THE EFFECTS OF USING COTTONSEED OIL VERSUS CORN OIL AS PART OF AN OVERALL DIETARY STRATEGY TO INCREASE VITAMIN E INTAKE IN HEALTHY PARTICPANTS.

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To the Dean of Graduate Studies and Research:

I am submitting herewith a thesis written by Jennifer Lynn Braun entitled " The effects of using cottonseed oil versus corn oil as part of an overall dietary strategy to increase vitamin E intake in healthy participants." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

V Imrhan
Major Professor (Vicky Imrhan, Ph.D)

We have read this thesis and
recommend its acceptance:

Accepted: Michael N Dreyer
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DEDICATION

This project would not have been possible without the help of some very important people. I would like to thank my committee members, Dr. King, Dr. Radcliffe, and Dr. Imrhan. First of all, Dr. Imrhan for putting up with my high stress level and making me figure out the challenging tasks of this project by myself. Also, Dr. Radcliffe for helping me through numerous revisions and answering several questions when I did not have a clue. Although we have never met face-to-face, I have learned so much from you. Next, I would like to thank my parents for supporting me through my entire college career. You both taught me that I could do anything I put my mind into; I have accomplished so much with your encouragement and praise. Last, I would like to thank the love of my life, Brian, for reminding me that "Everything is going to be all-right." Everything *is* "all-right" because you were by my side every step of the way!

ABSTRACT

THE EFFECTS OF USING COTTONSEED OIL VERSUS CORN OIL AS PART OF AN OVERALL DIETARY STRATEGY TO INCREASE VITAMIN E INTAKE IN HEALTHY PARTICPANTS.

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The purpose of this study was to assess the effects of foods made with cottonseed oil and corn oil on vitamin E intake and lipid levels. There were no significant changes found between the two groups in regards to change in vitamin E intake. There were no significant changes in cholesterol levels. There was however, a significant change in triglyceride level, with the corn oil group showing an increase and the cottonseed oil group showing a decrease. The change in total cholesterol trended downward in the corn oil group and upward in the cottonseed oil group. HDL-cholesterol levels decreased in both groups, while LDL-cholesterol showed a decrease in the corn oil group and increase in the cottonseed oil group. Findings from this study suggest that consuming foods made with cottonseed oil are acceptable. Other research is needed to draw definite conclusions on the oils affect on blood lipid values.

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CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) represents the number one cause of death in the United States. The disease kills about 950,000 people annually. In women alone, more die from cardiovascular disease than all cancers combined. However, the age-adjusted death rates from these diseases have been declining since the 1960's. This decline can be attributed to advances in medical treatment and emphasis on changing certain lifestyle factors that contribute to the development of these diseases, e.g., smoking, inactivity, and a high fat diet. Recently, studies have shown that antioxidant nutrients such as vitamin E may also reduce the risk of CVD (AHA, 1999).

The function of an antioxidant involves a complex process to protect cells against damage caused by activated oxygen. The antioxidant nutrients include the minerals copper, zinc, selenium, and manganese, as well as the vitamins C, A, E, and β-carotene. The reduction of oxygen is a normal process that occurs at all times in the body. An example would be the reduction of oxygen to water in the electron transport chain. In these processes, oxygen may gain an electron, which generates a free radical, which sets off a chain of events that produces even more highly reactive molecules. These molecules can cause damage to living systems because they can take electrons from any organic molecules in the cells, such as deoxyribonucleic acid (DNA), proteins, and polyunsaturated fatty acids (PUFA), which will cause further damage that can lead to

disease. Vitamin E acts as an antioxidant by donating an electron to the free radical, thus terminating the reactions and preventing additional damage to the cell (Diplock, 1991).

Vitamin E is a fat-soluble vitamin that occurs in eight different forms, or compounds, in nature. Its compounds fall into two classes, the tocopherols, with saturated side chains, and the tocotrienols, with unsaturated side chains. Vitamin E is found primarily in vegetable oils and grains, green plants, egg yolk, milk fat, liver, nuts, and vegetables (Kayden & Traber, 1993). Alpha-tocopherol is the most active form of the vitamin, and according to the latest RDA, only alpha-tocopherol is considered to be a source of vitamin E activity (Monsen, 2000).

Along with fatty acids, vitamin E requires bile acids for micelle formation to be absorbed into the gut. Pancreatic enzymes are thought to also be involved in this process. With normal dietary intakes, the absorption of vitamin E is about 70% efficient. However, it is thought that as the vitamin intake increases, the absorption decreases. After uptake into the enterocyte, vitamin E is secreted into chylomicrons, transported through the lymph, and then into the circulation. The tocopherol in the chylomicrons is then equilibrated with all plasma lipoproteins including the very-low-density lipoproteins (VLDL), the low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Some vitamin E is taken into tissues during chylomicron or VLDL hydrolysis by lipoprotein lipase (LPL), the endothelial-bound enzyme. However, most of the vitamin E is distributed to tissues with LDL by the LDL receptor-mediated uptake. There is no single storage organ for vitamin E; however, the largest amount is found in adipose tissue. Vitamin E has no specific plasma transport protein like other fat-soluble vitamins

but is mainly transported by plasma lipoproteins. Thus vitamin E levels correlate with the levels of lipoproteins, particularly LDL (Kayden & Traber, 1993).

The report by the Panel on Dietary Antioxidants and Related Compounds was released in the April 2000 with publication in May of that year. In that report, the previously recommended levels for vitamin E were increased for both adult women and men to 15 mg/day, which is equivalent to 22 international units (IU) of natural-source vitamin E or 33 IU of the synthetic form (Monsen, 2000.) Evidence suggests, however, that higher levels could be beneficial to human health. It has been shown that the amount of vitamin E needed to protect PUFA against damage by oxidation is at least 0.4-0.8-mg vitamin E per gram of PUFA. Amounts may be as high as 1.5mg/g when diets are high in levels of long-chain PUFA. Vitamin E intakes of at least 40 IU, or 27 alpha-TE, per day have been shown in human studies to be the least amount demonstrated to inhibit LDL oxidation; intake of at least 60 IU, or 40 alpha-TE, per day enhanced immune responses; and intakes of 200-400 IU, or 134-268 alpha-TE, decreased platelet adhesion to vessel walls. Furthermore, double blind, large population studies have shown that oral vitamin E intake as high as 3200 IU, or 2144 alpha-TE, per day has few side effects. Problems with large doses have only been shown in vitamin K-deficient individuals, who have experienced coagulation problems (Weber et al., 1997).

The effect of antioxidants in the prevention of heart disease is controversial. In a recent study looking at the antioxidant vitamins C, E, A, and β-carotene, vitamin E was the only antioxidant found to affect platelet function in healthy volunteers (Calzada, Bruckdorfer, & Rice-Evans, 1997). Numerous studies have looked at vitamin E to

determine the antioxidant's effect on diseases of the heart, and results have varied. A number of large-scale epidemiologic studies have found an inverse relationship between the risk of CVD and vitamin E intake (Marchioli, 1999). Other studies have found, however, that there is no association between high intake of vitamin E and the risk of having a heart attack (Rimm et al., 1993).

Surprisingly, limited data are available to assess population consumption, intake patterns, and trends in exposure to nutrient and non-nutrient antioxidants. The Centers for Disease Control and Prevention (CDC) and U.S. Department of Agriculture (USDA) monitor intake patterns of individuals through dietary intake and supplement use surveys. Non-nutrient antioxidants in the food supply can be assessed through the Food Additives Survey that the Food and Nutrition Board conducted for the Food and Drug Administration (FDA). Since 1909, the USDA has kept a record of the levels of foods and nutrients available for consumption in the US called the US Food and Nutrition Supply Series. Vitamin E levels in the food supply have increased since 1909, with the major source as being vegetable oils. However, this does not mean increased intake because these vitamin E sources may not be consumed (Woteki, 1995).

Research on dietary supplements has mainly focused on use. In 1992, one study found that 46% of the US population reported taking a vitamin or mineral supplement in the past year (Lino, Dinkins, & Bente, 1999). Another recent representative study found that approximately 40 to 47% of the US population use vitamin or mineral supplements at least occasionally (ADA, 2001). An average of 26% of adults takes a product containing

vitamin E. The median amounts of vitamin E provided by these supplements is 200% of the RDA (Woteki, 1995).

When looking at dietary patterns of vitamin E consumption from foods, intake increases in individuals with higher incomes and education levels, and is higher among Whites than Blacks (Woteki, 1995). Fats and oils is the number-one contributor of tocopherols, providing more than 20% of vitamin E in the US diet. Vegetables are the second largest contributors providing about 15%. Fruits only contribute 5% of vitamin E in the US diet (Murphy, Subar, & Block, 1990).

In a United Stated Department of Agriculture (USDA) population survey carried out in 1985, the Continuing Survey of Food Intakes by Individuals (CSFII), the mean daily intake of vitamin E by women age 20-49 was 7.0 alpha-TE. In addition, the Second National Health and Nutrition Examination Survey (NHANES II) were used to estimate the intake of vitamin E in the US. This showed that mean intakes of vitamin E were close to the existing RDA for both men (9.6 alpha-TE per day) and women (7.0 alpha-TE per day). However, median intakes were lower than the RDA with the value being 7.3 alpha-TE/d in men and 5.4 alpha-TE/d in women (Murphy, Subar, & Block 1990). The more recently published NHANES III found a similar trend in vitamin E intake (Weber et al., 1997).

The use of vegetable oils high in alpha-tocopherol is one way to increase individuals' intake of vitamin E. Cottonseed oil is one that is rich in tocopherols. Crude cottonseed oil contains about 1000 mg/kg tocopherols, but 1/3 of this is lost in refining. Cottonseed oil has been part of the American diet for over a century. It represents about

56% of the total domestic fat and oil supply. Cottonseed oil is extracted from cottonseed of the cotton plant. This oil is produced for food for both animals and man. In foods, cottonseed oil has a wide variety of uses commercially and domestically. It can be used as a salad oil, frying oil, or made into shortening or margarine for baked goods. The high vitamin E content of the oil contributes to stability, giving it a long shelf life (Jones & King, 1996).

Cottonseed oil is among the unsaturated oils. Consumption of this oil rather than more unsaturated oils and animal fats is considered to be an accepted way to reduce the dietary intake of saturated fatty acids. It has a 2:1 ratio of polyunsaturated to saturated fatty acids, with 70% of all the fatty acids being unsaturated (18% monounsaturated, 52% polyunsaturated, 26% saturated). Cottonseed oil is described as being "naturally hydrogenated" because of high levels of palmitic acid. This makes the oil stable without the additional processing or formation of trans fatty acids. When the oil is hydrogenated, the percentage of fatty acids that are monounsaturated actually increases. The use of cottonseed oil is a way to promote good health, as its high vitamin E content and fatty acid profile make it useful in promoting current health guidelines. There has, however, been very little, if any, research on the effects of cottonseed oil on, for example, the vitamin E status of human subjects (Jones & King 1996).

The purpose of this study, which was carried out with healthy, adult, volunteer participants, is to compare foods made with cottonseed oil (muffins, dressing, and bar cookies) to foods made with corn oil as a way to increase participants' vitamin E intake when these foods are used with other dietary sources of vitamin E. Participants' serum

blood lipid levels were analyzed to determine how their levels of cholesterol and lipids are affected by the dietary intervention. An additional purpose was to determine if volunteers would accept food products containing cottonseed oil. Corn oil was chosen as the oil for comparison because it has been widely used in human feeding studies. Like cottonseed oil, it is a good source of vitamin E and polyunsaturated fatty acids. Moreover, in animal studies, cottonseed oil has, relative to corn oil, has demonstrated to have a cholesterol lowering effect (Edwards & Radcliffe, 1995). Since acceptability of the products made with these oils will affect vitamin E intake, comparison of the acceptability of these two products was determined.

Statement of the Problem

In order to compare the practicality of increasing dietary vitamin E intake with cottonseed oil versus corn oil, the following were tested: The effects, in volunteer participants, of foods made with cottonseed oil or corn oil on vitamin E intake. In addition, the effect of foods made with cottonseed oil or corn oil on serum lipid levels was investigated, and the acceptability of food products made with cottonseed oil or corn oil as determined by the consumption of foods provided and the answers to a questionnaire. Food products made with one oil were considered more acceptable than those made with the other if 2/3 of products is consumed in greater quantities than that oil.

Null Hypotheses

1) Consuming foods made with cottonseed oil, relative to ones made with corn oil, will not increase the dietary intake of vitamin E.

2) Consuming foods made with cottonseed oil, relative to those made with corn oil, will not affect blood lipid levels in the participants.
3) Food items (muffins, bars, and dressing) made with cottonseed oil will not be as acceptable as ones made with corn oil.

CHAPTER II

REVIEW OF THE LITERATURE

Dietary Fat and Dietary Guidelines

Recommendations to the American public about the amount of fat they should consume daily are relatively new. Interest in dietary fat and its effects started in the 1950's when researchers began to show that dietary fat was one factor in atherosclerotic heart disease. Large epidemiologic investigations at this time also showed dietary fat levels in correlation to mortality from heart disease. Later in the 1970's, investigators began to find that it was not dietary fat alone that contributed to these diseases but the type of fat in the diet, particularly saturated fat (Kritchevsky, 1998).

The first dietary guidelines were published in the late 1950's (Table 1), only to be rewritten four years later to guidelines similar to those we have today (Table 2), which are aimed to provide advice for Healthy Americans, age 2 and over, about food choices that promote health and prevent disease. In 1968, the American Heart Association's Committee on Nutrition published eight dietary guidelines as follows:

> 1) Reduce animal fat, 2) decrease saturated fats and increase polyunsaturated fats, 3) reduce cholesterol, 4) maintain ideal body weight, 5) apply dietary recommendations early in life, 6) maintain the principles of good nutrition with change in diet, 7) adhere to dietary recommendations, and 8) make sound food habits a family affair (Kritchevsky, 1998).

In 1978, the committee added a recommendation to reduce the intake of sodium. A year earlier, the Senate Select Committee on Nutrition and Human Needs published their own dietary guidelines for Americans (Table 3). The Food and Nutrition Board of the National Academy of Sciences later published a 24-page booklet in 1980 entitled "Toward a Healthful Diet." Their five recommendations covered areas such as selecting a nutritionally adequate diet by consuming the appropriate servings of foods, selecting a wide variety of foods, maintaining an appropriate weight, reducing alcohol, sugars, and fats, and using salt in moderation (Kritchevsky, 1998). Since that time, the *Dietary Guidelines* have been consistent emphasizing seven distinct guidelines (Table 4). However, the most recently published *Dietary Guidelines of 2000* contain ten guidelines. The additional guidelines include food safety, a separate guideline for fruits and vegetables, and a new physical activity guideline (Table 5). They are based on three principles: aim for fitness; build a healthy base; choose sensibly (Kennedy & Davis, 2000).

The current recommendations for total fat intake in the United States is presently 30% or less of total energy, with saturated fat less than 10% of total energy. This percentage relates to fat intake relative to total energy obtained from all the macronutrients including protein and carbohydrates. Excess energy intake can be a result of excessive dietary fat intake and/or total energy consumption. Therefore, the goal of the current dietary guidelines is to reduce fat intake and not to counterbalance it with an increase in carbohydrate intake (Lichtenstein et al., 1998).

Table 1
American Heart Association Report (1957)

1. Diet may play an important role in the pathogenesis of atherosclerosis.
2. The fat content and total calories in the diet are probably important factors.
3. The ratio between saturated fat and unsaturated fat may be the basic determinant.
4. A wide variety of other factors beside fat, both dietary and non-dietary, may be important

Ref. Kritchevsky, 1998

Table 2
American Heart Association Report (1961)

1. Maintain a correct body weight.
2. Engage in moderate exercise, e.g., walking to aid in weight reduction.
3. Reduce intake of total fat, saturated fat, and cholesterol. Increase intake of polyunsaturated fat.
4. Men with a strong family history of atherosclerosis should pay particular attention to diet modification.
5. Dietary changes should be carried out under medical supervision.

Ref. Kritchevsky, 1998

Table 3

Dietary Goals for the United States (1977)

1. Increase carbohydrate consumption to account for approximately 55 to 60% of energy intake.

2. Reduce overall fat consumption from 40 to 30% of energy intake.

3. Reduce saturated fat consumption to account for about 10% of total energy intake; and balance that with polyunsaturated and monounsaturated, which should account for 10% energy intake each.

4. Reduce cholesterol consumption to about 300 mg/day.

5. Reduce sugar consumption by about 40% to account for about 15% of total energy intake.

Ref. Kritchevsky, 1998

Table 4

Dietary Guidelines for Americans

1990	1995
Eat a variety of foods	Balance the food you eat with physical activity-maintain or improve your weight.
Maintain a healthy weight	Choose a diet with plenty of grain products, vegetables, and fruits.
Choose a diet low in fat, saturated fat, and cholesterol	Choose a diet low in fat, saturated fat, and cholesterol
Choose a diet with plenty of vegetables, fruits, and grain products.	Eat a variety of foods
Use sugars only in moderation	Choose a diet moderate in salt and sodium
Use salt and sodium only in moderation	Choose a diet moderate in sugars
If you drink alcoholic beverages, do so in moderation	IF you drink alcoholic beverages, do so in moderation

Ref. Kennedy & Davis, 2000

Table 5
Dietary Guidelines 2000

Let the Pyramid guide your food choices
Aim for a healthy weight
Be physically active
Choose a variety of grains daily, especially whole grains
Choose a variety of fruits and vegetables daily
Keep food safe to eat
Choose food low in saturated fat and cholesterol and moderate in other fats
Choose beverages and foods to moderate your intake of sugars
Choose and prepare foods with less salt
If you drink alcoholic beverages, do so in moderation

Ref. Kennedy & Davis, 2000

Dietary fat is defined as triglycerides (fat and oils), phospholipids, and sterols (cholesterol). Associated nutrients include the fat-soluble vitamins (A, D, E, and K), and related compounds such as the carotenoids. The majority of dietary fat is triglyceride which is made up of three fatty acids esterfied to a glycerol molecule (Lichtenstein et al., 1998). Dietary fat plays an essential role in the functioning of the body. Utilization of fat requires proper digestion and absorption. Most dietary triglyceride digestion takes place in the lumen of the small intestine. However, the process begins in the stomach with the action of lingual lipase on medium and short chain fatty acids. The absorption of fatty acids in the gut requires bile salts for emulsification to be exposed to the enzymes of the gastrointestinal tract (Groff et al., 1995). The complete hydrolysis of triglycerides by the enzyme pancreatic lipase in the small intestine results in free fatty acids, monoglycerides, diglycerides, and glycerol. These particles, along with cholesterol, combine with bile slats to form negatively charged aggregates called micelles. The micelles have a small diameter thus allowing them access to the absorptive spaces of the small intestine. Once absorbed into the enterocyte, these components are reassembled into a triglyceride molecule, which is secreted into chylomicron particle, dumped into the lymphatic system, and eventually end up in the portal system. However, some components of dietary fat, such as the short-chain fatty acids, are absorbed directly into the portal circulation (Lichtenstein, 1998).

Fatty acids are classified according to many systems. Their chain length or the presence or absence of double bonds classifies most. According to chain length they are classified as either short (4 to 6 carbon atoms), medium (8 to 10 carbon atoms), or long-

chain (12 to 18 carbon atoms). When classified according to presence or absence of double bonds, saturated fatty acids contain no double bonds, and unsaturated fatty acids contain at least one double bond. Unsaturated fatty acids are further divided into monounsaturated (one double bond) and polyunsaturated (two or more double bonds). Unsaturated fatty acids are also classified by the position of the first double bond from the methyl end of the carbon chain. The fatty acid is an omega-3 fatty acid (n-3) if the first double bond starts at the third carbon and an omega-6 (n-6) if the first double bond starts at carbon number six (Lichtenstein et al., 1998).

Two of the unsaturated fatty acids are considered to be essential nutrients for humans. The essential fatty acids include the omega-6 fatty acid linoleic acid (18:2) and the omega-3 fatty acid linolenic acid (18:3). These fatty acids are essential because humans are unable to create fatty acids with double bonds beyond the ninth carbon from the carboxyl end of the compound. The inclusion of these fatty acids in the diet allows the body cells to create longer, more unsaturated fatty acids that are important in the functioning of the body. Examples include arachidonic acid (20:4), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6). These fatty acids are important for the formation of cell membranes and are precursors of the eicosanoids, essential physiologically active compounds (Groff, Gropper, & Hunt, 1996). As a result, the National Research Council of the National Academy of Sciences has proposed that essential fatty acids need to be consumed at levels of 1-2% of the total energy in the diet, when fat supplies 25-50% of the calories (Jones & King, 1996).

Dietary fat plays many important roles in the human body. It is both a concentrated and efficient source of energy with nine calories per gram and requiring minimal water for storage because it is hydrophobic. Body fat is an insulator against temperature extremes and protects vital organs from injury. In foods, fats act as tenderizers and carries flavor thus providing mouth feel. Dietary fat also enhances the absorption of the essential fat-soluble vitamin A, D, E, and K (Lichtenstein et al., 1998).

The USDA has been monitoring consumption patterns of Americans for over 40 years. Between 1965 and 1995 there has been a downward trend in total fat consumption as a percent of calories. Saturated fatty acid intake as a percentage of total energy has also declined during this same period. While this shows a decline of percentage of energy from fat, the actual fat intake of Americans has a different pattern. There is a decrease in the reported intake of grams of total fat and saturated fatty acids from 1965 to 1989/91. However, from 1989/91 to 1995, grams of total fat increased for all ages in male and for females to 50 years of age. In 1995 the reported intake of saturated fatty acids also increased for males and females 51 years and older. These data shows that Americans have been increasing both their energy *and* fat intake in recent years (Lichtenstein et al., 1998). Moreover, these surveys show that those whose diets met the US Dietary Guideline Recommendations for fat and saturated fat intake had lower overall fat intake. Additionally, lower saturated fat intake is associated with lower energy intake. Fried potatoes are the main sources of calories in those who consume a high amount of fat the surveys show (Kennedy, Bowman, & Powell, 1999).

Pathophysiology of Heart Disease

Cardiovascular disease is a general term for all disorders of the heart and blood vessels. Atherosclerosis is the main form of CVD. When the blood supply to the heart is obstructed, coronary artery disease (CAD) or coronary heart disease (CHD) results. In ischemic heart disease (IHD), the blood supply to any organ or tissue may be cut off.

Atherosclerosis is a form of arteriosclerosis in which vessel walls are hardened due to soft deposits of intraarterial fat and fibrin that harden over time. Atherosclerosis can take several forms, depending on location, age and genetics, and risk factors to which the individual may have been exposed. There are many theories on the development of this disorder, but there is general agreement in the basic steps in the process (Brashers et al., 1998).

In most hypotheses of atherogenesis, endothelial injury has been identified as the initial step. Although little is known about how this injury occurs, smoking, hypertension, diabetes, turbulent blood flow, increased fibrinogen, autoimmunity, and bacteria and viruses have been implicated. Once the injury has occurred, the dysfunction leads to the following series of pathological events:

1) The endothelial cells stop making normal antithrombotic and vasodilator substances such as nitric oxide and prostaglandins.
2) Growth factors are released that cause smooth muscle proliferation in the wall of the affected vessel.
3) Macrophages start sticking to the damaged endothelial surface because of the production of adhesion molecules.

4) The macrophage enzymes oxidize low-density lipoproteins (LDL).

This oxidation of LDL is an important step in the development of the fatty deposits and results in recruitment of more macrophages to the area. These macrophages then take up the oxidized LDL and deposit them into the intima of the vessel. The macrophages that are full of oxidized LDL are called foam cells and are a first pathologic finding in atherogenesis (Brashers et al., 1998).

Once these foam cells accumulate in significant amounts, they form a lesion called a fatty streak. When formed, fatty streaks produce oxygen radicals and cause immunologic and inflammatory changes resulting in advancing damage to the vessel wall. The next stage involves the incorporation of fibrous tissue and damaged smooth muscle cells into the area forming a fibrous plaque or fibroadenoma. This progresses to further endothelial dysfunction, necrosis of vessel tissue, and narrowing of the lumen (Brashers et al., 1998).

The plaque continues to develop and can ulcerate and rupture as a result of shear forces and necrosis of the vessel wall. Platelets then aggregate and adhere to the surface of the ruptured plaque, the coagulation cascade is initiated, and a thrombus forms over the lesion that may obstruct the lumen. The lesion is then known as a complicated lesion. This can occur rapidly and result in tissue death (Brashers et al., 1998).

Atherosclerosis is the most common cause of coronary obstruction. In CAD, the myocardial blood supply is diminished until deprivation impairs the heart muscle metabolism enough to cause ischemia, a state in which the cells are temporarily deprived of blood supply. Myocardial cells remain alive but cannot function properly. Continuous

ischemia or the complete blockage of the artery causes infarction and death of the heart muscle. This results in the often-fatal "heart attack." (Brashers et al., 1998).

Dietary Fat and Heart Disease

The relationship between fat intake and cardiovascular disease is complex and controversial. Current research has focused on CHD, but diet can play a role in other cardiovascular diseases. Results are not always consistent in these epidemiologic and experimental feeding studies. In addition, the specific fatty acid molecule, chain length, and degree of saturation all contribute to the development or reduction of the risk for heart disease (Nelson, 1998). Furthermore, another focus of the prevention of cardiovascular diseases is the relationship between diet and blood cholesterol levels in which an elevation is a major risk factor (Lichtenstein et al., 1998).

Plasma lipoproteins (particles containing lipids and protein, which make them water-soluble for transport in the blood) consist of the chylomicrons, VLDL, LDL, and HDL. After fatty acids are absorbed into the gut and transported to peripheral tissues by chylomicrons, the liver takes up triglyceride-depleted chylomicron remnants for repackaging. The liver then secretes VLDL into circulation. In circulation, the VLDL loose triglycerides and become cholesterol rich particles, which are then termed LDL. The LDL is the major cholesterol-carrying lipoprotein in circulation. The LDL tends to promote the development of CHD and plasma levels of LDL are a good predictor of CHD risk. On the other side, the HDL are shown to promote the removal of cholesterol form peripheral tissue to be transported back to the liver, thus high levels of HDL are inversely correlated with CHD risk (Lichtenstein et al., 1998).

Another theory that shows high HDL levels association with a reduction in CHD is that the concentration of atherogenic lipoproteins is inversely related to HDL levels: when triglyceride levels are elevated, HDL levels are low. In other words, a low HDL may increase the risk of CHD through the association with elevated concentration of triglyceride rich lipoproteins. In addition, studies have shown that HDL carries paraoxonase, an enzyme that can protect LDL from oxidation, which may be critical in the pathogenesis of atherosclerosis (Semenkovich, 1999).

The degree in which fatty acids contribute to the risk of CHD is complicated, and dietary fat intake alone may be a poor predictor of CHD risk. The saturated fatty acids laurate (12 carbons), myristate (14 carbons), and palmitate (16 carbons) contribute the most saturated fat in the western diet. The reduction of these fatty acids may reduce the risk of developing heart disease. Stearic acid (18 carbons) is an additional fatty acid in the diet that has not been shown to promote CHD (Nelson, 1998). These facts were shown in the recent Nurses' Health Study, in which over 80,000 women aged 34 to 59 years of age, with no known diseases have been followed since 1980. The study showed that with each increase of 5% of energy from saturated fat rather than carbohydrate, their risk of coronary disease rose 17% (Hu et al., 1997).

The role of unsaturated fatty acids in the development of CHD is less clear despite extensive study. Monounsaturated fatty acids, with oleic acid (18 carbons) predominating, were considered to have a neutral effect on blood cholesterol levels and CHD. However, recent studies have shown otherwise (Nelson, 1998). The study by Hu et al. found monounsaturated fat intake to be inversely related with heart disease risk. In

addition, metabolic studies show that replacing carbohydrates with monounsaturated fat raises HDL levels without effecting LDL levels (Hu et al., 1997). Other studies have shown however, that monounsaturated fatty acids do lower LDL levels (Groff et al., 1995). Monounsaturated fats are also shown to be resistant to oxidation, which is another factor in the development CHD (Hu et al., 1997).

Studies have also concluded that polyunsaturated fatty acids are beneficial in reducing CHD risk. Omega-6 polyunsaturated fatty acids are found in seed oils, and animal fats such as chicken, turkey, and pork are thought to lower cholesterol thus reducing CHD risk. However, their effect on reducing this risk of CHD has not been tested rigorously enough. Omega-3 polyunsaturated fatty acids are found in fish, leafy green vegetables, and a few seed oils. The role of these fatty acids in CHD is also unclear. They have been shown to lower plasma triglycerides, and reduce the risk of CHD (Nelson, 1998). In addition, on an equal weight basis they are more effective in lowering cholesterol than omega-6 fatty acids. Furthermore, omega-3 fatty acids have been shown to reduce platelet aggregation, which lessons the chance of a heart attack (Groff, Gropper, and Hunt, 1995). In population surveys, people who eat at least one meal of fatty fish per week suffered half the number of cardiac arrests than those who do not (Cerrato & Paul, 1999). However, other studies suggest otherwise showing no cholesterol lowering effect (Nelson, 1998). Taken together, large, epidemiologic studies have shown that polyunsaturated fatty acids have an overall inverse association between dietary intake and coronary disease. These metabolic studies show a cholesterol lowering effect with the use of vegetable oils rich in the polyunsaturated fatty acid, linoleic acid

when substituted for saturated fat. In addition, they show that diets high in polyunsaturated fats are more effective in lowering total serum cholesterol and coronary disease risk than a low-fat, high carbohydrate diet. This type of diet has been shown to *lower* HDL levels (Hu et al., 1998). Therefore, replacing saturated fat with monounsaturated and polyunsaturated fats may be more beneficial in preventing CHD than reducing overall fat intake.

The double bond of the unsaturated fatty acids can exist in two geometric forms, *cis* or *trans*. It has been shown that the form the fatty acid exists in may promote coronary diseases. Most naturally occurring fatty acids are found in the *cis* form in nature. However, dietary *trans* fatty acids are formed when seed oils are partially hydrogenated. The typical western diet, with many processed foods available that contain these partially hydrogenated oils, can contain high levels of *trans* fatty acids. Although it is not confirmed precisely, the average US diet may contain 8-15 grams per day (3%- 5% of total calories) of *trans* fatty acids (Nelson, 1998).

Like saturated fatty acids, epidemiologic studies by Hu et al. and others along with experimental feeding studies, show that *trans* fatty acids can increase LDL and decrease HDL. The Nurses' Health study showed that a reduction of 2% of calories from *trans* fatty acids could possibly reduce the risk of CHD by 53%. Therefore, there is a relationship between trans fatty acids and CHD in women. However, the actual amount of trans fatty acid intake for Americans needs to be measured more precisely before a strong conclusion can be made (Nelson, 1998).

Overall these studies suggest that the modification of the type of fat in the diet can be more effective in lowering heart disease risk than modification of fat intake as a whole. They show that a diet low in saturated fatty acids, *trans* fatty acids, with high levels of unsaturated fatty acids may be the best way to stabilize blood cholesterol levels and prevent coronary heart disease.

Antioxidants

The function of an antioxidant involves a complex process to protect cells against damage caused by activated oxygen. The antioxidant nutrients include the minerals copper, zinc, selenium, and manganese, and the vitamins C, A, β-carotene and E. The reduction of oxygen is a normal process that occurs at all times in the body. An example would be the reduction of oxygen to water in the electron transport chain. In these processes, oxygen gains an electron, which generates a free radical. This free radical sets off a chain of events that produce even more highly reactive molecules. These molecules can cause damage to living systems because they can take electrons from any organic molecule in their area, which will cause further damage and lead to disease. DNA, proteins, and polyunsaturated fatty acids are examples of molecules that may be attacked by these free radicals. The antioxidants nutrients work together by terminating the reactions and preventing additional damage to cells (Diplock, 1991).

Dietary antioxidants have been tested and associated with a decreased risk of several types of diseases. For example, high levels of the antioxidant selenium have been shown to prevent cancer in animals, and low intakes are associated with a high cancer incidence in humans. This is possibly the case because high levels of selenium are

suggested to be toxic to rapidly dividing tumor cells. However, no evidence exists between selenium and heart disease (Diplock, 1991).

Vitamin C or ascorbic acid is a water-soluble antioxidant that interacts with free radical in the watery part of the cells. This vitamin is the main plasma antioxidant and appears to be the first line of defense against free radicals. Vitamin C, which regenerates reduced alpha-tocopherol, has been shown to increase cholesterol excretion, improve endothelium-dependent vasodilation and reduce monocyte adhesion (Adams et al., 1999). However, large-scale studies on vitamin C and disease have mixed results (Slattery et al., 1995). Evidence has shown ascorbic acid to be protective against both cancer and CVD. This evidence comes from the fact that the incidence of these diseases is lower in populations with abundant intakes of leafy green vegetables and fruit. In addition, some epidemiological studies have shown an inverse relationship between dietary and blood levels of vitamin C and ischemic heart disease and cancer (Diplock, 1991). However, one large study of over 99,000 postmenopausal women, found vitamin C to not be associated with the lower risk of dying from coronary disease (Kushi et al., 1996).

Like above, high levels of beta-carotene, a vitamin A precursor which is carried in plasma and LDL, has been found to be associated with a lower incidence of cancer. However, the relationship between beta-carotene and heart disease is less clear. The role beta-carotene plays in regulating lipids has conflicting results from controlled studies. For example, plasma beta-carotene has been shown to be associated with an increase in HDL levels, but not total cholesterol or LDL levels and has also been shown to have no effect on triglyceride, total cholesterol, or HDL concentrations (Slattery et al., 1995).

Epidemiologic studies on the nutrient also have mixed results. No association was found between beta-carotene intake and CVD risk in the Nurses' Health Study and a study of 35,000 women followed for 8 years. However, in the Health Professional Study in which 39,000 men free of CHD were followed for 4 years, a 29% reduction of CHD was shown with high intakes of this antioxidant. Additionally, various small prospective studies looking at both men and women have found a protective effect (Marchioli, 1999).

Vitamin E

The antioxidant nutrient with the most promising evidence in the prevention of heart disease is vitamin E. Vitamin E is a fat-soluble vitamin that occurs in eight different forms in nature. Its compounds fall into two classes, the tocopherols, with saturated side chains, and the tocotrienols, with unsaturated side chains. Synthetic alpha-tocopherol, sold as vitamin E supplements, contains equal amounts of these eight different forms. Tocopherols and tocotrienols react more readily with free radicals than PUFA. Thus vitamin E is a chain breaking antioxidant that is the major lipid-soluble antioxidant in the plasma (Kayden and Traber, 1993).

Vitamin E is found primarily in vegetable oils and grains, green plants, eggs yolk, liver, nuts, and vegetables. Of the different forms of vitamin E, only the alpha-tocopherol form of the vitamin is maintained in the plasma. In addition, the only forms of alpha-tocopherol maintained in the plasma are *RRR*-alpha-tocopherol, the form that occurs naturally in foods, and the other *2R*-stereoisomeric forms of alpha-tocopherol, which is found in synthetic vitamin E. Furthermore, non 2 *R*-isomers of the synthetic form of vitamin E, the *all rac*-alpha-tocopherol, are not maintained in human plasma or tissues.

Appendix A shows the structures of these forms of alpha-tocopherol. All other naturally occurring forms of vitamin E (β, γ, and δ-tocopherols and the tocotrienols) are not counted toward meeting the vitamin E requirements because they are not converted to alpha-tocopherol by humans and poorly recognized by the liver for conversion to alpha-tocopherol (Food and Nutrition Board 2000).

The report by the Panel on Dietary Antioxidants and Related Compounds was released in the April 2000 with publication in May of that year. In that report, the previously recommended levels for vitamin E were increased for both adult women and men to 15 mg/day, which is equivalent to 22 international units (IU) of natural-source vitamin E or 33 IU of the synthetic form (Monsen, 2000.) This new level is based on the alpha-tocopherol form of vitamin E, which is a change from most recent recommendations for the reasons mentioned above (Food and Nutrition Board 2000).

Before 1980, the United States Pharmacopoeia (USP) defined one international unit (IU) of vitamin E activity as 1 mg of *all-rac*-alpha-tocopheryl acetate, and 1 mg *RRR*-alpha-tocopherol was calculated to be equivalent to 1.49 IU of vitamin E. After that year, one USP unit of vitamin E was still defined as having the activity of 1 mg *all-rac*-alpha-tocopheryl acetate, 0.67 mg *RRR*-alpha-tocopherol, or 0.74 mg *RRR*-alpha-tocopheryl acetate. Today, the IU is no longer recognized, but many fortified foods and supplements are still using the terminology while USP units are used in labeling of vitamin E supplements (Food and Nutrition Board 2000).

Therefore, vitamin E definition is limited to the *2 R*-stereoisomers forms of alpha-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-alpha-tocopherol) to establish recommended

intakes. Based on this, *all rac*-alpha tocopherol has one-half the activity of *RRR*-alpha-tocopherol found in foods or present with the other *2 R*-stereoisomeric forms (*RSR-, RRS- and RSS-)* of alpha-tocopherol in fortified foods and supplements. Thus to achieve the RDA of 15 mg/day of alpha-tocopherol, one can take in 15 mg/day of *RRR*-alpha-tocopherol or 15 mg/day of the *2R*-stereoisomeric forms of alpha-tocopherol (30 mg/day of *all rac*-alpha-tocopherol) or a combination of the two. The factors to convert *RRR*- and *all rac*-alpha-tocopherol and their ester bonds of vitamin E to USP units (IU) are given in Table 6 (Food and Nutrition Board 2000).

Evidence today suggests that levels higher than the current RDA could be beneficial to human health. Many studies are suggesting that the intake of vitamin E in much higher levels than the current recommendations are associated with the reduced risk of CVD, cancer, and enhancement of the immune response (Weber, Bendich, & Machlin, 1997).

There are limited reports of vitamin E deficiency in man. In experimental deprivation when only 2-3 mg tocopherol was provided per day for 1-2 years, there were no shown symptoms expect an increased susceptibility of erythrocytes to hemolysis. People with familial, inherited isolated vitamin E deficiency, however, were reported to have severe neurologic symptoms such as difficulty walking and talking. In addition, in people having fat malabsorption for 10-12 years, neurologic dysfunction was also seen in children with malabsorption, this neuromuscular disorder appeared in a shorter period

Table 6

Factors for Converting IU[1] of Vitamin E to Alpha-Tocopherol (mg) to Meet Recommended Intakes.

	USP[2] Conversion Factors		Molar Conversion Factors	Alpha-tocopherol Conversion Factors
	IU/mg	mg/IU	Umol/IU	mg/IU
Synthetic Vitamin E				
dl α-tocopheryl acetate	1.00	1.00	2.12	0.45
dl α-tocopheryl succinate	0.89	1.12	2.12	0.45
dl α-tocopherol	1.10	0.91	2.12	0.45
Natural Vitamin E				
d-α-toocpheryl acetate	1.36	0.74	1.56	0.67
d-α-tocopheryl succinate	1.21	0.83	1.56	0.67
d-α-tocopherol	1.49	0.67	1.56	0.67

Ref: Food and Nutrition Board, 2000

[1] IU: International Units

[2] USP: United States Pharmocopoeia

of time. Furthermore, deficiency of vitamin E has been reported to also affect immune response (Weber, Bendich & Machlin, 1997).

Surprisingly, limited data are available to assess population consumption, intake patterns, and trends in exposure to nutrient and non-nutrient antioxidants despite the interest in the role they play in disease prevention. Consumption of the nutrient antioxidants including vitamins E and C and the carotenoids, is usually assessed through the food supply data that the USDA keeps. The Centers for Disease Control (CDC) and the USDA monitor intake patterns of Americans through dietary intake and supplement use surveys. Exposure to non-nutrient antioxidants in the food supply can be assessed through the Food Additives Survey that the Food and Nutrition Board conducted for the Food and Drug Administration (FDA) (Woteki, 1995).

Research on dietary supplements has mainly focused on use. In 1992, one study found that 46% of the US population reported taking a vitamin or mineral supplement in the past year. Characteristics of those taking vitamin/mineral supplements include being female, being white, a high level of education, a high income, and advancing age. In addition, living in the west, consuming fruits and vegetables, playing sports, and having health problems were also associated with supplement use. Approximately 26% of adults take a product containing vitamin E (Lino et al., 1999). The median amount of vitamin E provided by these supplements is 200%, and the 95th percentile is 2860% the current RDA at that time (Woteki, 1995).

In the food supply, vegetable oils are the major source of vitamin E, accounting for 66% of the total. When looking at dietary patterns of vitamin E consumption from

our food supply, intake increases with higher incomes and education levels, and is higher among whites than blacks (Woteki, 1995). Fats and oils is the number one contributor of tocopherols, providing more than 20% of vitamin E in the U.S. diet. Vegetables are the second largest contributors providing about 15%. Fruits only contribute 5% of vitamin E in the U.S. diet, but when combined with vegetables, they provide more vitamin E then fats and oils (Murphy et al., 1990). Thus increasing food choices form fruits and vegetables would be a low-fat way for individuals to increase their vitamin E intake. However, despite public recommendations to increase fruit and vegetable intake, the majority of the population consumes far less than the recommended amounts. For example, in analysis of the second National Health and Nutrition Examination Survey (NHANES II), it was found that 83% of adults reported consuming vegetables and only 59% reported consuming fruit the day before the survey (Woteki, 1995).

In an USDA population survey in 1985, the Continuing Survey of Food Intakes by individuals (CSFII), the mean intake of vitamin E by women age 20-49 was 7.0 mg alpha tocopherol (Woteki, 1995). In addition, the Second National Health and Nutrition Examination Survey (NHANES II) was used to estimate the intake of vitamin E in the U.S. This showed that the mean intakes of vitamin E were close to the RDA for both men (9.6 mg per day) and women (7.0 mg per day). However, median intakes were lower with an intake of 7.3 mg per day in men and 5.4 mg per day in women (Murphy, Subar, & Block, 1990). The more recently published NHANES III found similar results; the mean intake was 10 mg TE in men (median 9.3 mg TE) and 8.6 mg TE in women (median 6.8 mg TE) (Weber, Bendich, & Machlin, 1997).

Vitamin E and Heart Disease

Vitamin E has been studied vigorously in the recent years to determine the effect it plays in the prevention and treatment of diseases of the heart. For years there has been a debate over the origin of the development of fatty deposits in the blood vessels of the heart. The "response-to-injury" hypothesis of atherosclerosis explains this process "as an inflammatory response to injury of endothelium, which leads to complex molecular and cellular interactions between cells derived from the endothelium, smooth muscle cells and several blood cell components" (Chan, 1998). This inflammation sets off the production of free radicals, which promotes the oxidation of LDL trapped in the endothelium. The products from this oxidation are bioactive, and they cause the release of cytokines, growth factors, and several surface adhesion molecules. These surface adhesion molecules are capable of bringing circulating monocytes and T-lymphocytes into the intima where monocytes develop into macrophages which eventually deve
into foam cells. As a result of the presence of growth factors and cytokines, sr
muscle cells divide in the intima, which results in the narrowing of the lur
This oxidized LDL can also inhibit the endothelial production of pros
nitric oxide, two vasodilators and inhibitors of platelet aggregatio
oxidized LDL and injury of the endothelium plays a role in th
atherosclerosis.

LDL is the key carrier of vitamin E in circulation
studies suggests that this oxidation of LDL can be supr
vitamin E. Taken together, studies demonstrate that

oxidation, inhibit proliferation of smooth muscle cells, inhibit platelet adhesion, inhibit the function of adhesion molecules, and cause the release of PGI2 (a vasodilator) (Chan, 1998). Thus the intake of vitamin E may be a major protection against the development of heart disease.

Although much of the evidence is supportive, results of epidemiological studies on vitamin E for the prevention of heart disease are controversial. One such study is the previously mentioned Nurses' Health Study that included over 87,000 middle-aged women free of disease. To determine the effect of the consumption of vitamin E on heart disease, the subjects completed medical history and dietary questionnaires to assess their intake of vitamin E from food and supplements for eight years. The results from this large prospective study showed a 40 percent less risk of major coronary disease after adjusting for other risk factors among women who took vitamin E supplements than in those who did not (Stampfer et al., 1993). Reduction was only seen in those who took supplements, not with multivitamin use. In addition, women who took vitamin E supplements for more than two years has less of a relative risk of coronary disease than those who took them for short periods of time (Marchioli, 1999). Furthermore, there was no association found between plasma levels of vitamin E and resistance to LDL oxidation among nonusers of supplements, but vitamin E supplementation increased the resistance. Therefore, this study shows that supplementation at levels higher than what is achievable by the diet may be needed to reduce LDL oxidation (Stampfer et al., 1993).

Similar results were found in men. The Health Professionals Follow-up Study included over 50,000 male health professionals aged 40 to 75 years. These participants

completed detailed questionnaires that assessed their medical history and intake of nutrients including vitamin E for four years. Like above, it was shown that higher intakes of vitamin E reduced the risk of coronary disease after adjusting for other risk factors associated with coronary disease. The results from this study show that men who take supplements have less a risk for coronary disease than those who do not (Rimm et al., 1993). Again, short-term use of vitamin E supplements (less than 2 years) was not associated with a reduced risk of heart disease events (Marchioli, 1999).

Other epidemiologic studies however, have shown that dietary intake of vitamin E may also reduce the risk of coronary disease. These studies show that areas where there is a low dietary intake of vitamin E have higher rates of CHD. One such study of over 35,000 postmenopausal women followed for 7 years found a 62% reduction in those with higher vitamin E intake from foods (Marchioli, 1999). Cross-sectional studies have shown mean plasma concentrations of vitamin E to be higher in regions with low coronary mortality. These results support the antioxidant theory of vitamin E and coronary disease but show the need for further research because other dietary and lifestyle characteristics can differ in the regions studied. Therefore, it cannot be definitively concluded that the low rate of cardiovascular disease is due to dietary intake of the antioxidant vitamin E; many other risk factors must be considered (Stampfer & Rimm, 1995).

Although the above epidemiologic studies show that supplementation with high amounts of vitamin E may reduce the risk for coronary disease, smaller research studies have shown that supplementation with low-dose vitamin E can also reduce this risk. One

such study looked at the effect of low-dose vitamin E supplementation on the susceptibility of LDL and HDL to oxidation. Human subjects (n= 8) ingested low-doses of vitamin E (150 mg/day for 1 week, and 300 mg/day for 3 weeks afterwards) in divided doses with meals. There was no intake of vitamins or medications before the study began. After supplementation, the vitamin E content of LDL and HDL increased. This study also showed that this low-dose of vitamin E protected LDL against oxidative modification and decreased the propagation rate or HDL oxidation. Therefore, it was shown here that low-doses of vitamin E could possibly be beneficial in halting atherosclerosis (Suzukawa et al., 1995). In addition, in another study looking for the smallest intake of vitamin E possible to protect LDL against oxidation, it was found that supplementation in humans with even 25 mg/day leads to significant resistance against oxidation. In contrast, this study found the progression of lipid oxidation in LDL was reduced only after intakes of high doses of vitamin E (400 and 800 mg/day) (Princen et al., 1995).

Contrary to the information presented thus far, another epidemiologic study found no association between serum vitamin E and heart attacks in a population with high vitamin E levels. In the population-based MONICA Augsburg cohort, 2023 men and 1999 women aged 25 to 64 years were studied in 1984 and then again in 1987/88. The relationship between serum vitamin E levels and the risk for myocardial infarction (MI) was assessed. There were no differences found in the means of vitamin E concentration in the cases of MI between the controls and the subjects. Nor was a difference found in the mean vitamin E/total cholesterol ratios. Although this association was not modified

by other risk factors, this study concludes that serum vitamin E concentrations were not associated with the myocardial infarction risk. However, this is possibly due to the high average level of vitamin E in the study population (Hense et al., 1993).

Overall, the above and other epidemiological studies suggest that long-term use (greater than two years) of vitamin E from both diet and supplements is associated with a lower risk of CVD events. Additional studies are ongoing to further assess the relationship between heart disease and antioxidants.

One new area of interest in vitamin E is the antioxidant's association with cancer, particularly prostate cancer. The interest in vitamin E as a supplement for prostate cancer came from the results of a Finnish study that showed a lower morbidity and mortality from the disease in men taking 50 mg of synthetic alpha-tocopherol per day. Furthermore, some studies looking at heart disease found that gamma-tocopherol may also play a role in prevention. Therefore, a recent study tested that ability of gamma-tocopherol in the control of the growth of a human prostate cancer. The results showed gamma-tocopherol to be superior to alpha-tocopherol in cell inhibition in vitro. This shows that both form of the vitamin should be thoroughly looked at in the future for the prevention of cancer and other chronic diseases (Moyad et al., 1999).

Cottonseed Oil

The use of vegetable oils high in alpha-tocopherol is one way to increase individuals' intake of vitamin E. Cottonseed oil is an example that is rich in tocopherols (See Table 7). Crude cottonseed oil contains about 1000-ppm tocopherols, but about 1/3

is lost in refining. The main tocopherol content in the crude oil comes from both gamma-tocopherol, which accounts for 58% and alpha-tocopherol, which accounts for 41% of the total. Refined cottonseed oil contains about 320-ppm, or 32-mg/100 g, alpha-tocopherol and about 313 gamma-tocopherols. If desired, naturally occurring tocopherols lost during refining can be replaced with additional tocopherols and antioxidants that were lost (Jones & King, 1996). Therefore, one tablespoon of cottonseed oil contains approximately 5 mg of vitamin E and like all oils supplies 120 kcal and about 14 grams of fat.

Cottonseed oil has been part of the American diet for over a century. This oil ranks third in volume behind soybean and corn oil representing 56% of the total domestic fat and oil supply. Cottonseed oil is extracted from cottonseed of the cotton plant, which produces food for both animals and man in addition to many industrial uses. In foods, cottonseed oil has a wide variety of uses both commercially and domestically. It can be used as a salad oil in mayonnaise and for salad dressings, sauces, and marinades. In addition, cottonseed oil can be used as cooking oil, frying oil or as a shortening or margarine in baked goods. In the US, 56% of cottonseed oil is used as a salad or cooking oil, 36% is used in baking or frying fats, and smaller amounts are used as margarine and other uses. Furthermore, the high vitamin E antioxidant content of the oil contributes to stability thus giving products a long shelf life (NCPA, 1999).

Table 7

Tocopherols of Cottonseed Oil (mg/kg oil or ppm)

	Type of Oil	
Tocopherol Isomers	Crude	Refined
α	402	320
β	1.5	--
γ	572	313
δ	75	--
Total tocopherols	1050.5	633

Note: α: alpha tocopherol; β: beta tocopherol; γ: gamma tocopherol; δ: delta tocopherol
Ref. Bailey's Industrial Oil & Fat Products, Fifth Edition ("Cottonseed Oil, 1996)

Cottonseed oil is among the most unsaturated oils and is considered an acceptable way to reduce saturated fat intake (Table 8). It has a 2:1 ratio of polyunsaturated to saturated fatty acids, with 70% unsaturated (about 18% monounsaturated, 52% polyunsaturated, and 26% saturated) (NCPA, 1999). However, the specific fatty acid profile depends on the variety of cotton grown, growing conditions, and analytical method used to determine the profile. Cottonseed oil is in the oleic-linoleic group of vegetable oils because these fatty acids make up about 75% of the total fatty acids (Table 9). Oleic acid makes up 22% and linoleic acid makes up 52% of the fatty acid content. Additional fatty acids include palmitic acid, which makes up 24%, linolenic acids that makes up less than 1%, and smaller amounts of saturated fatty acids (including stearic acid) (Jones & King, 1996).

Cottonseed oil is described as being "naturally hydrogenated" because of the high levels of oleic, palmitic, and stearic acids. With an iodine number of 103-116, cottonseed oil is a good choice for food preparation since it is stable without the additional processing or formation of trans fatty acids. With the level of saturated fatty acids at 26%, cottonseed oil would need less hydrogenation or could be used more often in food applications where the use of other vegetable oils would require hydrogenation (Jones & King, 1996). In addition, when it is partially hydrogenated its monounsaturated fatty acids actually increase. For example, when hydrogenated to an iodine value of 80, its fatty acid profile shifts to 50% monounsaturated, 21% polyunsaturated, and 29% saturated, which is well within current health guidelines (NCPA, 1999).

Table 8

Comparison of Fatty Acid Profiles (Weight %) of Selected Dietary Fats

	Saturated (%)	Polyunsaturated (% Linoleic/Linolenic)		Monounsaturated (%)
Vegetable oil				
Canola	6%	26%	10%	58%
Safflower	9%	78%	Tr	58%
Sunflower	11%	69%	-----	20%
Corn	13%	61%	1%	25%
Olive	14%	8%	1%	77%
Soybean	15%	54%	7%	24%
Peanut	18%	34%	-----	24%
Cottonseed	27%	54%	-----	19%
Palm	51%	10%	-----	39%
Coconut	92%	2%	-----	6%

Ref: Human Nutrition Information Service, USDA

Table 9
Fatty Acid Composition of Cottonseed Oil

Fatty Acid	Percent Weight
Myristic (14:0)	0.9
Palmitic (16:0)	25.2
Palmitoleic (16:1)	0.8
Stearic (18:0)	2.7
Oleic (18:0)	17.5
Linoleic (18:2)	52.6
Linolenic (18:3)	-----
Arachidonic (20:4)	-----

Ref: Bailey's Industrial Oil & Fat Products ("Cottonseed Oil", 1996)

Limited nutritional studies have been conducted on cottonseed oil intake. Most studies were performed on rats to compare dietary cottonseed oil intake to corn oil intake. In one such study, the rats utilized the cottonseed oil diet more efficiently than the corn oil diet. The cottonseed oil had a hypocholesterolemic effect on the rats when compared to the corn oil fed rats. Total serum cholesterol was found to be lower in the rats fed the cottonseed oil diet compared to the rats fed corn oil. In addition, rats fed corn oil has significantly higher triglyceride levels than the cottonseed oil fed rats. However, VLDL and LDL were significantly higher in the rats fed cottonseed oil compared to the ones fed corn oil (Hampden et al., 1983).

Another similar study in which cottonseed and corn oil were compared in regard to their effect on serum lipid levels in rats found lower serum levels of cholesterol in the rats fed cottonseed oil than corn oil. However, the type of oil consumed did not affect the serum triglyceride levels of the rats in this study (Edwards & Radcliffe, 1995). In addition, a more recent study looked at the lipid lowering effects of a mixture of dietary corn oil with cottonseed oil. Rats were fed diet of either corn oil, cottonseed oil, or a 1:1 mixture of the two. This study found no effect on the lipid profile of the rats fed the cottonseed oil/corn oil mixture, but total replacement of cottonseed oil for corn oil resulted in lower HDL and total cholesterol levels in the rats without effecting triglyceride levels (Radcliffe et al., 2001).

The above results cannot be explained by the difference in the fatty acid profile of the two oils because cottonseed oil has a higher level of saturated fatty acids and lower levels of monounsaturated and polyunsaturated fatty acids than corn oil. This effect

therefore may be a result of a constituent or constituents of the non-lipid matter of cottonseed oil. Therefore, further studies to determine the cholesterol lowering constitutes of cottonseed oil to determine the oils ability to reduce the development of heart disease is needed (Edwards & Radcliffe, 1995). To this date, the nutritional effect of cottonseed oil intake in humans has not been studied exclusively.

Corn Oil and Lipid Levels

Like cottonseed oil, corn oil provides essential fatty acids, vitamin E, and is a rich source of polyunsaturated fatty acids. Corn oil is a good source of linoleic acid, with the ratio of linoleic acid (n-6) to linolenic acid (n-3) approximately 5:1. Therefore, a tablespoon of the oil will satisfy the daily essential fatty acid requirement for a healthy child or adult (Hui, 1996).

Corn oil has been used over the years in research looking at the relationship of dietary fat to blood cholesterol levels. This is because prior to the 1950's, corn oil was the only oil readily available to investigators. As a result of its stability, taste, and applications, corn oil was used as the standard which others where compared when looking at cholesterol lowering ability.

When looking at all studies from 1957 to 1993, the mean of cholesterol lowering effect of corn oil was 16%. Fourteen of the studies looked at lipoprotein levels. In general, LDL levels lowered as total cholesterol did. As for HDL levels, the results were more variable. In short duration studies (less than 6 weeks), the trend was HDL lowering. However, studies with a longer duration showed no change in HDL levels. This cholesterol lowering ability of corn oil has not been attributed to its fatty acid profile, but

high levels of nontriglyceride compounds in the oil. These include plant sterols. The ability of plant sterols to lower cholesterol, especially β-sitosterol, has been convincingly demonstrated in recent years (Hui, 1996). Table 10 compares the fatty acid composition of dietary corn oil to cottonseed oil (Radcliffe & Edwards, 1995).

Table 10
Comparison of Fatty Acid Composition of Dietary Corn and Cottonseed Oils

Fatty Acid	Corn Oil Weight of fatty acids (%)[1]	Cottonseed Oil Weight of fatty acids (%)
Myristic, C14:0	Not detectable	0.9
Palmitic, C16:0	10.5	23.5
Palmitoleic, C16:1	Not detectable	0.7
Stearic, C18:0	2.1	2.0
Oleic, C18:1 (n-9)	27.6	18.4
Linoleic, C18:3 (n-6)	58.6	52.9
Linolenic, C18:3 (n-3)	0.8	0.2
Arachidic, C20:0	0.2	0.4
Eiosanoic, C20: 1	0.2	0.4
Bechenic, C22:0	Not detectable	0.6
%Total fatty acids		
Saturated	12.8	27.4
Monounsaturated	27.8	19.5
Polyunsaturated	59.4	53.1

[1] Values in weight percentages of fatty acids

Ref. Edwards & Radcliffe (1995)

CHAPTER III

METHODOLOGY

This study assessed the effects of foods made with cottonseed oil or corn oil on vitamin E intake as well as on serum lipid levels. The acceptability of food products made with cottonseed oil or corn oil was determined by the consumption of the provided foods and the answers to a questionnaire.

Participants: Volunteers (n=21) for this study were men and women (all over the age of 18 years). Prior exclusion criteria were known allergies to cottonseed oil, corn oil, or other ingredients in the foods provided, use of medications that may affect fat metabolism, or pregnancy. This is included in the pre-study questionnaire (Appendix B) and the consent form (Appendix C).

Participants were randomly divided into two groups, in which each was assigned a random code to ensure confidentiality. All food items provided and questionnaires were assigned by code, not name.

Experimental Design: Table 11 shows an outline for data collection within the study design. Twenty-one subjects were recruited through class announcements. The study was five weeks in duration. On week one, participants' blood was drawn (10 mL) after an overnight fast to determine their baseline total cholesterol (TC), HDL-cholesterol (HDL), LDL-cholesterol (LDL), and triglyceride (TG) levels. Samples were spun at 3500-x g

Table 11
Data Collection Protocol

Week 1[1]	Week 2	Week 3	Week 4	Week 5[2]
Orientation	Study Foods	Study Foods	Study Foods	Blood Draw
Blood Draw	Diet Record	Diet Record	Diet Record	Questionnaire 3
Diet Record	Questionnaire 2			
Questionnaire 1				

Note: Participants n= 21.

[1] Baseline data (Diet records, blood, Questionnaire 1)

[2] Final data (Diet records, blood, Questionnaires 2 and 3)

for five minutes to separate the serum, which was agitated and stored at 0° C until analyzed. Questionnaire one (Appendix B), covering participants' height, weight, usual food choices, amounts of foods consumed, food allergies, and supplement or medications taken, was administered at this time. Food records (Appendix D) were kept every day the week before supplemental food was provided to determine baseline vitamin E intake from both dietary sources and supplements.

Participants were then provided with foods containing high amounts of vitamin E for the three following weeks (weeks 2-4). The foods included muffins made with cottonseed oil and wheat germ (2 per week), sweet potato bars made with cottonseed oil (2 per week), and salad dressing made with equal parts cottonseed oil and fat-free honey mustard (2 tablespoons). Controls were provided with the same foods except that the dressing, muffins, and bars were made with corn oil. Data and estimated nutrient contents of the food products are given in Table 12. Recipes for the food products provided are shown in Appendix E. Participants were required to keep daily diet records each day for the duration of the study. They were given a sample diet record assist them with accuracy of recording their intakes (Appendix D). There was no limit on their other food choices.

To compare week one to weeks two through four, blood (10-mL) was drawn (after an overnight fast) at the beginning of week five to determine if TC, HDL, LDL, and TG levels had changed. The blood samples were again spun and separated and were stored at 0°C until analyzed. Diet records were then analyzed to determine if dietary vitamin E levels had increased in weeks two through four and if the provided foods

Table 12
Nutrient Content of Food Products (per Serving)

	Cocoa muffins		Carrot Muffins	
	CO	CSO	CO	CSO
Energy (kcal)	237	237	214	214
Protein (g)	3.09	3.09	4.50	4.50
Fat (g)	9.91	9.91	8.08	8.08
SFA[1] (g)	1.34	2.54	1.18	2.08
MUFA[2] (g)	2.33	1.75	1.96	1.52
PUFA[3] (g)	5.73	5.12	4.42	3.96
Alpha-tocopherol	2.11	4.00	1.77	3.19
Gamma-tocopherol (mg)	5.90	2.72	4.44	2.06
α-TE	2.89	4.45	2.36	3.53
	Sweet Potato Bars		**Salad Dressing**	
	CO	CSO	CO	CSO
Energy (kcal)	129	129	164	164
Protein (g)	1.30	1.30	0.12	0.12
Fat (g)	6.28	6.28	13.64	13.64
SFA (g)	1.18	1.78	1.74	3.54
MUFA (g)	1.77	1.48	3.30	2.43
PUFA (g)	2.97	2.66	8.00	7.07
Alpha-tocopherol	0.81	1.75	1.97	4.80
Gamma-tocopherol	3.41	1.83	8.84	4.08
α-TE	1.16	1.94	2.88	5.21

[1] SFA: Saturated fatty acids
[2] MUFA: Monounsaturated fatty acids
[3] PUFA: Polyunsaturated fatty acids

were consumed. Participants then completed post-questionnaire 3 (Appendix B) to evaluate the perception of the food products consumed, supplements taken, and any problems they had with consuming the provided foods.

Questionnaires: Participants, who were given an orientation on the study design, were asked to complete pre- and post-study questionnaires. The pre-study questionnaire (Questionnaire 1) (Appendix B) covered height and weight, usual food choices, amounts of foods consumed, food allergies, and supplements or medications taken. Questionnaire 2 was given to the participants with their diet records at the beginning of week two. Questionnaire 2 was a hedonic scale questionnaire used as another way to evaluate the acceptability of the provided foods. The scale ranged from scores of 1 (dislike extremely) to 5 (neither like nor dislike) to 9 (dislike extremely). Scores 6-9 were considered to be acceptable for the foods provided, and scores 1-5 was considered to be unacceptable for the foods provided. An additional determination of acceptability was if subjects consumed 2/3 or 66% of the food items provided. After the completion of the study, a post-study questionnaire was administered (Appendix B). Questionnaire 3 was used to obtain feedback regarding the study. This questionnaire covered the participants' thoughts on how the provided foods affected their normal food choices. Two additional questions were used to again determine the use of medications or supplements during the study period.

Human Subjects Review: Texas Woman's University's Human Subjects Review Committee (HSRC) approved this study on July 26, 1999 (Appendix F). All study data

were kept locked in the investigator's home and office. No access to data was available to anyone except the investigator and Thesis committee members.

Data Analysis: Nutrient intakes were calculated using the Minnesota Nutrient Data System, Research Version 4.0. Vitamin E intakes, both as alpha-tocopherol equivalents, mg alpha-tocopherol, and gamma-tocopherol before the study were compared with vitamin E intake levels during the study by using the Mann-Whitney statistical test. Additional dietary results and percent acceptability of the products were also compared using the above test. The level of significance was $p<0.05$. Data on height, weight, and age (obtained from the questionnaire) were expressed as means and standard deviation and expressed descriptively. TC was determined using a Stanbio™ Cholesterol_liquiColor® enzymatic colorimetric test (Appendix G). Determinations of HDL were done using the Sigma kit (Appendix G). Determination of TG was done using the Stanbio™ kit (Appendix G). Values for serum lipids were compared using the Mann-Whitney statistical test with the significance level $p<0.05$. Results obtained from the sensory analysis were compared by cross tabulation of the data. SPSS version 9.0 and 10.0 was used to aid in the above statistical analysis.

CHAPTER IV

RESULTS

Of the 21 participants enrolled in the study, ten in the corn oil (CO) group and 11 in the cottonseed oil (CSO) group, 18 completed the study. Two female participants dropped out and one female was eliminated. Data that were collected on these participants can be found in Appendix H. Of the participants who dropped out, one was a result of illness while the other was because of a damaged diet record. The participant who was eliminated was done so due to excessive amount of vitamin E and fat intake from supplements and oils (i.e. flaxseed, primrose oil). These removed participants were in the CSO group leaving ten in the CO group and eight in the CSO group completing the study.

The average age of participants was 31.9±12.38 years with a range of 22 to 55 years. There were 13 females and five males completing the study in which two males and eight females were in the CO group and three males and five females were in the CSO group. None of the females was post-menopausal. Tables 13 and 14 show the age, height, weight and gender of the participants according to group. There was no statistical significance found between the two groups in regards to age, height or weight. Please see Appendix I for details on the age, height and weight for each participant according to group.

Table 13

Demographic Data for the Participants Consuming Corn Oil (CO) Products for Four Weeks (n=10)[1]

	Age (years)	Height (in.)	Weight (lbs.)
Mean	29.50	67.3	142.2
Std. Deviation	11.02	1.77	13.66
Minimum	22	64	122
Maximum	55	70	168

[1] Group contained two males and eight females.

Table 14

Demographic Data for the Participants Consuming Cottonseed Oil (CSO) Products for Four Weeks (n=8) [1]

	Age (years)	Height (in.)	Weight (lbs.)
Mean	35	67.75	163.63
Std. Deviation	14.04	4.33	35.3
Minimum	22	62	120
Maximum	55	74	220

[1] Group contained three males and eight females.

Hypothesis 1: Consuming foods made with cottonseed oil, relative to ones made with corn oil, will not increase the dietary intake of vitamin E.

After controlling for supplements containing vitamin E by not including them in the final dietary analysis and adjusting for synthetic vitamin E added to foods (i.e., Total cereal, meal replacement products) according to the recent guidelines, both alpha-tocopherol equivalents and mg alpha-tocopherol before the feeding period (baseline) were compared with vitamin E intake levels during the feeding period. Please see Appendix J for the vitamin E intake of the two subjects who took supplements before the diet records were adjusted. In addition, gamma-tocopherol intake was determined as a result of the recent interest in the use of the compound in the prevention of prostate cancer. There were no significant differences found between the CO and CSO groups in regards to change in daily intake of vitamin E expressed as alpha-tocopherol equivalents, mg alpha-tocopherol, or gamma-tocopherol from the pre-feeding period to the post-feeding period. The significance level of each was found to be 0.328, 0.286, and 0.534 respectively.

The average change, standard deviation, and range of change in alpha-TE, mg alpha-tocopherol, and mg gamma-tocopherol between the CO group and CSO are shown in Table 15. The level of change was not found to be significant, although the change was higher for the CSO group than the CO group for both alpha-TE and mg alpha-tocopherol, however, the change in gamma-tocopherol was slightly higher for the CO group. The actual vitamin E intake values for each subject in both the corn oil and cottonseed oil groups are shown in Appendix K. The percent change of vitamin E in

Table 15

The Average Change in Alpha-TE, Alpha-Tocopherol, and Gamma-Tocopherol in Participants Consuming Corn Oil (CO) or Cottonseed Oil (CSO) Products (n=18) [1]

	CO Group	CSO Group
Alpha-TE (mg)		
Mean	-2.132	.6538
Range	-16.60-2.61	-7.88-7.52
Std. Deviation	6.22	4.41
Alpha-Tocopherol (mg)		
Mean	-2.3240	.5538
Range	-15.94-2.08	-7.77-7.26
Std. Deviation	5.91	4.27
Gamma-Tocopherol (mg)		
Mean	1.9540	.6075
Range	-6.03-10.96	-3.34-5.08
Standard Deviation	4.64	3.15

[1] Final minus baseline values expressed as mg/day.

each group can also be found in Appendix K. The mean percent change was positive for all three forms of vitamin E in the CSO group, but the mean percent change was found to be positive only for gamma-tocopherol in the CO group.

Hypothesis 2: Consuming Foods made with cottonseed oil, relative to those made with corn oil, will not affect blood lipid levels in the participants.

To determine the change in blood lipid levels between the CO group and the CSO group, each participant's blood was analyzed to determine his or her baseline TC, HDL, LDL, and TG levels before supplementation and again at the end of the feeding period. There were no significant changes in TC, HDL, or LDL. There was however, a significant difference in the change in the TG levels between the two groups, with the CO group showing an increase in TG level and the CSO group showing a decrease in TG level. Table 16 shows the significance level of the change in blood lipids between the CO group and CSO group from week one to week four.

Although not all changes in bloods lipid values were statistically significant, there was a difference between the two groups in the change of each value. The following table, Table 17, shows the differences between the two groups in regards to change in TC, HDL, LDL, and TG levels. Appendix L shows the actual change for each participant in the CO and CSO group. Both average change and the actual values of each participant's change in blood lipids are shown. There was a significant change in TG levels between the two groups with the CO group showing an increase and the CSO group showing a decrease. The average change in TC decreased in the CO group and

Table 16
Significance Level of the Change in Blood Lipids between the CO Group and CSO Groups from week 1 to week 4 (n=18)

	TC[1]	HDL[1]	LDL[1]	TG[1]
Significance (2-tailed)[2]	0.120	0.304	0.182	0.013

[1] TC: Total cholesterol
HDL: High-density lipoprotein
LDL: Low-density lipoprotein
TG: Triglycerides

[2] Mann-Whitney statistical test

Table 17

Average Change in Lipid Values in the CO group and CSO group[1]

	TC[2] Change	HDL[2] Change	LDL[2] Change	TG[2] Change
CO Group (n=10) Mean Range Std.Deviation	-7.30 (mg/dl)[3] -38-28 21.92	-5.10 (mg/dl)[3] -12-8 5.97	-6.50 (mg/dl)[3] -42-24 21.46	21.50 (mg/dl)[3] -8-97 32.33
CSO Group (n=8) Mean Range Std. Deviation	5.50 -26-33 17.07	-3.63 -8-3 3.38	10.25 -24-45 20.83	-9.63 -27-11 13.90

[1] Change was calculated by final minus baseline values.

[2] TC: Total cholesterol
HDL: High-density lipoproteins
LDL: Low-density lipoproteins
TG: triglyceride

[3] mg/dl: milligrams/deciliter

increased in the CSO. HDL levels trended downward in both groups, while LDL levels showed an average decrease in the CO group and average increase in the CSO group.

Hypothesis 3: Food Items (muffins, bars, and dressing) made with cottonseed oil will not be as acceptable as ones made with corn oil.

The acceptability of food products made with cottonseed oil and corn oil was determined by the consumption of foods provided and the answers to the administered questionnaires. The first method used to determine if food products made with the two oils were acceptable was if 2/3, or 66%, of the products were consumed in greater quantities than the ones made with the other oil.

There was no significant difference between the two groups in regards to the amount of products consumed, therefore, the food products were equally accepted between the two groups according to the amount of consumed. The following tables, Tables 18 and 19 show the percent of products consumed by the CO and CSO groups, respectively.

The participants in the CO group consumed more of the provided foods than those in the CSO group (Tables 18 and 19). Each group however, had an average intake of over 66%, therefore the products were considered acceptable in both groups.

After the feeding period, participants were provided with a questionnaire containing a hedonic scale as a second way to determine the acceptability of the food items provided (Appendix B). The scale ranged from scores of 1 (dislike extremely) to 5 (neither like nor dislike) to 9 (like extremely). Scores 6-9 was considered to be

Table 18
Percent of CO Foods Consumed by Participants (n=10) [1]

Subject	Dressing	Carrot Raisin Muffin	Chocolate Muffin	Sweet Potato Bar	All Foods
2	100	100	100	66	92
3	0	100	100	100	75
7	100	100	100	100	100
9	75	66	100	66	77
12	66	100	100	100	92
13	100	100	100	100	100
14	100	100	100	100	100
17	100	100	100	100	100
18	0	100	100	100	75
20	100	100	100	100	100
Group	74	97	100	93	91

[1] Products were considered acceptable if participants consumed >66%.

Table 19
Percentage of CSO Foods Consumed by Participants (n=8)[1]

Subject	Dressing	Carrot Raisin Muffin	Chocolate Muffin	Sweet Potato Bar	All Foods
4	100	100	100	100	100
5	66	100	100	100	92
6	100	100	100	100	100
8	0	17	35	66	30
10	66	33	33	33	41
11	66	66	100	66	75
15	100	100	100	100	100
21	66	100	100	100	91
Group	71	77	84	83	79

[1] Products were considered acceptable if participants consumed more than 66%.

acceptable for the foods provided and scores 1-5 was considered to be unacceptable for the foods provided. When comparing the hedonic scores between the two groups, similar results were found. While not all participants gave each product a score of 6 or more, the majority of participants rated the products as acceptable according to the guidelines established for this study. Tables 20-25 summarize the hedonic ratings for the CO and CSO products according to overall acceptability, taste and appearance. In addition, when asked if they would buy the products if available, every participant in the CSO group said yes, and all but two stated that they would purchase the food items in the CO group.

In regards to energy and fat intake by participants, there were no significant differences found in each group from week one to week four. Table 26 shows the average change in energy and fat between the two groups from the pre to post feeding period. Appendix M contains the details of nutrient change for all participants in each group. Table 27 shows the average energy and fatty acid intake for week one and weeks two through four for both the CO and CSO group.

Table 20

Appearance Ratings of Study Foods Containing Corn Oil (n=10)[1]

Product	Dislike Extremely (1)	(2)	(3)	(4)	Neither Like nor Dislike (5)	(6)	(7)	(8)	Like Extremely (9)	Mean Score[2]
Chocolate Muffin	-	-	-	-	2	-	4	2	2	7.2
Carrot Raisin Muffin	-	-	-	1	1	4	-	3	1	7
Dressing	-	-	-	-	2	1	4	2	1	6.3
Sweet Potato Bars	-	-	-	-	1	3	2	2	2	7.1

[1] Hedonic scale rating of 6-9 was considered acceptable and 1-5 was considered unacceptable.

[2] Mean score is the average score given by participants for each item based on appearance.

Table 21

Appearance Rating of Study Foods Containing Cottonseed Oil (n=8)[1]

Product	Dislike Extremely (1)	(2)	(3)	(4)	Neither Like nor Dislike (5)	(6)	(7)	(8)	Like Extremely (9)	Mean Score[2]
Chocolate Muffin	-	-	-	-	1	-	3	1	3	7.6
Carrot Raisin Muffin	-	-	-	1	1	1	1	1	3	7.1
Dressing	-	-	-	1	2	-	2	1	2	6.8
Sweet Potato Bars	-	-	-	-	1	-	2	1	4	7.9

[1] Hedonic scale rating of 6-9 was considered acceptable and 1-5 was considered unacceptable.

[2] Mean score is the average score given by participants for each item based on appearance.

Table 22

Taste Rating of Study Foods Containing Corn Oil (n=10)[1]

Product	Dislike Extremely (1)	(2)	(3)	Neither Like nor Dislike (4)	(5)	(6)	(7)	(8)	Like Extremely (9)	Mean Score[2]
Chocolate Muffin	1	1	-	1	-	1	1	5	-	6
Carrot Raisin Muffins	1	-	-	-	-	1	2	4	1	6.2
Dressing	1	1	-	1	-	-	1	6	-	6.2
Sweet Potato Bars	-	1	-	-	-	1	1	2	5	7.6

[1] Hedonic scale rating of 6-9 was considered acceptable and 1-5 was considered unacceptable.

[2] Mean score is the average score given by participants for each item based on taste.

Table 23

Taste Rating of Study Foods Containing Cottonseed Oil (n=8)[1]

Product	Dislike Extremely (1)	(2)	(3)	(4)	Neither Like nor Dislike (5)	(6)	(7)	(8)	Like Extremely (9)	Mean Score[2]
Chocolate Muffin	-	-	-	1	2	-	-	2	3	7.1
Carrot Raisin Muffin	-	-	-	-	-	2	1	3	2	7.6
Dressing	-	1	-	1	1	1	3	-	1	5.9
Sweet Potato Bar	-	-	-	-	-	-	-	3	5	8.6

[1] Hedonic scale rating of 6-9 was considered acceptable and 1-5 was considered unacceptable.

[2] Mean score is the average score given by participants for each item based on taste.

Table 24

Overall Acceptability of Study Foods Containing Corn Oil (n=10)[1]

Product	Dislike Extremely (1)	(2)	(3)	(4)	Neither Like nor Dislike (5)	(6)	(7)	(8)	Like Extremely (9)	Mean Score[2]
Chocolate Muffin	1	1	-	-	-	1	2	5	-	6.3
Carrot Raisin Muffin	1	-	1	-	-	-	2	4	1	5.9
Dressing	1	1	-	1	1	-	1	5	-	5.9
Sweet Potato Bars	-	1	-	-	-	1	2	1	5	7.5

[1] Hedonic scale rating of 6-9 was considered acceptable and 1-5 was considered unacceptable.

[2] Mean score is the average score given by participants for each item based on overall acceptability.

Table 25

Overall Acceptability of Study Foods Containing Cottonseed Oil (n=8)[1]

Product	Dislike Extremely (1)	(2)	(3)	(4)	Neither Like nor Dislike (5)	(6)	(7)	(8)	Like Extremely (9)	Mean Score[2]
Chocolate Muffin	-	-	-	1	1	1	1	1	3	7.1
Carrot Raisin Muffin	-	-	-	-	1	1	2	1	3	7.5
Dressing	-	-	-	1	1	2	3	-	1	6.4
Sweet Potato Bars	-	-	-	-	-	-	-	1	7	8

[1] Hedonic scale rating of 6-9 was considered acceptable and 1-5 was considered unacceptable.

[2] Mean score is the average score given by participants for each item based on overall acceptability.

Table 26

Average Change in Energy and Fatty Acid Intake Between the CO Group and CSO Group[1]

	Energy (Kcal)	TFA[2] (g)	SFA[2] (g)	MUFA[2] (g)	PUFA[2] (g)
Corn (n=8)					
Mean	9.4	5.2	0.49	1.6	2.7
Range	-362-538	-21-39	-10-7.98	-7-21	-2-8
Std. Dev.	284	15.7	4.8	7.7	3.1
Cottonseed (n=10)					
Mean	12.6	2.5	0.43	0.61	1.5
Range	-336-634	-11-18	-3-6	-6-6	-3-5
Std. Dev.	317	10.8	3.2	4.2	2.8

1 Results are calculated by final minus baseline values

2 TFA: Total fatty acids
SFA: Saturated fatty acids
MUFA: Monounsaturated fatty acids
PUFA: Polyunsaturated fatty acids

Table 27

Energy and Fatty Acid Intake for Week 1 and Weeks 2-4 for the Corn Oil and Cottonseed Oil Group (n=18)

	Week 1		Weeks 2-4 (Average)	
	CO	CSO	CO	CSO
Energy (kcal)				
Mean	1652	1732	1662	1745
Range	1104-2339	1005-2486	1095-2391	1035-2675
SD	424	473	446	523
Total Fat[1] (g)				
Mean	49	55	54	58
Range	21-78	26-91	28-106	25-88
SD	6	24	7	8
SFA[1] (g)				
Mean	16	18	17	18
Range	6-27	7-31	9-31	7-28
SD	6	3	6	8
MUFA[1] (g)				
Mean	18	20	20	21
Range	8-28	9-32	9-44	8-33
SD	7	9	10	9
PUFA[1] (g)				
Mean	10	12	13	14
Range	5-17	6-23	8-23	8-24
SD	4	6	4	5

[1] Total fat: Total fatty acids
SFA: Saturated fatty acids
MUFA: Monounsaturated fatty acids
PUFA: polyunsaturated fatty acids

CHAPTER V

DISCUSSION

The CO group and CSO group were relatively similar in regards to demographics. There were more females enrolled in the study than males as a result of recruitment at a mostly woman's college. In addition, most of the participants were nutrition students, dietetic interns, or faculty who were familiar with study participation and diet record procedure.

There are likely many reasons for limited change in vitamin E intake when comparing the CO group to the CSO group. One main explanation is that the amount of vitamin E in the supplemental foods was not enough to make considerable changes in each participant's overall dietary intake of vitamin E. For example, if a participant in the CO group ate all the supplemental foods provided, he or she would only have consumed 9.29 mg alpha-TE per week, or 1.32 alpha-TE per day, from the provided foods in addition to his or her regular dietary intake of the vitamin. Table 28 shows the total amount of vitamin E per week from the supplemental foods. In addition to above, one can see from table 28 that the cottonseed oil foods contained more alpha-TE and alpha-tocopherol, while the corn oil foods contained twice as much gamma-tocopherol.

Another possible reason for the low change in vitamin E intake is that the study participants were not limited in their food choices throughout the study. Each participant's intake if vitamin E likely varied prior to the feeding period and during the

Table 28
Total Amount of Vitamin E per Week Provided by the Supplemental Foods[1]

	Corn Oil Foods	Cottonseed Oil Foods
Total alpha-TE (mg)[2]	9.29	15.13
Total alpha-tocopherol (mg)	6.66	13.74
Total gamma-tocopherol (mg)	22.59	10.69

[1] Recipes were analyzed using the Minnesota Nutrient Data System, Research Version 4.0

[2] Numbers are in milligrams (mg)

feeding period. One specific example is that participant number 13 from the CO group ate a greater amount of Total Cereal, which is fortified with synthetic vitamin E, during the week prior to be provided with supplemental foods than during the feeding period. This equaled 24.11 mg alpha-tocopherol per day in week one when no study foods were provided and only 8.17 mg alpha-tocopherol per day during the feeding period. This participant did not consume as much Total cereal during the study period. Therefore, changes in food choices could have greatly affected the total amount of vitamin E consumed for each participant.

A third reason for the above results could be the use of the self-reporting dietary record. Studies have been conducted on the limitations of such records in which it is common for individuals to underestimate their intake up to 50%. The bias is usually found to be the greatest in obese subjects, however, weight conscious individuals have also been found to under report dietary intake (Schoeller, 1995). The participants in this study were mostly nutrition majors who were likely weight conscious which may have resulted in underreporting of food intake. Therefore, self-reporting dietary intake records should be interpreted with caution.

Finally, the nutrient database used in calculating nutrient intake and researcher error could result in inaccurate data. The database used for this study was the Minnesota Nutrition Data System for Research (NDS-R), which is a relatively accurate, and inclusive nutrient analysis software. The database contains over 16,000 foods, including 7,600 brand-name products. Ethnic foods are also included in the database. Although the program is comprehensive, it still lacks certain food items that participants consumed.

Researcher error also could result in inaccurate data. In this study one researcher took each participants diet record and entered foods item into the NDS-R system. This results in assumptions that must be made. Although participants were asked to be specific on the amount and contents of foods they ate, some diet records were not as complete as planned. For example, if a participant ate a mixed dish such as lasagna, each food item in the lasagna was broken down by the database system for accuracy. However, if the participant did not specify the type of cheese they used in the recipe, percent fat in the meat, or if oil was used in the sauce, the researcher has to make assumptions on these variables. Therefore, as mentioned above, results from dietary records and nutrient database systems must be interpreted with caution.

When looking at the vitamin E intake of participants from appendix K only 20% of the participants (two in the CO group and two in the CSO group) meet the new dietary reference intake of 15mg/day for vitamin E. Participants 12 and 13 from the CO likely meet the requirement as a result of each consuming Total cereal during the study period.

In the CSO group two participants meet the 15mg/day requirement as well. Participant 10 ate natural granola bars daily that were made canola oil, which could have added to her final vitamin E intake. In addition, participant 21 consumed meal replacement bars and shakes that were fortified with vitamin E. Therefore, those that met the RDA were consuming food items that had vitamin E added to them. Only one participant, participant 10, meet the RDA through diet by consuming several servings of food items that contain vitamin E such as natural granola bars made with canola oil and peanut butter.

This new requirement for vitamin E may be hard to meet with the typical American diet. A diet rich in fruits and vegetables and low in fat may not contain 15 mg of α-tocopherol per day, unless certain nuts, grains, and whole grains are consumed. Most participants in this study had a relatively low fat and energy intake (Appendix M). In addition, many manufactured food items contain vegetable oils that are rich in gamma-tocopherol such as corn and soybean oil that is now not counted toward meeting the vitamin E requirements (Traber 2001).

The change in TG level was the only lipid value found to be statistically significant with an increase in the CO group and a decrease in the CSO group. These results are consistent with the study cited by Hampden et al.(1983) in which rats fed corn oil had significantly higher triglyceride levels than cottonseed oil fed rats. However, the more recent studies by Radcliffe et al. (2001) found no effect on TG levels when rats were fed a diet of either CO or CSO.

While the results were not found to be statistically significant, the change in TC, HDL, and LDL demonstrated a pattern that was inconsistent with the previous cited animal studies (Radcliffe et al., 2001). In the present study, both CO and CSO had a lowering effect on HDL while TC and LDL trended downward in the CO group and upward in the CSO group. In the animal studies by Radcliffe et al. (2001) replacing CSO for CO resulted in a decrease in HDL and TC without affecting the non-HDL levels.

Cottonseed oil has a higher level of saturated fatty acids and lower level of monounsaturated and polyunsaturated fatty acids than CO. Palmitic acid (16:0) is found in greater amounts in CSO than CO, 23.5% and 10.5% respectively (Table 8), which is a

fatty acid thought to be capable of elevating serum cholesterol levels in humans. In addition, levels of linoleic acid (18:2, n-6) and oleic acid (18:1, n-9), fatty acids that have been found to lower serum cholesterol in humans, are lower in CSO than CO (Table 9) (Hu et al., 2001). Thus the effect of the individual fatty acids in the oils could be attributed to the lipid profile findings in this study.

The above results may be noteworthy; however; there are many components of oils that may have an effect on blood lipid levels in humans such as plant sterols and antioxidants. In addition, the participants in this study were not limited in their food choices during the study period. In the above animal studies, the oil and other nutrients were controlled. Therefore, controlling the fat and oil intake of human subjects may be one way to determine the effect of CO and CSO on blood lipid levels in humans.

Although the food products provided to the subjects contained a considerable amount of fat, the energy, total fat, saturated fat, monounsaturated fat, and polyunsaturated fat intake did not change notably from the pre to post period in each group as shown in Tables 26 and 27. This could be a result of participants substituting the provided food items for similar products that they would normally eat rather than in addition to their normal food intake.

Tables 18-25 show that the products were considered acceptable for most of the participants. When group cross tabulation was done on the hedonic scores, there was no significance found between the two groups in regards to acceptability. Scores of six or less could be the result of a lack of variety in food items provided. Although

participants were asked about their food preferences prior to the start of the study, those who gave low scores may have not liked muffins, dressing, or bars of any kind.

When looking at the final response by participants from questionnaire 3, most participants expressed that their normal food intake was not affected by the consumption of the provided food items and that they did not find it difficult to consume all the foods provided. Appendix N gives additional responses by participants from questionnaire 3.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this study was to compare the effects, in volunteer participants, of foods made with cottonseed oil or corn oil on vitamin E intake and serum lipid levels, and to determine the acceptability of food products made with cottonseed oil and corn oil.

Null hypothesis one, Consuming food products made with cottonseed oil relative to ones made with corn oil, will not increase dietary intake of vitamin E was not rejected. The study data show that there were no significant changes in vitamin E intake in participants consuming products containing cottonseed oil or corn oil for three weeks.

Null hypothesis two, consuming foods made with cottonseed oil, relative to those made with corn oil, will not affect blood lipid levels in participants was rejected. While the triglyceride level was the only change found to be significant between the two oils, total cholesterol, HDL, and LDL levels changed from the beginning (baseline) to the end of the study in both groups. The results found here were inconsistent with the previously cited animal studies (Radcliffe et al., 2001). Future studies therefore should control the oil intake or even total intake of each participant to allow for explicit results.

Null hypothesis three, food items (muffins, bars, and dressing) made with cottonseed oil will not be as acceptable as ones made with corn oil was rejected. Food items made with cottonseed oil were found to be as acceptable as ones made with corn oil. Participants in both groups were receptive to the food items provided. Most

consumed more than two-thirds of the products each week without affecting their macronutrient intake. In addition, hedonic scores were found to be similar in each group. Providing a wider variety of food items could prevent boredom and may allow for a better representation of the acceptance of food items made with cottonseed oil in future research.

The present study showed that consuming foods made with cottonseed oil, relative to ones made with corn oil, did not increase dietary intake of vitamin E in healthy participants. This could be a result of the limited amount of food products given to the participants and the fact that they were not restricted in their other food choices throughout the study. Therefore to correct for this, future study should provide a greater variety of food items and possibly control the oil and other food intake of each participant to allow for more definite conclusions to be drawn.

Many health benefits of vitamin E have been researched in the recent years. Based on the findings of this study, it can be assumed that foods made with cottonseed oil are an acceptable addition to the diet. Other research is needed to draw definite conclusions on the effect of the oil on blood lipid values and to determine the affects, if any, on plasma vitamin E levels. In addition, future study is needed to determine the effects of the intake of gamma-tocopherol as an antioxidant as a result of its possible relation to diseases such as prostate cancer.

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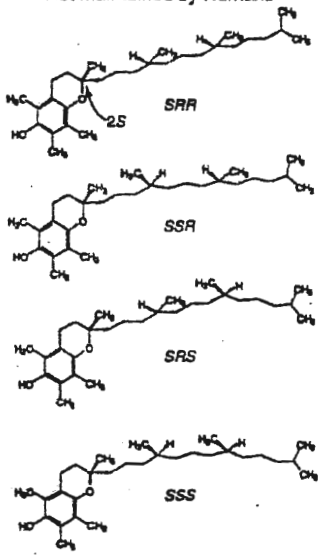
APPENDICES

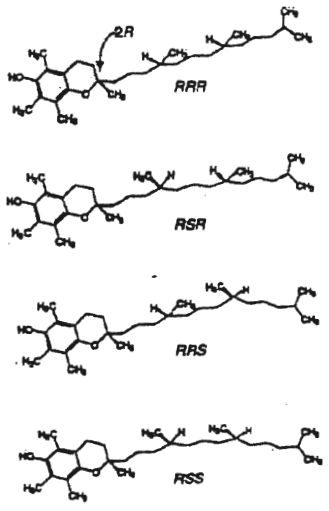
Appendix A

STRUCTURES OF ALPHA-TOCOPHEROL

2*S*-Stereoisomers of α-Tocopherol
Not Maintained by Humans



2*R*-Stereoisomers of α-Tocopherol
Maintained by Humans



Appendix B

QUESTIONNAIRES

Questionnaire One

The purpose of this questionnaire is to establish your usual food intake, food preferences, and if you qualify as a subject for the study. Remember that all the information you provide is confidential. Thank you for your participation.

Gender: M_____ F_____

Date of Birth: _____/_____/_____

Height:_____ Weight:_____ Are you pregnant: Yes_____ No _____

1) How many times a day do you normally eat?
One _____ Two _____ Three _____ Four _____ Five_____ Six_____

2) Which of the following foods do you normally eat? (please check all that apply)
Muffins_____ Salad Dressing_____ Wheat Germ_____
Sweet Potatoes _____ Other (specify)_____

3) Do you take vitamin/mineral supplements?
Yes _____ No_____ If yes, please specify _____
How often? One/day_____ Two/day_____ Three/day_____ Other_____

4) Are you allergic to any foods, or foods that contain cottonseed oil, corn oil, mustard, honey, eggs, whole wheat or white flour, sugar, chocolate, salt, baking powder, wheat germ, carrots, or raisins? Yes_____ No_____
If yes, please specify_____

5) Do you take any medications, specifically those whose action may be affected by fat intake? Yes_____ No_____ If yes, please specify_____
How may times per day?_____
Other (please specify)_____

Questionnaire 2

The purpose of this questionnaire is to determine your liking of the products provided.

Please check the appropriate line

1) How would you rate the following products **Overall?**

Chocolate Muffins: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Carrot Raisin Muffins: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Salad Dressing: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Sweet Potato Bars: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

2) How would you rate the **Taste** of the following products?

Chocolate Muffins: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike Extremely — Neither like nor dislike — Like extremely

Carrot Raisin Muffins: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Salad dressing: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Sweet Potato Bars: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

3) How would you rate the **appearance** of the following products?

Chocolate Muffins: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Carrot Raisin Muffins: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Salad Dressing: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike extremely — Neither like nor dislike — Like extremely

Sweet Potato Bars: ___ ___ ___ ___ ___ ___ ___ ___ ___

Dislike Extremely — Neither like nor dislike — Like extremely

4) Would you purchase these items if they were available commercially? Yes___ NO___

Questionnaire 3

The purpose of this questionnaire is get feedback on your thoughts about the study in which you participated. Remember all information is kept confidential Please answer all the questions.

1) Do you think that your normal food intake was affected by the consumption of the provided food items? Yes_____ No_____

If yes, please explain:

_____

_____

_____

_____

2) Did you find it difficult to consume all the food provided? Yes_____ No_____

If yes, Please explain:

_____

_____

_____

_____

3) Did you consume any vitamin/mineral supplements during this study?

Yes_____ No_____ How often? 1/day___ 2/day___ 3/day___ 4/day___ other___

Specify Brand: _____

4) Did you consume any medications during this study?

Yes_____ No_____ How often? 1/day___2/day___ 3/day___ 4/day___ other____

Please Specify:_____

Thank you for your participation!!!

Appendix C

CONSENT FORM

Advisor: Vicky Imrhan, Ph.D.

Texas Woman's University
Subject Consent to Participate in Research
The Effects of Using Cottonseed Oil Versus Corn Oil as Part of an Overall Dietary Strategy to Increase Vitamin E intake in Healthy Subjects

Date_____

I intend to participate in the study entitled, "The Effects of Using Cottonseed oil Versus Corn Oil as Part as an Overall Dietary Strategy to Increase Vitamin E Intake in Healthy subjects" at the TWU campus, Denton. I know that the principal investigator is Jennifer Braun, a master's student at TWU, and she may be reached at (972) 315-4009 or by calling the Nutrition department at (940) 898-2636. I can also call Vicky Imrhan, Ph.D. at (940) 898-2650.

I understand that this study involves research to compare the effects of cottonseed oil-containing foods and other foods containing vitamin E, to corn oil-containing foods on serum vitamin E and cholesterol levels for a four-week period.

I understand that I may be assigned to either a corn oil group receiving no treatment or a cottonseed oil group receiving treatment. I understand that the cottonseed oil group will be asked to consume 2 muffins made with cottonseed oil, 2 sweet potato bars, 2 T of wheat germ, and 2 T of dressing per week. This will be in addition to my usual food intake. I understand that the corn oil group will be asked to consume the same foods except the muffins and dressing will be made with corn oil.

I am not allergic to whole wheat or white flour, baking powder, sugar, eggs, carrots, raisins, chocolate, honey, mustard, sweet potatoes, cottonseed oil, corn oil, or wheat germ.

I understand that the potential risks of the study are mainly those associated with blood draws including swelling, bruising, discomfort, and infection. I understand that this risk will be reduced because a qualified registered nurse will be performing the blood draw. In addition enough time will be provided for my blood draw, and I will receive a small meal afterwards. Other risks include consuming disliked foods, the inconvenience of keeping a diet record and filling out questionnaires, and a loss of time. I understand that these risks will be reduced because I will be provided with detailed instructions on how to use the foods and fill out the forms.

I understand that the benefits of this study are free serum vitamin E and cholesterol determinations, the opportunity to experience original research, and the knowledge of my current dietary intake.

I understand that my data will be kept confidential and will be kept in a locked file cabinet in which the investigator will only have access. I understand that the data will be destroyed in four years (fall of 2003). I understand that my form with name will be kept separate from my data forms.

If I have any questions about the research study I should ask the researchers: their phone numbers are at the top of this form. If I have questions about my right as a subject or a way this study has been conducted, I may call Ms. Tracy Lindsay in the Office of Research & Grants Administration at (940) 898-3377.

The researchers will try to prevent any problem that could happen because of this research. I should let the researchers know at once if there is a problem and they will help me. I understand, however, that TWU does not provide medical services or financial assistance for injuries that might happen because I am taking part in this research.

I understand that my participation is voluntary, I may withdraw at any time, and that my refusal to participate will have no penalty and my identity will remain confidential. I understand that I may contact the principal investigator, Jennifer Braun, at any time at (972) 315-4009 for any questions I may have.

_____ _____

Signature Date

Address City State Zip

(_____)_____

Telephone Number

_____

Social Security Number

_____

Investigator's Signature

"The above consent form was read, discussed, and signed in my presence. In my opinion, the person signing did so freely and with full knowledge and understanding of its contents."

Appendix D

FOOD RECORDS

General Instructions for Keeping Record

- Please use black ink and print clearly
- Please keep this record every day for the duration of the study.
- Record each meal/snack RIGHT after it is eaten.
- Fill in the meal and place prepared for each meal or snack.
- In the place prepared column, write H for foods prepared at home, R for foods prepared in a restaurant and O for foods prepared in other places.
- Write each food or ingredient on a separate line.
- Skip a line after each meal or snack.
- If more space is needed for the same day, use the next page.
- Start each new day on a new page.
- Use the recipe pages (included) to describe homemade recipes.
- Use additional pages if needed and staple them to your food record.

Instructions for Recording Foods and Beverages

Please keep the following in mind when your write down the food you eat. (Please see attached sample)

- Fully describe foods, beverages, sauces, spreads, ect.
  Example: chicken thigh, skin not eaten; French dressing, low calorie.
- Write down brand names if you know them.
- Explain how foods are prepared. Example: Is meat fried, broiled, baked, breaded, ect.?
- Include foods prepared with fat; write down the kind of fat used.
  Example: fried in margarine (list brand name).
- Included foods you add at the table. Write these down on a separate line.
  Example: baked potato with
  1 TB (tablespoon) butter
- List each food or ingredient used in sandwiches as mixed dishes.
- Record exact amounts. Measure all foods in cups, tsp. (teaspoons), TB (tablespoons), or size in inches.

IF you have any questions please contact:
Jennifer Braun (972) 315- 4009 (h)

Please see the following page for some additional helpful hints

Additional Food Record Hints

The purpose of the food record is to provide a quantitative assessment of your food intake over the duration of the study.

Recording Procedure

1) All food and beverages consumed throughout the period must be recorded on the appropriate form. Include all snacks, condiments and spices, tonics, alcoholic beverages, candy, etc.
2) All snacks, meals, or beverages consumed away from home must also be recorded. (It may help to carry a small notepad).
3) Begin each new day on a new page of the record form. If a day uses more than one page, continue on the next page but start the new day at the top of a new page.
4) Record the day of the week and the date at the top of each page.
5) Record each item of a composite dish on a separate line. For example, a ham sandwich would be recorded as bread, ham, and mayonnaise, each on a separate line. (Please see example).
6) Record where (home, restaurant, office, etc.) and at what time each meal, snack, or drink was consumed.
7) If a vitamin or mineral supplements are used, list the amount taken each day, the brand name, and label information. If possible, the latter should read directly from the bottle used. You can include the label with the completed information.

How to describe foods and drinks

1) Method of cooking (e.g. roasted, stewed, fried, boiled, steamed).
2) Kind of food (e.g. raw or cooked; peeled or un-peeled; white or whole wheat; fresh, canned, frozen, or dried; whole, 2%, or skim milk).
3) Brand names of all processed foods wherever applicable (e.g. Kraft Macaroni and Cheese, Campbell's soup, Kellogg's corn flakes).
4) Include all condiments (e.g. pickles, sauces, catsup, mustard).
5) Provide as much label information as possible and the brand name of any unusual or special foods consumed.
6) If a recipe is used to make composite products such as casseroles, baked goods, sauces, etc., record, on the provided form, the complete recipe, giving the measured amount of each ingredient, the total number of servings, and the amount of the dish eaten by you.

How to record amounts of foods and drinks

1) Record the amounts of all food and beverages in the form they are consumed. For example, do not record the weight or size of a raw pork chop: instead, record the amount of a fried pork chop. (Example: 6 oz raw pork chop, 4 oz fried pork chop)
2) Record the amount of all leftovers on your plate after the meal; any remaining bones form meat, apple cores, or skin form the baked potato, etc.
3) Remove the peel from fruit such as bananas or oranges before recording the amount eaten.

EXAMPLE:

Breakfast:	Fried eggs	2
	Whole wheat toast	2 slices
	Butter	1 T
	Orange juice, calcium rich	1 cup
Snack:	Banana bread (recipe on back)	1 slice
	Skim milk	1 cup
Lunch:	White bread	2 slices
	Ham lunch meat, Butterball	2 slices
	Mayonnaise	1 T
	Yogurt, light, Dannon	6 oz
	Small apple, peeled	1
	Dr. Pepper	1 can

Place Prepared
H = Home
R = Restaurant
O = Other

Meal
B = B'fast
L = Lunch
D = Dinner
S = Snacks

Day: Sunday Date: 9/26/93

SAMPLE

	Meal	Place Prepared	Foods And Beverages	Amount
1	B	H	Orange Juice, unsweetened	2/3 cup
2			Oatmeal, quick cooking made with water	1/3 cup
3			Margarine, Mazola, stick	1 tsp
4			2% milk	1/2 cup
5			Brown Sugar	2 tsp
6			Coffee, decaffeinated	2 cups
7			Cream, half and half	2 TB
8			Toast, whole wheat	1 slice
9			Margarine, Mazola, stick	1 tsp
10			Multivitamin	1 pill
11			Vitamin C	1 pill
12				

Place Prepared
H = Home
R = Restaurant
O = Other

Meal
B = B'fast
L = Lunch
D = Dinner
S = Snacks

Day: Sunday Date: 9/26/93

SAMPLE

	Meal	Place Prepared	Foods And Beverages	Amount
1	L	H	Sandwich:	
2			Whole Wheat Bread	2 slices
3			Ham, boiled, deli (4"L x 4"W x 1/8th)	1 slice
4			Cheese, American Processed (3/4 oz/slice)	2 slices
5			Best Foods Mayonnaise	1 TB
6			Potato chips, ripple type	1 oz bag
7			Diet Coke with caffeine	1 can
8			Nabisco Oreo Cookies	2
9				
10	D	H	Beef Stew (see recipe page 34)	1 serving
11			Salad:	
12			Lettuce, romaine	1 cup

Place Prepared
H = Home
R = Restaurant
O = Other

Meal
B = B'fast
L = Lunch
D = Dinner
S = Snacks

Day: Sunday Date: 9/26/93

SAMPLE

	Meal	Place Prepared	Foods And Beverages	Amount
1			Tomato, peeled	1/4 med
2			Cucumber, sliced	2" piece
3			Hidden Valley Ranch dressing, regular	1 TB
4			Roll, white, yeast 2"W x 2"L x 1"th	1
5			Butter	2 tsp
6				
7	S	H	Chocolate Ice Cream, Dyrer's Grand	3/4 cup
8				
9				
10				
11				
12				

Appendix E

CSO AND CO FOOD RECIPES

CSO/CO Study Carrot Muffins

Mix the following ingredients together and bake at 375° for 21 minutes. Makes 8 muffins

1 ½ cup grated carrots

¾ cup unbleached white flour

¼ cup wheat germ

¼ cup stone ground whole-wheat flour

1/3 cup white sugar

½ cup raisins

¼ cup + 1 T CSO (or CO)

2 tsp. baking powder

¼ tsp. salt

1 medium egg

½ cup skim milk

1 tsp. cinnamon

CSO/CO Cocoa Muffins

Mix the following ingredients together and bake at 350° for 20 minutes. Makes 12 muffins.

¼ cup cocoa

¼ cup wheat germ

¾ cup unbleached flour

½ cup stone ground whole-wheat flour

1 cup white sugar

½ cup chopped walnut or dried cranberries

½ cup CSO (or CO)

1 tsp. baking soda

½ tsp. salt

1 tsp. ground cinnamon

1-cup cold water

1 T vinegar

Sweet Potato Bars

2 cups all-purpose flour

1 ½ cups sugar

2 tsp. baking powder

2 tsp. cinnamon

1 tsp. baking soda

¼ tsp. salt

¼ tsp. ground cloves

4 beaten eggs

1 16-ounce can sweet potatoes (puréed in a blender)

1 cup CO/CSO oil

Purchased cream cheese frosting

Combine flour, sugar, baking powder, cinnamon, soda, salt, and cloves. Stir in eggs, sweet potatoes, and oil until combined. Spread into a un greased 15X10X1-inch-baking pan. Bake at 350° for 25-30 minutes. Frost with cream cheese frosting. Makes 48 bars.

Dressing

1 tablespoon CO or CSO

1 tablespoons fat-free honey mustard dressing

Mix thoroughly

Appendix F

HUMAN SUBJECTS EXEMPTION FORM (TWU)

Texas Woman's University, Denton Campus
Application to Human Subjects Review Committee
Cover Page

Denton Mailing Address: Box 425619, TWU Station, Denton, TX 76204-5619
Denton Campus Mailing Address: Office of Research & Grants Administration, ACT 9th Floor
Phone #: 940-898-3377 Fax #: 940-898-3416 E-Mail: HSRC@VENUS.TWU.EDU

Use this form to describe your proposed procedures for all investigations that will involve human subjects. Submit the original and three copies of this Application to the Human Subjects Review Committee (HSRC) at the above address.

If any member of the HSRC should require additional information, the investigator will be so notified. Note that another review by the HSRC is required if your project changes or if it extends beyond one year from its date of approval.

Title of Study: The Effects of Using Cottonseed Oil Versus Corn Oil as Part of an Overall Dietary Strategy to Increase Vitamin E Intake in Healthy Subjects.

Name of Principal Investigator*: Jennifer L. Braun Phone: (972) 317-7802

Check Status of Principal Investigator: ☐ faculty ☒ student ☐ staff ☐ other _____

Address where correspondence is to be sent: 103 Glen Castle CT.
Highland Village, TX 75077

If the Principal Investigator is a student, provide the following student information:

[redacted] Department: Nutrition & Food Sciences

Name & Phone # of Research Advisor: Victorine Imhran Phd (940) 898-2650

Estimated beginning date of the study: September 1, 1999

Research being conducted for (check appropriate box):
☒ thesis ☐ student professional paper
☐ dissertation ☐ faculty research
☐ class project ☐ other _____

If this research is, or may be, supported by a grant or an outside sponsor, list name(s) of sponsor(s):

Texas Food and Fibers Commission

Required Signatures

_____ _____
Principal Investigator Date

_____ _____
Faculty Research Advisor (if applicable) Date

_____ _____
Dean, Department Head, or Program Director Date

Date Application Received by HSRC: _____

* Attach a separate cover page for each investigator. Correspondence will be sent only to investigator listed on front cover page. However, an approval letter will be generated and sent to each investigator for his/her records.

Appendix G

BLOOD LIPID PROCEDURE

Total Cholesterol Determination

(Standard kit, sigma standard)

1) Turn on water bath at 37° C
2) Label test tubes:
   a) Blank
   b) Standard
   c) Sample

*200mg/dl

Sample (use microcuvettes)

1) Take 30-ul of sample
3) Take 600-ul of reagent (must be at room temperature for 1 hour, so enzymes will work rapidly)
4) Vortex
5) Incubate for 15 min.
6) Read O.D.
7) Read O.D. 10 minutes later
8) Take maximum O.D. for each sample

*200mg/dl

Standard:

1) 30-ul of standard
2) 600-ul of reagent
3) Vortex
4) Incubate
5) Read at 5 minutes
   10 minutes
   15 minutes (with sample)
   25 minutes (with sample)
6) Take O.D. max for each sample

Calculation: $\frac{\text{O.D. Test}}{\text{O.D. Standard}}$ X 200 mg/dl

Determination of HDL

(Sigma kit)

Dilution =1.2 250-300 = 300/250 = 1.2
Sample (can use microcuvettes or tubes)

1) Take 250-ul of serum
2) 50-ul of pptng reagent
3) microfuge tube
4) vortex
5) stand 5 minutes
6) centerfuge at 3000 rpm in microfuge to 10 min
7) Supernatant, carefully
8) Take 60-ul of supernatant
9) 600-ul of reagent
10) Vortex
11) Incubate at 37°C for 15 minutes
12) Read O.D.
13) Read O.D. in 10 min
14) Take O.D. max

Standards:
Take 100-ul of standard and vortex with 300-ul of 0.85% saline OR 200-ul with 600 of saline = 50 mg/dl

1) Take 50-ul + 10-ul of saline
2) 600-ul of reagent
3) vortex
4) Read O.D. at 10 minutes
5) 15 minutes with sample

Calculation:

Calculation of (VLDL + LDL)C
(VLDL +LDL) = TC – HDL-c

$$\frac{\text{O.D. Test}}{\text{O.D Standard}} \times 50 \text{ mg/dl}$$

Determination of Triglycerides

1. Pipet into cuvets the following volumes (ml) and mix well.

	Reagent Blank (RB)	Standard (S)	Sample (U)
Activated Reagent	1.0	1.0	1.0
Standard	-	0.01	-
Sample	-	-	0.01

2. Incubate all cuvets at 37°C for 5 minutes, or incubate at room temperature for 10 minutes.

3. Read S and U vs. RB at 500 nm within 60 minutes.

$$\text{Serum TG (mg/dl)} = \frac{AU}{AS} \times 200$$

Appendix H

DATA COLLECTED ON ELIMINATED PARTICIPANT

Participant # 19, CSO group

1. Nutrient Data:

	Pre	Post
Energy (kcal):	1262	1096
Total fat (g):	41	43
Saturated fatty acids (g):	13	11
Monounsaturated fatty acids (g):	14	15
Polyunsaturated fatty acids (g):	8	13
A-TE (mg)	275	377
Alpha-tocopherol (mg)	274	377
Gamma-tocopherol (mg)	3.54	6.48

2. Blood Lipid Data:

Total cholesterol (mg/d)	156	194
LDL cholesterol (mg/dl)	94	117
HDL cholesterol (mg/dl)	72	64
Triglycerides (mg/dl)	70	66

3. Sensory Analysis

	Chocolate muffin	Carrot Raisin muffin	Dressing	Bar
Overall	5	7	7	8
Taste	7	7	7	7
Appearance	8	8	8	8

Appendix I

DEMOGRAPHIC DATA

Demographics of the CSO Group Participants

Participant #	Age (yrs)	Ht (in)	Wt (#)	Gender (M/F)
4	48	62	120	F
5	51	74	190	M
6	32	64	135	F
8	55	71	220	M
10	23	65	138	F
11	22	69	140	F
15	22	65	174	F
21	27	72	192	M

Demographics of CO Group Participants

Participant #	Age (yrs)	Ht (in)	Wt (#)	Gender (M/F)
2	31	68	148	F
3	23	65	150	M
7	23	68	145	F
9	25	67	150	F
12	23	68	124	F
13	22	67	135	F
14	27	67	135	F
17	43	64	122	F
18	23	69	145	F
20	55	70	168	M

Appendix J

VITAMIN E INTAKE BEFORE CONTROLLING FOR SUPPLEMENTS

Subjects 18 and 20 were both in the CO group. Each took a multivitamin supplement such as Centrum. The following is their vitamin E (prior to adjustment according to recent guidelines) intake before the supplement was taken out to the analysis:

	Pre (mg)	Post (mg)
1) Subject 18:		
Total A-TE:	23.98	25.49
Alpha-tocopherol:	23.44	24.63
Gamma-tocopherol:	4.36	6.75
2) Subject 20:		
Total A-TE:	30.15	32.76
Alpha-tocopherol:	28.42	29.83
Gamma-tocopherol:	16.14	27.10

Appendix K

VITAMIN E INTAKE OF WEEK 1 AND WEEKS 2-4

Actual Vitamin E intake of Week 1 and Weeks 2-4 in the CO Group (mg/day)

Subject	Week 1 (Actual)	Weeks 2-4 (Average)	%Change
2 α-TE	6.34	5.07	-20.0%
Alpha	4.96	3.92	-21.0%
Gamma	12.59	9.93	-21.1%
3			
α-TE	6.28	8.77	+39.6%
Alpha	4.64	6.72	+44.8%
Gamma	15.01	18.07	+20.4%
7			
α-TE	9.59	9.14	-4.7%
Alpha	8.56	7.82	-8.6%
Gamma	9.32	11.78	+26.4%
9			
α-TE	13.01	10.73	-17.5%
Alpha	10.49	8.84	-15.7%
Gamma	22.43	16.4	-26.9%
12			
α-TE	13.02	7.78	-40.2%
Alpha	16.58	6.52	-60.7%
Gamma	6.92	11.14	+17.6%
13			
α-TE	27.05	10.45	-61.4%
Alpha	24.11	8.17	-66.1%
Gamma	12.81	11.5	-10.2%
14			
α-TE	5.62	7.12	+26.7%
Alpha	4.64	5.57	+20.0%
Gamma	8.92	13.98	+56.7%
17			
α-TE	6.44	7.26	+12.7%
Alpha	4.74	5.33	+12.4%
Gamma	16.05	17.44	+8.7%
18			
α-TE	3.84	5.35	+39.3%
Alpha	3.29	4.48	+36.2%
Gamma	4.36	6.75	+54.8%
20			
α-TE	10.01	12.62	+26.1%
Alpha	8.28	9.68	+16.9%
Gamma	16.14	27.1	+67.9

Note: n=10. Note: α-TE: alpha-TE; Alpha: alpha-tocopherol; Gamma: gamma-tocopherol. Note: On average there was a –1.46% decrease in alpha-TE, a -4.18% decrease in alpha-tocopherol and a 19.43% increase in gamma tocopherol per day from week one to week four in the CO group.

Actual vitamin E intake of week 1 and weeks 2-4 in the CSO group (mg/day).

Subject	Week 1 (Actual)	Week 2-4 (Average)	%Change
4			
α-TE	5.94	13.46	+126.6%
Alpha	5.08	12.34	+142.9%
Gamma	6.75	8.80	+30.4%
5			
α-TE	9.13	9.96	+9.1%
Alpha	6.82	7.91	+16.0%
Gamma	20.7	18.28	-11.7%
6			
α-TE	7.29	7.25	+0.5%
Alpha	5.80	6.10	+5.2%
Gamma	13.60	10.26	-24.6%
8			
α-TE	9.62	7.95	-17.4%
Alpha	8.10	6.60	-18.5%
Gamma	13.87	12.48	-10.0%
10			
α-TE	17.36	20.03	+15.4%
Alpha	16.53	18.73	+13.3%
Gamma	5.91	10.21	+72.8%
11			
α-TE	13.24	5.36	-59.5%
Alpha	12.33	4.56	-63.0%
Gamma	8.19	6.96	-15.0%
15			
α-TE	10.74	13.96	+30.0%
Alpha	8.04	11.01	+36.9%
Gamma	24.69	26.5	+7.3%
21			
α-TE	17.58	18.16	+3.3%
Alpha	16.46	16.34	-0.7%
Gamma	8.14	13.22	+62.4%

Note: n =8. Note: α-TE : alpha-TE; Alpha: alpha-tocopherol; Gamma: gamma-tocopherol. Note: On average there was a 13.5% increase in alpha-TE, a 16.5% increase in alpha-tocopherol, and a 14% increase in gamma-tocopherol pre day from week one to week four in the CSO group.

Appendix L

LIPID PROFILE BEFORE AND AFTER THE FEEDING PERIOD

Lipid Profile Before and After the Feeding Period in the CO Group (mg/dl)

Subject	Pre Values	Post Values	Change
2 TC	168	166	-2
HDL	68	57	-11
LDL	85	86	+1
TG	73	115	+42
3			
TC	148	141	-7
HDL	45	44	-1
LDL	78	72	-6
TG	124	125	+1
7			
TC	182	165	-17
HDL	74	65	-9
LDL	96	87	-9
TG	60	65	+5
9			
TC	159	181	+22
HDL	55	49	-6
LDL	89	113	+24
TG	76	93	+17
12			
TC	135	163	+28
HDL	51	59	+8
LDL	69	88	+19
TG	74	80	+6
13			
TC	185	185	0
HDL	41	40	-1
LDL	128	130	+2
TG	78	74	-4
14			
TC	175	176	+1
HDL	56	52	-4
LDL	104	107	+3
TG	73	84	+11
17			
TC	199	170	-29
HDL	77	71	-6
LDL	110	89	-21
TG	59	51	-8
18			
TC	199	161	-38
HDL	52	40	-8
LDL	134	98	-36
TG	66	114	+48
20			
TC	180	149	-31
HDL	36	27	-9
LDL	111	69	-42
TG	167	264	+97

Note: n =10. Note: TC: Total cholesterol; HDL: High-density lipoprotein; LDL: low-density lipoprotein; TG: Triglyceride. Note: There was a –7.3mg/dl decrease in TC, a –4.7mg/dl decrease in HDL, a –6.5mg/dl decrease in LDL, and a +21.5 mg/dl increase in TG levels in the CO group.

Lipid Profile Before and After the Feeding Period in the CSO Group (mg/day)

Subject	Pre Values	Post Values	Change
4			
TC	173	162	-11
HDL	45	41	-4
LDL	90	109	+19
TG	69	60	-9
5			
TC	149	187	+38
HDL	73	65	-8
LDL	93	110	+17
TG	48	59	+11
6			
TC	207	212	+5
HDL	39	38	-1
LDL	146	157	+11
TG	111	84	-27
8			
TC	181	193	+12
HDL	40	35	-5
LDL	111	132	+21
TG	148	128	-20
10			
TC	173	147	-26
HDL	50	47	-3
LDL	111	87	-24
TG	59	66	+7
11			
TC	207	188	-19
HDL	49	52	+3
LDL	109	110	1
TG	136	132	-4
15			
TC	180	242	+43
HDL	55	50	-5
LDL	121	166	+45
TG	155	131	-24
21			
TC	179	149	-30
HDL	52	37	-15
LDL	109	94	-15
TG	103	92	-11

Note: n =8. Note: TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: triglyceride. Note: There was a +1.5mg/dl increase in TC, a –4.75mg/dl decrease in HDL, a +9.38mg/dl increase in LDL, and a –9.63mg/dl decrease in TG levels in the CSO group.

Appendix M

NUTREINT VALUES

CORN OIL GROUP

9913	PRE	POST
Energy	1877	1674
T.fat (g)	49.15	53.48
Sat	15.63	19.42
Mono	18.73	18.94
Poly	9.87	10.71
Total E	27.05 mg	10.45 mg
Alpha	24.11	8.17
gamma	12.81	11.5

9917	PRE	POST
Energy	1414	1428
T.fat (g)	58.01	51.34
Sat	17.71	14.86
Mono	23.24	18.33
Poly	13.05	13.86
Total E	6.44 mg	7.26 mg
Alpha	4.74	5.33
gamma	16.05	17.44

9903	PRE	POST
Energy	2339	2386
T.fat (g)	60.63	65.25
Sat	21.82	20.55
Mono	24.38	24.88
Poly	8.82	13.27
Total E	6.28 mg	8.77 mg
Alpha	4.64	6.72
gamma	15.01	18.07

9909	PRE	POST
Energy	2192	1950
T.fat (g)	78.21	57.11
Sat	26.97	16.89
Mono	27.95	20.52
Poly	17.1	15.22
Total E	13.01 mg	10.73 mg
Alpha	10.49	8.84
gamma	22.43	16.4

9920	PRE	POST
Energy	1853	2391
T. fat (g)	66.78	106.11
Sat	23.04	31.02
Mono	23.04	43.99
Poly	15.41	23.22
Total E	10.01 mg	12.62 mg

9914	PRE	POST
Energy	1289	1584
T.fat (g)	38.41	53.89
Sat	15.2	18.25
Mono	12.65	17.7
Poly	7.63	13.79
Total E	5.62 mg	7.12 mg
Alpha	4.64	5.57
gamma	8.92	13.98

9902	PRE	POST
Energy	1345	1095
T.fat (g)	40.93	36.52
Sat	12.48	11.39
Mono	14.14	11.99
Poly	11.19	10.16
Total E	6.34 mg	5.07 mg
Alpha	4.96	3.92
gamma	12.59	9.93

9912	PRE	POST
Energy	1272	1301
T.fat (g)	31.02	39.64
Sat	11.22	12.54
Mono	10.91	13.66
Poly	5.94	10.1
Total E	13.02 mg	7.78 mg
Alpha	16.58	6.52
gamma	6.92	11.14

9907	PRE	POST
Energy	1841	1479
T.fat (g)	43.22	48.25
sat.	11.78	12.9
mono	19.61	19.64
poly	8.56	11.93
Total E	9.59 mg	9.14 mg
alpha	8.56	7.82
gamma	9.32	11.78

9918	PRE	POST
Energy	1104	1332
T.fat (g)	21.3	28.14
sat	6.23	9.11
mono	7.95	8.92
poly	5.2	7.51
Total E	3.84 mg	5.35 mg

Alpha	8.28	9.68
gamma	16.14	27.1

alpha	3.29	4.48 mg
gamma	4.36	6.75

Cottonseed Group

9906	PRE	POST
Energy	1355	1186
T. fat (g)	53.07	41.88
Sat	17.2	14.75
Mono	19.18	13.32
Poly	12.32	10.58
Total E	7.29 mg	7.25 mg
Alpha	5.8	6.1
Gamma	13.6	10.26

9905	PRE	POST
Energy	2120	1883
T. fat (g)	76.67	82.14
sat	25	25.58
mono	31.52	32.78
poly	12.71	16.27
Total E	9.13 mg	9.96 mg
alpha	6.82	7.91
gamma	20.7	18.28

9908	PRE	POST
Energy	1774	1659
T. fat (g)	75.27	64.91
sat.	25.16	22.59
Mono	26.9	23.18
Poly	16.85	14.02
Total E	9.62 mg	7.95 mg
Alpha	8.1	6.6
Gamma	13.87	12.48

9915	PRE	POST
Energy	2486	2150
T.fat (g)	91.03	87.73
sat	30.71	27.64
mono	30.79	30.24
poly	23.09	23.56
Total E	10.74 mg	13.96 mg
alpha	8.04	11.01
gamma	24.69	26.5

9904	PRE	POST
Energy	1556	1551
T.fat (g)	29.33	36.32
Sat	9.37	10.25
Mono	10.18	12.95
Poly	7.17	9.94
Total E	5.94 mg	13.46 mg
Alpha	5.08	12.34
Gamma	6.75	8.8

9910	PRE	POST
Energy	1518	1817
T.fat (g)	34.32	49.44
Sat	12.11	16.32
Mono	12.35	18.24
Poly	6.33	10.65
Total E	17.36 mg	20.03 mg
Alpha	16.53	18.73
Gamma	5.91	10.21

9911	PRE	POST
Energy	1005	1035
T.fat (g)	25.77	25.39
Sat	6.5	6.56
Mono	8.92	8.1
Poly	7.8	8.38
Total E	13.24 mg	5.36 mg
Alpha	12.33	4.56
Gamma	8.19	6.96

9921	PRE	POST
Energy	2041	2675
T.fat (g)	54.48	72.31
sat	17.28	23.06
mono	21.41	27.3
poly	10.04	14.9
Total E	17.58 mg	18.16 mg
alpha	16.46	16.34
gamma	8.14	13.22

Appendix N

COMMENTS FROM QUESTIONNAIRE 3

The following are comments on the study given by participants on Questionnaire 3.

When asked if they found it difficult to consume all the food products:

> "I did not particularly like the salad dressing"
>
> "Sometimes the dressing made me sick to my stomach because of the oil in it."
>
> "Salad dressing, did not use dressing when eating the carrots as a snack, taste was OK, but the dressing was not used."
>
> "Sometimes I would forget about (the food products) or it would be hard to incorporate them into my diet. I would usually use them as a snack or muffins as breakfast."

When asked if they think their normal food intake was effected by the consumption of the provided foods"

> "The supplemental foods were very filling, so I did not eat as much fruit during my snack times."