The Effect of the Supplementation of Cranberry Seed Oil on the Lipid Profiles of Human Subjects.

by

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ABSTRACT

Heart disease is currently the number one cause of death for both men and women in the United States. Elevated cholesterol is a major contributor to heart disease. Maintaining optimal blood cholesterol levels can significantly reduce the risk for developing heart disease (American Heart Association, 2007).

Recently, food technologists have discovered that the by-products of cranberry processing are rich in antioxidants. Following this recent discovery, food engineers have started to extract the oil from cranberry seeds. Cranberry seed oil has an exceptional nutrient and antioxidant profile. It is the only edible oil that has a natural occurring omega-6 to omega-3 ratio of 1:1, and contains all eight isomers of vitamin E, plant sterols, phospholipids, and flavonoids; which all have been shown to reduce cholesterol. The beneficial ingredients that are found in cranberry seed oil are highly concentrated (Fruit Essentials, 2007).

The purpose of this study was to determine the effects of supplementing cranberry seed oil to a population who had borderline high to high total blood cholesterol levels (>200 mg/dl).

The effects would be measured by examining changes in lipid profiles of participants. A lipid profile test includes total cholesterol, LDL cholesterol, triglycerides, and HDL cholesterol. The purpose of this research study was to determine if there were significant reductions in total cholesterol, LDL cholesterol, and total triglycerides and significant increases in HDL cholesterol after supplementing with cranberry seed oil.

A total of 19 participants completed this research study, with 9 in the control group and 10 in the experimental group. Participants were randomly assigned to a control group or experimental group. Participants in the experimental group consumed one tablespoon of cranberry seed oil daily for eight weeks. Participants in the control group consumed one tablespoon of canola oil daily for eight weeks. At weeks one, four, and eight a certified laboratory technician administered lipid profile testing on each subject. Data from all lipid profile tests were analyzed using SPSS statistical software.

The results from the lipid profile test indicated that there were no significant differences between groups or within each group in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. Although not statistically significant, there were great improvements in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides after four weeks of supplementing cranberry seed oil. A decrease of 5.7 mg/dl in total cholesterol occurred in the experimental group after just four weeks of supplementing the cranberry seed oil. These results warrant further research.

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Chapter I: Introduction

Heart disease is the number one cause of death for both men and women in the United States (Centers for Disease Control and Prevention, 2007). High levels of saturated fat and cholesterol in the diet can cause blood cholesterol levels to increase (National Institute of Health, 2005). Excessive cholesterol builds up in the blood, thus leading to plaque formation and hardening of the arteries. This condition is called atherosclerosis. Atherosclerosis is the leading cause of heart disease, stroke, and heart attacks (U.S. Food and Drug Administration, 2004). Blood cholesterol levels are affected by diet, weight, and physical activity.

Emerging research suggests that flavonoids found in cranberries may have the ability to reduce the risk for heart disease. Flavonoids act as powerful antioxidants and can help reduce the risk of heart disease. Flavonoids are a sub-class of polyphenols and can be found in wide variety plants. Flavonoids are used in medical practice for their anti-inflammatory, anti-viral, and anti-allergic capabilities. Flavonoids also act as a powerful antioxidant by acting as a free-radical scavenger (Packer, & Evans, 1998).

Cranberries are a major contributor to Wisconsin's economy. According to the Economic Research Service of the United States Department of Agriculture, Wisconsin was the leading producer of cranberries in the year 2006. Wisconsin was responsible for 57% of the U.S. production (United States Department of Agriculture, 2007). The majority of harvested cranberries are further processed to make fruit juice. The processing of cranberry juice uses only 85% of the total cranberry. The other 15% of the cranberry consists of the skin, seeds, and pomace. This portion is often thought of as waste when in actuality is the most nutritious part of the fruit, since it is packed with

powerful antioxidants that have the potential to help fight heart disease (Fruit Essentials, 2006).

Recently, food engineers have been extracting oil from cranberry seeds. The cranberry seed oil that is obtained from extraction has an exceptional nutrient and antioxidant profile. Cranberry seed oil is the only edible oil found to have a naturally occurring ratio of 1:1 omega-6 polyunsaturated fatty acids (n-6 PUFA) to omega-3 polyunsaturated fatty acids (n-3 PUFA) (Fruit Essentials, 2006). Currently, Americans are thought to consume a diet with a ratio as high as 20:1 omega-6 polyunsaturated fatty acids (n-6 PUFA) to omega-3polyunsaturated fatty acids (n-3 PUFA). This imbalance has been linked to many illnesses such as heart disease, cancer, and diabetes (Simopoulos, 2006). In addition to its optimal omega-6 to omega-3 ratio, cranberry seed oil also contains all eight isomers of vitamin E, plant sterols, phospholipids, and flavonoids, which have been shown to help reduce cholesterol and thus considered a heart healthy fruit (Fruit Essentials, 2006).

Due to all the heart healthy components found in cranberry seed oil it is hypothesized that supplementing with cranberry seed oil has the ability to lower total cholesterol and blood LDL cholesterol, and to increase blood HDL choelsterol in human subjects.

Statement of the Problem

Heart disease is currently a major public health problem in the United States costing over 300 billion dollars in the year 2000 (Escott-Stump & Mahan, 2004). Alternate methods of drug therapies should be examined in order to reduce healthcare costs. The purpose of this study was to determine the effects of supplementing cranberry

seed oil to a population who had borderline high to high total blood cholesterol levels (>200 mg/dl) and to determine if the cranberry seed oil has the ability to significantly lower total cholesterol, LDL cholesterol, and total triglycerides and, to determine if it has the ability to increase HDL cholesterol.

Research Objectives

1. To determine if supplementing 1 tablespoon of cranberry seed oil each day for eight weeks would lower total blood cholesterol levels in human subjects.
2. To determine if supplementing 1 tablespoon of cranberry seed oil each day for eight weeks would lower low density lipoprotein (LDL) cholesterol levels in human subjects.
3. To determine if supplementing 1 tablespoon of cranberry seed oil each day for eight weeks would increase high density lipoprotein (HDL) cholesterol levels in human subjects.
4. To determine if supplementing 1 tablespoon of cranberry seed oil each day for eight weeks would decrease triglyceride levels in human subjects.

Assumptions of the Study

There are two assumptions in this research study. One assumption is that the participants advised to take 1 tablespoon of cranberry seed oil a day for eight weeks will take the whole dosage every day throughout the eight weeks. The second assumption being that the participants will not heat the cranberry seed oil above 250°F.

Definition of Terms

Antioxidant: type of phytochemical that defends against oxidative stress

Atherosclerosis: hardening of the arteries due to deposits of fatty substances, cholesterol, cellular waste products, calcium, and other substances in the inner lining of the arteries (American Heart Association, Inc., 2007)

Blood lipid profile: determined by a blood sample that usually consists of total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides

Cardiovascular Disease (CVD) disease of the heart with different forms including hypertension, coronary heart disease, stroke, rheumatic heart disease, or congestive heart failure.

Chylomicrons: large lipoproteins that transport lipids to adipose, cardiac, and skeletal tissues

Coronary Heart Disease (CHD): disease that involves the network of blood vessels surrounding the heart (Escott-Stump & Mahan, 2004)

Essential Fatty Acids: fats that cannot be synthesized in the body and must be consumed in the diet

Flavonoids: sub class of polyphenolic compounds that are distributed in plants (Packer & Rice-Evans, 1998)

Free Radical: atoms that contain unpaired electrons that are highly reactive

Functional Food: Any food that exerts health benefits beyond the traditional nutrients it contains

High Density Lipoprotein (HDL): type of lipoprotein that transports

cholesterol through the blood stream to the liver where it can then be excreted out of the body (Groff, Groupper, & Smith, 2005)

Low Density Lipoprotein (LDL): major cholesterol carriers in the blood. High levels of LDL in the blood are associated with coronary heart disease

Low Density Lipoprotein (LDL) Oxidation: initiated by enzyme-mediated and non-enzymatic mechanisms; a risk factor for atherosclerosis

Omega 3-Polyunsaturated Acid. an essential fatty acid that has a double bond on the third carbon from the omega end of the fatty acid

Omega-6 Polyunsaturated Fatty Acid: an essential fatty acid that has a double bond on the sixth carbon from the omega end of the fatty acid

Phytochemicals: a large group of non-nutrient compounds derived from plants that contain disease-preventing capabilities (Groff, Groupper, & Smith, 2005)

Phytosterol: plant sterols and are concentrated in the seeds of fruits and vegetables

Poyphenols: sub class of phytochemicals that include over 8,000 compounds that are thought to play many important roles in the body (Groff, Groupper, & Smith, 2005)

Limitations of the Study

There are two limitations of this study. The first limitation being that the subjects may not consume the appropriate amounts of their designated oil. The second limitation is that they may not follow the directions given to them stating not to heat the oil above 250°F.

Chapter II: Literature Review

History of Cranberries

Cranberries along with blueberries and grapes are one of the three fruits that are native to North America. Native Americans were the first to use the fruit in the human diet. The Native Americans introduced the fruit to English settlers in Massachusetts and cranberries were served at the first Thanksgiving feast. It is believed that the pilgrims named the berries, cranberries. The word cranberry was used to describe the fruit because of the small, pink blossoms that resembled the head of Sand hill cranes. Some also may say that the name was derived, because they are a favorite fruit of cranes (McNamee, 2007). Henry Hall, who was an American Revolutionary War Veteran, is thought to be the first to cultivate the cranberry for commercial production in the year of 1816. In 1871, the first association of cranberry growers was established. Other cranberry growers were quick to emulate Hall's techniques and the cranberries production increased rapidly throughout the 19th century (Jasperson, 1991). The cranberry market was almost completely eliminated when in 1859 Arthur S. Flemming, the secretary of the United States Department of Health, Education, and Welfare announced that the cranberry crop of 1959 contained traces of the herbicide aminotriazole. Cranberry growers lost millions of dollars. After the aminotriazole scare, the Ocean Spray Company spent a large amount of money in their research and development department in order to reinvent new cranberry products. This research produced cranberry apple juice blends and other cranberry juice blends which were introduced to the American market. During the 1980's and 1990's prices and production of the cranberry increased steadily (O'Donnell, 2007).

Cranberry Species and Cranberry Attributes

The majority of cranberries that are harvested in the United States are from the species of *Vacciunium macrocarpon.* This species of cranberries are known as the American cranberry or bearberry. This species of cranberries are native to the northeastern region of North America, eastern Canada, and the eastern United States. The leaves of the *V. macrocarpon* range in size from 10-20 mm long. The flavor of the *V. macrocarpon* can be described as a slightly apple-like taste (United States Department of Agriculture, 2007).

Cranberry Cultivation

Cranberries require very particular growing conditions. An acidic peat soil base, a top layer of sand and an abundant fresh water supply are the keys to a successful cranberry harvest (Ocean Spray, 2007). The only way cranberries can survive and flourish is with the proper combination of soils and water. Within the United States, Massachusetts, Wisconsin and Oregon provide for these ideal conditions and a growing season from May to October.

The cranberry plant is a low lying vine. These vines are set inside impermeable beds that are layered with sand, peat, gravel, and clay. In addition, glacial deposits naturally developed some beds. Beds are often referred to as "bogs" or "marshes". The bogs are further contained with dikes. Dikes are created by forming a ridge of soil around the perimeter of the bogs. These dikes enable the bogs to be flooded prior to harvesting the cranberries and to protect the vines during the winter season. Irrigation equipment is utilized to provide ample water to the cranberry vine for proper growth and at times for frost protection. It is very important to prevent frost because frost can ruin an

entire year's crop. Cranberry growers must monitor temperatures and sprinklers 24 hours a day. The beds are irrigated throughout the year in order for the soil to maintain proper moisture levels (Ocean Spray, 2007).

Harvesting of the Cranberry

In late September, or early October, cranberries are harvested when they reach a deep red color. The first step in the harvesting process is to flood the bog. This flooding of the bog causes the cranberries to float to the surface of the water. The floating cranberries are then collected and pumped or conveyed out of the bogs into waiting trucks (Burlington County Library System, 2007).

Dry Harvesting

Dry harvesting of cranberries may also be done. This process requires no water as opposed to the floating methods of harvesting. In dry harvesting the cranberries are taken off of their vines by the use of a mechanized picking machine (Burlington County Library System, 2007).

Processing of Cranberries

The majority of harvested cranberries are further processed to make fruit juice and other cranberry food products, 35% are processed into sauce products and 60% are processed into various fruit drinks (Vattem, Ghaedian, & Shetty, 2005). When cranberries are processed to make cranberry juice, only 85% of the total cranberry is used. The other 15% of the cranberry that is removed consists of the skin, seeds, and pomace (Fruit Essentials, 2006). This by product of pomace is mainly composed of the skin, flesh and seeds of the fruit. Traditionally the pomace has been used in animal feed; however, recent evidence shows that the pomace has the potential to be a cheap source of

natural antioxidants. Fruit pomace has been shown to contain especially rich levels of disease fighting phenolics (Vattem, Ghaedian, & Shetty, 2005). So the portion that was frequently thought of as waste product may actually be the most nutritious part of the fruit. It is packed with powerful antioxidants that have the potential to help fight heart disease (Fruit Essentials, 2006).

Cranberries to Consumer

Once the cranberries are harvested, they are shipped to various processing plants. At the plants, the berries are initially cleaned and then sorted by color and their ability to bounce. About 5% of all cranberries are packaged and distributed to the market as fresh fruit. The remaining 95% are processed into juice drinks, sauces, or sweetened dried products. These products are distributed to various markets across the country and are available for consumers to purchase and eat (Cranberry Marketing Committee, 2007). Studies have shown that an increased consumption of cranberries and cranberry food products have the potential to help reduce the risk factors for cardiovascular disease (Vattem, Ghaedian, & Shetty, 2005).

Cardiovascular Disease (CVD)

Cardiovascular disease has been the leading cause of death since the early 1900's. The various forms of cardiovascular disease include hypertension, atherosclerosis, coronary heart disease (CHD), stroke, rheumatic heart disease, and congestive heart failure. Cardiovascular disease is a great public health problem with costs reaching over 327 billion dollars in the year of 2000 (Escott-Stump & Mahan, 2004). The majority of deaths related to CVD are caused by CHD.

Coronary Heart Disease

Coronary heart disease (CHD) is the result of obstructed blood flow to the blood vessels that surround and serve the heart. This obstruction causes impaired and inadequate blood flow to the heart. The underlying cause for CHD is often attributed to atherosclerosis (Escott-Stump & Mahan, 2004).

Atherosclerosis

The beginning stages of atherosclerosis start during early childhood and can be defined as a process of thickening and narrowing of the arteries that is caused by accumulation of lipids (American Heart Association, Inc., 2007). There are many factors that contribute to the pathogenesis of atherosclerosis. Some major contributors include high blood cholesterol levels, oxidized low-density lipoprotein (LDL), high blood pressure, cigarette smoking, diabetes, obesity, and diets that are high in saturated fat and cholesterol. All of these contributors cause damage to the arterial walls. Atherosclerosis is an inflammatory response to these injuries (Escott-Stump & Mahan, 2004). Cholesterol is vital for the human body to work properly: however, too much cholesterol circulating in the blood can be harmful to the health of the heart.

Cholesterol

Cholesterol is a sterol, which means it is a combination of a steroid and an alcohol. It is essential for many functions in the human body including building and maintaining cell membranes, and aiding in creating bile that aids in the digestion of fats. It is a precursor of vitamin D and many steroid hormones. Cholesterol is lipid based so it is insoluble in the blood. For this reason, cholesterol uses lipoproteins as a transport mechanism in the blood. Cholesterol is transported from the liver and excreted out of the

body through bile acids and bile salts (Groff, Groupper, & Smith, 2005). Cholesterol is vital to the human body, but accumulation of cholesterol in the blood can be harmful. A blood test can be performed to determine an individual's lipid profile. Results of a lipid profile test can help a health care provider better assess potential health risks to patients relative to serum cholesterol levels.

Lipid Profile

A lipid profile usually consists of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and total triglycerides (TG). A desirable lipid profile is a total cholesterol level less than 200 mg/dl, LDL cholesterol less than 130 mg/dl, HDL cholesterol greater than 40 mg/dl, and triglyceride level less than 150 mg/dl.

Total Cholesterol

Total cholesterol is the accumulation of cholesterol found in all lipoproteins circulating in the blood stream. This includes low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low density lipoprotein (VLDL). Increased levels of total cholesterol are directly related to coronary heart disease (CHD). Age, diets high in saturated fat and cholesterol, genetics, increased body weight, and decreased physical activity are all major factors that contribute to elevated levels of total cholesterol.

HDL Cholesterol

HDL cholesterol is often known as the "good cholesterol". HDL cholesterol helps transfer the LDL cholesterol to the liver where it then is excreted out of the body through bile acids (Groff, Groupper, & Smith, 2005). It is believed that HDL cholesterol plays a role in protecting the heart. Physical activity and pharmological treatment of

fibrates and nicotinic acid have been shown to increase levels of HDL concentrations (Couillard et al., 2006). Fibrates are a class of carboxylic acids. Fibrates have been shown to be effective at increasing HDL cholesterol. Nicotinic acid is a form of the water soluble vitamin B^3. Nicotinic acid has been shown to improve all HDL concentrations in the blood (National Heart Lung and Blood Institute, 2007).

Triglycerides

Triglycerides are a highly concentrated source of energy. The majority of fat in the body is in the form of triglycerides. The lipoproteins that are rich in triglycerides include chylomicrons and VLDLs (Groff, Groupper, & Smith, 2005).

LDL Cholesterol

LDL cholesterol is often known as the "bad cholesterol" and is the primary carrier of cholesterol in the bloodstream. Increased levels of LDL cholesterol are often linked to an increase risk for CHD (Escott-Stump & Mahan, 2004). An accumulation of circulating LDL cholesterol in the blood can lead to increased levels of oxidation.

LDL Oxidation

Under normal conditions, LDL cholesterol circulates in the blood, but is not oxidized. Many environmental factors and stress to the body can affect the oxidation of LDL in the blood. Macrophage cells take up oxidized LDL cholesterol. When there is an abundance of oxidized LDL cholesterol, macrophage cells become overloaded with lipids. They then start to form foam cells. Foam cells create fatty streaks that can accumulate and cause plaque formation in the arteries, which lead to atherosclerosis (McEnemy & Young, 2001). Supplementations of antioxidants, such as vitamin E, have been suggested by some healthcare professionals in order to slow down the LDL

oxidation process and ultimately reduce the risk for developing cardiovascular disease (Groff, Groupper, & Smith, 2005). It is unknown as to what the precise mechanism of LDL oxidation is. However, it has been thought that an abundance of free radicals circulating in the blood may induce LDL oxidation (Carr, McCall, & Frei, 2000).

Free Radicals

Accumulation of free radicals in the blood stream is often linked to an increased risk for heart disease. Free radicals are molecules that possess at least one unpaired electron. Free radicals are produced when a non-radical species loses one electron, thereby leaving one unpaired electron. Free radicals that contain oxygen are often referred to as reactive oxygen species (ROS). ROS act as oxidizing agents and can be formed from exposure to harmful substances such as smog, ozone, chemicals, drugs, radiation, high oxygen, and many others (Halliwell, 2001). ROS can initiate chain reactions that have the potential to create hydrogen peroxide. Increased concentrations of ROS and hydrogen peroxide in the body can lead to cellular destruction. This is very damaging to the body and can lead to many different types of diseases, including CVD (Groff, Groupper, & Smith, 2005). It has been shown that increased intake of antioxidants can help reduce the amount of free radicals circulating in the blood (Couillard et al., 2005).

Antioxidants

An antioxidant is any substance that when present can significantly delay or prevent oxidation of a molecule in the human body (Halliwell, 2001). Our bodies produce antioxidants endogenously in order to protect ourselves against free radicals. Antioxidants function as inhibitors of both initiation and propagation steps of oxidation

leading to protection of oxidative damage. Antioxidants are great free radical scavengers and can be found as vitamins A, C, and E, proanthocyanins, and anthocyanins, which are obtained from wide variety of fruits, vegetables, and their seeds. Increased intake of fruits and vegetables has been linked to a decreased risk for many chronic diseases, including CVD (Bagchi, Sen, Bagchi, & Atalay, 2004). A diet rich in antioxidants and polyphenols has been shown to reduce the risk from many cardiovascular diseases (Manach et al., 2004).

Polyphenols

Polyphenols are a class of antioxidants found in a variety of plants. They are quite abundant and play an important role in the human diet (Manach, et al., 2004). Increased intake of polyphenols has been thought to prevent a variety of diseases that are linked to oxidative stress including CVD. Interest in polyphenols has been increasing rapidly due their strong antioxidant potential. Polyphenols help protect plants against biological and environmental stresses. Fruits such as apples, cranberries, grapes, raspberries, and strawberries are rich in polyphenolic compounds (Vattem et al., 2005). Polyphenols can be broken down into phenolic acids, flavonoids, lignans, and stilbenes (Manach et al., 2004).

Phenolic acid Chemistry of Cranberries

Cranberries contain hydroxy cinnamic acids, which are a form of phenolic acids. Hydroxy cinnamic acids contain both caffeic and quinic acids. The caffeic acid structure is illustrated in Figure 1. When caffeic and quinic acid are combined they form chlorogenic acid (Figure 2), which is found in a wide variety of fruits. Phenolic acids can contribute to sensory and nutritional qualities of fruits. Phenolic acids act as

antioxidants by acting as free radical acceptors. They also can help protect lipids both in cells and in food products (Packer & Rice-Evans, 1998).

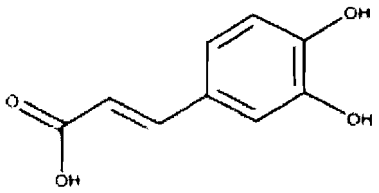

Figure 1. Caffeic Acid

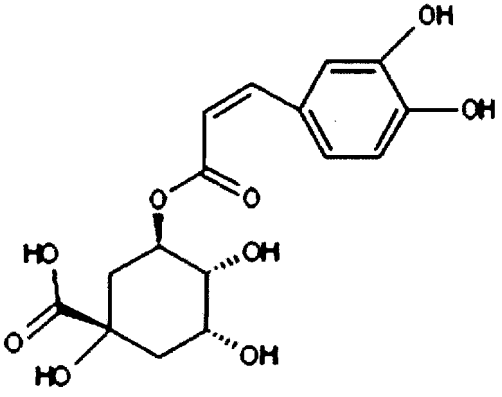

Figure 2. Chlorogenic acid

Flavonoid Chemistry of Cranberries

Flavonoids are a sub-group of polyphenols that are derived from plant products. Flavonoids are formed from the aromatic amino acids, phenylalanine and tyrosine, along with acetate units. Flavonoids are strong antioxidants due to their electron-donating properties. Flavonoids have at least two phenyl rings that are separated by a pyran ring. Their antioxidant capabilities depend on which part of a molecule has the most electron donating properties. Flavonoids also have the ability to scavenge free radicals through electron transfer reactions with the formation of stable intermediates (Reed, 2002). In most cases this is the B-ring of the flavonoid structure, and the A and C ring are most likely used for enzyme activation. This causes a maximum beneficial effect because the two ring structures can react independently (Packer & Rice-Evans, 1998). The cranberry flavonoids are members of three groups; anthocyanins, flavonols, and proanthocyanins. The A and the B ring attach by the three carbon bridge on the C ring (Figure 3). The numbers on the rings represent position where functional groups attach.

Anthocyanins

Anthocyanins are very abundant in edible berries. They are responsible for the pigment of the berry and serve as a natural antioxidant. Anthocyanins vary in the number and position of -OH groups, sugar groups, and other functional groups (Figure 4).

Studies have shown that anthocyanins found in berries are effective at reducing oxidative stress. Anthocyanins also act as an anti-inflammatory agent and help with platelet aggregation. These properties protect the heart by maintaining good blood flow (Zafra-Stone et al., 2007).

A research study examined the antioxidant potential of the anthocyanins found in OptiBerry extract that included fruit extracts from wild blueberry, billberry, cranberry, elderberry, raspberry seeds, and strawberry. They measured the antioxidant potential of each type of fruit extract in vitro by determining their oxygen radical absorbing capacity (ORAC) values. The cranberry fruit extract exhibited higher ORAC values than both elderberry and raspberry seed fruit extracts. OptiBerry extract received the highested ORAC value and exhibited superior antioxidant properties compared to vitamin C, E, and A. (Bagchi et al., 2004). Through this research it is thought that a combination of berry extracts exhibits the highest antioxidant capacity. This suggests that a combination of berry extracts, rather than one individual berry extract is optimal for reducing oxidative stress to the body.

Another research study examined the anti-atherosclerotic activity of anthocyanins when supplemented in hamsters. Hypercholesterolemic hamsters were fed 10 mg per 1 gram of body weight of a berry extract including fruit extracts from wild blueberry, billberry, cranberry, elderberry, raspberry seeds, and strawberry for 12 weeks. The hamsters that received the berry extract saw an 8% decrease in weight. The atherosclerotic index, which is the percentage of the aorta covered with foam cells, was significantly lowered by 36.6% in the hamsters that received the berry extract supplement. This research suggests that the berry extract may have the ability to reduce the incidence of atherosclerosis by its reduction in foam cell formation (Zafra-Stone et al., 2007).

Flavonols

Flavonols are the most common flavonoid found in foods. Flavonols are located mainly in the leaves and the outer parts of plants and can be found in both fruits and vegetables (Manach et al., 2004). One of the main representatives of flavonols is quercetin. Quercetin has two hydroxyl groups attached to the B ring (Figure 5). Compounds with dihydroxyl groups on the B ring have been shown to be more stable and exihibit antioxidant activity (Terao, Piskula, & Qing, 1994). Quercetin is found in cranberries and has been shown to be a very potent anti-oxidant (Manach et al., 2004).

A study that was conducted in Finland on 800 elderly men looked at the effects on the intake of quercetin and four other flavonols. The participants were followed for five years. The mean intake of flavonoids was found to be 20.1 millagrams per day (mg/d). The three primary flavonols consumed were quercetin (15.4 mg/d), kaempferol (3.6 mg/d), and myricetin (0.9 mg/d). The researchers determined that flavonol intake and mortality from coronary heart disease was inversely related. This suggests that a diet rich in flavonols may help reduce deaths related to CHD (Hertog et al., 1993).

Proanthocyanins

Proanthocyanins are also known as condensed tannins and are responsible for the astringency in fruits such as grapes, peaches, apples, pears, and berries (Manache et al., 2004). Proanthocyanins contain polymers that are made up of multiple anthocyanin units (Figure 6). Proanthocyanins have the highest degree of polymerization of all the flavonoids that are present in cranberries. Proanthocyanins have been thought to have up to 50 or more sub-units attached to their flavonoid ring structure. This increased degree

of polymerization has been linked to a greater ability to inhibit LDL oxidation (Cunningham, et al., 2001).

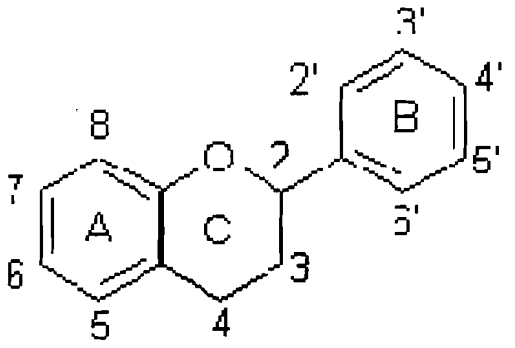

Figure 3. Ring structure of flavonoids

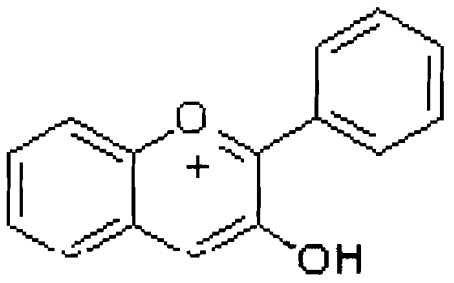

Figure 4. Anthocyanin chemical structure

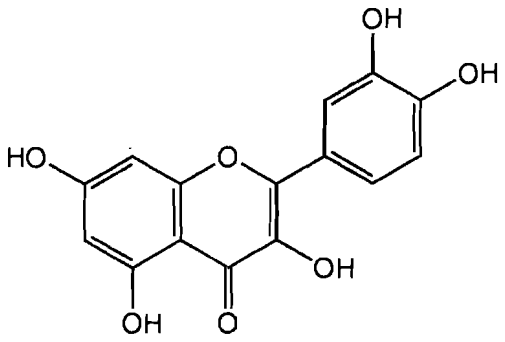

Figure 5. Quercetin's chemical structure

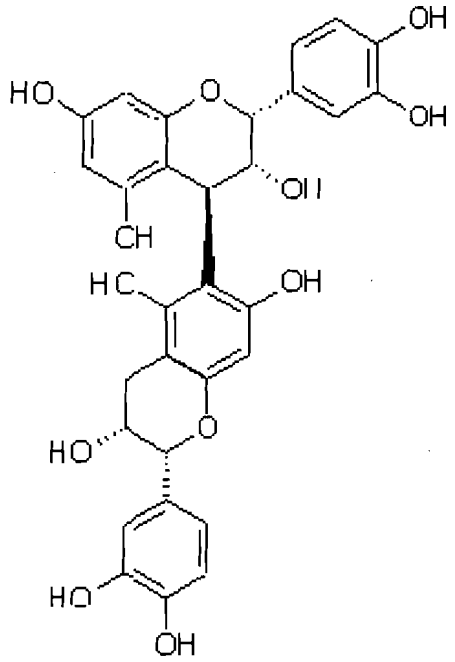

Figure 6. Proanthocyanin chemical structure

Flavonoids Inhibit LDL oxidation

An increase in levels of LDL oxidation in the body is a risk factor for the development of atherosclerosis. Inhibiting LDL oxidation helps prevent the development of atherosclerosis. When oxidized LDL accumulates inside the intima, which is the internal wall of the arteries of the heart, macrophage cells are produced and the macrophages then initiate the formation of foam cells. The formation of foam cells leads to atherosclerosis. Flavonoids are able to associate with LDL and be active inside the intima. Antioxidants that are able to associate and be active inside the intima have the greatest protective capabilities against atherosclerosis (Reed, 2002). A study that looked at the ability of cranberry extract to inhibit LDL oxidation in vitro showed that when LDL oxidation took place in the presence of the 1.00 ml 0.10% diluted cranberry extract, the formation of thiobarbituric acid reactive substances (TBARS) were decreased. TBARS are an end product of lipid oxidation and are often used as a marker for oxidative stress. Increased levels of TBARS correlate with increase risk for atherosclerosis. This study suggests that the cranberry extract has the ability to inhibit the oxidation of LDL particles thus possibly reducing the risk for developing atherosclerosis (Wilson, Porcari, & Harbin, 1998).

A similar study looked the ability of flavonoids fractions from cranberry juice powder to inhibit Cu^{2+} induced LDL oxidation in vitro. The study fractioned the cranberry juice powder into six separate fractions using high performance liquid chromatography (HPLC). The researchers looked at in individual components of hydroxy cinnamic acids, anthocyanins, and proanthocyanins and their ability to individually inhibit LDL oxidation. The study concluded that the hydroxy cinnamic

acids and anthocyanins did not significantly inhibit LDL oxidation, however the proanthocyanins found in the cranberry juice powder did significantly inhibit LDL oxidation. This was shown through an increased lag time of Cu^{2+}-induced LDL oxidation. The proanthocyanins in the cranberry juice powder had a lag time of 225 minutes compared to a lag time of 100 minutes in the control. This study also showed that the higher the polymerization of the proanthocyanins the greater the lag time of the Cu^{2+} induced LDL oxidation. This indicates that the degree of polymerization and the structure of the proanthocyanins influence the antioxidant capacity of the cranberries. Having an increased antioxidant capacity would help fight heart disease by reducing the amount of circulating free radicals in the blood stream. (Cunningham et al., 2001). All of the research conducted on the different classes of polyphenols found in cranberries suggest that they may help reduce the risk for developing heart disease and also aid in the treatment and therapy of heart disease patients. Recently, it has been discovered that the compounds found in cranberry seeds and pomace, which are by-products of cranberry juice and cranberry sauce processing could contain polyphenols that are more potent antioxidants for the body than the flavonoids found in processed cranberries. Therefore increased consumption of antioxidant rich cranberry seeds may be linked to decrease risk for heart disease.

Cranberry Seed Oil

Due to the discovery that cranberry seeds are rich in antioxidants, food engineers have recently started to extract the oil from the cranberry seed. The cranberry seed oil that is obtained from this extraction process has an exceptional nutrient and antioxidant profile. It is the only edible oil found to have a natural occurring 1:1 ratio of omega-6

polyunsaturated fatty acids (n-6 PUFA) to omega-3 polyunsaturated fatty acids (n-3 PUFA) (Fruit Essentials, 2006).

Engineering of Cranberry Seed Oil

The bioavailability of the powerful antioxidants found in cranberry seed oil is maintained by utilizing specialized extraction methods. Cranberry seed oil is extracted utilizing the cold pressed method. Cold pressed oil extraction takes place in a temperature controlled environment where the temperature is not allowed to exceed 120 degrees Fahrenheit, seeing that heat is not desirable during the pressing of the seeds, as it can destroy the powerful nutrients that are found in the seeds (Fruit Essentials, 2006). Cold pressing oils is gaining popularity due to consumer demand and a desire for safe and natural food products. The process of cold pressing oil requires no organic solvent so the end product is oil that is chemically contaminant-free (Damude & Kinney, 2007). Cold pressing also maintains the integrity of the antioxidant and fatty acid profile. In cranberry seed oil extraction, cold pressing ensures that all of the long chain proanthocyanins are maintained. Also, in order to reduce oxidation, nitrogen is pumped through the extractor every five seconds, thereby improving the stability and shelf life of the oil (B. Lager, personal communication, August 17, 2007). Cold-pressed oils have greater levels of natural antioxidants than oils that go through traditional oil presses, while still being able to maintain an acceptable shelf life and product safety levels. The large antioxidant levels in cold pressed oils are thought to contribute to a decrease risk for heart disease (Adams et al., 2003) Cranberry seed oil contains increased levels of omega-3 fatty acids, a 1:1 ratio of omega-6/omega-3 fatty acids, phytosterols, and all forms of the antioxidant vitamin E. Numerous studies have shown that each of these components

of cranberry seed oil have the ability to lower total blood cholesterol which indicates that cranberry seed oil has a strong potential to reduce cholesterol and in turn help fight heart disease. Omega-3,-6, and-9 fatty acids are essential fatty acids (EFAs). There are considered essential because they cannot be synthesized in the human body. Therefore humans must consume EFAs in their diet or as a supplement.

Essential Fatty Acids

Essential fatty acids (EFAs) have gained attention in recent years. There are three known essential fatty acids: omega-3, - 6 and -9. The fatty acids are name based on the position of the carbon to carbon double bond. Omega-3 fatty acids can be found in flaxseed and fish oils such as salmon, tuna, and lobster. Omega-6 fatty acids are found in enriched cereals and grains, vegetable oils, eggs, and poultry. Dietary sources of omega-9 fatty acids or oleic acid include olive and canola oils. Americans consume much more omega-6 fatty acids from vegetable oils and cereal grains than omega-3 fatty acids (Escott-Stump & Mahan, 2004).

American Diet

Various studies have reported that drastic changes have occurred in the human diet, especially over the past 150 years. The studies show major changes in the type of essential fatty acids and the antioxidants, vitamin C and vitamin E which we now consume. Centuries ago humans consumed lean meat, fish, green leafy vegetables, fruits, nuts, berries, and honey. These foods were the staples that the human's genetic nutritional requirements are based upon. These foods are also rich in omega-3 PUFA (Simpoulous, 1999).

Approximately 150 years ago, there was major change in the American diet with the advent of the large-scale production of vegetable oils. The inventions of the Expeller®, the continuous screw press, steam vacuum deodorization, and solvent extraction made large-scale production of vegetable oils more economical and efficient. There was a drastic increase in Americans consumption of omega-6 PUFA. This impacted Americans greatly by increasing their omega-6/omega-3 ratio (Simpoulous, 2002).

Omega-6/Omega-3 Polyunsaturated Fatty Acid Ratio

History suggests that humans evolved from consuming a diet very rich in omega-3 fatty acids. Over 40,000 years ago during the Paleolithic period, humans consumed a diet very low in trans-fat and saturated fat and with greater amount of green leafy vegetables and fruits than the Western diet today. The increased amounts of green leafy vegetables and fruits provided humans with more vitamin E, vitamin C, and other antioxidants. During the Paleolithic period, humans also consumed more calcium and potassium and less sodium. It has been thought that during the Paleolithic era humans consumed close to a 1:1 ratio of omega-6 to omega-3. Today, Americans as a whole are deficient in omega-3 polyunsaturated fatty acids (PUFA), but eat excessive amounts of omega-6 PUFA. The estimated omega-6/omega-3 PUFA ratio in the American diet today is 15-16:1 (Simpoulous, 1999). Even though the diet of humans has changed considerably, the genetic profile of humans has remained quite constant throughout the years changing as little as 0.005% in the last 10,000 years. The American diet today differs greatly from the diet that our genetic profile was selected from. Currently the

American diet has been hypothesized to be contributing to the increase in cardiovascular disease, cancer, inflammatory diseases, and autoimmune diseases (Simopoulos, 1999).

High levels of omega-3 PUFA and a low omega-6 PUFA/omega-3 PUFA ratio have been shown to have suppressive effects on these disease states. The recommended omega-6 PUFA/omega-3 PUFA ratio has been estimated to be 2:1 to 3:1; however, a 1:1 ration is optimal (Escott-Stump & Mahan, 2004). This is approximately is four-to- five times lower than what Americans are currently consuming. Recently, omega-3 PUFA has been supplemented in many commonly consumed food products such as butter spreads, eggs, poultry, and fish. It is important for Americans to decrease the omega-6/omega-3 PUFA ratio in order to decrease the risk of the diseases stated above.

Omega 3-Fatty Acids and Cardiovascular Disease

Several studies have concluded that there is an inverse relationship between omega-3 PUFA and mortality caused by coronary heart disease due to their hypolipidemic and antithrombotic effects. Omega-3 PUFA cardio protective effects have been thought to be attributed to two essential fatty acids, eicosapentaenoic acid (EPA) and docosahexanoid acid (DHA) (Bucher et al., 2002). Both EPA and DHA contain many health-promoting capabilities. DHA is a main component of human's cell membranes, such as retinal and brain membranes. It also has been thought to play an important role in cognitive development of infants and the mental health of adults. EPA has been shown to have many heart protecting benefits. EPA and DHA regulate many vital metabolic functions of the human body that include inflammatory responses, blood pressure control, and blood clotting (Damude & Kinney, 2007).

The link between n-3 supplementation of 2-10 g/d and reduced risk for coronary heart disease is supported through several different mechanisms. Omega-3 PUFAs reduce endothelium dysfunction by reducing sympathetic over activity, which is a risk factor for heart disease. A research study was conducted where men with CHD disease supplemented 5.1 g/d of n-3. After six months of supplementation participants showed enhanced vasodilatation, which relaxes the blood vessels and causes the blood to flow more freely throughout the body when n-3 was supplemented at 5.1 g/d for six months. They act as an anti-inflammatory by inhibiting monocyte adhesion and suppressing the inflammatory mediatory of thromboxane A_z. Thromboxane A_z is a prostaglandin hormone which induces platelet aggregation and vasoconstriction, which would decrease blood flow and increase clot formation (Bucher et al., 2002).

Phytosterols and Cholesterol Levels

Cranberry seed oil is rich in the phytosterols; stigmasterol, campesterol, and beta-sitosterol. Studies in humans on unrestrictive diets have shown that supplementing phytosterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption (DeQuattro, 2000).

Plant sterols have been shown to reduce LDL cholesterol as much as 20% when supplemented at 2 grams per day. Plant sterols have also been shown to affect non-lipid factors for atherosclerosis (Naruszewicz & Kozlowska-Wojciechowska, 2007).

A research study was conducted examining the effects of plant sterols on cholesterol levels in young adults. The subjects supplements 3g of plant sterols a day for eight weeks. The researchers found a 12% decrease in LDL concentration and also saw significant reductions in platelet aggregation. In a separate study plant sterols were

supplemented in humans at 3g/d. Researchers saw decreased levels of oxidized LDL by 20-22%. The mechanism of these actions is thought to be due to limitation of cholesterol absorption (Naruszewicz & Kozlowska-Wojciechowska, 2007).

A separate research study examined the effects of free phytosterols on plasma and liver cholesterol when given to gerbils that were fed a diet containing 0, 0.05, 0.1, 0.15, or 0.5% (g/100 g) cholesterol. The gerbils consumed a diet of 0.75% (g/100g) plant sterols. The study concluded that plant sterols effectively blocked cholesterol absorption and was effective in lowering total serum cholesterol and LDL cholesterol (Hayes, Pronczuk, Wjendran, & Beer, 2002).

Another study observed the effects of beta sitosterol supplementation in 15 young men who had suffered from previous heart attacks. The men were given 12-18 grams of beta sitosterol per day. The men in this study had sustained reductions in total serum cholesterol. When these observations were compared with control observations of effects of diet, weight maintenance, and placebos there was an increase in confidence that the reduction of total cholesterol was due to the beta sitosterol supplementation (Farquhar, Smith, & Dempsey, 1956).

Vitamin E

Cranberry seed oil contains all biologically active forms of vitamin E. Vitamin E is a fat soluble vitamin having eight different compounds or isomers. These eight compounds contain a phenolic functional group that is located on the head of the molecule and contains an attached phytyl side chain. There are two classes that the eight compounds are divided into; the tocopherols, and the tocotrienols. The tocopherols have saturated side chains with 16 carbons (Figure 7). Tocotrienols have unsaturated side

chains with 16 carbons (Groff, Groupper, & Smith, 2005). The chemical structure of all four tocotrienols is illustrated in Figure 8. Cranberry seed oil contains all 8 isomers of vitamin E.

Several clinical studies have concluded that 400-800 IU/d of vitamin E is an effective treatment for coronary artery disease. Vitamin E does this by reducing the liver's production of cholesterol, inhibiting LDL oxidation, and by being an effective anti-coagulant (Pedersen, 2000).

A large cohort study of both men and women with CVD was conducted. Researchers in this study found that supplementation of 800 IU of vitamin E along with other antioxidants reduced the susceptibilty of LDL to oxidation (Jial & Grundy, 1993).

A double blind study was conducted on patients with confirmed coronary heart disease. A total of 2,002 patients were randomly assigned to two separate groups ; an experimental group and a placebo group. The experimental group received a dose of vitamin E of either 400 or 800 IU for a total of 510 days. The results showed that vitamin E reduced non-fatal heart attacks by 77% (Crockcroft & Chowienczyk, 1996).

These combined research findings suggest that vitamin E may be effective at reducing the risk of atherosclerosis and coronary heart disease.

Even with these benefits mentioned to date there has been no published research conducted on the potential benefits of cranberry seed oil supplementation on cholesterol levels in human or animal subjects. It can be hypothesized that due to the components that are found in cranberry seed oil it would have the potential to effectively lower total cholesterol.

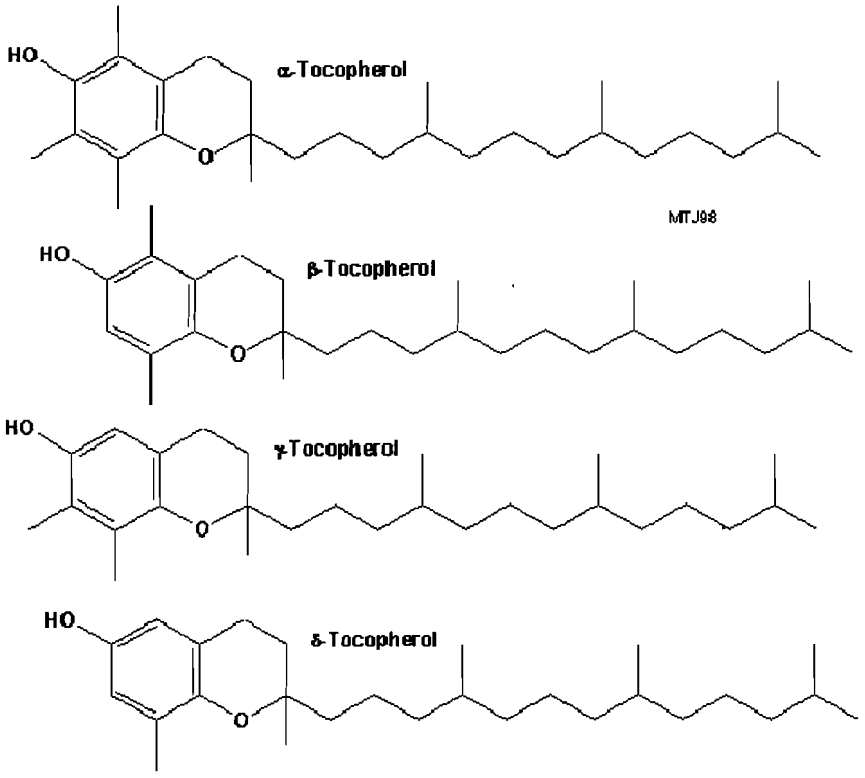

*Figure 7.*The chemical structure of all four forms of tocopherols

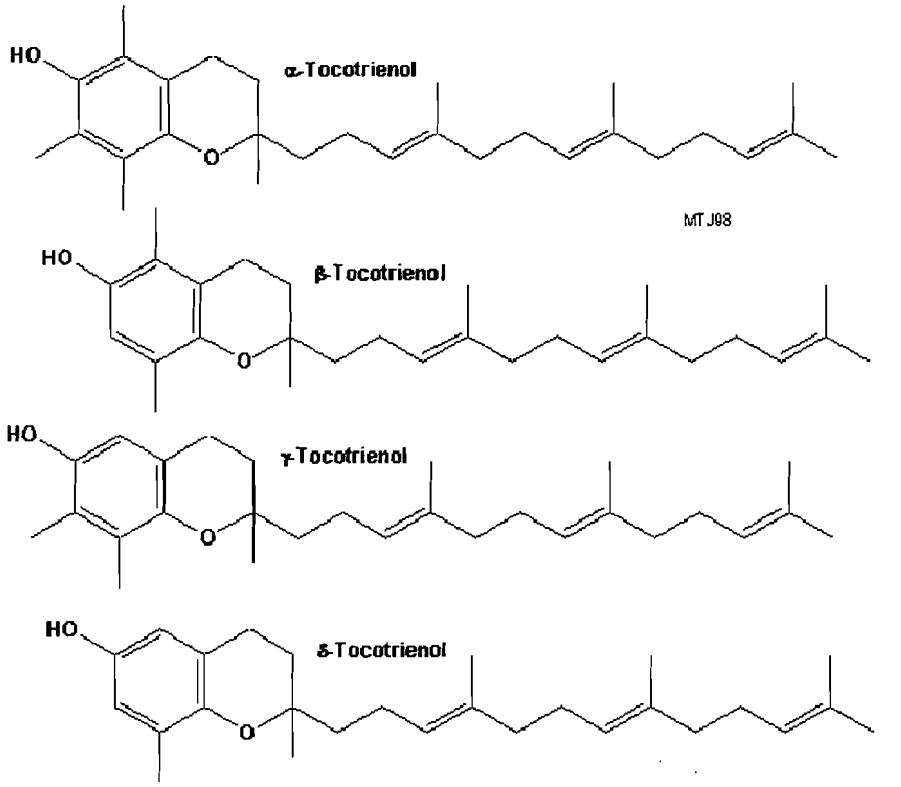

Figure 8. The chemical structure of all four forms of tocotrienols.

Chapter III: Methodology

The purpose of this research study was to determine if the consumption of cranberry seed oil would have an effect on human participant's lipid profiles, which would include total blood cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

Subject Selection and Description

A total of 19 participants were utilized in this study. The participants were volunteers that were selected after they responded to an email that was sent to the UW-Stout faculty and students outlining the study. Interested candidates needed to meet the qualifications of the study which included that they were 18 years of age or older , were not taking any cholesterol altering medications and had a total blood cholesterol level greater than 200 mg/dl. The interested candidates were informed about the details of the study through an informative email explaining the time, date and location of the initial blood draw. This e-mail can be found in Appendix B. Lipid profile tests were obtained for all interested participants prior to the start of the study through a blood draw by a certified laboratory technician. If the interested particpants had a total blood cholesterol level greater than 200 mg/dl, they were asked to continue the study by taking the cranberry seed oil. At four and eight week intervals, the participants were asked to return to the Nutrition Assessment Lab at UW-Stout for follow-up lipid profile testing.

Samples

The samples used in this study were canola oil and cranberry seed oil. Wesson® brand 100% canola oil from lot number 69084-FGC GF2566 was used in the control group for the full eight weeks of the study. The cranberry seed oil used in the experimental group was manufacutred by BGL, LLC (Wisconisn Rapids, WI). The first

4 week supply of the cranberry seed oil given to participants was from the lot number CBSDO-07248. The second 4-week supply of cranberry seed oil was from the lot number CBSDO-07290.

Equipment

All equipment used for the blood draw was inspected for possible defects and expiration dates. Equipment used included evacuated blood collection tubes, disposable vacutainer holders, gauze pads, tourniquet (single use – disposable – latex free), biohazard needle disposal unit, band-aids, cloth/tape, or stretch bandage wrap, latex-free gloves, and syringes.

Blood withdrawal procedure

Blood testing was completed prior to week one of the study and at both weeks four and eight of the study. The estimated time of each test session was five minutes for each individual. A certified laboratory technician from RCMC performed a blood draw on each participant using venipuncture. Venipuncture, or the puncturing of a vein with a needle, is the most common method of procurement of blood specimens for analysis. Venipunctures are performed whenever laboratory tests require serum, plasma, or whole blood (Nyland, 2007). The certified lab technician practiced standard safety precautions. The technician wore gloves and a lab coat while performing the venipunctures. The disposable holders and needles were immediately discarded in the appropriate vascusafe containers and the syringes and blood transfer devices were immediately discarded in the closest needle disposal container. The procedure for the venipuncture was as follows. The lab technician located the venipuncture site and the tourniquet was applied 3-4 inches above the intended venipuncture site. The patient was then asked to close their

hand. The venipuncture site was then cleaned with the use of 70% ispopropyl alcohol. The patient's arm was positioned so that it is fully extended at a 15-20 degree downward angle, which prevents backflow of blood from the tube. The cap of the needle was removed. The patient's arm was firmly taken by the lab technician with the use of the thumb and forefinger. The patient was given a warning statement before the venipunture was performed. The needle was aligned in the same direction as the vein and was facing upward. The tourniquet was released as soon as possible after the blood began to flow inside the tube. The tube was pushed into the end of the needle piercing the rubber stopper. The patient was then instructed to open his/her fist. As blood flowed into the collection tube following the puncture, the flow was monitored until collection was completed and blood ceased to flow. After the collection was complete the tube was removed from its holder. With gentle, steady pressure, the plunger was pulled back until the desired amount of blood was obtained. The blood was transferred from the syringe to the tubes using a blood transfer device. The needle was then removed from the syringe and the blood transfer device was attached to the syringe in order to fill evacuated tubes. After the venipuncture procedure was complete, all of the participant's blood samples were sent to be analyzed, and their lipid profiles were determined by a certified laboratory technican from Red Cedar Medical Center of Menomonie, Wisconsin.

Data Collection

In September, 2007 permission to conduct this research study was granted from the University of Wisconsin-Stout Institutional Review Board. The participants were 19 volunteers that were recruited through the UW-Stout faculty and student email. Upon

response, interested participants were given information regarding details of they study and the date and time of the first initial blood draw.

All participants signed a consent form prior to the initial blood draw, which indicated testing procedures, confidentiality, potential risks, and benefits of the research study. Assurance that the all the participants' participation was voluntary was stated and reinforced through the consent form, email, and all three blood draw dates.

All interested participants were asked to have an intial blood draw administered by a certified laboratory technican from Red Cedar Medical Center in order to determine their lipid profile. Participants were advised to have not ingested food, caffeine, or alcohol twelve hours prior to the draw. The initial blood draw took place in the Nutrition Assesment Lab located in room 423 of the Home Economics building at the University of Wisconsin-Stout. Participants whose blood cholesterol was not over 200 mg/dl were informed that they did not fit the desired qualifications for the study. If participants were unable to attend the scheduled blood draw they were allowed to go to Red Cedar Medical Center of Menomonie, Wisconsin at a time that was convienient for them.

The study was a randomized blind-controlled clinical trial. All qualified participants were randomly placed into a control group or an experimental group. The participants were unaware of which group that they were in. The control group was to supplement canola oil and the experimental group was to supplement cranberry seed oil. All participants were educated on the study. They all received an illustrated brochure explaining cholesterol, cranberries, and cranberry seed oil (Appendix C). The participants were then given a four week supply of their designated oil and asked to ingest one tablespoon per day. The participants were also asked to refrigerate the oil and

not to heat the oil above 250°F. At four and eight weeks both the control and experimental groups were asked to return to the Nutrition Assessment Lab at UW-Stout for follow-up for lipid profile testing. At the four week follow up blood draw all participants received an additional 4-week supply of their designated oil.

Data Analysis

All of the participants lipid profile tests were analyzed at Red Cedar Medical Center, Menomonie, Wisconsin. The lipid profile results from weeks one, four, and eight were entered into Statistical Package for Social Science (SPSS) statistical software to interpret all of the data obtained. Means and standard deviation for lipid profile were calculated at week one, four, and eight.

Chapter IV: Results

The purpose of this study was to determine the effects of supplementing cranberry seed oil to a population who had borderline high-to-high total blood cholesterol levels (>200 mg/dl). The effects would be measured by examining lipid profile test of human participants. A lipid profile test includes total cholesterol, LDL cholesterol, triglycerides, and HDL cholesterol. The purpose of this research study was to determine if there were significant reductions in total cholesterol, LDL cholesterol, and total triglycerides and significant increases in HDL cholesterol after supplementing cranberry seed oil.

Initially, 22 interested participants underwent lipid profile testing. After determining if the participants met the qualification of having a total cholesterol > 200 mg/dl there were a total of 20 participants. All 20 participants showed up for the four week follow up blood draw. Only 19 participants showed up for the final blood draw.

There were a total of 19 participants who completed the study with 9 being in the control group and 10 being in the experimental group. At weeks one, four, and eight of the study data for lipid profile results was collected. Paired sample t tests and independent sample t tests were determined. There were no significant changes in lipids within each group or between groups in any comparison when significance was set at 0.05.

Lipid Profile

The National Cholesterol Education Program (NCEP) within the National Institute of Health (NIH) recommends that the desirable level of total cholesterol is <200 mg/dl. The NCEP classifies a total cholesterol of 200-240 mg/dl as borderline high and a total cholesterol of >240 mg/dl as high. An optimal LDL concentration according to the NCEP is <100 mg/dl, and classifies 100-130 mg/dl as near optimal, 130-159 mg/dl as

borderline high, 160-189 as high, and ≥ 190 as very high. The NCEP recommends a concentration of HDL cholesterol over 40 mg/dl (National Institute of Health, 2005). Triglyceride levels are classified normal being < 150 mg/dl, borderline high being 150-199 mg/fl, high being 200-499 mg/dl, and very high as ≥ 500 mg/dl (Escott-Stump & Mahan, 2004).

Total Cholesterol

The mean week one total cholesterol value for the experimental group supplemented with cranberry seed oil was 240.6 mg/dl with a 5.7 mg/dl decrease at week four, compared to a mean week one total cholesterol value of 233.2 mg/dl for the control group having 3.2 mg/dl increase in total cholesterol at week four (Table 1). The mean week eight total cholesterol value for the experimental group was 247.9 mg/dl, compared to the mean week eight total cholesterol value of 241.2 mg/dl in the control group (Figure 9).

There were no significant reductions in total blood cholesterol for the human subjects who were in the experimental groups. However, subjects in the experimental group did see slight improvements after week four of cranberry seed oil supplementation. There also were no significant differences in total cholesterol between the control and experimental groups during any time period.

LDL Cholesterol

The mean week one LDL cholesterol value for the experimental group was 149.3 mg/dl with a 5.4 mg/dl decrease to 143.9 mg/dl at week four and a decrease of 1.4 mg/dl to 147.9 mg/dl at week eight of cranberry seed oil supplementation. The control group had a mean week one LDL cholesterol value of 146.3 mg/dl with a 4 mg/dl increase to

150.3 mg/dl at week four and a 6.7 mg/dl increase to 153 mg/dl at week eight (Figure 10).

There were no significant reductions ($p<0.05$) in LDL cholesterol in those supplementing cranberry seed oil, however, it was noted that there were slight reductions in LDL cholesterol. There also were no significant differences in LDL cholesterol between the control and experimental groups.

HDL Cholesterol

The mean week one HDL cholesterol value for the experimental group was 60.6 mg/dl with a 3.7 mg/dl increase to a mean HDL cholesterol value of 64.3 mg/dl at week four and a 1.5 mg/dl increase to a mean HDL cholesterol value of 62.1 mg/dl at week eight. The control group demonstrated no change in HDL cholesterol value at week four (65.1 mg/dl), but did have a slight increase of 1.5 mg/dl at week eight (Figure 11).

There were no significant increases in HDL cholesterol for the experimental group, however it was noted that there were slight increases in HDL cholesterol. There also was no significant difference in HDL cholesterol between the control and experimental groups.

Triglycerides

The mean week one triglyceride value for the group supplemented with cranberry seed oil was initially 156.2 mg/dl, followed by a 21.6 mg/dl decrease to 134.6 mg/dl at week four, and a 5.0 mg/dl decrease to 151.2 mg/dl at week eight (Figure 12). The control group, supplemented with canola oil, had a mean week one triglyceride value of 110.8, which decreased by a 4.2 mg/dl to 106.6 mg/dl at week four, and slightly increased by 0.1 mg/dl at week eight to 110.9 mg/dl (Figure 12).

There was no significant ($p<0.05$) reduction in triglyceride concentration for the experimental group supplemented with cranberry seed oil, but there were slight triglyceride reductions. There also were no significant differences in triglycerides levels between the control and experimental groups.

Table 1.

Adjusted Mean (M) and Standard Deviation (SD) Results of Lipid Profile for Control and Experimental Groups[1]

	Week 1		Week 4		Week 8	
	Control	Experimental	Control	Experimental	Control	Experimental
Total Cholesterol (mg/dl)	233.2 ± 27	240.6± 20	236.4 ± 27	234.9 ± 20	241.2 ± 43	247.9 ± 40
LDL cholesterol (mg/dl)	146.3 ± 26	149.3 ± 21	150.3 ± 23	143.9 ± 19	153.00 ± 34	147.9 ± 18
HDL cholesterol (mg/dl)	65.1 ± 14	60.60 ± 16	65.11 ± 15	64.3 ± 16	66.56 ± 17	62.1 ± 16
Triglycerides (mg/dl)	110.8 ± 41	156.20 ± 56	106.6 ± 42	134.60± 65	110.9 ± 38	151.20± 71

[1] Control N = 10; Experimental N = 11; HDL = high density lipoproteins; LDL = Low density lipoproteins

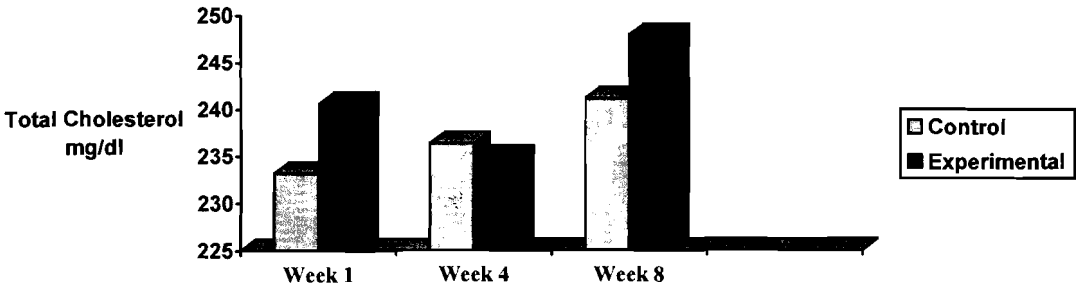

Figure 9. Mean total cholesterol values in (mg/dl) at weeks one, four, and eight

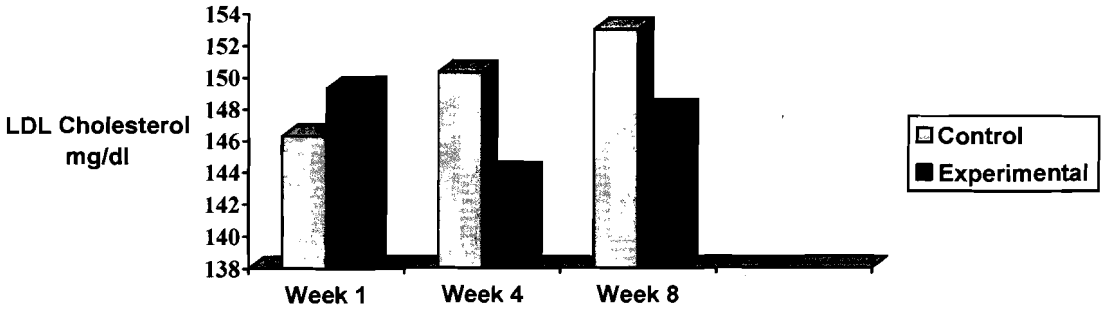

Figure 10. Mean LDL cholesterol values in (mg/dl) at weeks one, four, and eight
LDL= low density lipoprotein

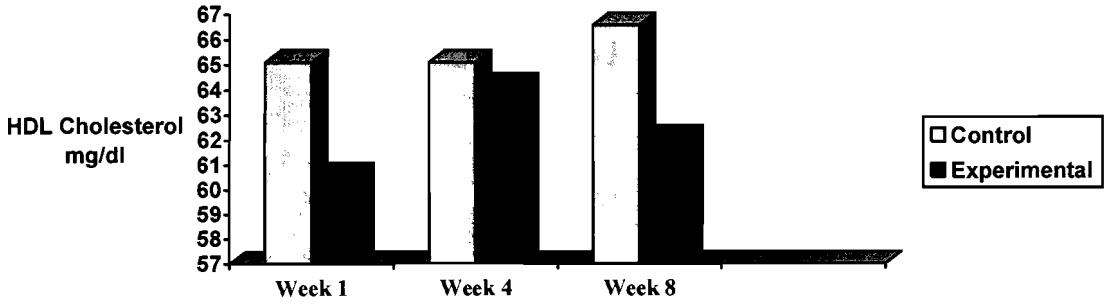

Figure 11. Mean HDL cholesterol values in (mg/dl) at weeks one, four, eight
HDL= high density lipoprotein

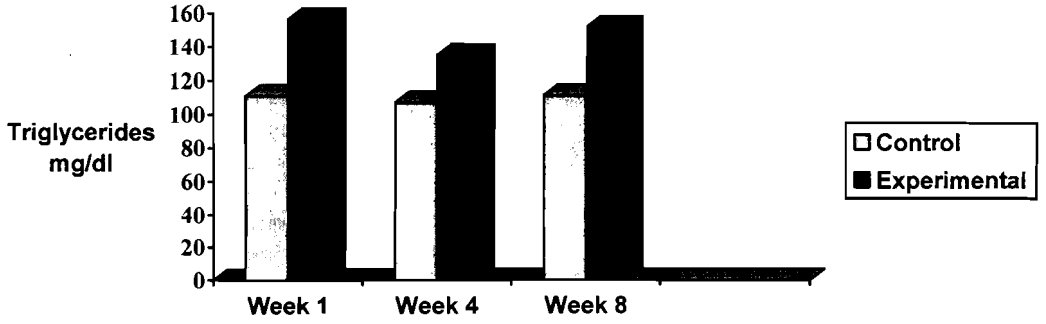

Figure 12. Mean triglyceride values in (mg/dl) at weeks one, four and eight

Chapter V: Discussion

The purpose of this research study was to determine the effect of the consumption of cranberry seed oil on the lipid profiles of human subjects with borderline high-to-high cholesterol, which would include total blood cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

Total Cholesterol

Though not statistically significant ($p<0.05$), there were noticeable reductions in total cholesterol in the experimental group after just four weeks of consuming the cranberry seed oil. At week four, there was an average of 5.7 mg/dl decrease in the experimental group, compared to a 3.2 mg/dl increase in the control group. One subject in the experimental group had 34 mg/dl (13.6%) reduction in total cholesterol at week eight. .

LDL Cholesterol

Though not statistically significant, there were also vast reductions in LDL cholesterol in the experimental group at weeks one and eight. The was an average decrease in LDL cholesterol of 5.4 mg/dl in the experimental group, compared to an average 4 mg/dl increase at week four in the control group. At week eight the experimental group had a 1.4 mg/dl decrease, while the control group had an average increase of 6.7 mg/dl at week eight. An individual subject in the experimental group had a 24 mg/dl (12.1%) decrease in LDL cholesterol at week eight.

HDL Cholesterol

Even though there were no significant increases in HDL cholesterol, vast improvements were found in the experimental group after four weeks of cranberry seed oil consumption. There was an average increase of 3.7 mg/dl in the experimental group

at week four compared to no change in HDL cholesterol at week four. In addition, a subject in the experimental group had an 11 mg/dl (18%) increase in HDL cholesterol at week eight of the study.

Triglycerides

Although not statistically significant, there were also great improvements in triglycerides of the subjects that were in the experimental group. There was an average decrease of 21.6 mg/dl in triglycerides in the experimental group at week four, compared to only a 4.2 mg/dl decrease in the control group at week four. The experimental group saw an average decrease of 5 mg/dl at week eight, compared to a 0.1 mg/dl average increase at week eight in the control group. Additionally, a subject realized a 46 mg/dl (25.7%) decrease in triglycerides at week eight.

Conclusions

Through analysis of the results of lipid profile tests, no significant changes within each group or between each group were observed with significance set at $p < 0.05$. Though not statistically significant, there were slight improvements in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides of subjects in the experimental group at week four. However, subjects' lipid profile results declined at week 8. This is thought to be due to non-compliance in supplementing the one-tablespoon per day of cranberry seed oil of the subjects in the experimental groups.

In addition, it is difficult to make any accurate inferences about the data due to the large standard deviations in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. The standard deviation at week eight was 40.1 mg/dl for total cholesterol, 17.7 mg/dl for LDL cholesterol, 16.3 mg/dl for HDL cholesterol, and 70.7 mg/dl for

triglycerides. These large standard deviations suggest a large variance in the results of subjects' lipid profile tests. Due to the large standard deviations, the vast improvements in some individuals' lipid profile tests went unnoticed. One subject in the experimental group had 34 mg/dl (13.6%) reduction in total cholesterol at week eight. Another subject in the experimental group had a 24 mg/dl (12.1%) decrease in LDL cholesterol at week eight. Another subject in the experimental group had an 11 mg/dl (18%) increase in HDL cholesterol at week eight. Additionally, a subject realized a 46 mg/dl (25.7%) decrease in triglycerides at week eight in the experimental group.

Cranberry seed oil is thought to be an alternative method to statins, bile acid sequestrants, nicotinic acid, and fibrates for improving lipid profile test results. Studies using statins have shown a 20-60% reduction in LDL cholesterol and have been effective at lowering triglycerides. Bile acid sequestrants are often used in conjunction with statin drugs and have been shown to lower LDL cholesterol by over 40%. Nicotinic acid has shown to be effective at lowering total cholesterol, LDL cholesterol (10-20%), and triglycerides (20-50%) while increasing HDL cholesterol (15-35%). Fibrates have been shown to lower triglycerides (20-50%) and increase HDL cholesterol (10-15%) (National Heart Lung and Blood Institute, 2007).

Even though no significant changes in lipid profile tests were reported between groups or within groups at a significance level of $p<0.05$, great findings were found. There was an average of 5.7 mg/dl decease in total cholesterol, an average of 5.4 mg/dl decrease in LDL cholesterol, an average of 3.7 mg/dl increase in HDL cholesterol, and an average of 21.6 mg/dl decrease in triglycerides in the experimental group after four weeks. This data suggest that the supplementation of cranberry seed oil has a great

potential to act as a natural antioxidant and help optimize blood cholesterol levels, particularly for an initial duration of up to four weeks supplementation as demonstrated as in this study. Therefore, data from this study warrants further investigation into the possible in vivo cholesterol lowering abilities that cranberry seed oil exhibits.

Limitations

One of the limitations of this study was its small sample size. A total of three participants were lost throughout the duration of the study. Two participants were eliminated after the week one-lipid profile testing because they did not meet the qualification of having a total cholesterol concentration of > 200 mg/dl. Another participant did not show up for the final lipid profile testing. The researcher was unable to contact the participant so they were eliminated from the research study. An optimal sample size of 55 for both control and experimental groups was calculated at a 5% confidence level and a beta error level of 50% using a sample size calculator (DSS Research, 2006). This means that there would have to be a very large difference between the control and experimental group in order to be considered significant at the level of 0.05.

Another limitation of the study was that the subjects may not have taken the oil in the correct amount diligently throughout the duration of the study. Through personal conversations with the subjects, the researcher became aware that some subjects forgot to take the oil 1-5 days out the course of eight-week study. Subjects in the experimental group also indicated to the researcher that the oil was of great distaste and that they had a hard time taking it every day. Subjects also admitted that they had to chase down the oil with high fat and sugar foods to ward off or mask the strong flavor of the oil. This may

have increased the total fat, saturated fat, and cholesterol consumption in the subjects', thus possibly affecting the results of their lipid profile testing.

An additional limitation was that the cranberry seed oil supply for weeks four to eight came from a separate lot number than the cranberry seed oil supplied for weeks one to four. The results of the lipid profile test of subjects in the experimental group looked positive at week 4 and then went in a negative direction at week eight. The difference in lot number of the cranberry seed oil could have possibly played a role.

Through observations with subjects in the control group, the researcher was made aware that some subjects were trying to lose weight and adopt a healthy lifestyle through nutrition and exercise. The change in their lifestyle could have influenced their lipid profiles test results as well.

Recommendations

1. Conduct a research study examining the effects of cranberry seed oil supplementation on human subjects with borderline high-to-high cholesterol levels utilizing a larger sample size with a minimum of 55 subjects in both the control and experimental groups. This would help to reduce the standard deviation and variance of the data.
2. Conduct a research study examining the effects of cranberry seed oil supplementation on human subjects with borderline high-to-high cholesterol levels with a longer duration, a minimum of 12-weeks. This would strengthen the validity of the study.
3. Conduct a research study examining the effects of cranberry seed oil supplementation on human subjects with borderline high-to-high cholesterol

levels with subjects in the experimental group supplementing cranberry seed oil in the capsule form. This would improve subject compliance.

4. Conduct a research study examining the effects of cranberry seed oil supplementation on human subjects with borderline high-to-high cholesterol levels and have the subject supplement the oil for 1-4 weeks. Then have no supplement from weeks 5-8, and then have the subjects go back to supplementing the oil for weeks 9-12 to reduce subject boredom in compliance with the research study.
5. Conduct a research study examining the effects of cranberry seed oil supplementation on human subjects with borderline high to high cholesterol levels and have the subjects fill out a food frequency questionnaire prior to the start of the study and at week four and eight of the study to determine if changes in diet patterns affect lipid profile results.
6. Analysis of the fatty acids found in cranberry seed oil using high performance liquid chromatography (HPLC) to determine exact amounts of omega-3, omega-6, and omega-9 fatty acids in the cranberry seed oil prior to subject consumption of the oil to ensure that the quality and consistency of the cranberry seed oil is maintained.

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Appendix A: Consent to Participate in UW-Stout Approved Research

Megan Eno Cynthia Rohrer

612-483-5469
8151 33rd Ave. S. E unit 806
Bloomington, MN 55425

715-232-2088
Food/Nutrition Department
University of Wisconsin-Stout
Menomonie, WI 54751

The objective of this research study is to support the hypothesis that adding cranberry seed oil to the diet results in decreasing total cholesterol, LDL cholesterol, and triglycerides and increasing HDL cholesterol. You will be taken to the Nutrition Assessment Lab located in the Home Economics' building at UW-Stout after reading and signing the consent form in order to be screened for total cholesterol. If your total blood cholesterol fits within the range of 200-240 mg/dl and you fit the rest of the qualifications for the study you will then be placed in either the control or experimental group and receive an initial blood draw by a certified laboratory technician from Red Cedar Medical Center located in Menomonie, WI. At both four and eight weeks into the study you will return to Nutrition Assessment Lab located in the Home Economics' building at UW-Stout for follow up blood draws.

There are no potential social or emotional risks associated with this research study. One potential physical risk would be the possibility of experiencing slight discomfort with the blood draw. You must be eighteen years of age or older and not taking any cholesterol altering drugs. You will be able to withdraw from this research study at any time without having any penalties.

This research study will take place over an eight week time period. Laboratory assessment and testing will take place before week one and after weeks four and eight. The approximate time for each assessment and testing is fifteen minutes.

This research study in completely confidential, no names, or identification numbers will be reported in the final thesis paper write up.

Participation in this research study is completely voluntary. You may choose to withdrawal and not participate at any point throughout the study without penalty.

Through participating in this research study you must agree to release the University of Wisconsin-Stout from any potential liability related your voluntary involvement in this study.

The University of Wisconsin-Stout's Intuitional Review Board (IRB) reviewed and approved this research study. If you may have any questions or concerns about this

research study feel free at anytime to contact the Investigator, Megan Eno (612-483-5469, enom@uwstout.edu), Advisor, Cynthia Rohrer (715-232-2088, rohrerc@uwstout.edu), or the IRB administrator, Sue Foxwell (715-232-2477, foxwells@uwstout.edu).
By signing this consent form you are agreeing to participate in the research project entitled, "The effects of cranberry seed oil on lipid profiles of human subjects."

_____ _____

Signature Date

Appendix B: Email to Interested Participants

Dear Interested Research Study Participant,

I would greatly appreciate your participation in my research study.

The study is over an eight week time frame. There is a control and experimental group. The control group would consume one tablespoon of canola oil everyday for eight weeks. The experimental group would consume a tablespoon of the cranberry seed oil everyday for eight weeks.

Blood work would be completed prior to the start of the study and at weeks four and eight into the study in order to determine lipid profiles for all participants. I am planning on having a lab technician from Red Cedar Medical Center come out to the Nutrition Assessment Lab located in room 423 of the Home Economics building the morning of October 9th for the first initial blood draw. The lab tech would also come out at weeks four and eight of the study to perform follow up blood draws on November 6th and December 4th. If it doesn't work for you to come on these days you would be able to go to Red Cedar Medical Center in Menomonie, WI to receive your blood draws.

Attached is the consent form. You can sign this and bring it to your first blood draw on October 9th. You can also pick up all of the materials for the study at this time.

I will send you a reminder email about the initial blood draw
Feel free to contact me with any questions or concerns
Look forward to hearing from you.
Thanks so much for your interest,

Megan Eno
612-483-5469
enom@uwstout.edu

Appendix C: Participant Educational Brochure

Research Study

Everyday for the next 8 weeks you will take 1 Tbsp of your assigned oil. You can take the oil anytime throughout the day.

You can consume the oil by:

- *Dipping a piece of bread in it.*
- *Drizzling it over cooked vegetables.*
- *Spreading it on pancakes or French toast.*
- *Adding it to your favorite salad.*
- *Just make sure not to cook with it or heat it. This may effect the beneficial components of the oils.**

Blood draws

- *You will return to the Human Assessment Lab on Tuesday, November 6th, and Tuesday, December 4th between 8-11 a.m. for your 4-week and 8-week follow up blood draws.*
- *You also have the option to receive your blood draws at Red Cedar Medical Center located in Menomonie, WI.*

Questions?

Please do not hesitate to contact me anytime throughout the study with any questions or concerns.

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University of Wisconsin-Stout Research Study

Cranberries: Building a Healthy Heart!

Megan Eno
Cynthia Rohrer, PhD

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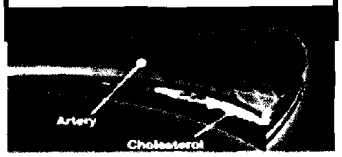
Cholesterol



- Cholesterol plays an essential role in the human body. The body needs cholesterol for digesting dietary fats, making hormones, and building cell walls.
- Too much cholesterol in the blood can damage the arteries.
- Excess cholesterol build up in the blood can lead "plaque" in the linings of the blood vessels. This condition is called atherosclerosis.
- Atherosclerosis is a health condition that if not taken care of can cause a heart attack. Having an optimal cholesterol profile can reduce your risk for heart disease. (FDA, 2007)

Types of Cholesterol

Low Density lipoprotein (LDL) cholesterol also known as the "bad" cholesterol. LDL cholesterol is the type of cholesterol that can build up in the blood and cause plaque formation that leads to atherosclerosis

High density lipoprotein (HDL) cholesterol- also known as the "good" cholesterol
HDL cholesterol transports cholesterol back to the liver where it then can be eliminated from the body.

Heart Healthy Cranberry Research

- Emerging research suggests that consumption of cranberries may have a protective effect on cardiovascular disease.
- Cardiovascular disease is the number one killer currently in the United States. (American Heart Association, 2007) Cholesterol and obesity are major contributors to cardiovascular disease.
- New laboratory studies suggest that antioxidant properties that are found in cranberries may increase HDL "good" cholesterol and have cardio protective effects in humans.
- Cranberry seed oil is currently gaining popularity in the U.S. market. Cranberry seed oil is made out of the by product of cranberries when they are processed for juices, jams, and jellies.
- Cranberry seed oil is unique in that it has an optimal ratio of omega-3-, 6, and 9 fatty acids. The cranberry seed oil contains higher concentrations of tocopherols and tocotrienols (forms of the antioxidant vitamin E) than whole cranberries.
- Vitamin E has been found to decrease LDL cholesterol and help prevent the risk of having a heart attack. (American Heart Association, 2007)

Delicious Cranberry Seed Oil Recipes

Cranberry Seed Oil Vinaigrette

- 1 Tbsp red-wine vinegar
- 2 tsp frozen cranberry juice concentrate
- 1/8 tsp salt
- 1/3 c. cranberry seed oil

Nutrition Information
81 kcal, 9g Fat, 0 g Carbohydrate
Uses salad dressing or bread dipper

Cranberry Chicken Salad

2-3 c. of chopped boneless skinless chicken breast (can also use canned chicken breast)
2 Stalks of chopped celery
5 Tbsp mayonnaise
1/2 c. craisins
1/4 c. pine nuts
Sprinkle with parmesan cheese
Drizzle with cranberry seed oil to taste.
Mix all ingredients until blended.
Great in a sandwich, over a bed of lettuce, spread on crackers, or delicious eaten plain.