Anthocyanins and Cardiovascular Disease
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II. Abstract

The leading cause of mortality worldwide for both men and women is due to cardiovascular diseases. Many factors can contribute to cardiovascular disease including high, oxidized LDL-cholesterol levels, low HDL-cholesterol levels, hypertension, and CVD-related biomarkers. Anthocyanins in food products have been found to decrease multiple factors that contribute to cardiovascular disease, ultimately due to their antioxidant effect. Basu et al. conducted a randomized, single-blinded controlled study in 66 obese men and woman who were diagnosed with metabolic syndrome. Each participant was required to drink 1 cup of a freeze-dried blueberry mixture twice a day. At the end of eight weeks, the systolic and diastolic blood pressures of the participants decreased (P=0.003 and P=0.04, respectively) in addition to a decrease in plasma-oxidized lipids, and MDA and HNE oxidative biomarkers. Ruel et al. studied the effects of low-calorie cranberry juice cocktail on 31 sedentary men. The men drank a placebo cranberry juice during a four-week run-in period followed by the consumption of the real cranberry juice cocktail in three phases: 125 ml/d, 250 ml/d and 500 ml/day each for three weeks. The number of plasma-oxidized lipids were reduced at 250 ml/d (P value= <0.05), and at 500 ml/d (P value= <0.001). HDL-cholesterol levels significantly increased, and sVCAM-1 and sICAM-1 levels decreased. In a randomized placebo-controlled trial, Zhu et al. tested the effect of anthocyanin capsules on 150 hypercholesterolemic participants for 24 weeks. The participants consumed four-80 mg capsules a day, receiving anthocyanins from bilberries and black currants. A P value of <0.001 was observed for hsCRP and a P value of 0.005 for sVCAM-1 for biomarkers of inflammation. In addition, the anthocyanin capsules had an effect on both HDL and LDL-cholesterol levels, increasing HDL-cholesterol levels by 14% and decreasing LDL levels by 10.4%. All three of the studies support the association of anthocyanins with decreased risks of cardiovascular disease.
III. Introduction

The hypothesis that will be tested in this review article is the consumption of anthocyanins is associated with decreased risk of cardiovascular disease.

Cardiovascular disease is the leading cause of mortality in both men and women worldwide (1). One in every four deaths in the United States results from some type of cardiovascular disease (1). Heart attacks, strokes, arrhythmia, heart failure and heart valve conditions result from various types of cardiovascular diseases. Coronary artery disease is the most prominent cardiovascular disease, killing over 380,000 people annually (1). Therefore, it is vital to study factors associated with a higher risk of cardiovascular disease. People consume anthocyanins on a daily basis, but may just not be aware of the positive effects they can have in their body, including the ability to decrease these risks.

Figure 1. The process of atherosclerosis
Cardiovascular disease is the process of atherosclerosis, which is the buildup of plaque in the arteries (see Figure 1) (2). There are many risk factors that contribute to an increased risk of cardiovascular disease, which include but are not limited to high blood pressure, increased low density lipoprotein (LDL)-cholesterol, decreased high density lipoprotein (HDL)-cholesterol, increased biomarkers of inflammation and oxidative stress, obesity and diabetes (3). The plaque buildup narrows the artery, which allows less blood and oxygen to flow through freely. Plaque buildup starts out as a fatty streak, then turns into a fibrous plaque, and ultimately becomes a complete occlusion thrombus (2). Any damage to the endothelium wall can make room for oxidized LDL-cholesterol to enter the artery and buildup. (2). The smaller and denser the LDL-cholesterol becomes, the more damage occurs in the artery (3). The smaller the LDL particles are, the more capable they are of fitting into the artery. Once the small, dense LDL particles are inside the artery they can rupture and travel throughout the artery, and are later capable of causing a blood clot (3).

Anthocyanins are water-soluble pigments found in foods that are red, blue or violet in color (4). Some examples of food products that contain the highest amount of anthocyanins are chokeberries, which can contain up to 1000 mg of anthocyanins/100 g, eggplant, which can contain up to 750 mg of anthocyanins/100 g, blueberries, which can contain up to 497 mg of anthocyanins/100 g, and cranberries, which can contain up to 200 mg of anthocyanins/100 g (5). Since they are water-soluble, they easily spill out when juice is made. They are made up of one or two sugar units bonded to an anthocyanidin, the sugar typically being glucose or galactose (4). Anthocyanins usually are present in foods in groups of 20 or 30 (4). Anthocyanins are unstable to heating, the
presence of mineral ions, changes in pH, oxygen, and enzymes, and will frequently change color due to any of these processes (4). The increase of hydroxyl groups on an anthocyanin increases the blue color of the product, and the increase of methyl groups increases the red color (4).

Anthocyanins are natural antioxidants, and have properties that include reacting with free radicals and peroxides to destroy the process of oxidative rancidity (6). Anthocyanins have protective effects against LDL-cholesterol oxidation, because they do not allow your LDLs to react with free radicals in the body. They are also cardiovascular protective due to their anti-inflammatory effects in the body, which can slow to progression of atherosclerosis (7).

There are many terms used in this scientific review article that need to be defined. The studies assess blood pressure, serum lipid levels, and biomarkers for inflammation and oxidative stress. Normal blood pressure is defined as 120/80, pre-hypertension is defined as 120-139/80-89, and hypertension is defined as ≥ 140/90 (8). Serum lipids assessed include HDL and LDL cholesterol. HDL-cholesterol levels should be ≥60, and LDL-cholesterol levels should be < 100 to be considered in the normal range (9). Biomarkers of inflammation include C-reactive protein (CRP), soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and IL-1β (10). Biomarkers of oxidative stress include malondialdehyde (MDA), hydroxynonenal (HNE), myeloperoxidase (MPO), and oxidized lipids (10). All of these are significant biomarkers to assess the levels of oxidative stress and inflammation in the body.
Enzyme-linked immunosorbent assay (ELISA) kits are used in the studies to assess the oxidative stress and inflammation biomarkers. Antibodies and substrates for enzymes are added to the solutions placed in well plates to cause a color change in that solution (10). The amount of color change accurately determines the biomarker amount in the sample (10). The third study focuses on participants with hypercholesterolemia, which is defined as having high cholesterol in the blood: 200-239 mg/dL is borderline high, >240mg/dL is high (11).

IV. Review of Research Studies

A. Article 1


The purpose of this study was to see if blueberry supplementation could adequately improve features of metabolic syndrome, by testing if the anthocyanins in blueberries caused a decrease in biomarkers of lipid and lipoprotein oxidation and inflammation.

The study was a randomized, single-blinded controlled study with duration of eight weeks. It involved 66 obese men and women who were 50.0 ± 3.0 y. Participants were required to have at least three components of metabolic syndrome, which included a fasting glucose rate ≥ 100 mg/dL, blood pressure ≥ 130/85 mmHg, triglyceride level ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL in men and < 50 mg/dL in women, and a waist circumference of ≥40 inches in men and ≥ 35 inches in women. Participants were excluded from the study if they were taking any medications for chronic diseases, taking
antioxidants, smoked, consumed > 1-2 alcoholic beverages a day, or were pregnant. Subjects were randomly assigned to the blueberry or placebo group.

Two different types of freeze-dried blueberries totaling 50 grams were formulated with 480 ml of water. Each participant in the blueberry group had to drink 1 cup (or 240 ml) of the blueberry drink in the morning, and then another cup (240 ml) six to eight hours later. Participants logged food records to make sure they were not consuming any food products that could interfere with the absorption of the anthocyanins. Body weight, height, waist circumference and blood pressure were taken at weeks four and eight. Blood samples were also taken at weeks four and eight to test for fasting glucose, insulin, total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol levels. Oxidative biomarkers MDA, HNE, oxidized-LDLs, and MPO were collected through serum plasma samples and separated by centrifugation. ELISA kits were used to assess inflammation biomarkers: CRP, sVCAM-1, and sICAM-1.

A decrease in both diastolic and systolic blood pressure was reported. Systolic blood pressure dropped $7.8 \pm 2.50$ mmHg ($P$ value=0.003), and diastolic dropped $2.5 \pm 1.10$ mmHg ($P$ value=0.04). Waist circumference and body weight were slightly decreased, but the value was not statistically significant. For biomarkers of oxidative stress, oxidized lipids had the biggest decrease, with a $P$ value reported of <0.009. MDA and HNE were significantly decreased as well, with a $P$ value of <0.005 (see Table 1). For biomarkers of inflammation, SICAM-1 and sVCAM-1 were both slightly decreased, but their values were not statistically significant. No other changes occurred in the lipid profile or biomarkers for inflammation or oxidative stress.
The findings of this study show an association between consumption of blueberries and a decrease in blood pressure and two of the major biomarkers for oxidative stress. Though not all of the variables of the study were altered, based off of these findings, consuming 50g of freeze-dried blueberries/d containing 742 mg of anthocyanins can decrease risk of cardiovascular disease.

There were many advantages to this study. First of all, both men and women were used so there was no bias towards one gender. The study used a placebo group to compare the blueberry results to. Lastly, the study isolated one food product, blueberries, as the only source of anthocyanins. In addition there were some limitations to consider. There was a large dropout rate for the study: 27% in the blueberry group and 28% in the placebo group. Therefore, only 48 participants completed the study, so the sample size was not very large. Another limitation was that none of the serum lipid levels decreased (or increased), and serum lipids levels are big factors in contributing to cardiovascular disease.

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Table 1. Changes in plasma biomarkers of oxidative stress and inflammation

<table>
<thead>
<tr>
<th>Variables</th>
<th>0–8 wk</th>
<th>Blueberry</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CRP, ng/l</td>
<td>0.2 ± 0.50</td>
<td>0.4 ± 1.50</td>
<td></td>
</tr>
<tr>
<td>sICAM-1, ng/l</td>
<td>−0.1 ± 0.02</td>
<td>0.0 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>sVCAM-1, ng/l</td>
<td>−0.1 ± 0.04</td>
<td>0.0 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/l</td>
<td>0.0 ± 0.01</td>
<td>0.0 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Adiponectin, μg/l</td>
<td>0.0 ± 0.01</td>
<td>0.0 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>ox-LDL, μ/L</td>
<td>−30.0 ± 4.00*</td>
<td>−9.6 ± 9.50</td>
<td></td>
</tr>
<tr>
<td>MPO, μg/l</td>
<td>2.5 ± 5.00</td>
<td>−2.4 ± 8.50</td>
<td></td>
</tr>
<tr>
<td>MDA and HNE, μmol/l</td>
<td>−0.2 ± 0.03*</td>
<td>−0.1 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

1 Data are means ± SE. *Different from control, P < 0.01.
disease. There were also side effects that occurred in participants, including nausea, vomiting, diarrhea and constipation due to the large amount of fiber in the mixture. This may be a sign that the anthocyanin mixture (or mg of anthocyanins) may not be able to be consumed on a daily basis without adverse effects.

**B. Article 2**


The purpose of this randomized controlled study was to test the effects of consuming different doses of low-calorie cranberry juice cocktail (CJC) on oxidized LDL concentrations and cell adhesion molecules in men.

Thirty-one sedentary men between the ages of 18 and 70 participated in this study. The subjects had inclusion criteria, which included a BMI between 25 and 35, a waist circumference ≥ 90 cm, LDL-cholesterol levels between 3 and 5 mmol/L, and subjects were not allowed to use medications that had anti-inflammatory effects or that could affect lipid metabolism. Participants were excluded from the study if they consumed more than 15 g of alcohol/day, had a chronic use of supplements, or had any chronic diseases such as cardiovascular disease or diabetes. The study used Ocean Spray® 125 ml ready-to-drink containers with no sugar added.

The study began with the subjects participating in a four-week run-in period in which they consumed 500 ml/day of cranberry flavored low-calorie placebo juice. After the run-in period, the participants were subjected to three different four-week long phases of consuming the CJC. Phase one consisted of consuming 125 ml/day, phase two
consisted of consuming 250 ml/day, and phase three consisted of consuming 500 ml/day. To keep the liquid constant, placebo juice was added to the CJC so that each drink contained the same amount of fluid (375 ml for phase 1, 250 ml for day 2, 0 ml for day three).

Each time the participants visited the study site, blood pressure was tested and blood sample were taken. Plasma VLDLs were measured by centrifugation, and HDL and LDL-cholesterol concentrations were determined from the VLDLs. ELISA kits were used to sICAM-1, sVCAM-1 and oxidized lipid levels.

Plasma oxidized LDL-cholesterol levels were significantly decreased at phase 2 with the consumption of 250 ml/d, with a P value <0.05 and at phase 3 with the

Figure 2. Changes in Plasma Oxidized LDL concentrations with increasing amount of CJC
consumption of 500 ml/d, with a P value <0.001 (see Figure 2). Systolic blood pressure was recorded as being decreased only with the consumption of 500 ml/d (P<0.03). The cranberry juice cocktail did have an affect on HDL-cholesterol concentrations, increasing significantly at both phase 2 and 3 (P<0.001). SVCAM-1 and sICAM-1 levels were found to be significantly decreased at phase 3 (P value <0.05 and P value <0.0001, respectively). LDL-cholesterol and diastolic blood pressure had decreased, but not to a statistically significant value. The study also looked at the different levels of oxidized LDLs, sVCAM-1 and sICAM-1 levels between the participants with metabolic syndrome (9) and those without (21). Both oxidized LDLs and sICAM-1 levels were decreased in men with or without metabolic syndrome, but sVCAM-1 levels were only significantly decreased in those without metabolic syndrome.

This study concludes that consuming increasing amount of cranberry juice (mostly 500 ml/d) can have sizeable effects on oxidized lipid levels, sVCAM-1 and sICAM-1 levels, and is associated with lowering systolic blood pressure. The findings also showed a significant increase in HDL-cholesterol levels, which was not seen in the first study. This study was significant in that it compared the decrease in oxidative stress biomarkers in both those with metabolic syndrome and those without, and for the most part, they remained constant. People can see from this study that one does not have to have a chronic disease in order for anthocyanins to decrease cardiovascular risk factors.

One of the advantages of this study was that it used increasing amounts of CJC, so it was evident at which phase effects could be seen. The study was also consistent with the amount of fluid each participant was receiving, even when different millimeter amounts of CJC were being consumed. This allowed no room for error with differing
hydration statuses between the phases. Lastly, the study compared the oxidative stress biomarkers of those with and without metabolic syndrome, which was also not seen in the first study. One of the limitations of the study was that it only used males, not females. There was also a small sample size of only 31 men, with a wide age range of 18-70. Additionally, though the study contained a four-week run-in period, it did not contain a placebo group to directly compare the results of the CJC group to.

C. Article 3


The purpose of this study was to explore the effects of anthocyanin capsules on inflammatory biomarkers that are involved in the progression of atherosclerosis. In addition, the study was performed to explore the effects of the anthocyanin capsules on LDL and HDL-cholesterol levels.

One hundred and fifty hypercholesterolemic subjects aged 40 - 65 participated in the randomized, double blind, placebo-controlled trial. The length of the study was 24 weeks. Inclusion criteria included having a fasting plasma glucose level between 200 and 310 mg/dL. Participants were not allowed to have a history of cardiovascular disease, diabetes, hypertension, thyroid disorders, or have a history of smoking or drugs. Before the study began, participants were subjected to a seven-day run-in period in which no consumption of any food or drink that contained anthocyanins was allowed. After the run-in period, subjects were randomly assigned to the placebo or the anthocyanin group.
The anthocyanin group consumed four 80 mg anthocyanin capsules a day containing anthocyanins from bilberries and black currants. Two capsules were taken at a time: two after breakfast and two after dinner for a total of 320 mg. The placebo group took four placebo capsules a day. Participants were to remain their normal diet and lifestyle. They came back every four weeks for measurements including body weight, waist and hip circumference, and blood pressure. At weeks 12 and 24 they were required to fast overnight and get blood samples taken in the morning. A three-day 24-hour diet recall was done by each of the participants also at weeks 12 and 24 to assess dietary habits.

Related to serum lipid levels, HDL-cholesterol was increased by 14%, and LDL-cholesterol was decreased by 10.4% (P=0.036 and P=0.030, respectively). Total cholesterol and triacylglycerol levels were decreased in both the placebo and the anthocyanin group, so the results were not significant. (See Table 2.) Results for the inflammatory biomarkers were also assessed. HSCRP levels significantly decreased at

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 73)</th>
<th>Anthocyanin (n = 73)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 24 wk</td>
<td>Mean change, % (95%CI)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.48 ± 0.84 6.25 ± 0.83</td>
<td>-3.6 (-7.8 to -0.6)</td>
<td>0.556</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>6.45 ± 1.02 6.18 ± 0.82</td>
<td>-2.9 (-6.3 to -0.5)</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.24 ± 0.21 1.23 ± 0.20</td>
<td>-0.9 (-5.2 to 3.4)</td>
<td>0.036</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>1.22 ± 0.23 1.37 ± 0.22</td>
<td>14.0 (7.9 to 20.2)</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>3.29 ± 0.47 3.30 ± 0.52</td>
<td>0.3 (-2.9 to 3.5)</td>
<td>0.030</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>3.36 ± 0.58 3.01 ± 0.41</td>
<td>-10.4 (-14.8 to -6.0)</td>
<td>0.462</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>2.41 (1.47 to 2.70)</td>
<td>-3.2 (-7.6 to -1.2)</td>
<td></td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>2.45 (1.53 to 2.74)</td>
<td>-4.8 (-9.8 to -0.2)</td>
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</tbody>
</table>

Table 2. Changes in Lipid Profile at Baseline and 24 Weeks.
21.6% (P=0.001) as well as the sVCAM-1, which had a 12% decrease (P=0.005). IL-1β levels were decreased as well, but the value was not statistically significant.

The results of the study determined that anthocyanin capsules had an effect on several different variables that could lead to an increase in cardiovascular disease. Anthocyanin capsules were not tested in the two previous studies mentioned; they only used anthocyanins from food products. This study allows there to be more interpretation about the way in which anthocyanins can be consumed, yet still remain beneficial.

The study had many advantages. The sample size was large, containing 150 participants. In addition, the study happened over a period of 24 weeks, which is a long duration. Serum lipid levels and inflammatory biomarkers were both examined. In addition, there were significant results for both LDL and HDL-cholesterol, unlike the two previous studies. The hsCRP value also was significantly decreased. The hsCRP value is said to be the best biomarker for inflammation (12). No adverse effects were reported from consuming the anthocyanin capsules four times a day. The main limitation to the study was that anthocyanin capsules were used, not actual food items. This is a limitation because many people receive their anthocyanins directly through food products, not through capsules.

V. Conclusion

The first study concluded that blueberries have a cardio-protective role due to their effect on decreasing blood pressure, oxidized LDL-cholesterols, and lipid peroxidation. The second study determined that consuming low-calorie cranberry juice cocktail decreased circulating sICAM-1 and sVCAM-1, in addition to increasing HDL-
cholesterol levels. Study three concluded that anthocyanin capsule supplementation reduced serum levels of CRP, sVCAM-1, and IL-1β, but most importantly improved lipid profiles for both LDL and HDL-cholesterol. Oxidized lipid levels were improved in two of the studies. In two of the studies HDL-cholesterol levels were increased. Additionally, two of the studies determined that biomarkers of inflammation were decreased. The anthocyanins in each study showed an increase or decrease in at least two variables that were tested in each study. The studies mentioned in this review paper all correlated with each other. Overall, all three studies were associated with decreased risk of cardiovascular disease, which supports my hypothesis.

**VI. Future Research**

For future research, I think that studies should be done to test whether the combination of certain anthocyanins in food products has an overall better effect than one anthocyanin alone. Though the third study mentioned that the capsules used a combination of bilberries and black currants, it would be beneficial to see the combination of anthocyanins in actual food products. Researchers could also look into whether certain anthocyanins are directly correlated to one or two variables. For instance, if blueberries are mainly associated with decreasing blood pressure, or if cranberries are mainly associated with increasing HDL-cholesterol levels. Additionally, research could be done to see if one anthocyanin color (more methyl or hydroxyl groups) has more benefits over the other colors.
References


