IDL exceeds the level of TG, the IDL is officially recognized as LDL. LDL is comprised of 46% cholesterol, 22% PL, 21% protein, and 11% TG (48), and an elevated concentration is considered the primary risk factor for CHD.

HDL functions in opposition to LDL, as LDL transports cholesterol away from the liver to tissues, and HDL carries excess cholesterol back to the liver to be excreted in bile or stored for later use (47). This function of HDL is referred to as reverse cholesterol transport, and is considered to be protective against CHD. HDL contains the highest quantity of protein and the lowest quantity of TG, and is comprised of 50% protein, 26% PL, 20% cholesterol, and 4% TG (48).

Figure 2. Metabolic pathway of lipoproteins. Adapted from Olson, R. E., 1998. (48). CM indicates chylomicron, FFA indicates free fatty acids, LCAT indicates lecithin-cholesterol acyltransferase, LPL indicates lipoprotein lipase, A, B, C, and E indicate apolipoprotein A, B, C, and E.
Triglycerides account for approximately 95% of dietary fat and their main function is to provide energy to body tissues. They are also the primary storage form of fat in adipocytes. Although the association between elevated LDL cholesterol and CHD risk is well established and understood, the relationship between elevated TG and CHD has been somewhat more ambiguous. However, many studies suggest that elevated concentrations of TG increase CHD risk independently of cholesterol levels (23, 49). Cullen conducted a meta-analysis of 17 prospective research studies in which HDL and LDL cholesterol were controlled for, pooling nearly 11,000 women and more than 46,000 men, and found that an 88 mg/dL increase in TG concentration significantly increased CVD risk by 37% in women and 14% in men (p < 0.05) (49). These results support the premise that hypertriglyceridemia is an independent risk factor for CHD.

**Dyslipidemia**

Dyslipidemia is a condition characterized by an independent, elevated concentration of LDL-cholesterol and TG and a reduced concentration of HDL-cholesterol (singly or in combination), which increases the risk of developing CHD (23). The NCEP is an organization established to create evidence-based guidelines for the clinical prevention, treatment, and management of dyslipidemia (23). The NCEP has defined optimal total cholesterol levels as < 200 mg/dL, borderline high as 200 – 239 mg/dL, and high as > 240 mg/dL. Optimal LDL cholesterol is defined as < 100 mg/dL, near optimal as 100 – 129 mg/dL, borderline high as 130 – 159 mg/dL, high as 160 – 189 mg/dL, and very high
LDL cholesterol was determined to be $\geq 190$ mg/dL (Table 1). Serum LDL-cholesterol values exceeding 100 mg/dL are considered to be atherogenic, with higher LDL cholesterol concentrations positively correlating with an increased occurrence of CHD. Therefore, reductions in LDL cholesterol levels are considered to be protective against CHD. However, NCEP targets for LDL-cholesterol reductions become progressively stringent as the number of CHD risk factors in a dyslipidemic individual increase. There is an inverse relationship between CHD risk and HDL cholesterol concentration, and high levels are considered to be protective against CHD. The NCEP has defined low HDL cholesterol as $< 40$ mg/dL and high HDL cholesterol as $\geq 60$ mg/dL (Table 1).

Elevated TGs are also an independent risk factor for CHD and impact serum LDL and HDL cholesterol concentrations. NCEP has set the optimal TG level at $< 150$ mg/dL, borderline high as 150 – 199 mg/dL, and very high TG level as $\geq 500$ mg/dL (Table 1).

**TABLE 1**

*Lipid Classifications*

<table>
<thead>
<tr>
<th>Lipid, mg/dL</th>
<th>Below optimal</th>
<th>Optimal</th>
<th>Near optimal</th>
<th>Borderline high</th>
<th>High</th>
<th>Very high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>n/a</td>
<td>$&lt; 200$</td>
<td>n/a</td>
<td>200 - 239</td>
<td>$&gt; 240$</td>
<td>n/a</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>n/a</td>
<td>$&lt; 100$</td>
<td>100 - 129</td>
<td>130 - 159</td>
<td>160 - 189</td>
<td>$\geq 190$</td>
</tr>
<tr>
<td>TG</td>
<td>n/a</td>
<td>$&lt; 150$</td>
<td>n/a</td>
<td>150 - 199</td>
<td>200 - 499</td>
<td>$\geq 500$</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>$&lt; 40$</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>$\geq 60$</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Adapted from the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report, 2002. (23).

Because dyslipidemia plays a pivotal role in the development of CHD, it is necessary to understand the risk of developing dyslipidemia, the actions that can
be taken to lower such risk, and the etiology of the condition. The Framingham Offspring Study was conducted between 1971 and 2001 to determine the risk of developing dyslipidemia over a thirty year period, and included 4701 men and women between the ages of 30 and 59 years at baseline. The study sample included participants with and without various forms of dyslipidemia, but participants who experienced myocardial infarction or TG exceeding 400 mg/dL were excluded in the statistical analysis. An examination and fasting blood draw was performed approximately every 4 years during the study period, and both the prevalence and the risk of developing dyslipidemia was determined in the sample population. The prevalence of borderline-high LDL cholesterol (defined as ≥ 130 mg/dL and/or lipid modifying drug use) was determined to be 24% in women and 44% in men in the 30-34 age group, 36% in women and 57% in men in the 40-44 age group, and 51% in women and 61% in men in the 50-54 age group. The prevalence of high LDL cholesterol (defined as ≥ 160 mg/dL and/or lipid modifying drug use) was demonstrated to be 6% in women and 16% in men in the 30-34 age group, 12% in women and 24% in men in the 40-44 age group, and 24% in women and 29% in men in the 50-54 age group (50). The prevalence of low HDL cholesterol (defined as < 40 mg/dL) was less dependent upon age and was determined to be approximately 40% in men and 10% in women. Cobain et al. determined that the risk of developing borderline high LDL cholesterol after 30 years was approximately 69% for women and 81% for men in the 30-34 age group at baseline, 81% for women and 82% for men in the 40-44 age group, and
83% for women and 82% for men in the 50-54 age group. The 30-year risk of developing high LDL cholesterol was demonstrated to be approximately 43% for women and 52% for men aged 30-34, 55% for women and 57% for men aged 40-44, and 57% for women and 59% for men aged 50-54 at baseline. The risk of developing low HDL cholesterol after 30 years was determined to be approximately 27% for women and 67% for men in the 30-34 age group, 29% for women and 65% for men in the 40-44 age group, and 28% for women and 67% for men in the 50-54 age group at baseline. After 30 years, approximately 20-40% of the sample population developed a combination of high LDL and low HDL cholesterol, putting the participants at significant risk for developing CHD (50). Although the generalizability of study results to the entire American population is limited due to a lack of geographic and ethnic diversity within the sample population, this study clearly demonstrates that dyslipidemia poses a significant risk to public health and must be dealt with.

**Dyslipidemia and Inflammation in the Etiology of Atherosclerosis**

Hypercholesterolemia may result in the accumulation, oxidation, and modification of lipids in the vascular endothelium, leading to endothelial dysfunction, chronic inflammation, and CVD (51). It has become increasingly evident that chronic inflammation is involved in the atherosclerotic process. Atherosclerosis refers to the accumulation of lipids, calcium, and connective tissue within the arterial endothelium that initiates plaque formation and the thickening of arteries, subsequently producing the stenosis and/or thrombosis (51).
associated with CHD. The pathogenesis of atherosclerosis is initiated by elevated levels of LDL-cholesterol that accumulate in the arterial intima and are subject to oxidation by reactive oxygen species (ROS) (52). Endothelial dysfunction also occurs in these early stages of atherosclerosis, as a variety of mechanisms (including reactivity with ROS) contribute to the reduced production and bioactivity of the vasodilator NO (28). In addition to regulating vascular tone, endothelial NO also acts to reduce leukocyte adhesion to endothelial cells, the ability of smooth muscle to proliferate in forming plaques, and the aggregation of platelets, all of which are important mechanisms in the process of atherogenesis and will be described in more detail below.

The reduced capacity of NO to regulate the vascular endothelium is accompanied by an elevated expression of adhesion molecules, which permit the increased binding of monocytes and T lymphocytes to the endothelium and entry into the arterial intima (28). Once in the intima, the monocytes differentiate into macrophages and internalize oxidized LDL to become foam cells, subsequently secreting pro-inflammatory cytokines such as TNF-α and IL-1β (28). T lymphocytes also become activated in response to oxidized LDL, secreting pro-inflammatory cytokines and interacting with macrophages to elicit the production of the tissue factor matrix metalloproteinase (MMP), which in turn functions to increase the inflammatory response (28). This continuous cycle of oxidation, endothelial injury, and inflammation stimulates vascular smooth muscle cells to proliferate and produce collagen and elastin, ultimately protruding into the intima.
Additional factors promote the death of smooth muscle cells and macrophages, which accumulate as necrotic debris and contribute to inflammation (51). The collagen and elastin produced by smooth muscle cells create a fibrous cap over the growing arterial lesion that is susceptible to rupture, potentially producing a thrombus that could result in blood vessel occlusion. The growing plaque and its fibrous cap may also cause blood vessel stenosis, potentially accompanied by ischemia. Elevated levels of LDL-cholesterol and the modification that occurs as a result of oxidation play a primary role in the pathology of atherogenesis (52). By promoting mature macrophage transformation, provoking MCP-1 production by endothelial cells, enhancing foam cell formation and oxidized LDL-cholesterol uptake, and stimulating monocyte expression of pro-inflammatory cytokines, oxidized LDL-cholesterol initiates and promotes the inflammatory process associated with the development of CVD (52).

Figure 3. Oxidized LDL acts to promote inflammation and atherosclerosis. Adapted from Kris-Etherton, P.M. et al., 2004. (53).
**Functional Foods**

Chronic disease development and the inflammatory and oxidative processes underlying their pathogenesis can be modified by several behavioral factors, one of which is diet. Functional foods are those that contain bioactive components that are believed to provide physiological benefits beyond what basic nutrients are expected to supply, promoting health and/or preventing the development and progression of many diseases (54). Epidemiological studies have clearly demonstrated an association between increased fruit and vegetable intake and a reduced risk of CVD, heart disease, and type 2 diabetes (2-4), and it is plausible that the beneficial effects of fruit and vegetable consumption may be attributed to the specific phytochemicals that these foods contain. Polyphenols are a diverse class of plant-derived compounds found in fruits and vegetables that are bioactive agents, conferring a wide variety of potential physiological benefits (5). Polyphenols are comprised of 2 or more hydroxyl groups attached to a benzene ring and can be divided into 5 major subclasses: cinnamonic acids, chalcones, flavanoids, procyanidins, and anthocyanins (5). Anthocyanins are a group of water-soluble pigments that give orange, blue, red, and purple fruits, vegetables, and flowers their characteristic colors, and are present in high levels in black currants, purple grapes, strawberries, raspberries, blue berries, and cherries (6, 55). The literature has identified over 500 different anthocyanins, which are composed of the aglycone anthocyanidin linked to a sugar to create a more stable glycoside that is water-soluble (56).
anthocyanidin, as shown in Figure 4, consists of a flavilium or 2-phenyl-
benzopyrilium cation, and anthocyanins are differentiated primarily based on the
number and position of the methoxyl and hydroxyl groups attached to the base
(56). The most frequently encountered anthocyanins in fruits and vegetables
include delphinidin, pelargonidin, malvidin, petunidin, peonidin, and cyanidin
(Figure 4) (56). Studies have demonstrated that anthocyanins may function as
antioxidants and diminish the formation of free radicals, favorably impact
dyslipidemia, and reduce inflammation (6, 57, 58).

![Figure 4](image)

Figure 4. Structures of 6 anthocyanins common in fruits and vegetables. Adapted
from de Pascual-Teresa, S. et al., 2010 (56).

**Anthocyanins**

Many studies suggest that anthocyanins may offer protection against the
formation of free radicals and oxidative stress (6, 9, 57, 59), and this is
particularly important because the generation of free radicals is thought to
contribute to the pathology of chronic disease (60). Antioxidant enzymes and
compounds neutralize the oxidation of cells and their constituents by free radicals.
ROS are continuously created as a byproduct of metabolism and can damage body
systems if antioxidants are unable to neutralize them (9). When such an imbalance
between ROS production and antioxidant availability occurs, the free radicals can attack lipids (as happens in LDL-cholesterol oxidation), proteins, and even DNA, resulting in the destruction of cell membranes and the impairment of cellular functions (9). Limiting the exposure of LDL-cholesterol to free radicals by improving antioxidant defenses may reduce its subsequent oxidation, thereby decreasing the role of LDL-cholesterol in promoting atherogenesis. A portion of the high antioxidant capacity of anthocyanins is thought to be attributed to their unique structure, which consists of multiple hydroxyl groups that can donate electrons to neutralize a radical (61). The antioxidant activity of anthocyanins has been reported to increase as the number of hydroxyl groups increases, and to decrease with the glycosylation of anthocyanidins (59). Direct antioxidant activity by anthocyanins has also been attributed to their capacity to chelate metals such as Fe (II), which catalyzes lipid oxidation (62). The resulting Fe (II) complexes of anthocyanins are inert and unable to engage in lipid peroxidation but purportedly retain their radical scavenging abilities (63).

The ability of anthocyanins to neutralize free radicals and chelate metal ions has been demonstrated at high concentrations in vitro (8, 9, 16, 63). The actual plasma concentrations of anthocyanins and their metabolites that occur in vivo are much lower due to relatively low absorption, and this may alter their capacity to act by these mechanisms. While both the parent compound and many of its degradation products have been shown to retain their direct radical scavenging activity following absorption (9), a study conducted by Kay et al. (64)
determined their absorption rate to be only 0.15% of the initial dose. The low bioavailability and rapid elimination of anthocyanins and their metabolites in vivo have led some researchers to suggest that the increased anti-oxidant activity observed in many studies may be better explained by an ability to inhibit oxidation through indirect rather than direct mechanisms. It has been proposed that anthocyanins may upregulate the expression of certain antioxidant and detoxifying enzymes, which in turn function to directly neutralize free radicals and protect against the harmful effects of oxidative stress (65). SOD and Gpx are two such antioxidant enzymes that have demonstrated an increase in activity in response to anthocyanin consumption (10, 66, 67).

Although research has shown anthocyanins to protect against oxidative stress and attenuate the inflammatory response, these phytochemicals have also been demonstrated to modulate inflammation in other ways. Cyclooxygenase (COX) is an enzyme that catalyzes the conversion of arachidonic acid to pro-inflammatory prostaglandins and thromboxanes. The two major isoforms of COX include COX-1 and COX-2, with COX-1 being expressed in a variety of tissues and COX-2 being induced at sites of inflammation, including within macrophages and mast cells following stimulation by pro-inflammatory cytokines (7). NSAIDs such as aspirin, ibuprofen, and Advil reduce inflammation by inhibition of the COX-1 and 2 pathways, and several studies have reported that anthocyanins also have the capacity to inhibit COX-enzyme activity (8-10). In addition to the COX-1 and 2 enzymes, TNF-α, IL-6, CRP, MCP-1, and NO (generated by iNOS) are
biomarkers that also stimulate the inflammatory response. Studies have demonstrated that these pro-inflammatory cytokines, chemokines, and messenger molecules are significantly reduced following anthocyanin consumption (13, 14, 66, 68), thereby providing additional mechanisms for the anti-inflammatory effects associated with anthocyanins.

There is research to suggest that anthocyanins may also have a beneficial effect on dyslipidemia. Studies using both human and rodent models have reported an increase in HDL (67, 69), and reductions in total cholesterol (12, 67, 70), LDL cholesterol (67, 69, 70), and triglycerides (12, 67, 70) following the consumption of anthocyanin-rich tart cherries. There are several proposed mechanisms for the positive effect of anthocyanins on serum lipid parameters. One such explanation involves the inhibition of CETP in reverse cholesterol transport. It has been shown that anthocyanins act to significantly lower the activity of CETP, resulting in an increase in HDL-cholesterol and a decrease in LDL-cholesterol (69). In addition, anthocyanin supplementation has been demonstrated to induce cholesterol efflux from macrophages and foam cells in mice, which may act as a potential mechanism in humans to further aid in reverse cholesterol transport (71). Anthocyanins may also function to improve serum lipid homeostasis by stimulating the upregulation of the LDL receptor (LDLR) (57). LDLR regulate cholesterol homeostasis by eliminating LDL-cholesterol from circulation. A higher level of functional LDLR will generally correlate with a greater clearance of LDL-cholesterol from the blood. It has been demonstrated
that anthocyanin supplementation induces the expression of LDLR genes in a
dose dependent manner, leading to an increase in the number of LDLR on cell
surface membranes (57).

**Anthocyanins in Tart Cherries**

Anthocyanins are particularly high in cherries and highly concentrated in
the skin of the fruit, accumulating during the ripening process (55, 72). Cherries
consist of more than 100 different species, of which the “sweet” and “sour” (tart)
cherry species are perhaps the most recognized (72). Sweet and tart cherries are a
good source of many vitamins, minerals, and phytochemicals, but tart cherries
contain considerably more total phenolics, which has been partially attributed to
the higher content of anthocyanins in tart cherries (72). One of the most
frequently encountered anthocyanin in cherries is cyanidin-3-glucoside (see
Figure 4), and the quantity of anthocyanins in cherries produced by various
cultivars has been measured at 30-79 mg of cyanidin-3-glucoside equivalents
(CGE)/100g in sweet cherries and 45-109 mg CGE/100g in tart cherries (72). The
scientific designation for tart cherries is *Prunus cerasus* L., and although they are
vitamin and nutrient rich, they are not calorically dense (Table 2).
TABLE 2

*Tart cherry nutrient composition*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>78 g of tart cherries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>36</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>0.34</td>
</tr>
<tr>
<td>Protein, g</td>
<td>0.71</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>8.54</td>
</tr>
<tr>
<td>- Dietary fiber, g</td>
<td>1.2</td>
</tr>
<tr>
<td>- Sugar, g</td>
<td>6.99</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>0.21</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>10</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>1</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>7</td>
</tr>
<tr>
<td>Pottassium, mg</td>
<td>96</td>
</tr>
<tr>
<td>Vitamin A, IU</td>
<td>674</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>0.7</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Adapted from USDA Commodity Food Fact Sheet for Schools & Child Nutrition Institutions (73).

Because anthocyanins are relatively unstable pigments, their concentration in tart cherries can be altered by many physical and environmental factors (72, 74). Increasing temperature and pH during storage and processing greatly influence the rate and degree of anthocyanin degradation (74). Various anthocyanin degradation products exhibit varying degrees of activity within the body (74). Upon consumption, the anthocyanins from tart cherries are absorbed in the intestine (aglycones such as cyanidin can be absorbed directly whereas glycosylated forms must be hydrolyzed prior to absorption), and then conjugated in the intestine and liver by methylation, sulfation, and glucuronidation. The conjugated anthocyanin derivatives are then attached to albumin and transported.
through the blood to peripheral tissues where they are needed before being eliminated in the bile and/or urine (72).

Kay et al. conducted a study using a sample population of 3 healthy men to determine the profile and pharmacokinetic properties of metabolites produced following the consumption of cyanidin-3-glycoside (a major anthocyanidin in tart cherries) and found that glucuronidation was the primary metabolic pathway for anthocyanin metabolism, followed by methylation. Glucuronidated and methylated metabolites were detected at levels twice that of the intact (parent) compound, and the absorption of these compounds over a 24-hr period was found to be extremely low at 1072 mcg, or 0.15% of the initial dose. The appearance of the parent compound and metabolites in the serum reached a peak at 2.8 hrs and the maximum excretion in the urine was observed at 3.7 hrs, indicating a very rapid absorption and elimination. Although anthocyanin absorption was low, the sample size in the study was very small (n = 3) and many anthocyanin metabolites have yet to be identified (64), prohibiting definitive conclusions.

Additional research on anthocyanin metabolites as they exist in circulation is necessary to determine their capacity to perform biological activities. Amidst the mounting evidence demonstrating the many health benefits potentially conferred by anthocyanins, the high anthocyanin content of tart cherries makes them a very attractive functional food for lowering disease risk. Many studies have been conducted using in vitro, animal, and human models to determine the
effects of tart cherry supplementation on oxidation, inflammation, and
dyslipidemia.

**Tart Cherry Supplementation, Oxidation, and Inflammation In Vitro**

Increased levels of oxidative stress are intimately related to inflammation and the pathology of chronic disease. Much research has been conducted *in vitro* to determine the potential of compounds derived from tart cherries to function as antioxidant and anti-inflammatory agents. Wang et al. (9) conducted an *in vitro* study to compare the anti-inflammatory and antioxidant activities of the 3 major anthocyanins from tart cherries (cyanidin 3-glucosylrutinoside, cyanidin 3-rutinoside, and cyanidin 3-glucoside) and their aglycon (cyanidin) to the activity of commercial products. The anthocyanin compounds were assayed for antioxidant activity based on the reduction of fluorescence observed when free radicals produced by a pro-oxidant (Fe$^{2+}$) degrade a phospholipid bound to a fluorescent probe. Cox-1 and -2 anti-inflammatory activities of the various commercial NSAIDs and the anthocyanin compounds were determined through the use of prostaglandin endoperoxide H synthase-1 and -2 isozymes (PGHS-1 and -2) and their capacity to convert arachidonic acid to prostaglandins. At 2 mM concentrations, the activities of all three anthocyanins and cyanidin were comparable to the commercial antioxidants BHA and BHT, and were far superior to α-tocopherol (vitamin E). Although the 3 major anthocyanins showed little anti-inflammatory activity, cyanidin was observed to have COX-1 and -2 anti-
inflammatory activity superior to aspirin, but less than the NSAIDs ibuprofen and naproxen (9).

Seeram et al. (74) conducted a similar study by characterizing and determining the anti-inflammatory and antioxidant activities of the degradation products. The 3 major anthocyanins and cyanidin were added to McCoy’s 5A medium supplemented with 10% fetal bovine serum to isolate and characterize the degradation products. Protocatechuic acid, 2,4-dihydroxybenzoic acid, and 2,4,6-trihydroxybenzoic acid were determined to be the primary degradation products. Fluorescent spectroscopy was again used to compare the antioxidant capacity of the anthocyanin compounds to commercial products, and COX-1 and -2 assays were conducted by adding 10 µL of arachidonic acid to an enzyme preparation using ram seminal vesicles and a preparation from insect cell lysate to determine anti-inflammatory activities. At a concentration of 100 µM, the degradation products were not observed to have Cox-1 and -2 inhibition activities compared to Naproxen, Celebrex, Ibuprofen, or Vioxx. However, the antioxidant activity of 50 uM of protocatechuic acid was comparable to BHT, BHA, and the commercial antioxidant tert-butylhydroquinone (TBHQ).

Mulabagal et al. (16) analyzed the antioxidant and anti-inflammatory capacity of whole extracts of cherries, examining the ability of several varieties of sweet and tart cherries to inhibit lipid peroxidation (LPO) and the COX-1 and -2 enzymes. LPO inhibitory activities were determined by fluorescence spectroscopy as previously described, where oxidation was initiated by the addition of the pro-
oxidant Fe$^{2+}$ to the LPO assay. To evaluate the biological activity of both water and lipid-soluble cherry compounds, methanol and ethyl acetate cherry extracts were assayed at a concentration of 250 µg/mL. The percent peroxidation inhibition was evaluated using water and DMSO as controls, and BHA, BHT, and TBHQ as positive controls. The percentage of COX-1 and -2 enzyme inhibition was also determined using water and DMSO as controls, and 60µM aspirin, 32 nM Vioxx, and 26 nM Celebrex were tested as positive controls. Tart cherry extracts of the Balaton and Montmorency varieties demonstrated antioxidant capacity, inhibiting LPO by 38 to 59% respectively. Extracts of the Balaton and Montmorency tart cherries also showed anti-inflammatory activity, with water extracts having a greater capacity than the methanol or ethyl acetate extracts to inhibit the COX-1 and -2 enzymes. The percent inhibition of COX-1 and -2 enzymes by water extracts of Balaton and Montmorency cherries was measured at 91 and 87%, and 84 and 76%, respectively.

**Tart Cherry Supplementation, Oxidation, and Inflammation in Rats**

Although results obtained from many *in vitro* studies suggest that compounds derived from tart cherries possess antioxidant activity and may have the capacity to function as anti-inflammatory agents, *in vivo* research is necessary to evaluate the biological activities of tart cherry derivatives following absorption and metabolism. Studies using rodent models have been conducted with this aim. Saric et al. (10) investigated the antioxidant activity and anti-inflammatory potential of tart cherry juice in male mice. The mice were allocated into a control
group, which received a diet of commercial food pellets, and two experimental
groups, which each received commercial food pellets supplemented with 10% or
50% Brix concentrated tart cherry juice. Each group consisted of 10 mice, which
were fed their respective diets for a two-week period prior to testing. To
determine the antioxidant efficacy of tart cherry juice, total SOD, Gpx, and
catalase (CAT) activity were examined in the brain, liver, and blood (erythrocyte
lysates), and LPO activity was measured in the liver and brain. To evaluate the
effect of tart cherry juice on COX-2 activity, 12 days before testing all mice were
injected with incomplete Freund’s adjuvant to elicit the inflammatory
autoimmune disease adjuvant-induced arthritis (AIA), and then COX-2 inhibitory
capacity was assessed in the peritoneal macrophages of the mice. The results of
the study showed that tart cherry juice increased antioxidant activity in the liver
and blood, although the brain was unaffected. Both the 10% and 50%
concentrations of tart cherry juice significantly increased SOD activity in the
blood, and the 50% concentration significantly increased SOD activity in the liver
versus control. Gpx activity was also significantly increased in the liver at both
the 10% and 50% cherry juice concentrations. No significant increase in CAT
activity was observed in any of the assessed tissues. LPO was found to
significantly decrease in the liver at the 10% concentration, although no effect
was demonstrated in the brain at either the 10% or 50% concentration. Tart cherry
juice was observed to oppose inflammation through inhibition of the COX-2
enzyme, as the 10% concentration decreased COX-2 activity by 33% and the 50% concentration decreased COX-2 activity by 41% versus the control.

He et al. (66) conducted a similar study in male Sprague Dawley rats to analyze the antioxidant and anti-inflammatory effects of anthocyanins extracted from tart cherries. As in the Saric et al. (10) study, SOD activity was assessed to partially determine antioxidant potential and injections of complete Freund’s adjuvant were used to induce AIA. The rats (12 per group) were randomly divided into a normal group, an AIA group, and an AIA group each receiving a low (10 mg/kg), medium (20 mg/kg), and high (40 mg/kg) oral dosage of tart cherry anthocyanins. Anti-oxidative impact was determined by measuring total anti-oxidative capacity (T-AOC), SOD, and malondialdehyde (MDA) in the blood, and anti-inflammatory effects were evaluated by measuring serum TNFα and prostaglandin E2 (PGE2) in the right hind paw (site of Freund’s adjuvant injection). The anthocyanins from the tart cherry juice had antioxidative effects, as T-AOC and SOD significantly increased in all dosage groups and MDA was significantly reduced. Anti-inflammatory effects were also observed as TNF-α and PGE2 levels declined with increasing doses of anthocyanins, reaching significance at 40 mg/kg.

In a 2009 study conducted by Seymour et al. (13), inflammation was assessed in male Zucker fatty rats following tart cherry supplementation. Rats were allocated into experimental and control groups, each containing 12 rats. The experimental group was fed a higher fat diet supplemented with freeze-dried tart
cherry powder, and the control group received a diet equivalent in calories and macronutrient content, but without the cherry powder. Following a 90 day period, tart cherry supplementation was associated with a significant reduction in the inflammatory cytokines TNF-α and IL-6 in both the plasma and the retroperitoneal fat. In the plasma, TNF-α and IL-6 were measured at 26 ± 4 and 191 ± 22 pg/mL, respectively, in the treatment group and 43 ± 5 and 289 ± 31 pg/mL, respectively, in the control group, corresponding to decreases of 40% and 44%. TNF-α and IL-6 expression in the retroperitoneal fat was reduced by 3.2 and 3.6-fold in the treatment group. A reduction in TNF-α and IL-6 gene transcription was also observed in rats consuming tart cherry powder, with mRNA levels declining by 2.9-fold and 4.5-fold.

**Cherry Supplementation, Oxidation, and Inflammation in Humans**

Although limited in number, in vivo studies using cherries have also suggested effectiveness in increasing antioxidant status and reducing inflammation in humans. Traustadottir et al. (11) investigated the effect of tart cherry juice supplementation on oxidative stress in a placebo-controlled crossover study using a population of 12 healthy older adults, where oxidative damage was measured as changes in the amount of plasma F₂-isoprostane following forearm ischemia-reperfusion (I/R). The participants were normal and overweight men and women (BMI of 19-29 kg/m²) aged 61-75. At baseline all subjects underwent I/R and were randomly assigned to either placebo or tart cherry juice. In the first arm of the study, the treatment group consumed 8 ounces of tart cherry juice twice
daily for 14 days and underwent a second I/R, followed by a 4-week washout period. In the second arm of the study, the I/R trial was repeated a third time, and then subjects consumed 8 ounces of a placebo beverage twice daily for 14 days and completed a fourth I/R. Subjects initially assigned to the control group followed the same protocol but consumed the cherry and placebo beverages in the reverse order. Urine was collected during the last 5 days of each study arm to measure markers of oxidative damage, including isoprostanes, DNA, and RNA. Blood was drawn subsequent to each I/R trial and was also drawn 15, 30, 60, 120, 180, and 240 minutes after each trial. Tart cherry juice supplementation decreased oxidative damage following I/R, as $F_2$-isoprostane levels in the blood were significantly reduced compared to placebo. In addition, urinary markers of oxidatively damaged DNA (measured as 8-OHdG) and RNA (measured as 8-oxo-G) were also significantly reduced. However, markers of oxidized proteins and lipids were unaffected by the intervention.

Kelley et al. (14) conducted a study to examine the effects of cherry consumption on markers of inflammation in humans, but used Bing sweet cherries instead of tart cherries. The study participants included 18 healthy men and women aged 45-61 with a BMI between 20.0 and 30.0 kg/m$^2$. The study was divided into three distinct segments comprised of an 8-day baseline period free of dietary intervention, a 28-day intervention period where 300 g of cherries were consumed by each subject daily, and a 28-day post-intervention period free of dietary intervention. Fasting blood draws occurred on day 0 and 7 of baseline, day
21 and 35 of the intervention period (14 and 28 days after cherry consumption began), and day 64 of the post-intervention period (28 days following the termination of cherry consumption). Plasma NO, CRP, IL-6, TNF-α, and regulated upon activation, normal T-cell expressed, and secreted (RANTES) were each among the 45 different inflammatory markers analyzed. Although the majority of the inflammatory markers evaluated were not affected by the intervention, circulating concentrations of NO, CRP, and RANTES significantly decreased by 18%, 25%, and 21%, respectively, by day 28 of the study. Following the discontinuation of cherry consumption, concentrations of CRP and NO partially returned to their baseline levels, suggesting the reductions were caused by cherry supplementation.

In a small pilot study, Martin et al. (75) evaluated the effect of a cherry intervention on many of the same biomarkers of inflammation as those analyzed by Kelley et al (14). Participants included 10 overweight and obese healthy men and women aged 24 to 60, each having a BMI between 27.3 and 41.6 kg/m². The study was a 10-wk randomized, crossover, placebo-controlled intervention where the subjects were initially assigned to either a treatment group or a control (placebo) group. Participants consumed either 8 ounces of 100% tart cherry juice or a placebo beverage equivalent in macronutrient and caloric content daily for a 4-week period, underwent a 2-week washout period, and then switched to the alternate beverage. Fasting blood draws were performed at baseline (week 0) and at the end of each study arm (following weeks 4, 6, and 10) to measure ESR,
hsCRP, IL-6, IL-10, TNF-α, and MCP-1. Erythrocyte sedimentation rate (ESR), a measurement of chronic inflammation, was significantly reduced following cherry juice consumption compared to placebo. At study conclusion, TNF-α and MCP-1 were determined to be significantly lower in those subjects starting the study drinking tart cherry juice than those initially assigned to consume placebo.

**Figure 5.** Many in vitro and in vivo studies have demonstrated the potential of tart cherries to exert anti-inflammatory and antioxidant effects protecting against the development of chronic disease. Adapted from Ferretti, G. et al., 2010 (72).

**Tart Cherry Supplementation and Dyslipidemia in Rats**

In addition to the studies determining the antioxidant and anti-inflammatory capacity of tart cherries, research has also been conducted to evaluate effects on dyslipidemia. Studies using rat models have suggested that tart
cherries may have a favorable impact on blood lipid profiles. In the previously described 2009 study by Seymour et al. (13), Zucker fatty rats were not only monitored for fluctuations in inflammatory markers following tart cherry powder supplementation, but also for alterations in serum lipid levels. Subsequent to the 90-day intervention period, whole blood was obtained for analysis and the total cholesterol and triglycerides of rats consuming the tart cherry diet were found to be significantly lower than that of the control group (185 ± 15 and 241 ± 18 mg/dL compared to 207 ± 14 and 284 ± 23 mg/dL, respectively).

In an earlier study, Seymour et al. (12) investigated the effect of a tart cherry-enriched diet on serum lipid values in male Dahl-Salt Sensitive (Dahl-SS) rats and derived results similar to the 2009 study. Unlike Zucker fatty rats, Dahl-SS rats are prone to developing hyperlipidemia without being obese. The rats were randomly divided into two groups of 12, with the treatment group receiving a diet supplemented with 1% freeze-dried whole tart cherry powder by weight, and the control group receiving a calorie and carbohydrate equivalent diet free of cherry powder. Following a 90-day intervention period, whole blood was collected for lipid analysis and total serum cholesterol and triglyceride concentrations were significantly lower in rats receiving the tart cherry powder compared to rats in the control group (75 ± 4 and 28 ± 3 mg/dL versus 87 ± 5 and 34 ± 3 mg/dL, respectively).
Cherry Supplementation and Dyslipidemia in Humans

Research evaluating the effect of tart cherries on serum lipid levels in humans is extremely limited and the characteristics of the selected subject samples are highly variable between studies, making it difficult to directly compare study outcomes. Many of the studies that have been conducted have also demonstrated contradictory results, contributing to the ambiguity associated with the effect of tart cherries on dyslipidemia in humans. Ataie-Jafari et al. (70) conducted a pilot study in a diabetes clinic in Iran to determine the effect of tart cherry juice on the blood lipid profiles of 19 women diagnosed with type-2 diabetes. The women were otherwise healthy, reporting no additional chronic diseases, and did not use insulin. For a period of six weeks, subjects consumed 40 g or 3 Tbsp of concentrated tart cherry juice daily. Fasting blood draws were performed to determine concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides at baseline (week 0) and at the completion of the study (week 6). Although no significant changes were observed in HDL or triglyceride levels following tart cherry juice supplementation, a significant reduction did occur in total cholesterol and LDL cholesterol, with levels decreasing from 213.9 to 193.2 mg/dL and 118.4 to 103.6 mg/dL, respectively.

The effect of tart cherry juice on serum lipid concentrations was also examined in the previously described pilot study by Martin et al (76). In this study, inflammatory markers and blood lipid levels were measured in 10 overweight and obese participants consuming tart cherry juice and placebo for 4
weeks each, separated by a 2-week washout period. Fasting blood samples were acquired at baseline and following each arm of the study period (a total of 4 blood draws were performed) to determine serum levels of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. In contrast to the results reported by Ataie-Jafari et al., no significant reduction was observed in total cholesterol and LDL cholesterol concentrations in participants consuming tart cherry juice, but a significant reduction in triglyceride levels was noted, which declined 14% from 147 ± 55 to 127 ± 45 mg/dL. No change in HDL cholesterol concentration was observed.

In the previously cited study by Kelley et al. (14), the consumption of 300 g of Bing sweet cherries daily over a period of 28 days was associated with a reduction of certain biomarkers of inflammation in 18 healthy normal weight, overweight, and obese men and women. However, this study also examined the effects of Bing sweet cherries on serum lipid concentrations and reported outcomes different than those observed by Ataie-Jafari et al. (70) and Martin et al (76). Kelley et al. (14) did not report the actual serum lipid concentrations in their published study, but stated that lipid levels did not significantly decrease in response to cherry supplementation. Although each of the three studies reported incongruent conclusions regarding the effect of cherries on serum lipid concentrations in humans, the varying characteristics of the subject samples and sample sizes used makes direct comparison difficult. Ataie-Jafari et al. (70) used a sample of 19 diabetic women, Kelley et al. (14) recruited 18 healthy normal
weight and overweight participants, and Martin et al. (76) included 10 healthy overweight and obese individuals. Because Kelley et al. (14) used a study sample of healthy, non-obese subjects and did not report the baseline lipid values, it is possible that no changes were observed in serum lipids because participants’ values were normal to begin with and were therefore less susceptible to improvement through intervention.

Cherry Study Outcomes and the Need for Further Research

Several pharmaceutical drugs are fairly effective in combating inflammation and reducing blood lipid levels, but these are frequently associated with many devastating and undesirable side effects, creating a demand for safe and natural anti-inflammatory and lipid-lowering agents. In recent years, tart cherries have been cited as a functional food that may have the capacity to reduce the oxidative damage, inflammation, and dyslipidemia associated with the pathogenesis of chronic disease. Several studies have documented the antioxidant properties of tart cherries both in vitro and in vivo, but there are much fewer studies exploring the effects of tart cherries on inflammation and dyslipidemia. Much of the research that has been conducted in these areas has used rodents, which possess different metabolic characteristics than humans. Therefore, results obtained from such studies may not be particularly applicable to humans. Of the few studies that have analyzed the effects of tart cherry juice on inflammation and serum lipid values in humans, the results have been contradictory and the characteristics and size of the subject sample has varied. There is a need for
further research in this area, especially among non-diabetic, overweight and obese individuals that may have a higher probability of suffering from inflammation and dyslipidemia. Therefore, it would be advantageous for further research to explore the responsiveness of large sample size of such participants to a tart cherry juice intervention.
Chapter 3

Methodology

Research Participants

This research study enlisted 36 overweight and obese men and women from the greater Phoenix area who were at least 18 years of age. Volunteers were recruited through email announcements, flyers mailed to departments at the ASU Tempe campus, and flyers placed throughout Arizona State University (ASU) campuses and in local businesses. The participants were classified as overweight or obese based on a BMI $\geq 25.0$ kg/m$^2$. For participation in the study, a minimal body fat of 20% for men and 25% for women were required. In addition, individuals were non-smokers, were not lactating or pregnant at any time during the study, were not taking lipid-lowering or thyroid medications, were not taking non-steroidal anti-inflammatory drugs (NSAIDS) regularly (as defined as 3 or more 600 mg doses per week or more than one 80 mg baby aspirin daily), and were generally healthy and free of chronic diseases such as type 1 or 2 diabetes, inflammatory bowel disease, arthritis, and liver disease. In addition, to be eligible for study participation it was required that the volunteers be weight-stable for 6 months prior to the start of the study and throughout the study duration.

Potential participants were initially asked a series of questions via telephone to see if they met basic inclusion requirements (Appendix A). Candidates who qualified for the study were scheduled for their first appointment and, upon arrival, were read a disclosure statement (Appendix B). Participants
provided informed consent to the protocols of the research that were approved by the Institutional Review Board (IRB) at ASU (Appendix C). Upon signing the consent form, each participant was asked to complete a brief medical history questionnaire, which consisted of questions regarding the use of medications and dietary supplements, as well as questions regarding the diagnosis of certain medical conditions (Appendix D). This questionnaire was used to confirm that potential candidates met the inclusion criteria.

**Study Design**

This study was a 4-week randomized, crossover, single-blinded, placebo-controlled intervention. A diagram of the study can be found in Appendix E. The participants were randomly assigned into a placebo (control) or treatment group on day one of the study. Those assigned to the treatment group consumed 8 ounces of 100% tart cherry juice daily for 4 weeks, followed by a 4-week washout period, and then consumed 8 ounces of a non-anthocyanin containing placebo beverage similar in calorie and carbohydrate content for 4 weeks. Subjects initially assigned to the placebo (control) group underwent the same protocol but consumed the cherry and placebo beverages in the reverse order. All participants were required to abstain from the consumption of foods that are considered rich sources of anthocyanins over the 12-week study period. A list of these foods was provided to participants (Appendix F).

A fasting blood draw occurred on Day 1 of the study (baseline) and then every 4 weeks thereafter (at the completion of weeks 4, 8, and 12). Subjects were
given instructions on completing a dietary record that reflected food intake over a 24-hour period and were to submit one 24-hour dietary record (Appendix G) for each week of the study (4 records at each visit). All participants filled out the shortened version of the International Physical Activity Questionnaire (Appendix H) at the completion of each 4-week study arm (reflecting the previous 7 days of activity prior to each visit). It was assumed that the dietary intake and physical activity level reported at each visit reflected the average during the previous study arm. Anthropometric and body composition data were collected at each visit (blood draw) in addition to blood pressure and pulse measurement.

**Dietary Intervention**

The caloric and carbohydrate content of the tart cherry and placebo beverage were very similar, and this was expected to prevent weight differences caused by the different dietary interventions (Appendix I). The placebo beverage served as the control because it is anthocyanin-free. Information regarding placebo preparation and tart cherry juice preparation can be found in Appendix J. Tart cherry juice or placebo were provided at the visit preceding each 4-week study arm period and participants were provided measuring cups and instructions on how to measure and store juices. As a measure of compliance, the subjects were expected to return all empty and remaining bottles of juice at the completion of each study arm.

Participants were required to fill out one 24-hr dietary record for each week of the study and, on the week of the blood draw, the dietary record reflected
consumption on the day prior to the draw. This permitted the assessment of dietary changes that may have occurred throughout the study and also enabled the tracking of any unexpected blood measurements that were not diet related. The dietary records were analyzed by Food Processor Nutrition and Fitness Software (ESHA, version 8.5; Salem OR) to determine whether subjects were altering their diets and to determine compliance with instructions to drink 8 oz of placebo or tart cherry juice and to avoid anthocyanin-rich foods.

**Anthropometric Measurements**

Anthropometric measurements were obtained at the baseline visit and at each visit (blood draw) following the completion of weeks 4, 8, and 12. Height was recorded at baseline, and weight, waist circumference (WC), blood pressure, and pulse were recorded at baseline and every 4 weeks thereafter. In addition, body composition (body fat percentage, fat mass, fat-free mass, and total body water) and basal metabolic rate was measured by bioelectrical impedance (BIA) using a TBF 300A Tanita Body Composition Analyzer (Tokyo, Japan), and BMI was recalculated at each visit (Appendix K).

**Blood Analyses**

Blood samples were drawn from the antecubital vein by a trained phlebotomist at the ASU Polytechnic Campus Health Sciences Center or the ASU Tempe Campus Clinical Research Unit at baseline of the study and at the completion of weeks 4, 8 and 12. Prior to each blood draw, the participants had fasted for at least 8 hours. EDTA-treated whole blood was immediately used to
determine ESR by the Westergren method (77) (Appendix L) using the FisherBrand Disposable Dispette 2 Blood Sedimentation System (Thermo Fisher Scientific, Pittsburgh, PA). Plasma from the blood samples were then separated by centrifugation at 4°C and 3,300 rpm for 15 minutes and divided in aliquots (derived from serum separator, EDTA, and heparin tubes). The aliquots were frozen until a later date when blood lipids, fasting glucose, insulin, hsCRP, IL-6, IL-10, TNF-α, and MCP-1 were analyzed. Total cholesterol, triglycerides, LDL-C, HDL-C, fasting glucose, and hsCRP were measured directly using the COBAS C 111 blood chemistry analyzer (Roche Diagnostics, Indianapolis, IN) and VLDL-C was calculated as TG/5. Insulin was analyzed using an Immulite 1000 automated chemiluminescent blood chemistry analyzer (Diagnostic Products Corporation, Siemens, Deerfield, IL) according to manufacturer’s instructions (78, 79). IL-6, IL-10, TNF-α, and MCP-1 were analyzed by a standard sandwich enzyme-linked immunosorbent assay (ELISA) using Single Analyte ELISArray kits (SABiosciences Corporation, Bethesda, MD) (Appendix M).

**Statistical Analyses**

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 18.0, 2010. All values are expressed as mean ± standard deviation (SD). According to sample size calculations based on a study conducted by Volek, et al. (80), 15 participants were determined to provide an 80% power to detect a 23 mg/dL (30%) reduction in triglycerides at an alpha level of 0.05. The sample size for this study was 26. Differences were considered significant at
P<0.05. A histogram and Shapiro-Wilk’s test were used to test the variables for normality, and the following data were logarithmically transformed to achieve a normal distribution: CRP, IL-6, IL-10, Insulin, and HOMA. The non-parametric data for BMI was inversely transformed to achieve a normal distribution. Anthropometric, inflammatory, and plasma lipid values were subsequently analyzed using two-way analysis of variance (ANOVA) with repeated measures. Each ANOVA test assessed data by group (treatment) and by time (pre/post) to determine an interaction effect. Dietary and physical activity data from individual treatment groups were compared with the respective controls using paired-samples t-tests, and data determined to be non-parametric were analyzed via the Wilcoxin Signed Rank Test.
Chapter 4

Results

**Tart Cherry Juice Composition**

Tart cherry juice was used as the treatment intervention in this study because it is a significant source of anthocyanins. HPLC was used to establish the anthocyanin profile of 100% tart cherry juice, which appears in Figure 6. The predominant anthocyanin pigment in tart cherries, cyanidin-3-glucosylrutinoside, is represented by the peak at 12.026 minutes. Other prominent peaks include those at 13.516 minutes, 13.985 minutes, and 16.946 minutes, which correspond to cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-rutinoside, three additional anthocyanins present in tart cherry juice. The peak at 19.595 minutes could not be identified.

![HPLC anthocyanin profile from 100% tart cherry juice.](image)

**Figure 6.** HPLC anthocyanin profile from 100% tart cherry juice.
The total polyphenol concentration of the tart cherry juice and placebo beverages were each determined by the Folin-Ciocalteu method, and tart cherry juice was demonstrated to contain a significantly higher concentration of phenolics ($p < 0.05$) than the placebo (Figure 7).

![Figure 7](image)

**Figure 7.** Total phenolics of the placebo and treatment beverages as determined by the Folin-Ciocalteu method. *Statistical significance $p < 0.05$.

**Participant Characteristics**

Although 36 individuals initially enrolled in this study, 26 participants completed it in its entirety, including 8 males and 18 females. Of the ten participants that dropped out, 2 were lost to follow-up and one participant dropped out for each of the following reasons: time constraints, feeling mistreated, disliking the taste of the juice, weight gain that was attributed to the juice, hospitalization for unrelated issues, a broken foot, an adverse reaction subsequent to placebo consumption, and heartburn following juice consumption.
Table 3 portrays the participant characteristics at study baseline along with the corresponding reference ranges. Based upon BMI values, 14 participants were categorized as overweight and 12 as obese. The mean age was 41 years, ranging from 22 to 61, and the mean BMI was $31.3 \pm 6.3 \text{ kg/m}^2$, ranging from $25.0$ to $51.3 \text{ kg/m}^2$.

**Anthropometrics**

Anthropometric measurements were recorded at baseline and following weeks 4, 8, and 12. The effects of time and treatment on BMI, body fat percentage, and WC were evaluated by two-way repeated measures ANOVAs (Table 4). There were no significant interaction effects ($p > 0.05$) for BMI, body fat percentage, or WC. There was a statistically significant main effect of treatment for WC ($p < 0.05$), as the placebo measurements were slightly higher than the tart cherry measurements, and there was also a statistically significant effect of time on BMI ($p < 0.05$), as the post-treatment values were higher than the baseline values following both the placebo and cherry interventions. However, there was no significant change in BMI, body fat percentage, or WC when comparing the two treatment groups over time.
## TABLE 3

Baseline characteristics of participants\(^1\)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Reference Range</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n(m/f))</td>
<td>26 (8/18)</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>41 ± 11</td>
<td></td>
</tr>
<tr>
<td>BMI, (kg/m^2)</td>
<td>31.3 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Body fat, (kg/m^2)</td>
<td>37.11 ± 9.99</td>
<td></td>
</tr>
<tr>
<td>Biomarker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, (mg/dL)</td>
<td>&lt; 200</td>
<td>191.6 ± 40.4</td>
</tr>
<tr>
<td>LDL cholesterol, (mg/dL)</td>
<td>&lt; 100</td>
<td>107.7 ± 29.8</td>
</tr>
<tr>
<td>VLDL cholesterol, (mg/dL)</td>
<td>&lt; 30</td>
<td>28.76 ± 27.3</td>
</tr>
<tr>
<td>HDL cholesterol, (mg/dL)</td>
<td>≥ 40(^2)</td>
<td>42.1 ± 12.0</td>
</tr>
<tr>
<td>Triglycerides, (mg/dL)</td>
<td>&lt; 150</td>
<td>143.8 ± 136.5</td>
</tr>
<tr>
<td>TG/HDL CVD risk ratio</td>
<td>&lt; 3</td>
<td>4.25 ± 6.23</td>
</tr>
<tr>
<td>TC/HDL CVD risk ratio</td>
<td>&lt; 4</td>
<td>4.97 ± 2.21</td>
</tr>
<tr>
<td>LDL/HDL CVD risk ratio</td>
<td>&lt; 3</td>
<td>2.76 ± 1.10</td>
</tr>
<tr>
<td>Fasting blood glucose, (mg/dL)</td>
<td>&lt; 100</td>
<td>92.7 ± 7.03</td>
</tr>
<tr>
<td>Insulin, (\mu U/mL)</td>
<td>5.0 - 20.0</td>
<td>12.95 ± 6.98</td>
</tr>
<tr>
<td>QUICKI(^3)</td>
<td>≥ 0.312</td>
<td>0.336 ± 0.035</td>
</tr>
<tr>
<td>HOMA(^3)</td>
<td>&lt; 4</td>
<td>2.986 ± 1.651</td>
</tr>
<tr>
<td>CRP, (mg/L)</td>
<td>&lt; 3</td>
<td>3.75 ± 3.99</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>0 – 30(^6)</td>
<td>18.25 ± 10.75</td>
</tr>
<tr>
<td>IL-6, (pg/mL)</td>
<td>0 - 5.0</td>
<td>93.98 ± 111.64</td>
</tr>
<tr>
<td>IL-10, (pg/mL)</td>
<td>1.3 - 15.6</td>
<td>38.04 ± 25.16</td>
</tr>
<tr>
<td>MCP-1, (pg/mL)</td>
<td>163 - 438</td>
<td>242.92 ± 161.09</td>
</tr>
<tr>
<td>TNF-(\alpha), (pg/mL)</td>
<td>0 - 16.6</td>
<td>N/A(^7)</td>
</tr>
</tbody>
</table>

1. Values are mean ± SD
2. Men: ≥ 40 mg/dL
   Women: ≥ 50 mg/dL
3. Values are based on \(n = 25\), \(n = 14\), and \(n = 4\) due to data being below the assay detection limits
4. Men: ≤ 50 yr: 0 - 15 mm/h, > 50 yr: 0 - 20 mm/h
   Women: ≤ 50 yr: 0 - 20 mm/h, > 50 yr: 0 – 30 mm/h
5. Below limit of detection for assay
TABLE 4

Effect of cherry and placebo interventions on anthropometrics$^1$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, $kg/m^2$</td>
<td>31.4 ± 6.4</td>
<td>31.6 ± 6.5$^a$</td>
<td>31.3 ± 6.4</td>
<td>31.4 ± 6.6$^a$</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>38.2 ± 8.2</td>
<td>38.5 ± 8.3</td>
<td>38.7 ± 8.4</td>
<td>38.6 ± 8.1</td>
</tr>
<tr>
<td>WC, cm</td>
<td>99.3 ± 14</td>
<td>99.1 ± 14</td>
<td>98.6 ± 14$^b$</td>
<td>98.6 ± 14$^b$</td>
</tr>
</tbody>
</table>

$^1$ Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD, n = 26. No significant difference for the interaction (time and treatment) or main effect of treatment. $^a$Main effect of time was significant at $P < 0.05$. $^b$Main effect of treatment was significant at $P < 0.05$.

$^2$ Data were inversely transformed (1/variable) to achieve normality; values reported untransformed, mean ± SD.

**Dietary Intake**

One 24-hour food record was submitted at baseline and for each week of the study period (13 total). The average daily amount of each nutrient consumed during the placebo and cherry intervention is shown in Table 5. Though their diets were uncontrolled, subjects were asked to maintain their usual intakes throughout the study to maintain their original body weight, as fluctuations could confound results. Paired-samples t-tests were conducted to compare nutrient consumption between the treatment group (cherry) vs. the control group (placebo).

Although no significant difference ($p > 0.05$) in the consumption of energy, carbohydrate, sugar, total fat, polyunsaturated fat, saturated fat, soluble fiber, or omega-3 fatty acids occurred between the placebo and treatment interventions, a trend toward significance ($0.05 < p \leq 0.10$) was observed for
protein and monounsaturated fat intake, which were slightly higher during the tart cherry juice intervention compared to the placebo intervention (90 ± 40 and 17.9 ± 7.4 g/d vs. 83 ± 35 and 15.5 ± 7.4 g/d, respectively). In addition, the consumption of trans fat was significantly higher (p < 0.05) during the tart cherry intervention compared to the placebo intervention (0.47 ± 0.61 g/d vs. 0.22 ± 0.38 g/d) (Figure 8).

**TABLE 5**

<table>
<thead>
<tr>
<th>Nutrient composition during cherry and placebo interventions (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Energy, kcal/d</td>
</tr>
<tr>
<td>Protein (^2), g/d</td>
</tr>
<tr>
<td>Carbohydrate, g/d</td>
</tr>
<tr>
<td>Sugar, g/d</td>
</tr>
<tr>
<td>Total fat (^2), g/d</td>
</tr>
<tr>
<td>Monounsaturated fat, g/d</td>
</tr>
<tr>
<td>Polyunsaturated fat (^2), g/d</td>
</tr>
<tr>
<td>Saturated fat (^2), g/d</td>
</tr>
<tr>
<td>Trans fat (^2), g/d</td>
</tr>
<tr>
<td>Total fiber (^2), g/d</td>
</tr>
<tr>
<td>Soluble fiber, g/d</td>
</tr>
<tr>
<td>n-3 fatty acids (^2), g/d</td>
</tr>
</tbody>
</table>

\(^1\) Data analyzed using paired t-tests. Values are mean ± SD, n = 25. \(^a\)Significance was observed at P < 0.05; Trend toward significance was observed at \(^b\)P 0.1 ≤ P > 0.05.

\(^2\) Wilcoxin signed rank test was used when data were determined to be abnormal.
Figure 8. Trans fat intake was significantly higher during the cherry intervention as compared to the placebo intervention. * Statistical significance $p \leq 0.05$.

Physical Activity

The average physical activity level of each participant was accounted for via the collection of the IPAQ questionnaire at the end of each study arm, with activity being expressed as the metabolic equivalent of tasks (METs). The number of METs performed is an expression of the energy cost associated with physical activity, and the average MET-minutes/week expended by subjects during the tart cherry juice and placebo intervention is displayed in Table 6. There was no significant difference ($p > 0.05$) in MET-minutes/week between the treatment and placebo arms, demonstrating that participant physical activity levels remained relatively constant.
TABLE 6

Physical activity during cherry and placebo interventions

<table>
<thead>
<tr>
<th>Physical Activity</th>
<th>Placebo</th>
<th>Cherry</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET-minutes/week²</td>
<td>2193.16 ± 2023.58</td>
<td>2256.35 ± 2496.66</td>
</tr>
</tbody>
</table>

¹ Data analyzed using paired t-tests. Values are mean ± SD, n = 25.
² Wilcoxon signed rank test was used when data were determined to be abnormal.

Lipids and CVD Risk Ratios

Lipid samples from venous blood draws were obtained at baseline and following weeks 4, 8, and 12. The effects of time and treatment on total cholesterol, LDL-C, VLDL-C, HDL-C, and triglycerides were evaluated by two-way repeated measures ANOVAs (Table 7).

TABLE 7

Effect of cherry and placebo interventions on Plasma Lipids

<table>
<thead>
<tr>
<th>Plasma lipids, mg/dL</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>192 ± 44</td>
<td>196 ± 43ᵃ</td>
<td>187 ± 33</td>
<td>196 ± 35ᵃ</td>
</tr>
<tr>
<td>LDL-C</td>
<td>111 ± 32</td>
<td>113 ± 31ᵃ</td>
<td>112 ± 30</td>
<td>120 ± 31ᵃ</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>28 ± 28</td>
<td>32 ± 43</td>
<td>26 ± 14</td>
<td>26 ± 15</td>
</tr>
<tr>
<td>HDL-C²</td>
<td>42 ± 10</td>
<td>42 ± 10</td>
<td>42 ± 8</td>
<td>43 ± 7</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>140 ± 137</td>
<td>161 ± 216</td>
<td>129 ± 68</td>
<td>128 ± 74</td>
</tr>
</tbody>
</table>

¹ Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD, n = 26. No significant difference for the interaction (time and treatment) or main effect of treatment. ²Main effect of time was significant at P < 0.05.
² Normality was achieved following the removal of one outlier; Therefore, values are based on n = 25, mean ± SD.
No significant interaction effects (p > 0.05) or treatment effects were observed for any of these measurements, although there was a statistically significant effect of time (p < 0.05) on total cholesterol and LDL-C, as the post-intervention values were higher than the corresponding baseline values following both the placebo and cherry arms. However, there was no significant change in total cholesterol, LDL-C, VLDL-C, HDL-C, or triglycerides when comparing the two treatment groups over time. CVD risk ratios were calculated based on lipid values obtained from each blood draw (Table 8), and these values were also analyzed via two-way repeated measures ANOVAs. There were no significant interaction effects between time and treatment (p > 0.05) or for the main effects of time and treatment for the TG/HDL, TC/HDL, or LDL/HDL risk ratios, demonstrating that each ratio remained relatively stable between treatment groups over time.

**TABLE 8**

<table>
<thead>
<tr>
<th>CVD risk ratio</th>
<th>Placebo Pre</th>
<th>Placebo Post</th>
<th>Cherry Pre</th>
<th>Cherry Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG/HDL</td>
<td>4.09 ± 6.28</td>
<td>5.01 ± 10.14</td>
<td>3.29 ± 2.20</td>
<td>3.20 ± 2.45</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>4.79 ± 2.22</td>
<td>4.91 ± 2.40</td>
<td>4.53 ± 1.38</td>
<td>4.62 ± 1.39</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2.73 ± 1.07</td>
<td>2.76 ± 1.01</td>
<td>2.73 ± 1.05</td>
<td>2.85 ± 1.03</td>
</tr>
</tbody>
</table>

1 Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD, n = 26. No significant difference for the interaction (time and treatment) or main effects of time and treatment.
Glucose, Insulin, and Insulin Sensitivity

Fasting glucose samples obtained from venous blood draws occurring at baseline and following weeks 4, 8, and 12 were assessed by two-way repeated measures ANOVAs (Table 9), and no significant interaction effect (p > 0.05) or main effect of treatment was reported. The main effect of time was determined to be significant (p < 0.05) as the post-intervention measurements were higher than the corresponding baseline measurements subsequent to both the tart cherry juice and placebo arms. However, there was no significant change in fasting glucose when comparing the two treatment groups over time.

**TABLE 9**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Cherry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>93.6 ± 7.2</td>
<td>97.5 ± 10.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD, n = 26. No significant difference for the interaction (time and treatment) or main effect of treatment. <sup>a</sup>Main effect of time was significant at P < 0.05.

Fasting insulin was also measured at each of the 4 blood draws and was used along with fasting glucose measurements to calculate the quantitative insulin-sensitivity check index (QUICKI) and the homeostasis model assessment (HOMA), which are both indices of insulin sensitivity. Fasting insulin, QUICKI, and HOMA were each analyzed via two-way repeated measures ANOVA (Table 10) and no significant interaction effect or main effect of time or treatment was observed (p > 0.05). Although no significant alteration in fasting insulin or the
indices of insulin sensitivity occurred between the two treatment groups over time, the baseline values obtained from the QUICKI and HOMA equations indicate that the participants of this study are approaching insulin resistance and the associated pre-diabetic state.

**TABLE 10**

Effect of cherry and placebo interventions on insulin and markers of insulin sensitivity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>13.25 ± 9.75</td>
<td>15.64 ± 9.96</td>
<td>17.07 ± 12.61</td>
<td>19.72 ± 19.75</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.125 ± 2.44</td>
<td>3.913 ± 2.81</td>
<td>3.922 ± 2.76</td>
<td>4.952 ± 6.05</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.337 ± 0.03</td>
<td>0.328 ± 0.04</td>
<td>0.330 ± 0.032</td>
<td>0.320 ± 0.032</td>
</tr>
</tbody>
</table>

1 Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD, n = 24. No significant difference for the interaction (time and treatment) or main effects of time and treatment.

2 Data were logarithmically transformed (log variable) to achieve normality; values reported untransformed, mean ± SD.

**Inflammatory Cytokines and Biomarkers**

In addition to blood lipids, fasting glucose, and fasting insulin, cytokines and inflammatory biomarkers were also measured using samples obtained from venous blood draws occurring at baseline and following each 4-week placebo and tart cherry juice intervention. The cytokines evaluated included IL-6, IL-10, and TNF-α, and the inflammatory biomarkers assessed included CRP, ESR, and MCP-1. Of 26 subjects tested, only 14 produced samples with IL-6 levels above the detection limit of the assay and only 4 provided samples having IL-10 levels
above the assay detection limit. No samples were determined to contain TNF-α concentrations above the detection limit of the assay, and therefore this cytokine was eliminated from analysis. The small number of viable measurements obtained for IL-6 and IL-10 were evaluated using a two-way ANOVA (Table 11) to compare the effect of time and intervention on each variable, and the interaction effect was determined to be insignificant for each ($p > 0.05$). In addition, no significant difference for the main effects of time and treatment were observed ($p > 0.05$).

**TABLE 11**

<table>
<thead>
<tr>
<th>Cytokine, pg/mL</th>
<th>Placebo Pre</th>
<th>Placebo Post</th>
<th>Cherry Pre</th>
<th>Cherry Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 2, 3</td>
<td>78.6 ± 113.6</td>
<td>230.8 ± 394.7</td>
<td>89.3 ± 138.3</td>
<td>197.9 ± 264.3</td>
</tr>
<tr>
<td>IL-10 2, 4</td>
<td>34.6 ± 25.0</td>
<td>47.1 ± 39.8</td>
<td>24.9 ± 19.9</td>
<td>20.1 ± 16.5</td>
</tr>
<tr>
<td>TNF-α 5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

1 Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD. No significant difference for the interaction (time and treatment) or main effects of time and treatment.

2 Data were logarithmically transformed (log variable) to achieve normality; values reported untransformed, mean ± SD.

3 Values are based on $n = 14$ due to samples being below the assay detection limit

4 Values are based on $n = 4$ due to samples being below the assay detection limit

5 All samples below assay detection limit
The inflammatory biomarkers CRP, ESR, and MCP-1 were also assessed via two-way ANOVA, and the values preceding and subsequent to each 4-week intervention are displayed in Table 12. Analysis revealed no significant difference (p > 0.05) for the interaction (time and treatment) effect or main effects of time and treatment on CRP, ESR, or MCP-1 levels, demonstrating that statistically there was no appreciable change in the concentrations of these inflammatory biomarkers between the tart cherry juice and placebo intervention over time.

TABLE 12

<table>
<thead>
<tr>
<th>Marker</th>
<th>Placebo</th>
<th>Cherry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>CRP, ng/mL</td>
<td>4.04 ± 4.30</td>
<td>3.83 ± 3.90</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>19.6 ± 13.2</td>
<td>21.9 ± 13.7</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>250 ± 164</td>
<td>276 ± 131</td>
</tr>
</tbody>
</table>

1 Data analyzed using two-way repeated measures ANOVA. Values are mean ± SD, n = 26. No significant difference for the interaction (time and treatment) or main effects of time and treatment.

2 Data were logarithmically transformed (log variable) to achieve normality; values reported untransformed, mean ± SD.

Although the change in MCP-1 between the pre and post-cherry and pre and post-placebo interventions was not determined to be statistically significant via two-way ANOVA, MCP-1 increased by more than 26 pg/mL after the placebo intervention and decreased by more than 18 pg/mL after the tart cherry intervention.
Chapter 5

Discussion

The purpose of this study was to assess the effectiveness of 100% tart cherry juice in improving biomarkers of dyslipidemia and inflammation in overweight and obese subjects. It was hypothesized that the consumption of 8 oz of cherry juice daily over a 4-week period would result in a measurable improvement in both the lipid and inflammatory profiles of study participants without disrupting glucose homeostasis. Data analysis comparing the placebo and treatment interventions over time did not show a statistically significant improvement in any of the lipid or inflammatory biomarkers. However, there was a statistically significant increase in dietary trans fat during the treatment arm compared to the placebo arm and, although unlikely, it is possible that this alteration may have confounded study results.

A specific aim of this study was to evaluate the effect of tart cherry juice consumption on lipid parameters, including total cholesterol, LDL-C, VLDL-C, HDL-C, TG and the calculated CVD risk ratios TC/HDL, TG/HDL, and LDL/HDL. The outcome of this study, demonstrating no significant effect of cherry juice on lipid biomarkers and risk ratios, is both supported and refuted by the literature, as previous research studies conducted using cherries have found conflicting results. Two previously cited studies conducted by Seymour, et al. (12, 13) reported a significant decrease in total cholesterol and triglycerides in Dahl-SS and Zucker fatty rats following 90 days of freeze-dried tart cherry
consumption. These findings were not in agreement with the results of this study, and the different outcomes may have derived from differences in the study designs and subject populations, as 90 days of treatment may be more likely to elicit a significant alteration in blood lipids, and Zucker and Dahl-SS rats display an extremely high affinity for developing characteristics of metabolic syndrome and may therefore be more susceptible to a treatment response. Improvement observed in the lipid profile of the fatty Zucker rats may also have been a function of the significant weight loss that was observed over the intervention period. In addition, rats and human have different metabolic characteristics, making results from rodent models potentially less applicable to humans. Rats absorb less dietary cholesterol than humans, have significantly higher HDL-C levels, and also lack CETP, making them resistant to atherogenesis and a poor experimental model for use in lipid-related research (81).

Previous research studies analyzing the effect of cherry consumption on lipid parameters in humans have reported a diverse array of outcomes, which may also be partially attributed to differences in the study designs and subject populations employed between interventions. The lack of significant effect observed on the blood lipid profile in this study was in agreement with results reported by Kelley et al. (14) following 28 days of Bing sweet cherry consumption, where no changes were measured in total cholesterol, LDL-C, VLDL-C, HDL-C, TG, or the TC/HDL CVD risk ratio. The study conducted by Ataie-Jafari et al. (70) evaluating the effect of 3 TBSP of tart cherry juice...
concentrate daily over a period of 6 weeks on blood lipids in 19 type 2 diabetic Iranian women also reported no effect on blood lipid concentrations. However, a significant decrease was detected in total cholesterol and LDL-C when considering only hyperlipidemic subjects. Alternatively, in the pilot study by Martin et al. (76), no effect was observed on concentrations of total cholesterol, LDL-C, VLDL-C, or HDL-C following the daily consumption of 8 oz of tart cherry juice for 4 weeks, but a significant reduction was observed in TG.

Potential explanations for discrepancies in the outcomes of this study and the Ataie-Jafari et al. study (70) might be based on differences in subject lipid profiles. The subject population used in this study possessed baseline lipid values within the healthy reference ranges, with the exception of LDL-C, which was only slightly elevated (107.7 mg/dL) and considered near optimal according to the NCEP (23). The population that experienced a significant reduction in total cholesterol and LDL-C in the Ataie-Jafari et al. study was hyperlipidemic, having total cholesterol and LDL-C pre-treatment values of 213.9 and 118.4 mg/dL, respectively, and demonstrated more susceptibility to the cherry juice intervention than the population displaying healthy levels. Pre-treatment total cholesterol and LDL-C values in this study were 187.3 and 111.5 mg/dL, respectively, leaving less room for improvement. Another factor that may have contributed to significance in the Ataie-Jafari et al. study may be attributed to the research design, as the treatment duration was longer (6 weeks vs. 4 weeks) and the daily
dosage of concentrated juice was slightly higher (3 TBSP vs. 2.66 TBSP) than in this study.

Similar to the Ataie-Jafari et al. study (70), a possible explanation for different outcomes between this study and that of Martin et al. (76) in respect to TG may also be attributed to disparities in the blood lipid levels of each subject population, as Martin et al. reported much higher pre-treatment TG values (147 vs. 129 mg/dL). In addition, Martin et al. used 10 participants, which may have enabled TG outcomes to become skewed. No significance was observed in respect to total cholesterol, LDL-C, VLDL-C, and HDL-C, which were generally within the optimal reference ranges and were each very similar to the values of this study, thus contributing to evidence that higher serum lipids may be necessary to achieve significant changes.

Only one of these studies reported significance in regard to the TC/HDL, TG/HDL, or LDL/HDL CVD risk ratios (non-significance was reported in TC/HDL by Kelley et al. (14)), but achieving significance in these parameters is dependent upon alterations in individual TC, LDL-C, HDL-C, or TG concentrations. This study did not demonstrate statistical differences in any of the ratios, which is not surprising due to the lack of change observed in the lipid biomarkers. Had participants displayed sufficient hyperlipidemia, possessing lipid values and CVD risk ratios well above normal reference ranges, evidence from previous research suggests that there would have been more room for alteration.
and significance may have been derived from increased susceptibility to a cherry intervention.

A second aim of this study was to evaluate the effects of tart cherry juice on the inflammatory profile. Analyses of IL-6, hsCRP, MCP-1, ESR, and IL-10, showed no statistically significant improvement following the administration of treatment compared to placebo over time. Similar to the contradictory outcomes derived from studies analyzing the effect of a cherry intervention on blood lipid parameters, previous research evaluating the effects of cherries and anthocyanin-rich fruit juices on biomarkers of inflammation have yielded inconsistent results.

Contrary to the lack of effect observed on IL-6 levels in this study, the Seymour et al. study (13) conducted in Zucker fatty rats demonstrated a significant reduction in retroperitoneal and plasma IL-6 concentrations, as well as a significant decline in IL-6 mRNA production following consumption of freeze-dried whole tart cherry powder. Contrasting results may have been a function of the significant total and retroperitoneal fat loss that occurred during the treatment intervention of the Seymour et al study as opposed to the lack of weight-loss observed in this study. Adipocytes generate approximately 1/3 of plasma IL-6, and concentrations have been shown to positively correlate with fat mass (82). In addition, visceral adipose depots express more IL-6 than other areas due to increased macrophage infiltration (29), making the significant retroperitoneal fat loss observed in the Zucker rats a likely explanation for the corresponding IL-6 reduction. Other previously described factors that may explain the different
outcomes achieved between these studies are the much longer intervention period (90 days) and the higher dosage of anthocyanins provided in the Seymour et al. study, the high affinity for obesity and systemic inflammation that may increase treatment susceptibility in Zucker rats, and the different metabolic properties of rats and humans that limit applicability of research outcomes obtained using rodent-models to humans.

Alternatively, studies analyzing the effect of a cherry intervention on IL-6 concentrations in humans have supported the findings of this study, as no statistically significant effects on IL-6 were reported by Kelley et al. (14) or Martin et al. (75). However, research using anthocyanin-rich fruit juice as a treatment intervention has derived conflicting results in respect to inflammatory biomarkers and IL-6. While the phenolic profile between individual fruits may differ somewhat and produce slight variations in anti-oxidant and anti-inflammatory capacities, blueberries and bilberries are similar to cherries in that they are high in anthocyanin content. Although making direct comparisons between the effects of different fruits on the inflammatory profile has its limitations, comparing outcomes of studies using fruit-juice derived interventions has the benefit of a similar food matrix, unlike comparisons made between juice and whole fruits or purified anthocyanin-rich extracts.

Basu et al. (83) conducted a randomized placebo-controlled study evaluating the effect of daily blueberry juice consumption (equivalent to 350 g of fresh blueberries) on inflammatory biomarkers over a 8-wk period in 48 obese
subjects with metabolic syndrome, and Karlsen et al. (84) conducted a similar randomized placebo-controlled study to determine the effect of 330 mL of daily bilberry juice consumption on inflammatory parameters over a 4-wk period in 62 participants at elevated risk of CVD. These studies obtained contradictory results, as Karlsen et al. demonstrated a significant increase in IL-6 levels and Basu et al did not. Results of studies analyzing the effect of cherries and anthocyanin-rich fruit juices on other inflammatory biomarkers, such as CRP, also derived conflicting results, as Kelley et al. observed a significant 25% reduction in CRP (14), Martin et al. observed a non-significant 16% increase in levels (75), Karlsen et al. reported a significant reduction in levels (84), and Basu et al. reported no significant change in CRP levels (83). Similar to findings by Martin et al and Basu et al., no statistically significant reduction in CRP concentration was demonstrated in this study. However, a 19.5% decline in CRP was observed subsequent to cherry juice consumption compared to a 5.2% decline following placebo, and it is likely that significance was not achieved partially due to insufficient power conferred by large standard deviations, ranging from 3.8 to 7.1 ng/mL.

There are several potential explanations for the different outcomes achieved by this study and those reported by Kelley et al. (14) in respect to CRP and Karlsen et al. (84) in respect to both CRP and IL-6. The whole cherries used by Kelley et al. may have had more anthocyanins and a higher capacity to reduce CRP than the cherry juice used in this study, as the skin of whole cherries are
reported to contain the majority of the anthocyanins with the fruit (55, 72).

Similarly, the form of intervention used by Karlsen et al. may have conferred an increased capacity to reduce CRP and IL-6 than that used in this study, as the phenolic profiles and content of bilberries and cherries differ. Although some cherry varieties are reported to contain more anthocyanins than bilberries (1.3 – 6.8 mg/g vs. 4.5 mg/g), cherries contain far fewer total phenolics than bilberries (2.4 – 8.8 mg/g depending on variety vs. 10.4 mg/g) (85). The higher amount of total phenolics present in bilberries, or an increased ability of anthocyanins to synergistically function with these additional polyphenols, may have caused or contributed to the significant reduction observed in CRP and IL-6 in response to the bilberry juice intervention as opposed to the tart cherry intervention. In addition, the daily dosage of bilberry juice was higher (330 mg/dL or approximately 11 oz juice) than the dosage of cherry juice (8 oz), thereby potentially conferring a greater effect on CRP and IL-6 levels depending on the concentration of each juice.

Another possible explanation for the different outcomes achieved by this study and that of Karlsen et al. (84) in respect to CRP is based on certain characteristics of each study population and the ability of these characteristics and other exogenous factors to impact CRP levels. CRP has previously been described as an acute-phase reactant protein produced by the liver during inflammatory processes and other diseases (86), but levels have also been reported to escalate in response to a variety of factors, including environmental pollution and irritants,
second hand smoke, specific dietary patterns, minor tissue injuries, low levels of
physical fitness, and the use of estrogen and progesterone containing supplements
such as birth control and hormone replacement therapies (87). Of the 26
participants who completed this study, 18 were female and of these 4 reported
taking some form of contraceptive or estrogen supplement, which may have
driven CRP levels up slightly and reduced the potential lowering effect conferred
by tart cherry juice. In contrast, the female participants in the Karlsen et al. study
were at least 12-months post-menopausal and therefore not taking any form of
birth control, and were also not engaging in hormone replacement therapy. Had
participants in this study not been permitted to take estrogen and progesterone
containing supplements, perhaps CRP levels would have been more responsive to
treatment and significance may have been reached. Kelley et al. (14) also noted
significant reductions in CRP but did not publish data regarding the use of
estrogen-containing supplements, preventing consideration of this factor during
the interpretation of study results.

The population from which subjects were selected for this study was
assumed to be high risk for low-grade inflammation due to classification as
overweight or obese based on BMI calculations and the association of adipose
tissue with elevated cytokines. High baseline CRP, IL-6, and IL-10 concentrations
substantiated this assumption, although levels of MCP-1 and ESR were within the
normal reference ranges. Only 14 of the 26 subjects had IL-6 values at the
minimal level of detection for the assay, and within these 14 participants the mean
concentration was $94 \pm 111.6$ pg/mL. Even when including the 12 additional
subjects by substituting in the minimum value possible for each, IL-6 was still
above the normal reference range. Similarly, baseline CRP levels were also above
normal. The high corresponding levels of IL-6 and CRP make sense when
considering the inflammatory process, as CRP synthesis and release from
hepatocytes is primarily dependent upon circulating levels of IL-6. In turn, CRP
has been demonstrated to contribute to the cyclic nature of inflammation by
increasing IL-6 production by monocytes (88). Because high baseline levels of
inflammatory biomarkers provide substantial room for improvement, it is
interesting that no significant reduction was observed in IL-6 or CRP
concentrations. Therefore, levels too low for improvement via treatment is not a
viable explanation for lack of effect.

Very few studies have reported the effect of a cherry or anthocyanin-rich
fruit juice intervention on levels of MCP-1, IL-10, or ESR, but those that have
done so generally agreed with the results of this study, demonstrating no
statistically significant reduction in these biomarkers. As an extremely potent
chemokine, MCP-1 mediates inflammation and atherogenesis by attracting
monocytes to inflammatory lesions and helping to initiate their endothelial
binding (89). Not only does MCP-1 function to influence downstream pathways
and exacerbate the inflammatory response by attracting chemical mediators to
sites of damage, but its synthesis and release is in turn influenced by the
concentration of other cytokines and has been associated with high CRP levels
It is interesting to note that Kelley et al. (14) and Karlsen et al. (84) observed no effect on concentrations of MCP-1, even though they observed significant reductions in other inflammatory biomarkers, such as CRP. It is also interesting that in this study, baseline levels of CRP and IL-6 were above the normal reference ranges, yet no correlation was seen with MCP-1 values, as these were within the normal reference range.

Although cherry juice consumption did not result in a statistically significant reduction of MCP-1 in this study, a non-significant reduction of 6.3% was observed following the treatment arm compared to a 10.4% increase following the placebo arm. As with CRP, this non-significant result may be partially attributed to insufficient power conferred by the large standard deviations around the mean values, which ranged from 131 to 216 pg/mL.

Nonetheless, non-significance in MCP-1 is generally consistent with the findings from previous studies, including those by Kelley et al. (14) and Karlsen et al. (84). Martin et al. (75) also did not detect a significant reduction in MCP-1 when comparing the treatment and placebo arms of the crossover study, but a significant decrease was observed when subjects were grouped by study beverage (n = 4). This may indicate the need for a longer washout period. Additionally, conclusions based on stratification resulting in small sample sizes are more prone to a type 1 systematic error.

Although Martin et al. (75) also noted a significant difference in ESR when comparing the first arm only, no significance was observed in ESR across
both intervention arms, concurring with the results of this study. ESR measures the rate at which RBCs settle in the blood, and inflammatory states elevate blood protein causing RBCs to descend more quickly over a given period of time. Therefore, this inflammatory parameter is non-specific in nature and, similar to CRP, may be influenced by a variety of exogenous factors, such as menstruation, anemia, oral contraceptives, vitamin A, and certain pharmaceutical drugs (86). As previously noted, 4 of the participants in this study reported taking some form of oral contraceptive, and 2 of the participants reported experiencing periodic episodes of anemia. Theoretically, if the literature were to have supported the effectiveness of tart cherry juice in decreasing ESR, one explanation for the non-significance noted in this study may have been a reduced capacity of the juice to elicit significant lowering effects due to ESR elevation by participant contraceptive use and anemia. Other explanations might include limited susceptibility to a significant treatment response conferred by the normal baseline ESR values in this study, or the inability of ESR to respond due to a lack of necessary time and sensitivity, as ESR is an extremely slow responder and less sensitive than other indicators of inflammation (86).

Just as no significant alterations were observed in any of the inflammatory parameters IL-6, CRP, MCP-1, and ESR, no change was observed in IL-10 concentrations when comparing the tart cherry juice and placebo interventions over time. Kelly et al. (14), Karlsen et al. (84), and Martin et al. (75) corroborated this finding, each determining their anthocyanin-rich intervention to have no
effect on IL-10 levels. IL-10 is an anti-inflammatory cytokine that typically 
elevates and declines along with levels of pro-inflammatory cytokines, being up-
regulated in response to increased inflammation in order to down-regulate it (91). 
Therefore, because no statistically significant changes were observed in the other 
inflammatory markers in this study, it is not unexpected that no significant change 
was elicited in IL-10. In addition, because baseline CRP and IL-6 levels were 
determined to be high, it makes sense that baseline IL-10 concentrations were also 
elevated.

This study was based on the hypothesis that tart cherry juice consumption 
would improve biomarkers of dyslipidemia and inflammation without disrupting 
glucose homeostasis. Fasting glucose, insulin, and the insulin-sensitivity indices 
HOMA and QUICKI were expected to remain constant throughout the study, as 
demonstrated by numerous published studies evaluating the effect of an 
anthocyanin-rich intervention in humans (14, 70, 83, 92, 93, 93, 94). As 
anticipated, this study noted no significant alteration in fasting glucose, insulin, or 
the insulin-sensitivity indices and is consistent with previous research.

WC, BMI, and body fat percentage were measured throughout the study to 
monitor any changes in weight or adiposity that might confound study results. 
None of these variables significantly changed throughout the 12-week study 
period as expected, demonstrating that energy expenditure, energy intake, and 
macronutrient consumption remained relatively consistent during each arm of the 
intervention. No statistically significant alteration was observed in physical
activity level as measured by MET-minutes/week, and there was no significant
difference between calorie consumption or intake of carbohydrates, sugar, protein,
total fat, monounsaturated fat, polyunsaturated fat, saturated fat, soluble fiber, or
omega-3 fatty acids within the placebo and treatment arms.

However, the consumption of trans fat was determined to be significantly higher during the cherry intervention as compared to the placebo intervention (p < 0.05), despite subjects being instructed to maintain their usual dietary habits throughout the entire study period. The significant difference between trans fat intake during the placebo and treatment arms is important because trans fat has been reported to have a variety of adverse effects on risk factors for chronic disease, including those related to inflammation and dyslipidemia (95). Although they occur naturally in small quantities in meat and dairy products, most trans fats are produced commercially via the partial hydrogenation of unsaturated oils, which renders oils solid at room temperature by converting some of the cis bonds to trans bonds (96). The modified physical properties of trans fats makes them more desirable for use in the cooking and manufacturing of processed foods, but also negatively alters the biological effects of the fats upon consumption (95). A meta-analysis examining the effects of trans fats on blood lipids and systemic inflammation determined increased trans fat intake to be associated with increased total cholesterol, LDL-C, triglycerides, TC/HDL ratio, and decreased HDL-C, as well as increased IL-6, CRP, TNF-α, and MCP-1 possibly through effects exerted on monocytes and macrophages (95-97). There is also some evidence to suggest
that high trans fat consumption may increase post-prandial insulin, glucose, and visceral fat accumulation (95).

When determining the potential for the significant differences detected in trans fat levels between the cherry juice and placebo arms of the study to have a confounding effect on results, several issues must be considered. First, the potential of these differences to be valid should be explored. Of the 25 participants who turned in complete dietary data, 64% reported consuming more trans fat during the cherry arm than the placebo arm. In addition, there were no outliers to potentially skew the mean. Based on the randomized, single-blind, placebo-controlled, crossover design of the study, it is perplexing that the subjects would randomly increase their trans fat intake by more than twice as much during the cherry arm as compared to the placebo, especially when they were instructed to maintain their usual dietary habits throughout the entire study. In addition, there is no evidence for a biological mechanism to account for this observation, such as an increased craving for trans fats conferred by tart cherry juice. Alternatively, a behavioral explanation for the significant deviation in intake may be more feasible, as several subjects reported disliking the taste of the cherry juice and may have been drinking it along with high trans fat-containing comfort foods to increase palatability. Another more likely possible explanation would be that the variation in levels is spurious, and simply occurred by chance (type 1 statistical error).
When deliberating the validity of these differences, the weaknesses associated with 24-hour food records and the food processor program used to analyze nutrient data must each be taken into account. Inaccurate reporting of food consumption is a considerable problem across research studies. When documenting food intake over a 24-hr period, the most common error made is under or over-reporting, which produces serious bias in total energy, macronutrient, and micronutrient estimates (98). Approximately 12 to 44% of subjects using estimated food intake methods have been demonstrated to significantly underestimate consumption, frequently as a result of memory lapses, inability to adequately judge portion sizes, and/or embarrassment or shame (98). The quality of programs used for entering, analyzing, and quantifying nutrient data, as well as the database through which the program derives nutrient information, can also affect the validity of the nutrition data obtained. For this study, dietary records were analyzed by Food Processor Nutrition and Fitness Software (ESHA, version 8.5), which was made available in 2005. The database through which the program runs is incomplete in reference to trans fat, which is continually being updated as new data becomes available and as new versions are released. Disclosure of trans fat values on manufactured product labels were not required until January 1, 2006, and data sources such as the USDA generic data, voluntary labeling data, and fast food data are still exempt from this requirement. As of 2011, only 50% of the software’s database foods included information for trans fat. Because this study used the 2005 software version, the database was
significantly more incomplete, seriously calling into question the validity of the trans fat values provided.

However, if it were assumed that the levels of trans fat derived from the Food Processor software were accurate, another factor that would need to be explored is biological relevance. In Europe and North America, the average intake of trans fat is between 5 and 10 g/day when consuming a typical 2000 kcal diet, or between 2% and 4% of total energy intake (95). However, the American Heart Association recommends that trans fat be limited to less than 1% of daily energy intake, or less than 2 g/day on a 2000 kcal diet (99). Therefore, the levels of trans fat reported during both the placebo and cherry intervention arms are well under recommendations at 0.22 g and 0.47 g each. This amounts to only 0.1% and 0.2% of subjects’ total daily energy intake and, even though a mere 2% increase in trans fat has been associated with a 23% increase in CVD risk (96), the intake during both the cherry and placebo interventions are far too low to be considered biologically relevant or even significantly different from one another when analyzed as total energy intake.

Although the actual values reported in this study are likely to be too low due to largely incomplete trans fat data provided by the Food Processor database, it is possible that the proportion of trans fat consumption occurring during the treatment arm compared to the placebo arm is valid. If this is the case, but each of the values is actually significantly higher, the differences could be biologically relevant and a confounding effect on the inflammatory and blood lipid variables
may have occurred. If this had occurred, pro-inflammatory biomarkers and total cholesterol, LDL-C, VLDL-C, TG, and the CVD risk ratios may have been driven up, and the anti-inflammatory biomarker IL-10 along with HDL-C may have been driven down. However, this scenario is unlikely primarily because of the error introduced by the Food Processor software combined with that of participant food record misreports.

Although this study found no evidence to suggest that consuming 8 oz of tart cherry juice daily will improve biomarkers of dyslipidemia and inflammation in a population of overweight and obese subjects, a target population at greater risk may have produced a different set of results. Participants in this study were assumed to have high risk for dyslipidemia and inflammation based on high BMI and body fat measurements. However, if these inclusion criteria had been set higher, such as requiring a minimal BMI of 30.0 or 35.0 kg/m\(^2\) and a minimal body fat of 30% for men and 35% for women, the subject sample may have been hyperlipidemic or demonstrated an even greater level of inflammation and been more responsive to treatment. Excluding potential subjects based on the use of progesterone and estrogen-containing supplements may also have increased treatment response, particularly in respect to CRP and ESR. An attrition rate of 20% was assumed prior to the study, yet 28% of the participants dropped, producing a smaller sample population than planned. Perhaps the implementation of higher and more rigorous inclusion criteria combined with the recruitment of a larger subject sample may have elevated and tightened the serum lipid and
inflammatory characteristics, narrowing the standard deviation in parameter values and increasing the potential of measurements to achieve significance. Although compliance was determined by the return of empty and remaining bottles, it is possible that participants poured juice out instead of drinking it, and therefore overall compliance may be unknown. It was also assumed that the self-reported activity levels provided were correct and that the 24-hr dietary recalls accurately reflected dietary intake. Whether reported values and differences in trans fat consumption were legitimate or not and whether confounding may have occurred is unlikely but subject to conjecture. Clearly, further investigation evaluating the effects of a tart cherry juice intervention on biomarkers of dyslipidemia and inflammation is warranted.
Chapter 6

Conclusions and Applications

The growing prevalence of chronic disease within the United States and globally has become an epidemic, killing millions of people each year. Inflammation and dyslipidemia are intimately associated with the etiology of many chronic diseases, most notably CVD, which is the leading cause of death in America. Studies evaluating the effect of anthocyanin-rich interventions on serum lipid and inflammatory profiles have demonstrated beneficial effects. Because tart cherries are high in anthocyanins and total phenolics, it was hypothesized that the daily consumption of 8 oz of tart cherry juice would significantly improve biomarkers of dyslipidemia and inflammation.

However, data comparing concentrations of total cholesterol, LDL-C, VLDL-C, HDL-C, TG and the CVD risk ratios TC/HDL, TG/HDL, and LDL/HDL, as well as IL-6, hsCRP, MCP-1, ESR, and IL-10 across time and intervention showed no statistically significant changes in any of the markers. Possible explanations include a reduced susceptibility to treatment conferred by a study cohort possessing lipid and inflammatory values too near normal to be significantly altered, the relatively high use of progesterone and estrogen-containing supplements driving certain inflammatory biomarkers up, and the large variation in subject characteristics producing standard deviations too high in many variables to show significant alterations. Other possible explanations for the lack of effect observed include the unknown compliance of participants regarding juice
consumption and the potential of the treatment dose to be too low in anthocyanin concentration to elicit a response.

Another issue that may have influenced the effect of tart cherry juice on the blood lipid and inflammatory variables is trans fat consumption. Trans fat intake was observed to be significantly higher during the cherry arm as compared to the placebo arm (p < 0.05), causing the potential of confounding to be called into question. However, this scenario is very unlikely due to the high probability that the incomplete Food Processor program database produced inaccurate trans fat values.

Although no significant effects were observed on the lipid and inflammatory profiles of the participants drinking tart cherry juice, valuable information was obtained that may be useful in establishing future recommendations for cherry juice consumption. For example, although the cherry juice contained approximately 27 grams of sugar, fasting plasma glucose and insulin levels were not significantly affected. In addition, a 1% reduction was observed in triglycerides following cherry juice consumption compared to a 14% increase following placebo, even though the cherry juice contained slightly more sugar than the placebo beverage. These observations suggest that tart cherry juice may be a safe beverage option for those seeking to prevent the development of dysglycemia and high triglycerides, and may be a healthy alternative to drinking non-anthocyanin containing sugary beverages such as fruit punch. Furthermore, although this study does not demonstrate the capacity of tart cherry juice to
improve lipid and inflammatory profiles already abnormal or approaching abnormal, it is possible that tart cherry juice consumption may be an important preventative measure against the development of dyslipidemia, inflammation, and chronic diseases associated with such conditions. While important applications have been derived from this study, further research is necessary to better investigate the impact of tart cherry interventions on biomarkers of dyslipidemia and inflammation.
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APPENDIX A

PHONE SCREEN INCLUSION CRITERIA FORM
Inclusion Criteria Check Sheet

1. Generally healthy    Yes  No
2. Over 18 years of age    Yes  No
3. Pregnant     Yes  No
4. Diagnosed with diabetes   Yes  No
5. Diagnosed with other medical condition or disease such as inflammatory disease
   Yes  No
6. Ht:_____Wt: _____Calculated BMI: _______ (completed by investigator >25.0 kg/m^2)
7. % Body Fat _____________ Unknown
8. Inflammatory condition such as rheumatoid arthritis or gout?      Yes  No
   If so, are you taking medications for the condition? ______________
9. Take medications and/or supplements?   Yes  No
   If so, what meds/supplements? ______________________________________
10. Take NSAIDS (such as aspirin, ibuprofen, or advil) fairly consistently      Yes       No
11. Smoker     Yes  No
12. Wt stable for 6 months    Yes  No
13. Exercise more than 3 hrs/wk?      Yes  No
14. Plan to lose wt in the next 12 wks    Yes  No
15. On cholesterol lowering meds     Yes  No
16. Do you have high cholesterol (>200 mg/dL)?  Yes No  Unknown
   Total-C:_________ LDL-C:_________ HDL-C_________
17. Do you have high triglycerides (>150 mg/dL)?  Yes No  Unknown
18. Liver Disease     Yes  No
19. Do you have a problem fasting for 8 hrs prior to blood draws?  
   Yes  No
20. Do you have a problem with drinking tart cherry juice, or a similar beverage, every day for 28 days?  
   Yes  No
21. Are you already drinking pomegranate juice, cherry juice, or a similar beverage?  
   Yes  No
22. Do you have a problem with providing venous blood samples from your arm and a saliva swab from your mouth?  
   Yes  No
APPENDIX B

DISCLOSURE STATEMENT
VERBAL SCRIPT/DISCLOSURE STATEMENT

Hello, my name is (Katie Coles). I am a Human Nutrition graduate student at Arizona State University Polytechnic campus. I am collecting information for a study about the effects of 100% tart cherry juice on chronic disease biomarkers. You have been selected for inclusion in this project based on your willingness to participate in this study and your consistence with inclusion criteria.

The information I gather here will only be used for this study and will not be published or shared with the public. I will only include your name on this disclosure statement and thereafter, you will be assigned an unidentifiable code. In other words, every effort will be made to protect the confidentiality of the information you provide. You are not required to participate in this project.

There will be no benefits for you personally if you participate in this project other than the experience. If you do choose to participate, your weight, height, and waist circumference will be measured on each of the 4 visits, you will be asked to fill out a brief dietary questionnaire once per week and a physical activity log daily, and you will be asked to consume 8 oz of tart cherry juice or placebo daily for 8 of the 12 weeks of the study. A total of 4 blood draws will be performed; 1 blood draw at the first visit and then 1 every 4 weeks over the 12-week study period. Each visit will last approximately 30 minutes.

Will you be willing to participate in this project? Can you come to ASU Poly? If not, would you be willing to visit ASU in Tempe? If at any time during the study you wish to stop, please inform me and we will not continue.

Do you understand? Do you have any further questions?

Thank you very much for your time and participation.
APPENDIX C

INFORMED CONSENT
CONSENT FORM
Effects of Tart Cherry Juice on Chronic Disease Biomarkers

INTRODUCTION
The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

RESEARCHERS
Keith R. Martin, Ph.D., MTox, an Assistant Professor of Nutrition at Arizona State University, and Katie Coles, graduate student in the Nutrition Program, have invited your participation in a research study.

STUDY PURPOSE
The purpose of this research study is to determine if anthocyanin-rich tart cherry juice will affect plasma lipid levels and inflammatory marker levels in humans.

DESCRIPTION OF RESEARCH STUDY
You have indicated that you are at least 18 years of age, a non-smoker, not pregnant or lactating, and have not been diagnosed with diabetes. We are seeking adults who currently have a BMI of at least 25.0 kg/m². You will need to keep your diet and exercise routine the same throughout the study. This study will involve 4 visits to either the Health Sciences Center at the ASU Polytechnic Campus in Mesa, AZ or the Clinical Research Unit (CRU) in Tempe, AZ following an 8-hour fast. You will be asked to complete a brief medical history questionnaire once a week and a physical activity log daily. At each visit, height, weight, and waist circumference will be recorded. We will also collect a swab of your inner cheeks of your mouth. Visits should last approximately 30 minutes, and at this time, participants will have their blood drawn and receive 4 weeks of beverages to be consumed on a daily basis. If you forget to consume the beverage you will need to notify the investigators of the study. If you say YES, then your participation will last for 12 weeks. Approximately 30 subjects will be participating in this study.

RISKS
You may feel pain at the site of the needle insertion or experience mild bruising and faintness. As with any research, there is a possibility of other risks that have not yet been identified.

BENEFITS
This study will provide information regarding cherry juice consumption as it relates to cardiovascular disease and inflammation within the body.

NEW INFORMATION
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY
All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. In order to maintain confidentiality of your records, Dr. Martin will utilize subject codes that will be placed on all data obtained. A master copy of the codes will be kept in a locked filing cabinet away from all data samples collected. All confidential information will be disposed of by shredding or incineration at the termination of the study.
WITHDRAWAL PRIVILEGE
You are permitted to withdraw from the study at any time and for any reason. Deciding to withdraw from the study will not affect you in any manner.

COSTS AND PAYMENTS
The researchers want our decision about participating in the study to be absolutely voluntary. They recognize that your participation may pose some inconvenience (i.e. traveling and time commitment). In order to thank you for your time, a $10 gift card will be provided after each blood draw (four total draws).

COMPENSATION FOR ILLNESS AND INJURY
If you agree to participate in the study, your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT
Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Martin; 6950 East Williams Field Road, Mesa, Arizona 85212 or call at (480) 727-1925.

If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any of the risks involved. Remember, your participation is voluntarily. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given (offered) to you.

Your signature below indicates that you consent to participate in the above study.

__________________________________________  ____________________ ____________  ____________
Subject’s Signature                  Printed Name     Date

__________________________________________  ____________________ ____________
Contact Phone Number                  Email

INVESTIGATOR’S STATEMENT
“"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document.”

Signature of Investigator _________________________ __  Date _____________
APPENDIX D

MEDICAL HISTORY FORM
Medical History Questionnaire

Age: _______

Gender (circle one):  M  F

Ethnicity (please circle):  Hispanic or Latino  Not Hispanic or Latino

Race: (please circle):  American Indian/Alaska Native   African-American   White
Native Hawaiian/Other   Pacific Islander   Asian   Other

1. Do you regularly take prescribed and/or over-the-counter medications or dietary supplements?  
   Yes  No

   If yes please list:

<table>
<thead>
<tr>
<th>Medication/Supplements</th>
<th>Frequency</th>
<th>Reason and duration</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

2. Have you ever been diagnosed with any of the following conditions?

   Diabetes       Yes   No
   Thyroid problems Yes   No
   Kidney disease  Yes   No
   Cancer         Yes   No
   Liver disease   Yes   No
   Atherosclerosis Yes   No
   Rheumatoid arthritis Yes   No
   High cholesterol Yes   No
   High blood pressure Yes   No
   Gastrointestinal problems Yes   No

   Other illness(es):  ________________________________
APPENDIX E

RESEARCH DESIGN
8 oz cherry juice daily

Washout Period

8 oz placebo beverage

Blood Draw 1

Blood Draw 2

Blood Draw 3

Blood Draw 4
Anthocyanin-rich foods and beverages to avoid during the 7-week Cherry Study being conducted at the ASU Nutrition Department

- Red grapes and juice
- Pomegranates and juice
- Berries such as blueberries, cranberries, raspberries, strawberries, blackberries, and Acai berries and juices
- Egg plant
- Red cabbage
- Red wine
- Dark chocolate
- Most fruits that are darkly colored in the peel and the flesh

If there are questions, please contact:

Katie Coles
Masters of Science graduate student
ASU Nutrition
(480) 727-1412
APPENDIX G

24-HOUR FOOD RECORD
### 24-hour Dietary Recall

<table>
<thead>
<tr>
<th>Food</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
</tbody>
</table>

Participant ID #: __________
Date: ___________________
APPENDIX H

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ days per week

   □ No vigorous physical activities ➔ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   _____ hours per day
   _____ minutes per day

   □ Don’t know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate physical activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ days per week

   □ No moderate physical activities ➔ Skip to question 5
4. How much time did you usually spend doing moderate physical activities on one of those days?

____ hours per day
____ minutes per day

☐ Don’t know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

____ days per week

☐ No walking → Skip to question 7

6. How much time did you usually spend walking on one of those days?

____ hours per day
____ minutes per day

☐ Don’t know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

____ hours per day
____ minutes per day

☐ Don’t know/Not sure

This is the end of the questionnaire, thank you for participating
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Placebo</th>
<th>100% tart cherry juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories, kcal</td>
<td>92.2</td>
<td>106.4</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>23.0</td>
<td>26.6</td>
</tr>
<tr>
<td>Protein, g</td>
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<td>1</td>
</tr>
<tr>
<td>Fat, g</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
APPENDIX J

PLACEBO PREPARATION AND INGREDIENTS
Placebo Beverage Preparation

Each subject is to be provided with 8 bottles of prepared Placebo to take home and will be instructed to freeze 4 bottles and store the remaining 4 bottles in his or her refrigerator to be used per instructions.

Ingredients:

- SmartWater (8 bottles per participant)
- Dextrose
- Fructose
- Black Cherry flavored Kool-Aid
- Lemon Powder
- McCormick’s Red Food Coloring
- McCormick’s Blue Food Coloring

Procedure:

1. Pour 3 bottles SmartWater into gallon pitcher.
2. Weigh 145g dextrose into food weigh boat. Add to pitcher.
3. Weigh 145g fructose into food weigh boat. Add to pitcher.
4. Measure ½ teaspoon red food coloring. Add to pitcher.
5. Add 6 drops blue food coloring.
6. Add 3 packets black cherry flavored Kool-Aid.
7. Add 3 packets lemon powder.
8. Stir to mix well.
9. Use funnel to add 28oz. prepared tart cherry juice into each of the bottles.
10. Cap and clean bottles.
11. Store in freezer.
12. Provide 8 bottles of prepared placebo to subject.
APPENDIX K

ANTHROPOMETRY AND ESR
Anthropometry and ESR

Week 1
Date:________
Weight:______________ Blood Pressure:______
Height:______________ Pulse:______________
Calculated BMI:_______ Sed. Rate:___________
Waist Circumference:_____

Week 4
Date:________
Weight:______________ Blood Pressure:______
Height:______________ Pulse:______________
Calculated BMI:_______ Sed. Rate:___________
Waist Circumference:_____

Week 8
Date:________
Weight:______________ Blood Pressure:______
Height:______________ Pulse:______________
Calculated BMI:_______ Sed. Rate:___________
Waist Circumference:_____

Week 12
Date:________
Weight:______________ Blood Pressure:______
Height:______________ Pulse:______________
Calculated BMI:_______ Sed. Rate:___________
Waist Circumference:_____
APPENDIX L

WESTERGREN METHOD
Procedure for Westergren Method

Adapted from Thermo Fisher Scientific

1. Shake filling reservoir downward to force pre-loaded saline to the bottom of the reservoir. Keep upright and remove reservoir cap.

2. Add 1 mL EDTA-treated whole blood to the level of the filling line.

3. Replace cap.

4. Mix the saline and EDTA-treated whole blood by performing a minimum of 8 inversions.

5. While holding the reservoir with one hand, hold the Dispette tube at the 180mm mark with the other hand and penetrate the cap membrane.

6. Continue to insert the Dispette tube until it reaches the bottom of the reservoir. The blood should rise up the Dispette tube until it reaches or surpasses the grommet at the zero level.

7. Place the full Dispette assembly in a stand and ensure that the tube is at 90 degrees ± one degree to the horizontal.

8. Record the blood level in millimeters following one hour of sitting.
APPENDIX M

ELISA PROTOCOL
Single Analyte ELISA Procedure

Adapted from SABiosciences Corporation

The Single ELISAArray Kits measure the amount of an individual chemokine or cytokine using an ELISA, and each 96-well plate is coated with a protein-specific capture antibody. Therefore, the same general measurement technique is used to quantify all chemokines and cytokines, but the capture antibody used will differ.

Procedures:

1. Prepare your experimental samples and replicate serial dilutions of the Antigen Standard.
2. Pipette 50 µL of Assay Buffer into each well.
3. Transfer 50 µL of standards and/or samples to the appropriate wells of the ELISA strips.
4. Gently shake plate for 10 seconds and incubate for 2 hours at room temperature.
5. Wash the ELISA wells:
   a. Decant well contents and add 350 µl × Washing Buffer.
   b. Gently shake plate for 10 seconds and decant.
   c. Blot array upside down on absorbent paper to remove any remaining buffer.
   d. Repeat wash 2 more times.
6. Pipette 100 µL of Detection Antibody solution and incubate for 1 hour at room temperature.
7. Wash ELISA wells as previously described.
8. Pipette 100 µL Avidin-HRP solution to wells. Incubate for 30 minutes in the dark at room temperature.
9. Wash ELISA wells 4 more times.
10. Add 100 µL of Development Solution to each well and incubate the plate for 15 minutes in the dark at room temperature.
11. Add 100 µL of Stop Solution to each well. The color should change from blue to yellow.
12. Read the absorbance at 450 nm within 30 minutes of terminating the reaction. Subtract readings at 570 nm from the reading at 450 nm if wavelength correction is available.