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Can Cranberry Supplementation Reduce Risks for Diabetes?

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CAN CRANBERRY SUPPLEMENTATION REDUCE RISKS FOR DIABETES?

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An Abstract of the Thesis Presented
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Diabetes affects approximately 250 million people worldwide and health care costs related to diabetes equal approximately $98 billion each year. Aldose reductase has been shown to contribute to the side effects of diabetes including kidney disease, nerve disease, and retinopathy. Cranberries contain anthocyanins and other flavonoids that have been shown in vitro to inhibit the enzyme aldose reductase and to inhibit protein glycosylation. It is believed that daily cranberry supplementation could reduce side effects of diabetes.

Twenty-seven adults with type 2 diabetes were recruited for this 12 week double-blind, placebo-controlled study. Fasting blood analysis was done at weeks 0, 6, and 12. The blood analyses included: cholesterol (total cholesterol, triglycerides, HDL, LDL, and percent HDL), glycosylated hemoglobin (HbA_{1C}), blood glucose, insulin, fructosamine, aldose reductase activity, and hexanal. The subjects were asked to take 6 capsules each day

that contained either spray-dried cranberry powder or a placebo. The placebo consisted of cellulose and artificial food coloring, as well as sucrose, fructose, magnesium hydroxide, and ascorbic acid in the same concentrations as in the cranberry powder.

Improvements were seen in blood glucose levels, HbA_{1C}, fructosamine, insulin, total cholesterol, triglycerides, and percent HDL for some subjects. Glucose levels were significantly lower ($p = 0.036$) at week 12 in subjects who had diabetes more than 5 years in the cranberry group compared to the placebo group. At week 6, in subjects less than 50 years of age, blood glucose levels decreased significantly ($p = 0.030$) in the cranberry group compared to the placebo group. HbA_{1C} levels decreased significantly ($p = 0.031$) in the cranberry group at week 6 in subjects who had diabetes more than 5 years. Fructosamine levels improved significantly in patients less than age of 50 in the cranberry group compared to the placebo group from week 0 to week 6 ($p = 0.027$). Triglyceride and total cholesterol levels were significantly lower ($p = 0.013$ and $p = 0.007$ respectively) in the cranberry group than the placebo group in subjects who had diabetes more than 5 years.

Cranberry supplementation may be beneficial for individuals with diabetes. More research is needed in subjects with poor glycemic control and to determine proper dosages.

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INTRODUCTION

BACKGROUND INFORMATION ON DIABETES MELLITUS

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (DM) defines diabetes as "a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both" (1). Insulin is a hormone that is produced by the beta cells of the pancreas and is necessary to utilize carbohydrate in the body. Insulin increases oxidation, glycogenesis (production of glycogen), and lipogenesis (production of fat from dietary carbohydrate), and helps to make possible the diffusion of glucose into cells of the body (2). There are many side effects associated with chronic hyperglycemia resulting from DM. The side effects include cardiovascular disease, macrovascular and microvascular disease, retinopathy, nephropathy, and neuropathy (1). Symptoms of DM include significant weight loss, polyphagia (excessive hunger), polydipsia (excessive thirst), polyuria (excessive urination), and blurred vision (1, 2).

TYPES OF DIABETES MELLITUS

DM can be categorized as gestational DM, type 1 DM, type 2 DM, or other. The focus of this research project was type 2 DM. In gestational DM glucose intolerance occurs during pregnancy, and usually returns to normal after childbirth. Up to 60% of women with gestational DM will develop type 2 DM within 5 to 15 years (2).

Type 1 DM, or insulin dependent DM, usually occurs at an early age (less than 30 years of age), however, the disease may occur at any age. In type 1 DM there is autoimmune destruction of the beta cells of the pancreas, which halts insulin production. Glucose cannot be utilized without insulin, so glucose is excreted in the urine. Patients with type 1 DM experience significant weight loss, polyphagia, polydipsia, polyuria, and ketoacidosis (accumulation of ketone bodies due to excessive breakdown of fat). Exogenous insulin is necessary to prevent death in patients with type 1 DM (2).

Approximately 97% of people with diabetes have type 2 DM, previously known as non-insulin dependent DM (3). Type 2 DM may occur at any age, but occurs most often in individuals over the age of thirty. Endogenous insulin secretion may be depressed, elevated, or normal, and there is a decrease in insulin sensitivity in the body. With a decrease in insulin sensitivity, more insulin is needed to control blood glucose levels in the body (2). The need for more insulin results in higher than normal levels of insulin in the blood in patients with type 2 DM. Type 2 DM has been linked to a sedentary lifestyle, poor nutritional habits, and being overweight (4); however, people who maintain a healthy weight may develop the disease. Individuals with type 2 DM may or may not experience excessive hunger, thirst, and urination, and are unlikely to experience ketoacidosis (2). A patient may have type 2 DM for many years without being diagnosed because blood glucose levels may be elevated without causing symptoms severe enough to be noticed by the

patient (1). The preferred treatment for type 2 DM is lifestyle change, which includes improved diet and exercise patterns. These changes are often difficult for patients to maintain, so drug therapy may become necessary.

Oral medications that increase insulin secretion or improve insulin sensitivity may be prescribed when diet and exercise changes are not successful. Oral hypoglycemic drugs include sulfonylureas, meglitinides, biguanides, thiazolidinedione (TZDs), and alpha-glucosidase inhibitors. Sulfonylureas increase insulin production by stimulating the pancreas. Chlorpropamide, tolazamide, acetohedamide, and glipizide are examples of sulfonureas. Repaglinide is a meglitinide, which increases insulin production by stimulating the pancreas. Metformin (Glucophage) is a biguanide which increases insulin sensitivity and decreases liver production of glucose. Rosiglitasone (Avandia) and pioglitazone (Actose) are TZDs which improve insulin sensitivity and improve carbohydrate metabolism. Precose and Glyset are alpha glucosidase inhibitors which work by decreasing glucose absorption in the small intestine (5). Type 2 DM may be treated with insulin in severe cases. Insulin therapy is not usually necessary for survival, but is necessary to control blood glucose levels and prevent side effects and complications of diabetes in approximately 40% of patients (2, 6).

DIAGNOSIS OF DIABETES MELLITUS

Screening for DM may be completed if a person has risk factors for the disease, a family history of the disease, or shows symptoms of the disease. It is recommended that all individuals over the age of 45 be tested for diabetes every three years. According to the Expert Committee on Diagnosis and Classification of DM, there are three criteria for diagnosis of DM. The first is symptoms of diabetes with non-fasting plasma glucose greater than 200 mg/dL (11.1mmol/L). The second criteria for diagnosing DM is a fasting plasma glucose level greater than 126 mg/dL (7.0 mmol/L) (1, 6). This value was changed in 1998 from 140 mg/dL (7.8 mmol/L) to 126 mg/dL (7.0 mmol/L) in order to diagnose the disease earlier and prevent the many side effects and complications of the disease (6, 7). The third criteria for diagnosis of DM is a plasma glucose level greater than 200 mg/dL (11.1 mmol/L) two hours after an oral glucose tolerance test (1, 6). Elevation of blood glucose levels above 200 mg/dL (11.1 mmol/L) are associated with increased incidence of diabetic complications (7).

COST OF DIABETES MELLITUS

In 1997 there were approximately 124 million people worldwide that had been diagnosed with DM, and this number is increasing at an alarming rate (3). In the period from 1990 to 1998 there was a 33% increase in the number of Americans diagnosed with type 2 DM (4). With DM being diagnosed at 126 mg/dL instead of 140 mg/dL starting in 1998, the increased incidence of DM

likely continued since 1998. The increased incidence of obesity in the United States is a factor that likely contributed to the increased incidence of DM. Type 2 DM is being diagnosed at much earlier ages. As early as 10 years ago type 2 DM was a disease only found in adults, but at present as many as 300,000 children in the United States have the disease (4). It is important to find a treatment to reduce the side effects of diabetes to decrease health care costs and improve quality of life for patients with DM.

Persons with diabetes are three times more likely than non-diabetic persons to be hospitalized, which leads to increased healthcare costs (3). Risk of death is also higher in people with diabetes than non-diabetic persons (Table 1).

Table 1: Relative Risk of Death for People with DM

Age	Risk Of Death
Age 44 or younger	4.2 times higher
Age 45 - 64	4.4 times higher
Age 65 or older	3 times higher

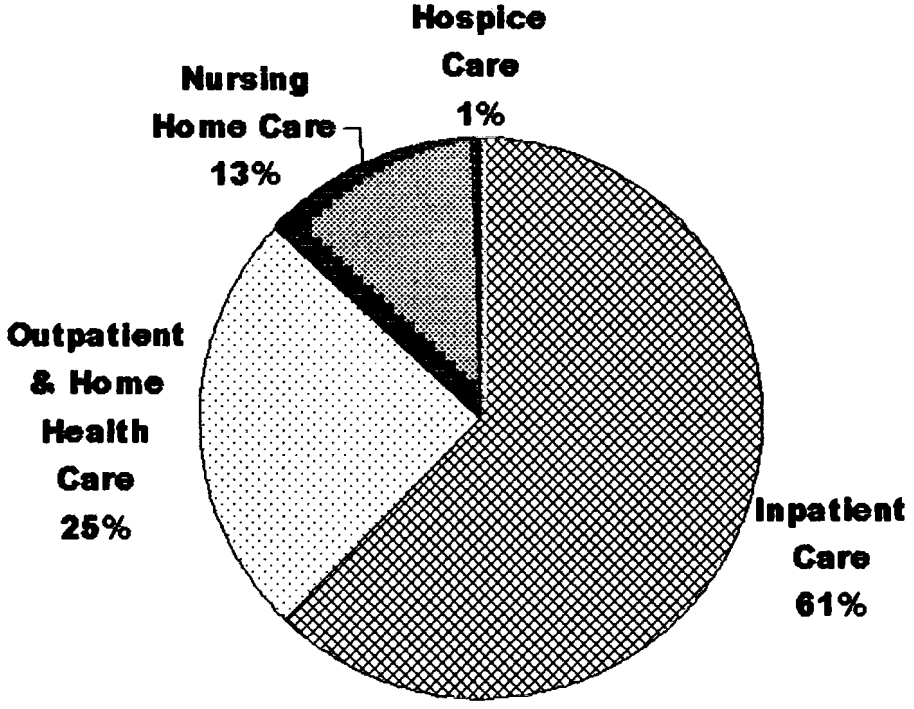
(3)

Patients with DM have higher healthcare costs. Both direct and indirect costs are problematic. Direct costs include physician visits, hospital stays, medications, etc. Indirect costs include sick days, loss of income, loss of employment, and related expenses resulting from the disease (3). According

to the National Institute of Health, total cost of diabetes in 1997 was $98 billion. Direct medical costs were responsible for about $44 billion dollars and indirect costs were about $54 billion dollars (6).

The American Diabetes Association evaluated the direct costs attributed to DM in the United States in 1997 (8). Sixty-two percent of expenditure for care of patients with DM was spent on inpatient care, 24.7% was spent on outpatient services and home health care, 12.5% was spent on nursing home care and 0.5% was spent on hospice care (Figure 1).

Figure 1: Healthcare Expenditures for Patients with DM



(8)

Researchers also found that expenditures for health care in people with DM increase as the patients age. Sixty-five percent of healthcare costs were for patients 65 years of age or older. Indirect costs are also higher in individuals with DM. Patients with DM lost an average of 8.3 days from work compared to 1.7 days for individuals without diabetes. Results of this study indicate that health care costs are significantly higher for individuals with diabetes than for those who do not have the disease.

Increased healthcare costs have been linked to increased glycosylated hemoglobin (HbA_{1c}) levels (3). Serum HbA_{1c} is an indicator of blood glucose control. A study by Gilmer et al. (9) investigated the cost of poor glycemic control for a Health Maintenance Organization (HMO). The subjects recruited for the study were all members of the HMO who had diabetes and were continuously enrolled in the HMO for the 4 year period of the study. Researchers found that when HbA_{1c} levels were greater than 7%, healthcare costs increased 10% for each percent increase in HbA_{1c}. Results of the study also indicated that in patients with heart disease and hypertension in addition to diabetes, there was a significant decrease in health care cost associated with a one percent decrease in HbA_{1c} levels (Table 2).

INTENSIVE TREATMENT VS TREATMENT OF COMPLICATIONS

There are four major studies that have looked at the cost effectiveness of better blood glucose control compared to treating the complications that occur

Table 2: Decreased Cost Associated with 1% Decrease in HbA_{1c} Levels

Decrease In HbA_{1c}	Cost Savings Per Year
10 to 9%	$4,116
9 to 8%	$3,090
8 to 7%	$2,237
7 to 6%	$1,504

(9)

as a result of years of hyperglycemia. These studies include the Diabetes Control and Complications Trial (DCCT), Stockholm Study, the United Kingdom Prospective Diabetes Study (UKPDS), and the Kumamuto Study. Results of these studies suggest that intensive treatment of blood glucose levels costs more than traditional treatments, but reduce complications of DM. This leads to an overall cost reduction for DM patients with intensive treatment (3, 10)

The DCCT (11, 12, 13) was a randomized clinical trial that compared the effects of conventional therapy to intensive therapy in patients with type 1 diabetes. Fourteen hundred forty one subjects with type 1 diabetes were followed for approximately 6.5 years. Participants were divided into two groups; one group received conventional therapy and the other intensive therapy. Subjects in the group receiving conventional therapy received one or two insulin injections daily. Patients receiving intensive therapy received three

or more insulin injections daily to maintain blood glucose levels as close to normal as possible. Results of the study indicated that subjects in the intensive treatment group had significantly lower average HbA_{1C} levels than the group receiving conventional treatment. This indicates that overall blood glucose levels were significantly lower in the intensively treated group. There was a 54% decreased risk of developing retinopathy with intensive treatment than conventional treatment. Risk of developing microalbuminemia (protein loss in the urine) decreased 34% with intensive treatment. At five years, the risk of developing neuropathy was decreased by 69% with intensive treatment when compared to conventional treatment. Results of this study indicated that there is a decrease in side effects and complications of diabetes when blood glucose levels are more tightly controlled (11, 12, 13).

In 1998, Turner published a review on the results of the UKPDS (14). The main objective of the UKPDS was to determine if intensive control of blood glucose levels in patients with DM would reduce risks for the side effects and complications of DM. Five thousand one hundred two patients with type 2 DM were recruited to participate in the study. Fifty percent of the subjects had signs of tissue damage related to diabetes at the beginning of the study. Initially all patients received diet therapy. The subjects were then randomized into one of four treatment groups. One group was treated with conventional therapy with dietary treatment, another group received intensive treatment with chlorpramide, another group received intensive treatment with glyburide,

and the final group received intensive insulin therapy. An additional group of obese individuals received metformin. In all intensive treatment groups the goal of therapy was to achieve fasting glucose levels less than 108 mg/dL. Results indicated that intensive treatment was successful in lowering blood glucose concentrations, and that all methods of intensive therapy tested were equally effective in controlling blood glucose levels. Intensive treatment of diabetes can decrease blood glucose levels, which will decrease side effects and complications of the disease (14, 15).

ACCOMPANYING CONDITIONS

There are many complications associated with development of DM including: cardiovascular disease, stroke, neuropathy, nephropathy, and retinopathy (16). The UKPDS has shown a reduction of microvascular complications associated with DM, and a reduction of myocardial infarction with increased control of blood glucose levels (16).

Cardiovascular Disease

Diabetes increases a patient's risk of developing heart disease. A man with diabetes has double the risk of developing heart disease than a man without the disease, and a woman with diabetes has four times greater risk of death than a woman without the disease (4,7). The number one cause of death among people with diabetes is cardiovascular disease (CVD). Diabetes is an

independent risk factor for CVD (4). Up to 80% of individuals with type 2 DM will die from CVD (17).

The San Antonio Heart study, conducted by Wei et al. (18) was a 7 to 8 year study investigating the effect of hyperglycemia on risk of developing CVD in 4,875 subjects. At baseline there were 471 subjects with diabetes and 4,404 non-diabetic subjects. An additional 27% of subjects developed diabetes during the follow-up period. Researchers found that hyperglycemia is an independent risk factor for CVD, and that risk for CVD increases as blood glucose levels increase. It was also noted that TG levels increased and HDL cholesterol decreased with increased plasma glucose levels.

Turner et al. (19) analyzed data from the UKPDS for risk factors for coronary artery disease (CAD) related to diabetes. Results indicated that risk factors for CAD were elevated levels of LDL cholesterol, triglycerides, HbA_{1C}, fasting plasma glucose, and systolic blood pressure, and low levels of HDL cholesterol. There was a 1.57 fold increase in risk for CAD with each 1 mmol/L increase in LDL cholesterol and a 0.15-fold decrease in risk for CAD with each 0.1 mmol/L increase in HDL cholesterol. This study also found an 11% increase in risk of CAD for each 1% increase in HbA_{1C}.

Oxidation of LDL is a causative factor of atherosclerosis (20). It is believed that antioxidants may have a protective effect and reduce the oxidation of

LDL. Flavonoids are antioxidants, and it has been shown that there is an inverse relationship between intake of dietary flavonoids and coronary heart disease (20, 21).

Neuropathy

Neuropathy is damage to the nervous system caused by chronic hyperglycemia (22). Approximately 60 to 70% of people with diabetes have some degree of neuropathy. Neuropathy may lead to impaired sensation in the hands or feet, which can result in foot ulcers and amputation of extremities. In the United States more than 50% of lower limb amputations are the result of diabetes, with approximately 67,000 amputations being performed yearly on people with diabetes. Autonomic neuropathy may lead to gastrointestinal, genitourinary, cardiovascular or sexual dysfunction (1, 6, 23). Symptoms of neuropathy include tingling or numbness in the extremities, decreased ability to feel hot or cold, and impaired reflexes (23).

In a study published in 1997, Adler et al. (23) investigated risk factors for diabetic peripheral sensory neuropathy. Subjects included 778 U.S. veterans, of which 78% were white and 98% were male. Monofilament testing was used to determine presence of neuropathy. Patients were tested at baseline and were followed up within 2 to 3 years. Results indicated that height, age, HbA_{1c} level, and history of lower-extremity ulcer are all independent risk factors for diabetic neuropathy.

Nephropathy

The primary cause of renal failure is DM. Approximately 10% of people with renal failure has diabetes (24), and diabetes is responsible for approximately 40% of new cases of end-stage renal disease (6). Several factors may be responsible for nephropathy in patients with DM. Oxidative stress may damage the kidneys due to the increase in reactive oxygen species associated with hyperglycemia and glycosylation of proteins (25). A high concentration of aldose reductase in the inner medulla of the kidney leads to the buildup of sorbitol, which can damage the kidneys (26). Many patients with type 2 DM have high blood pressure, which increases stress on the kidneys. Table 3 lists the stages in the progression of diabetic kidney disease.

Table 3: Stages of Nephropathy

Stage	Characteristics	Description
I	Hyperfiltration	Blood flow through kidneys increases
II	Microalbuminemia	Small amounts of albumin lost in urine
III	Clinical Albuminemia	Increased amounts of albumin and other protein lost in the urine
IV	Advanced Clinical Nephropathy	Decreased glomerular filtration rate (<75 mL/minute)
V	End Stage Kidney Disease	Glomerular filtration rate <10 mL/minute

(27)

Retinopathy

Diabetic retinopathy is a side effect of both type 1 and type 2 DM, and risk of developing diabetic retinopathy increases with duration of the disease (28) Diabetic retinopathy is the leading cause of blindness in adults and is responsible for 12,000 to 24,000 new cases of blindness each year (6). Early characteristics of retinopathy include macroaneurisms (ballooning out of walls of blood vessels), hemorrhages, and hard exudates (leakage of proteins and lipids from the blood into retina). There are several mechanisms that lead to diabetic retinopathy. The first is macular edema or fluid in the retina, which results from buildup of hard exudates. The second is growth of new blood vessels on the retina. The third is hemorrhaging from capillaries into the eye (28).

Cataracts also lead to vision deficits in patients with DM. There is a high concentration of aldose reductase in the lens of the eye; therefore, when blood glucose levels are elevated, sorbitol accumulates in the lens. The high concentration of sugar alcohol causes water to be pulled into the lens, which leads to vision deficits. Inhibition of the enzyme aldose reductase may reduce the vision impairments caused by DM.

LINK BETWEEN DIET AND DIABETES MELLITUS

The increase in incidence of type 2 DM is likely due to changes in dietary habits. There have been numerous studies looking at the dietary habits of

patients with type 2 DM (29,30,31). A decline in consumption of whole grains, fruits, and vegetables and an increase in consumption of highly processed convenience foods and high fat fast food have been shown to increase risk for developing type 2 DM.

Uusitupa et al. (32) investigated the effect of diet and exercise in preventing or delaying onset of type 2 DM in patients with impaired glucose tolerance (IGT) in the Finnish Diabetes Prevention Study. Five hundred twenty three subjects with IGT were recruited for this ongoing controlled trial. The treatment group was counseled to improve diet and exercise habits. The advised diet contained greater than 50% of kcals from carbohydrates, less than 30% from fat, less than 300 mg of cholesterol, and at least 15 grams of fiber per day. After 1 year, subjects in the treatment group had significantly lower 2 hour glucose levels, fasting glucose levels, and 2 hour insulin levels than the control group. Results of this study suggest that there is a decreased risk of developing type 2 DM with changes in diet and exercise habits.

Ford and Mokdad (33) investigated whether consumption of fruits and vegetables had a preventive effect for DM. Researchers followed 9,665 subjects for approximately 20 years, and mean daily intake of fruits and vegetables was recorded. Results indicated that the mean number of fruits and vegetables eaten daily was lower in the group that developed DM. There

was also a lower percentage of people who consumed 5 or more servings of fruits and vegetables daily in the group that developed DM.

A study published in 2002 by van Dam et al. (29) looked at the association between dietary intake and risk for developing type 2 DM. This study was a prospective cohort study in which 42,504 professional men were followed for 12 years (1986 to 1998). A food frequency questionnaire was given every 4 years and was used to determine foods commonly eaten by subjects, portion sizes consumed, and how often specific foods were consumed. Every 2 years participants were given a questionnaire about "exposures and newly diagnosed diseases." Subjects were grouped into one of two dietary patterns, either prudent or western. The prudent dietary pattern was characterized by a high consumption of whole grains, fruits, vegetables, and fish. The western dietary pattern was characterized by a high consumption of red meat, processed meat, French fries, high fat dairy products, sweets and desserts, and refined grains. Results of this study indicated that the risk of developing type 2 DM increased in participants with the western dietary pattern, and decreased with consumption of the prudent dietary pattern.

A study conducted by Ekblond et al. (31) looked at the effects of dietary intake on urinary glucose excretion, which is a predictor of type 2 DM. The cross-sectional study took place between December 1993 and April 1997, and 27,146 Danish men and 29,861 women participated in the study. All

participants filled out a food frequency questionnaire and a lifestyle questionnaire with questions about smoking, alcohol intake, physical activity and medical history. Subjects who experienced urinary glucose excretion during the study were compared to subjects who did not. Investigators found that persons with glucosuria tended to eat more meat and less fruits and vegetables, cereal grains, and poultry than subjects who did not experience glucosuria.

MONITORING OF BLOOD GLUCOSE LEVELS

Control of blood glucose levels can be monitored by measuring blood glucose concentrations, fructosamine levels or glycosylated (glycated) hemoglobin (HbA_{1c}) levels. Measurement of serum glucose levels is an indication of current blood glucose control (1). The fructosamine assay measures glycosylated albumin or glycosylated total proteins. According to Armbruster (34), fructosamine is a "ketoamine" which is produced by a non-enzymatic reaction between a sugar and a protein. The sugar is normally glucose and the protein normally albumin. Measurement of fructosamine levels is an indicator of blood glucose control in the 2 to 3 weeks prior to the test (34). Glycosylated hemoglobin is a hemoglobin protein that a glucose molecule has covalently attached itself to. These proteins are more susceptible to oxidative damage than normal hemoglobin molecules. Serum HbA_{1c} levels are an indication of blood glucose control in the previous 3 months. Because the life span of a red blood cell is 3 months, it takes this long for all of the hemoglobin

molecules in the body to turnover. HbA_{1C} and fasting glucose tests are the methods that are currently recommended to monitor treatment of diabetes by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1). Treatment goals for individuals with type 2 DM are listed in Table 4.

Table 4: Methods of Monitoring Treatment of DM

Indicator	Presence Of Hyperglycemia	Normal Values
Blood glucose levels	Immediate	3.9 to 7.0 mmol/L
Serum Fructosamine	Previous 2 to 3 weeks	1.6-2.6 mmol/L
Serum HbA_{1C}	Previous 3 months	Less than 6%

(1, 35)

PROTEIN GLYCOSYLATION

Protein glycosylation (or glycation) is a direct non-enzymatic reaction between a reducing sugar and the amino group of a protein (36). This is a non-enzymatic reaction where the glucose molecule covalently attaches itself to a reactive amino group. The rate at which this reaction occurs is dependent on blood glucose concentrations. These glycated proteins are precursors to advanced glycation products. Non-enzymatic glycation of proteins is also damaging to the body because this increases the rate of free radical production 50-fold (37).

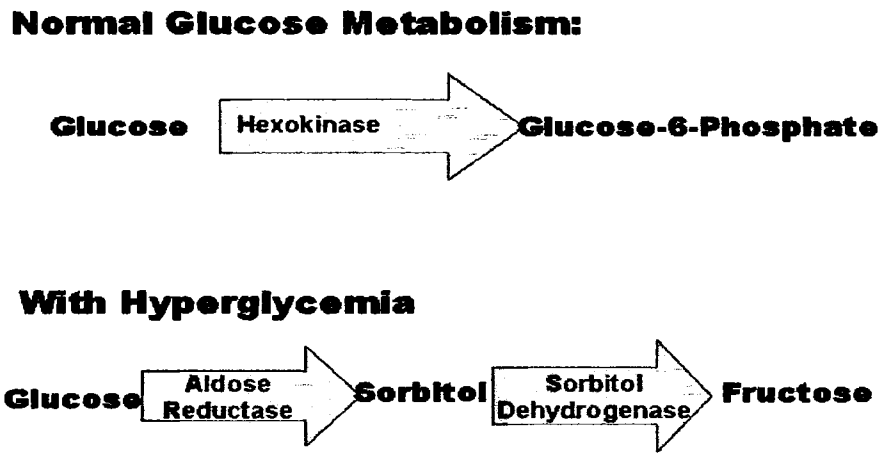
Many proteins in the body experience glycosylation when blood glucose levels are elevated. Proteins susceptible to protein glycosylation include hemoglobin, red blood cell membrane proteins, albumin, collagen, myelin, and proteins in the lens of the eye. Protein glycosylation is an oxidation reaction; therefore, it is believed that antioxidants are effective in preventing this reaction (36).

ALDOSE REDUCTASE

Aldose reductase is known to be the cause of many of the complications of type 2 DM including cataracts, retinopathy, nephropathy, and neuropathy. Aldose reductase is an enzyme found in the body that is responsible for the reduction of glucose to sorbitol in the polyol pathway (Figure 2) (37). This reaction occurs when D-glucose is converted to sorbitol by aldose reductase. This pathway is only active when blood glucose concentrations are elevated (38). Hexokinase is an enzyme that converts glucose to glucose-6-phosphate. Glucose-6-phosphate is the form of glucose that is used by the cells of the body. When hexokinase becomes saturated due to hyperglycemic conditions, aldose reductase is activated to convert excess glucose to sorbitol. Sorbitol cannot be used in the body as a fuel source, so it needs to be converted to something useable. The enzyme sorbitol dehydrogenase converts sorbitol to fructose; however, concentrations of sorbitol dehydrogenase do not increase in proportion to concentrations of sorbitol, so sorbitol builds up in the tissues. The fructose that is converted from sorbitol may also damage proteins due to

enzymatic fructosylation of proteins. The fructose may be converted to 3-deoxyglucosone, which is a precursor to advanced glycation end products. This pathway also leads to production of free radicals for autoxidation of glucose, hydrogen peroxide (H_2O_2), and superoxide molecules ($O_2\cdot$) (37).

Figure 2: Polyol Pathway



(37)

If an increase in aldose reductase is responsible for many of the side effects of diabetes, including neuropathy, nephropathy, and retinopathy, then finding a way to inhibit this enzyme may decrease the side effects of DM and improve quality of life for people with DM (36).

In a study published in 2002, Obrisiva et al. (39) investigated the effect of sorbinil, an aldose reductase inhibitor, on rat peripheral nerve function, metabolism and antioxidant defense. When a dose of 65 mg/kg of body weight of sorbinil was given, there were significant improvements in

neurovascular function and nerve conduction. An improvement in metabolic abnormalities was also noted. Lipid peroxidation was increased in diabetic rats compared to the control group, but the lipid peroxidation was arrested with administration of sorbinil.

Hamada et al. (40) investigated the effect of epalrestat, a known aldose reductase inhibitor, on the accumulation of end products of glycation. Blood was drawn from 38 subjects with type 2 DM. Sixteen of the subjects had been taking 150 mg of epalrestat daily for 2 months and 38 had not been treated with the drug. Sorbitol, fructose, triglycerides, carboxymethyl-lysine (CML), triosephosphates, erythrocyte 3-deoxyglucosone (3-DG), and the thiobarbituric acid-reactive substance (TBARS) assay were measured. TBARS are products of the decomposition of hydroperoxides. Sorbitol and fructose are products of the polyol pathway, and CML, triosephosphates, 3-DG are advanced glycation end products. Results indicated that sorbitol, fructose, triosephosphates, TBARS and 3-DG levels were significantly lower in subjects receiving epalrestat than in patients not receiving the drug. There were no changes in plasma glucose or HbA1c levels. These results suggest that inhibition of the enzyme aldose reductase can reduce the end products of protein glycosylation.

A study conducted by Vincent et al. (38) investigated the ability of vitamin C to inhibit aldose reductase. Human erythrocytes collected from normal

volunteers were used for the study. The erythrocytes were incubated with increasing concentrations of glucose from 5 to 40 mM, and sorbitol concentrations increased progressively with the increasing concentrations of glucose. Ascorbic acid was then added and was found to decrease intracellular sorbitol concentrations by 25 to 45 %.

Pfeifer et al. (22) conducted a review of research completed investigating the efficacy of aldose reductase inhibitors (ARI) on the side effects of diabetes. Of 32 clinical trials on the effect of ARI on distal symmetrical polyneuropathy, 59% showed positive results on at least one measure. Most often motor nerve conduction velocity was improved by treatment with an ARI. Of 15 trials conducted on the effect of ARI on autonomic neuropathy, 5 showed improvement in autonomic neuropathy. These trials indicate that ARI may be a promising treatment to reduce side effects in patients with DM, but due to the number of studies that have not shown positive results, more research needs to be completed.

ALTERNATIVE TREATMENTS

Use of complementary and alternative medicine (CAM) has become popular recently for treatment and prevention of disease. CAM is defined as use of therapies and treatments that are not used in conventional medicine (41). Several research studies have investigated the used of CAM in treatment of diabetes.

Egede et al. (41) analyzed data collected in the Medical Expenditure Panel Survey (MEPS) conducted in 1996. The MEPS collected data on CAM use, medical conditions, health insurance, demographic information, and health care expenditures for US citizens. Egede et al. analyzed data on demographics, medical conditions and CAM use. Results of the survey indicated that patients with DM were more likely to use CAM than patients with no chronic diseases. The CAM therapies most often used by patients with DM were nutritional advice, spiritual healing, herbal remedies, massage therapy, and meditation training.

Ryan et al. (42) conducted a survey investigating the use of CAM in patients with diabetes. Five hundred two patients with diabetes and 201 non-diabetic control subjects were surveyed. Results indicated that 78% of patients with DM were taking prescription medications compared to 63% of non-DM patients. When use of over-the counter supplements (OTCS) were compared, researchers found that 44% of patients with DM compared to 51% of non-DM patients were taking OTCS. Thirty-one percent of patients with DM were using CAM compared to 37% of non-DM subjects. CAM supplements used by subjects with DM included garlic, Echinacea, glucosamine, chromium, ginkgo biloba, cayenne and St. John's Wort. Only patients with DM reported taking chromium, which is believed to improve blood glucose control. Researchers also found that patients with DM spent approximately the same amount on over-the-counter supplements and alternative medicine as they did on

prescription medications ($23.53 per month for OTC and CAM supplements compared to $28.51 for prescription medications). Subjects with DM used CAM supplements and OTCS in addition to prescription medications, rather than replace prescription medications with CAM supplements and OTCS.

ANTHOCYANINS AND FLAVONOIDS

Anthocyanins and flavonoids are chemicals found in fruits, vegetables, nuts, seeds, stems, flowers, tea, and wine that are powerful antioxidants. These compounds have been shown to improve resistance of LDL to oxidation, protect against oxidative damage, decrease protein glycosylation and affect enzyme activity (36). Fresh berries are a rich source of flavonoids and anthocyanins. Lipid oxidation is believed to be a causative factor in cardiovascular disease. Antioxidants, such as anthocyanins and flavonoids found in fruits and vegetables, can help protect against lipid oxidation and therefore protect against cardiovascular disease.

Protein Glycosylation

Camire et al. (43) investigated the ability of fruit anthocyanins to inhibit aldose reductase in-vitro. Fruit powders of raspberries, blueberries, cranberries and grapes were analyzed as well as the flavonoid quercetin and anthyocyanin aglycones cyanidin, delphinidin and malvidin. Aldose reductase was inhibited 10 to 52% by fruit powders. Raspberries were most effective at inhibiting aldose reductase followed by blueberry and cranberry powders. Grape

powder was the least effective inhibitor of aldose reductase. Over 90% of the aldose reductase was inhibited by quercetin, cyanidin and delphinidin, and only 70% was inhibited by malvidin.

Keenoy and De Leeuw (44) investigated the effect of Daflon, a flavonoid preparation containing 90% diosmin and 10% hesperidin, on protein glyosylation and antioxidant status. Twenty-eight subjects with type 1 DM participated in the 3-month double-blind, placebo-controlled study. Blood glucose and HbA_{1C} levels were measured to determine blood glucose control and protein glycosylation. Antioxidant capacity was measured by determining reduced glutathione (GSH) and gluthione peroxidase content in red blood cells. Blood glucose levels remained constant during the course of the study, but HbA_{1C} levels decreased significantly with flavonoid treatment. No significant differences in antioxidant activity were found between the flavonoid group and the placebo group.

Asgary et al. (36) conducted a study which looked at the effect of the flavonoids quercetin, rutin, and kaempherol on protein glycosylation in vitro. The optimal concentration and incubation time of flavonoids to reduce protein glycosylation was investigated. A colorimetric method was used to determine the degree of hemoglobin glycosylation in the presence of each flavonoid. Results of the study indicated that all three of the flavonoids had a preventive effect on protein glycosylation (Table 5). The preventive effect of the

flavonoids on protein glycosylation was dependent on the concentration of the flavonoid. Quercetin had the greatest effect on protein glycosylation and kaempherol had the least effect. Additional studies need to be completed to determine whether flavonoids have the same effect on protein glycosylation in vivo (36).

Table 5: Inhibition of Hemoglobin Glycosylation by Flavonoids

Flavonoid	0.5 µg/mL	5 µg/mL	10 µg/mL
Rutin	11%	27%	42%
Quercetin	3%	37%	52%
Kaempherol	10%	12%	15%

(36)

In 2000, a study by Jankowiski et al. (45) investigated the effects of anthocyanin pigments from red wine on diabetes. The study used 80 rats divided into 4 groups: a control group, a group receiving streptozotocin to induce diabetes, a group receiving intragastric anthocyanins, and a group receiving streptozotocin and anthocyanins. Glucose concentrations in blood and urine increased in the rats after streptozotocin was injected, but was decreased in the animals receiving anthocyanins. There was a substantial decrease in blood and urine glucose, a reduction in free radical production and a decrease in the concentration of products of unsaturated fatty acid

oxidation in blood and urine in the group of rats receiving streptozotocin and anthocyanins.

Lipid Oxidation

In 1997 Laplaud et al. (20) conducted a study looking at the antioxidant effect of blueberry extract on human LDL in vitro. Plasma was obtained from 6 fasting adult Caucasians, and the LDL was isolated by ultracentrifugation. Copper chloride was used to induce LDL oxidation. A lipoperoxide assay, a TBARS assay, and relative electrophoretic mobility (REM) were performed on the LDL to determine the amount of oxidation with the addition of differing amounts of blueberry extract. The results indicated that the blueberry antioxidants are effective in inhibiting the oxidation of LDL at concentrations as low as 15 µg/mL.

In 1998, Heinonen et al. (46) conducted a study looking at the effect of polyphenolics found in berries on oxidation of LDL and liposomes. The berries chosen for this study included blackberries, red raspberries, sweet cherries, blueberries, and strawberries. In this study LDL oxidation was completely inhibited by all berries except strawberries and blueberries. Berry extracts were successful in inhibiting 27 to 68% of hydroperoxide formation and 60.5 to 92.6% of hexanal formation.

In 1999, Gabrielska et al. (47) conducted a study looking at the effect of anthocyanin pigments extracted from chokeberry, honeysuckle and sloe on lipid oxidation in vitro. Lipid oxidation was induced in the liposome membrane by ultraviolet radiation. The amount of oxidation was evaluated by a TBARS assay. HPLC was used to evaluate the end products of lipid membrane oxidation. The results of this study indicated that the anthocyanins found in chokeberry, honeysuckle and sloe are protective against free radical oxidation of the lipid membrane.

Many research studies have been completed investigating the effect anthocyanins and flavonoids have on DM. The results indicate that these compounds have a protective effect on lipid oxidation and protein glycosylation in vitro. More research needs to be completed to determine if the same effects occur in vivo.

CRANBERRIES

Cranberries (*Vaccinium macrocarpon*) have been believed to be useful to prevent disease and treat illness for hundreds of years. Native Americans used cranberries to treat wounds and blood poisoning, and American sailors ate cranberries to prevent scurvy. The leaves of the cranberry plant have been used to treat diarrhea, urinary disorders and diabetes. Most research has been focused on the ability of cranberry juice to prevent and treat urinary tract infections (48). Cranberries are widely used as a treatment for urinary

tract infections, even though conclusive documentation has not been published.

Composition

In a study published in 2001, Prior et al. (49) analyzed the anthocyanin content of blueberries and cranberries. Researchers found that the four major anthocyanins that are responsible for the red color of cranberries are cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside, and peonidin-3-arabinoside. Cranberries also contain smaller amounts of the anthocyanin cyanidin-3-glucoside and peonidin-3-glucoside (Table 6).

Table 6: Percent of Anthocyanins found in Cranberries

Anthocyanins	% Of Total Anthocyanins
Peonidin-3-galactoside	32.2
Cyanidin-3-galactoside	25.2
Cyanidin-3-arabinoside	23.7
Peonidin-3-arabinoside	15.8
Cyanidin-3-glucoside	1.1
Peonidin-3-glucoside	0.9

(49)

In an article published in 2001, Kahkonen et al. (50) reported the phenolic and anthocyanin content of berries and apples. Cranberry (*Vaccinium oxycoccus*)

samples were extracted by acetone extraction and then dried and frozen for up to 1 week before being analyzed. The cranberries contained 397 mg of anthocyanins per 100g of dry weight of cranberries. The flavonoid content of the analyzed sample was 200 mg/100g dry weight.

Hakkinen et al. (51) investigated the content of the flavonoids quercetin, myricetin and kaemphferol in berries including cranberries. *Vaccinium oxycoccos*, a species of wild cranberry, was used in this study. Researchers found large amounts of quercetin (83 to 121 mg/kg) and myricetin (74 to 142 mg/kg) in the cranberry samples, but kaemphferol was not found. Cranberry had the second highest total flavonoid content (157 to 263 mg/kg) of the berries analyzed, second only to bog whortleberry (184 mg/kg).

Bioactivity of Cranberry Components

Pedersen et al. (52) conducted a study comparing the antioxidant capacity of cranberry and blueberry juice in the plasma of nine female volunteers. Researchers used ferric reducing antioxidant value (FRAP value) as one measure of plasma antioxidant activity, and electron spin resonance spectroscopy (ESR) to determine the ability of the plasma to donate a hydrogen atom or electron to a free radical. Results indicated that consumption of blueberry juice did not significantly increase concentrations of total phenols or vitamin C in the plasma of the volunteers. Researchers question how readily the phenols are absorbed in the gut. Consumption of

cranberry juice did lead to significant increases in total phenols and a 30% increase in serum vitamin C concentration. Researchers question if the increase of total phenols in serum following cranberry juice consumption is primarily due to the increased vitamin C levels.

Wilson et al. (53) conducted a study looking at the ability of cranberry extract to protect against low density lipoprotein oxidation. LDL was separated from the serum of 5 male volunteers and used for the study. Oxidation of the LDL samples was promoted and cranberry extract was added to each sample at concentrations of 0.00%, 0.10%, 0.010%, or 0.005%. After incubation, the samples were tested for oxidation by TBARS and electrophoresis. Results indicated that TBARS were inhibited at a 0.10% dilution and electrophoretic movement was reduced significantly when up to 0.05% dilution of cranberry extract was used. In this study cranberry inhibited LDL oxidation in-vitro.

Wang and Jiao (54) investigated the protective effect of cranberries, blackberries, blueberries, raspberries, and strawberries against superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and singlet oxygen (O_2). Researchers found that the ability to inhibit oxidation by these radicals varied by cultivars, but all cranberries tested inhibited greater than 54% of $O_2^{\bullet-}$, greater than 55% of H_2O_2, greater than 59% of OH, and greater than 7% of O_2.

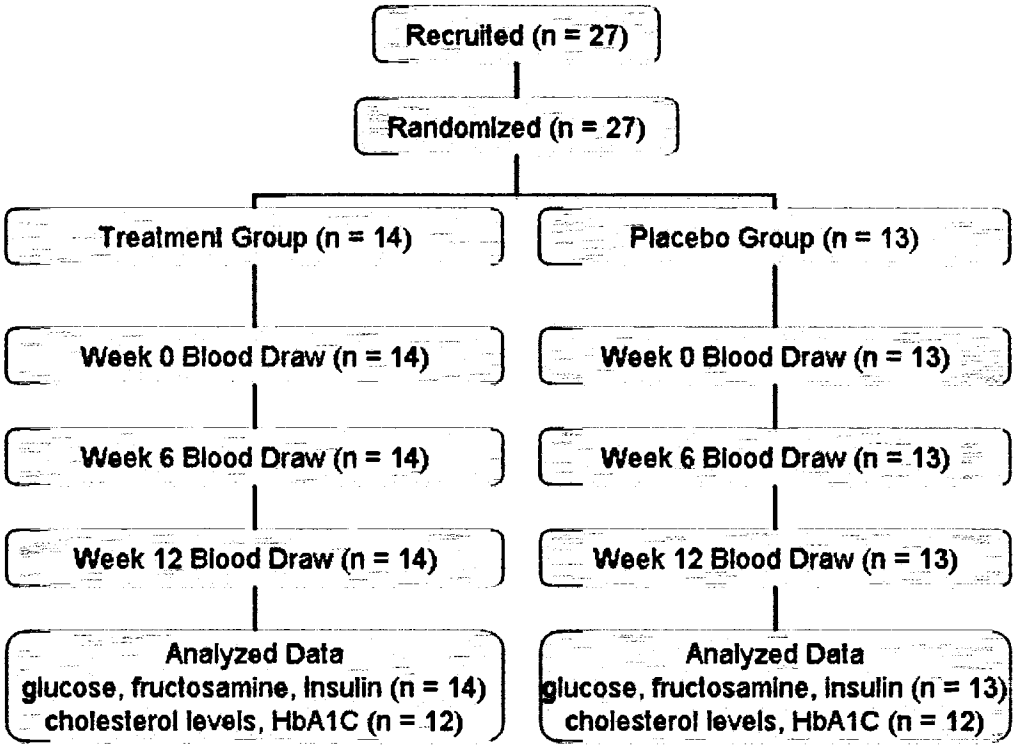
There has been a great deal of research investigating the effects of cranberry anthocyanins and flavonoids in vitro. It is possible that cranberry supplementation may decrease the protein glycosylation and lipid oxidation that contribute to the side effects of DM. The anthocyanins and flavonoids in cranberry may inhibit the enzyme aldose reductase, and therefore may inhibit the buildup of sorbitol in tissues of the body.

MATERIALS AND METHODS

STUDY DESIGN

Twenty-seven adults with type 2 DM, controlling their disease with diet and exercise, were recruited for this twelve week, double blind, placebo controlled study. Subjects were recruited from ads placed in the Bangor Daily News and in the Maine Perspective. Fliers were put up at local stores and health clubs, and notices were posted on the First Class University of Maine computer conference. Patients taking oral medications or insulin to control blood glucose levels were not invited to participate in the study. The University of Maine Protection of Human Subjects Review Board approved the study. Subjects were randomly assigned to either the cranberry group or the placebo group. Participants were asked to fill out an informed consent form (Appendix A), a health history questionnaire (Appendix B), and a food frequency questionnaire (Appendix C) at the first blood draw. Instructions were provided on the front page of the questionnaires, and someone was available to answer questions while subjects were filling out the questionnaires. During the study, participants were asked to take 6 capsules daily filled with either the cranberry powder or the placebo, and have blood drawn at 0, 6, and 12 weeks (Figure 3). Blood samples were taken after an overnight fast. A stipend of $50 dollars was given to each subject after each of the three blood draws for a total of $150.

Figure 3: Study Design



CRANBERRY TREATMENT

Size 00 gelatin capsules (00-coni-shape) (Feton Chemical and Pharmaceutical Company, Ramsey, NJ) were filled with Decas Cranberry Juice Concentrate Powder 90 (product code 06054), which was generously provided by Decas Cranberry Products (Wareham, MA). A Feton model 101 capsule filling machine was purchased from Feton Chemical and Pharmaceutical Company (Ramsey, NJ).

The capsules were opened by the filling machine, and were then filled with the powder. The powder was tamped down three times to ensure that the

capsules were filled completely. The machine then closed the capsules. Ten empty capsules were weighed with a Sartorius Analytical balance (model A2005, Sartorius Corporation, Edgewood, NY), and the total was divided by 10 to get the average weight of one capsule. The weight of an empty capsule was 0.12 grams. The average weight of the filled capsules was 0.64 grams. Each capsule of cranberry powder contained approximately 0.53 grams of Decas Cranberry Juice Concentrate Powder 90. The cranberry powder is 90% cranberry juice, so the weight of actual cranberry juice in each capsule is 0.48 grams.

The subjects in the treatment group were given cranberry capsules that contained the equivalent of eight ounces (240 mL) of cranberry juice cocktail, since that beverage is the major form of cranberry consumed in the United States. To calculate the amount of cranberry powder each subject needed daily, it was first important to know that liquid commercial cranberry juice cocktail contains approximately 27% cranberry juice. The Decas Cranberry Juice Concentrate Powder 90 is 90% cranberry juice. Nutrition information from Nutritionist 5 Nutrient Database (First DataBank, San Bruno, CA) was used to calculate the amount of cranberry in commercial juice. Each 8 ounce serving of cranberry juice cocktail weighs 253 grams. There are 216 grams of water and 34.2 grams of sugar in each 253-gram serving of juice. The water and sugar in the juice were subtracted from the total weight; 2.8 grams of cranberry juice are present in each 8 ounce serving of cranberry juice. The

cranberry powder is 90% cranberry juice, so the weight of cranberry powder in each capsule (0.53 grams) was multiplied by 90% to get the amount of cranberry juice in each capsule (0.48 grams). With 0.48 grams of actual cranberry juice in each capsule, six (5.89) cranberry capsules were required to equal an eight ounce serving of cranberry juice. Table 7 displays the nutritional information for cranberry powder.

Table 7: Nutritional Analysis of Cranberry Powder*

Nutrient	Amount per 3 gram Serving
Calories	10.8 kcals
Carbohydrate	2.7 g
Sugars	1.2 g
Potassium	27.3 mg
Vitamin C	3.2 mg
Calcium	2.1 mg
Magnesium	183.4 mg

* Nutrition information provided by Decas Cranberry Products Wareham, MA.

SUGAR ANALYSIS OF CRANBERRY POWDER

High performance liquid chromatography (HPLC) analysis was performed on the Cranberry Juice Concentrate Powder 90 using a Hewlett Packard HP 3394 Integrator (Palo Alto, CA), a Waters Associates Differential Refractometer, model R401 (Milford, MA), a Shimadzu LC06A isocratic pump

(Braintree, MA), a Primesphere NH_2, 5μ, 250 x 4.6 mm HPLC column (Phenomenex Inc. Torance, CA), and a VICI model E 60 injector (Valco Instruments Co Inc. Houston, TX). Acetonitrile (HPLC grade, A998-4) and water (HPLC grade, W5-4) for the HPLC analysis were purchased from EM science (Gibbstown, NJ). SIGMA ultra fructose (F2543) and sucrose (51174) were purchased from SIGMA Chemical Company (St Louis, MO). Anhydrous dextrose (D16-500) was purchased from TT Baker Chemical Company (Phillipsburg NJ).

High-performance liquid chromatography was used to determine the percentage of glucose and fructose in the Decas Cranberry Juice Concentrate Powder 90. This information was necessary to ensure that the placebo contained the same concentrations of glucose and fructose as the treatment. The mobile phase was 75% acetonitrile and 25% water, and a flow rate of 1.5 mL per minute was used. Five grams of Decas Cranberry Juice Concentrate Powder 90 was mixed with enough ethyl alcohol (ETOH) to make a 50 mL solution. The standard was mixed using 0.0786 grams of fructose, 0.0782 grams of dextrose, 0.0743 grams of sucrose and enough 85% ETOH to bring the solution up to 100 mL. Twenty-five μL of standards and sample solutions were injected into the HPLC system. The chromatogram of HPLC sugar analysis of cranberry powder is located in Appendix D.

The following equation was used to calculate the amount of each sugar per mg of cranberry powder:

$$\text{Glucose or fructose} = \left(\frac{\text{peak height-sample (cm)}}{\text{peak height-std (cm)}}\right) \times (\text{conc of std mg/mL}) \times \left(\frac{\text{50mL (total volume of sample)}}{\text{0.5 grams (sample wt)}}\right)$$

conc = concentration std = standard wt = weight

The average of two analyses was used to determine the percentage of glucose and fructose in the Cranberry Juice Concentrate Powder 90.

PLACEBO

To ensure that any results were an effect of the cranberry powder and not the concentration of sugar, magnesium or ascorbic acid, it was important to ensure that the placebo contained the same concentrations of glucose, fructose, magnesium hydroxide, and ascorbic acid as the cranberry treatment. HPLC analysis of the Decas Cranberry Juice Concentrate Powder 90 indicated that each gram of the powder contained 57.6 mg (6%) of fructose and 184.2 (18%) mg of glucose. The Decas Cranberry Juice Concentrate Powder 90 is processed using magnesium hydroxide. Each gram of the cranberry powder contains 61.13 mg (6%) of magnesium. The Decas Cranberry Juice Concentrate Powder 90 contains 1 mg of ascorbic acid per gram of powder, which makes it 0.1% ascorbic acid.

A total of 4000 grams of the placebo powder was made. Purified cellulose (grade: just fiber, BH 65FCC, International Filler, North Tonawanda, NY) was used as the base of the placebo. The composition of the placebo is listed in Table 8. Magnesium hydroxide powder (USP Grade) was generously provided by Watson Foods Co. Inc. (West Haven, Ct). Dextrose corn sugar (020010-102, Corn Products International, Bedford Park, IL), fructose (95-603, ADM Corn Processing, Decatur, IL), and Now fine ascorbic acid powder (0792, Now Foods, Glendale Heights, IL) were purchased from Natural Living Center (Bangor, ME). Red (E1248) and violet (E0680) food coloring were purchased from Lorann Oils (Ramsey, NJ). Placebo capsules were filled using the same methods as the cranberry capsules and were approximately the same weight as the cranberry capsules.

Table 8: Composition of Placebo Powder

Component	Amount (Grams)	Amount (%)
Cellulose	2396	59.9
Glucose	729	18
Fructose	240	6
Magnesium Hydroxide	240	6
Red Food Coloring	200	5
Violet Food Coloring	200	5
Ascorbic Acid	4	0.1

BLOOD ANALYSIS

All blood draws were done at the phlebotomy laboratory at Cutler Health Center at the University of Maine, Orono, by Sally Hall, American Society of Clinical Pathology (ASCP) Medical Technologist, Amy Kelly, ASCP Medical Technologist, and Andy Phellen, ASCP Medical Technologist. The tubes of blood were allowed to settle for half an hour before being centrifuged. For each patient, one tube of blood was sent to Affiliated Laboratory, Bangor, Maine and one tube was taken to Dr Camire's laboratory in Holmes Hall, University of Maine. Total cholesterol, triglycerides, LDL, HDL, percent HDL, fasting glucose, and HbA_{1C}, levels were analyzed by Affiliated Laboratory, insulin levels were analyzed at Associated Regional and University Pathologists Inc. (ARUP), and fructosamine levels, aldose reductase activity, and hexanal concentration were analyzed by myself at the Holmes Hall laboratory. Single values per subject per blood draw were reported by Affiliated Laboratory.

Fasting Blood Glucose

Fasting blood glucose is a measure of how well the patient's blood glucose levels are being controlled at the present time. This analysis is accepted by the American Diabetes Association as a standard method to diagnose and to monitor treatment of DM. A decrease in blood glucose levels from baseline to week 12 for patients in the treatment group would indicate that cranberry supplementation had an effect on blood glucose levels. Analysis of fasting

glucose was done at Affiliated Laboratory in Bangor, Maine using the oxygen electrode method and a Beckman Coulter LX20 Pro clinical system (Fullerton, CA).

Glycosylated Hemoglobin

Glycosylated hemoglobin is a useful measure of the effectiveness of treatment to control blood glucose levels in the previous 3 months (36). A value less than 6% is considered normal. The treatment goal for a person with diabetes is to have an HbA_{1c} level less than 7% (1). Levels over 7% have been shown to increase incidence of side effects and complications of diabetes. This analysis was chosen because a change in HbA_{1c} levels between baseline and week 12 in the treatment group would indicate a decrease in protein glycosylation with cranberry supplementation. Blood analysis of HbA_{1c} levels was completed at Affiliated Laboratory in Bangor, Maine using the BioRad Variant II HPLC method.

Serum Insulin

Insulin is a hormone necessary for the utilization of carbohydrate. As insulin sensitivity decreases in patients with type 2 DM, the body produces more insulin in an attempt to control elevated blood glucose levels (2). Some oral medications used to treat diabetes increase pancreatic insulin secretion. Normal blood insulin levels are 5-35 mmol/L (30 – 210 pmol/L). Blood analysis of insulin levels were completed at ARUP (Salt Lake City, Utah)

using a chemiluminescent immunoassay. ARUP provided interpretive data with the results.

Cholesterol and Triglyceride Levels

Diabetes is an independent risk factor for CAD. Elevated total cholesterol, LDL cholesterol, and triglyceride levels as well as low levels of HDL cholesterol are additional risk factors for coronary artery disease. Patients with diabetes have higher total cholesterol, LDL cholesterol, and triglyceride levels and lower HDL levels that the general population (19). Due to the relationship between diabetes and CAD it is important to control cholesterol and triglyceride levels in patients with diabetes. It is especially important to control levels of LDL cholesterol because LDL can become glycated with elevated blood glucose levels, and these glycated LDL molecules are easily oxidized (19). Table 9 shows the 2001 National Heart, Lung and Blood Institute recommendations for optimal cholesterol levels.

Table 9: Recommendations for Cholesterol Levels

Cholesterol	Recommendation (mg/dL)	Recommendation (mmol/L)
Triglycerides	< 150	1.7
Total Cholesterol	<200	5.2
LDL	<100	2.6
HDL	40 to 60	1.0 to 1.6

(55)

Serum levels of total cholesterol, triglycerides and HDL were analyzed at Affiliated Laboratory utilizing a Beckman Coulter LX20 Pro clinical system and time endpoint methodologies. LDL and percent HDL were calculated using the results of the total cholesterol and HDL cholesterol (55). The equation used to calculate LDL is:

LDL (mg/dL) = total cholesterol – HDL cholesterol – TG / 5

Percent HDL is calculated by the following equation:

Percent HDL = HDL / total cholesterol * 100

When total cholesterol level was greater than 200 mg/dL (5.2 mmol/L), direct LDL was analyzed using the Beckman Coulter LX20 Pro clinical system and a liquid selective detergent method.

Fructosamine

Fructosamine levels are a measure of glycosylated albumin, and are an indicator of blood glucose control in the 2 to 3 weeks prior to the test (34). Normal values for fructosamine in patients who do not have diabetes are 1.6 to 2.6 mmol/L. The normal range for patients with diabetes is 2.1 to 5.0 mmol/L (35)

Fructosamine Kits (Procedure No.465) were purchased from Sigma Diagnostics (St. Louis, MO). A Spectronic 20D+ spectrometer from Spectronic Instruments (Rochester, NY) and a heat block (Fisher Scientific Company, Fair Lawn, NJ) were used to perform the analysis. Fisher brand micro cuvettes (14-385-938) were purchased from Fisher Scientific Company (Fair Lawn, NJ).

The SIGMA Fructosamine Kit (Procedure No.465) was used for analysis of serum fructosamine levels. To complete the analysis, the fructosamine reagent solution was first heated to 37°C. The spectrophotometer was set to a wavelength of 530 nm and the absorbance was zeroed using deionized water as a reference. The concentration of the fructosamine calibrator solution was recorded from the label on the bottle. Micro-cuvettes were numbered allowing for 1 cuvette for the calibrator and enough cuvettes to analyze each sample in duplicate. For the calibrator, 1.0 mL of reagent and 0.1 mL of fructosamine calibrator solution was pipetted into cuvette number 1. For each sample, 1.0 mL of reagent solution and 0.1 mL of the appropriate serum sample was pipetted into each of the other cuvettes. Each cuvette was then mixed by covering with para film and inverting gently. All cuvettes were incubated at 37°C for 10 minutes. After exactly 10 minutes the absorbance of each cuvette was recorded as initial A. Tube number 1 was recorded as Initial A^{cal}, and each of the other cuvettes was recorded as Initial A^{test}. All cuvettes were then incubated for exactly 5 more minutes (for a total of 15 minutes) and

the absorbance of each cuvette was recorded as Final A. Cuvette number 1 was recorded as Final A^{cal}, and the each of the other cuvettes was recorded as Final A^{test}. The following calculation was performed for each sample (35):

$$\text{Fructosamine (mMol/L)} = \frac{\text{Final } A^{test} - \text{Initial } A^{test}}{\text{Final } A^{cal} - \text{Initial } A^{cal}} \times \text{Concentration of Calibrator}$$

Aldose Reductase Activity

Sample Preparation

The method used to analyze aldose reductase activity was derived based on a method by Renner et al (56). To perform the deproteinization of the samples 100µL of serum, 100µL of 0.69 mM dulcitol (DO-0256 SIGMA Chemical Company, St. Louis, MO), and 1mL of absolute ethyl alcohol (200 proof, 64-17-5, Pharmaco Products Inc, Brookfield, CT) was added to a 1.5 mL centrifuge tube. The dulcitol was used as an internal standard. This mixture was vortexed and was then centrifuged using a Beckman Microfuge II centrifuge, at 400 XG for 10 minutes. The supernate was collected and the precipitate was discarded. The supernate was evaporated under nitrogen until dry. Each sample was then derivitized by adding 100µL of 0.49 butyl-boronic acid (16, 324-4 Aldrich Chemical Company, Milwaukee, WI) in ethanol to the dried sample. The mixture was then mixed in an ultrasonic bath for 10 minutes. An external standard using 6.86 mM sorbitol (S- 7457 SIGMA Chemical Company, St. Louis, MO) instead of serum was mixed and injected for each subject using the method described above.

Gas Chromatography

Gas chromatography was performed using a Hewlett Packard HP-6890 gas chromatograph (CG) with ChemStation (Agilent. Burlington, MA), using a Restek RTX1 Crossbond 100% dimethyl polysiloxane 30 m, 0.32mmID, 0.25 μm DF CG column (Restek Bellefonte, PA). The software used for GC analysis included ChemStation revision A.08.03 (Agilent, Burlington, MA) and Teklink 7000 version 2.00 (Tekmar-Dorhman, Cincinnati, OH). Two microliters of the prepared sample was injected directly into a Hewlett Packard HP-6890 CG. Inlet temperature was 250°C with a 100:1 split ratio and 9.52 psi and a total flow of 195 mL/minute. The initial oven temperature was 60°C. The temperature was increased 45°C per minute to 180°C and held for 1 minute. The temperature was then increased by 10°C per minute to 250°C and held for 3 minutes. Total run time was 14.67 minutes and equilibration time was 5 minutes. The retention time of the sorbitol was 13.032, and the retention time of the dulcitol was 13.336. Appendix E contains a sample chromatogram from the analysis of sorbitol.

Hexanal

Headspace analysis was performed using a Hewlett Packard HP-6890 CG with Chem station software (Agilent, Burlington, MA) and a Tekmar 7000 Headspace Auto sampler (Tekmar-Dohmann, Cincinnati, OH) using a Restek Stabilwax Crossbond Carbowax PEG 30 m, 0.32mmID, 1.0 μm DF CG column (Restek Bellefonte, PA). The software used for headspace analysis

included ChemStation revision A.08.03 (Agilent, Burlington, MA) and Teklink 7000 version 2.00 (Tekmar-Dorhman, Cincinnati, OH). Headspace vials, caps and teflon septa were purchased from Tekmar-Dorhman (Cincinnati, OH). Standards were prepared from hexanal (98% 11.560-6, Aldrich Chemical Co, Milwaukee, WI).

Headspace

Rapid headspace gas chromatography (GC) was used to measure hexanal, which is a product of lipid peroxidation. Elevated levels of hexanal indicate poor antioxidant status and a high level of lipid oxidation. Decreased levels of hexanal indicate increased antioxidant status. Hexanal could be used as a marker of compliance in this study because the cranberry powder has high antioxidant activity. Subjects who consistently take the cranberry capsules should have decreasing levels of hexanal during the study. The method used was based on work completed by Frankel et al. (57, 58). External standards were run each day the samples were analyzed. Five standards were analyzed to form a standard curve for analysis and consisted of a 1 mL sample of 50 μM, 25 μM, 12.5 μM, 6.25 μM and 0μM hexanal. For each sample of serum, 1 mL of serum was placed in a 22 mL headspace vial and sealed. Vials were stored in the refrigerator at 4°C until ready to be analyzed. Each sample was placed in the carrousel when the previous sample was loaded. Samples were held at a temperature of 50°C for 2 minutes. Platen temperature was 100° C, platen equilibrium time was 5 minutes, and sample equilibrium time was 10

minutes. The sample was mixed for 1 minute at a power of 1, and then allowed to stabilize for 1 minute. The vial pressurization time was 0.25 minutes, the pressure equilibrate time was 0.25 minutes, the loop fill time was 0.25 minutes and the loop equilibrate time was 0.20 minutes. The sample loop temperature was 65° C and the line temperature was 65° C. One injection was completed per sample and the GC cycle time was 15 minutes.

Gas Chromatography

Initial GC temperature was 50°C, and initial time was 2 minutes. The temperature was increased 5°C per minute to 65°C. Inlet temperature was 225°C with a split ratio of 100: 1 and a pressure of 11.9 psi. The run time was 15 minutes. Split flow was 259.6 mL/min and total flow was 265.4 mL per minute. Detector temperature was 180°C with a hydrogen flow of 40.0 mL per minute and an air flow of 450 mL per minute. The headspace above a 1 mL sample was analyzed for hexanal. SYSTAT 9 (version 9, Chicago, IL) statistical software was used to calculate a standard curve using external standards discussed previously. The following equation was used to calculate the amount of hexanal in each sample:

mmol of hexanal = (Area of sample * Area of Curve) + constant.

STATISTICS

All statistical analysis was completed using SYSTAT analytical software (version 9, Chicago IL). The general linear model and Fisher's Least Significant Difference test were used for analysis with gender, years since diagnosis of DM, initial blood glucose, and age group used as factors. Statistical analysis was not completed on information from the health history or food frequency questionnaires.

RESULTS

SUBJECT DEMOGRAPHICS

Twelve women and 15 men participated in the Cranberry Diabetes Study. There were 14 subjects in the cranberry group and 13 in the placebo group. Ages of the subjects ranged from 20 years to 80 years. The mean age of subjects in the study was 55 years. Subjects had been diagnosed with DM anywhere from 4 months to 408 months prior to participation in the study. Only ten of the twenty-seven subjects had initial fasting blood glucose levels greater than 7.0 mmol/L (range 4.4 to 13.2 mmol/L). A fasting blood glucose level less than 7.0 mmol/L indicates that blood glucose and DM were well controled at the beginning of the study.

Nine of the subjects were not taking any prescription medications during the study. Other subjects were taking antihyperlipidemic, antihypertensive, antianxiety, antidepressant and antiarthritic medications. Nineteen of the subjects exercised 2 or more times per week, one exercised once per week and seven exercised rarely or never. Tables 10 and 11 have complete subject demographic information.

Analysis of the food frequency questionnaires indicated that only three subjects consumed 3 servings of fruits and 3 servings of vegetables daily (Tables 12 and 13). Average intake of fruit was 1.6 ± 1.3 servings per day,

Table 10: Cranberry Group Health History Questionnaire Results

Subject	Gender	Age (years)	Initial Blood Glucose (mg/dL)	Months Since Diagnosis	Prescription Medications	Over the Counter drugs	Supplements	Exercise	Type of Exercise	Family History of DM
CR01	F	34	< 7.0	16 (0-2 years)	Antacid Antitussive ACE Inhibitor Thyroid Hormone Antihyperlipidemic Antiarthritic	N/A	N/A	Rarely	Walking	N/A
CR02	F	66	< 7.0	24 (0-2 years)	ACE Inhibitor Thyroid Hormone	Advil	Multi vitamin Vitamin C	2x week	Aerobics PT	Grandfather
CR04	M	49	>7.0	84 (>5 years)	N/A	N/A	Multi vitamin	3x week	Weights	Mother Grandfather
CR05	F	75	>7.0	67 (>5 years)	Antihyperlipidemic Antihypertensive	Aspirin	Multi vitamin	3x week	Walking Swimming Line dancing	N/A
CR07	M	46	< 7.0	72 (>5 years)	Antihypertensive (2)	N/A	N/A	3x week	Walking	N/A
CR09	M	69	< 7.0	408 (>5 years)	Diuretic Antihypertensive(2) ACE Inhibitor Anitarrhythmic Anticoagulant	N/A	Fish oil	3x week	Walking, Aerobics Swimming	Grandmother
CR10	M	49	< 7.0	65 (>years 5)	Antidepressant Antihypertensive	Aspirin	N/A	5x week	Bicycling Weights	Father
CR11	M	80	>7.0	24 (0-2 years)	Antihyperlipidemic Antihypertensive	Baby aspirin	Vitamin A Vitamin C Vitamin E	N/A	N/A	N/A
CR13	M	54	< 7.0	24 (0-2 years)	N/A	N/A	N/A	Rarely	N/A	Grandmother
CR15	M	57	>7.0	18 (0-2 years)	N/A	N/A	N/A	Daily	Walking	Siblings
CR16	F	49	>7.0	134 (>5 years)	N/A	N/A	N/A	Daily	Yoga Walking	N/A
CR17	F	49	< 7.0	10m (0-2 years)	Antihypertensive Antidepressant	N/A	Calcium	2x week	Swimming Walking,Yoga	N/A
CR23	F	56	>7.0	36 (2-5 years)	Thyroid Hormone ACE Inhibitor	Aspirin	Vitamin E Vitamin D	1x week	Gym	Mother
CR26	M	59	< 7.0	36 (2-5 years)	Antihyperlipidemic	Aspirin	Multi vitamin, B6 folic acid B12	Daily	Walking	N/A

N/A = Not applicable

Table 11: Placebo Group Health History Questionnaire Results

Subject	Gender	Age (years)	Initial Blood Glucose (mmol/L)	Months Since Diagnosis	Prescription Medications	Over the Counter Drugs	Supplements	Exercise	Type of Exercise	Family History of DM
CR03	M	64	< 7.0	18 (0-2 years)	Antacid	N/A	Multi vitamin Gincoba Calcium	5x week	Walking	Grandfather Brother
CR06	M	54	>7.0	48 (2-5 years)	N/A	N/A	N/A	Rarely	Walking	Mother
CR08	M	34	< 7.0	210 (>5 years)	N/A	N/A	ThermaChrom	2x week	Walking	Grandfather
CR12	M	42	>7.0	46 (2-5 years)	N/A	N/A	N/A	Daily	Train horses	N/A
CR14	F	67	>7.0	60 (2-5 years)	Anti-asthma	aspirin	Glucosamine Calcium Vitamin E	N/A	N/A	Siblings
CR18	F	57	< 7.0	18 (0-2 years)	Antiulcer	Tylenol Ibuprofen	Multi vitamin	3-4x week	Walking	Mother Grandmother
CR19	F	58	< 7.0	36 (2-5 years)	Anti-osteoporosis	Baby aspirin	Multi vitamin Calcium Vitamin E	3-4x week	Walking Nordic track	N/A
CR20	F	58	< 7.0	28 (2-5 years)	Thyroid Hormone Potassium chloride Antihypertensive (2)	N/A	Multi vitamin	2x week	Bicycling	N/A
CR21	M	62	< 7.0	18 (0-2 years)	N/A	N/A	N/A	Daily	Cardio Weights Golf 2x	N/A
CR22	F	20	< 7.0	12 (0-2 years)	N/A	N/A	Multi vitamin Calcium	3x week	Walking Rowing Bicycling	Mother
CR24	M	44	< 7.0	4 (0-2 years)	Antihyperlipidemic Antidepressant Anticonvulsant ACE Inhibitor	N/A	Multi vitamin	Rarely	Walking Nordic track Weights	Father Siblings
CR25	M	56	>7.0	132 (>5 years)	Antidepressant Antianxiety ACE Inhibitor Antihyperlipidemic Antihypertensive	Aspirin Benadryl	N/A	3-4x week	Walking	Mother
CR27	F	67	< 7.0	16 (0-2 years)	Antiarthritic	N/A	Omega 3 fatty acids Calcium	3x week	Walking	N/A

N/A = Not applicable

Table 12: Cranberry Group Food Frequency Questionnaires

Subject	Fruit		Vege		ETOH	CJ	Red Fruit				Total Fruit				Red Vege				Total Vege			
	D	W	D	W	Year		D	W	M	Y	D	W	M	Y	D	W	M	Y	D	W	M	Y
CR01	2	10	1	8	--	--	--	--	--	40	--	14	4	65	--	--	--	6	--	6	6	98
CR02	3	21	3	21	--	24	--	10	9	15	2	20	26	36	--	2	--	2	5	21	4	2
CR04	2	14	2	14	--	12	--	2	3	4	2	3	6	8	--	--	1	--	--	5	8	2
CR05	1	8	3	21	--	3	--	2	5	26	2	11	14	67	--	--	1	--	--	20	19	6
CR07	2	14	1	7	--	52	--	2	2	7	1	3	5	15	--	--	1	1	--	8	4	6
CR09	--	--	--	--	--	1	1	2	4	2	2	3	3	8	--	--	1	--	3	4	6	4
CR10	--	--	--	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	7	--	--
CR11	No Data																					
CR13	1	6	3	21	--	365	1	2	--	--	7	5	1	--								
CR15	2	10	1	6	--	1	2	1	1	4	2	1	2	5	--	--	--	--	2	8	3	--
CR16	--	3	--	3	156	2	--	--	3	37	--	3	11	66	--	--	1	--	2	5	7	1
CR17	2	10	2	10	--	10	--	9	5	22	--	25	13	22	--	--	--	2	1	15	9	20
CR23	2	--	2	2	--	--	--	--	1	6	1	6	1	12	--	3	--	--	1	10	5	--
CR26	--	3	3	--	104	2	--	--	1	30	--	2	2	95	--	--	12	--	--	10	12	10
Average	1	8	2	9	20	43	0	2	3	15	1	7	7	31	0	0	1	1	1	10	7	20

* Servings / time period
D = Day
Vege = Vegetables,
W = Week
CJ = Cranberry Juice
M = Month
ETOH = Alcoholic beverages
Y = Year

Table 13: Placebo Group Food Frequency Questionnaires*

Subject	Fruit		Vege		ETOH	CJ	Red Fruit				Total Fruit				Red Vege				Total Vege			
	D	W	D	W			D	W	M	Y	D	W	M	Y	D	W	M	Y	D	W	M	Y
CR03	4	28	5	35	--	--	--	--	--	--	4	3	-	-	--	1	--	--	--	14	--	--
CR06	--	1	1	5	3	24	--	--	4	20	1	--	5	24	--	--	--	--	--	3	4	2
CR08	2	5	2	5	--	2	2	3	23	3	2	9	6	4	--	--	--	--	2	1	3	
CR12	5		2		--	--	--	2	--	--	3	17	3	--	--	--	2	--	--	24	4	--
CR14	1	5	2		--	--	--	4	2	5	--	10	8	6	--	1	--	--	--	7	3	3
CR18	2	20	2	16	53	365	1	--	39	--	1	2	197	--	--	--	2	--	--	13	104	--
CR19	3	15	3	15	28	12	--	--	3	13	--	10	14	23	--	--	--	2	--	15	7	20
CR20	--	2	20	100	--	--	--	--	1	29	--	1	3	45	--	--	--	--	4	22	4	--
CR21	--	--	2	--	--	--	--	1	1	--	1	1	1		--	--	--	--	2	1	--	--
CR22	2	14	2	14	149	--	-	--	4	20	--	9	10	45	--	--	--	--	--	9	7	15
CR24	2	10	1	5	30	30	--	--	2	81	--	--	2	845	--	--	--	2	--	1	10	22
CR25	2	10	2	10	--	--	--	--	2	--	1	6	2	--	--	--	--	--	--	7	--	--
CR27	--	--	3	--	42	--	--	--	--	32	--	--	2	37	--	--	--	--	1	25	8	--
Average	2	8	4	16	23	33	0	1	6	16	1	5	19	78	0	0	0	0	1	11	12	5

* Servings / time period
D = Day
Vege = Vegetables
W = Week
CJ = Cranberry Juice
M = Month
ETOH = Alcoholic beverages
Y = Year

and average vegetable intake was 2.8 ± 3.7 servings per day. Seventeen of the subjects consumed cranberry juice at least once per year (range 0 to 365 servings per year). The average consumption of cranberry juice was 35 ± 98 servings per year. Ten of the subjects drank alcohol (range 0 to 156 servings per year).

Cranberry Group

The cranberry group consisted of six females and eight males (Table 10). Ages ranged from 34 to 80 years. The mean age of subjects in the cranberry group was 57 years. Eight of the subjects in the cranberry group were over the age of 50. Six of the subjects had initial blood glucose levels greater than 7.0 mmol/L, and eight had normal blood glucose levels. Six of the subjects had been diagnosed with DM less than 2 years before the study (range 10 to 24 months). Two subjects had been diagnosed with DM 2 to 5 years prior to participation in the study (both 36 months). The remaining six subjects in the cranberry group had been diagnosed with diabetes more than 5 years prior to participating in the study (range 65 to 134 months). The mean number of months since diagnosis for the cranberry group was 73 ± 102 months. Four of the subjects in the cranberry group exercised less than once per week, ten exercised more than twice per week, and four of the ten who exercised more than twice per week exercised 5 or more days per week.

Four of the subjects in the cranberry group were not taking any prescription medications during the study. Eight subjects were taking a diuretic, an angiotensin converting enzyme (ACE) inhibitor, or another type of antihypertensive drug. Four of the subjects were on cholesterol lowering drugs. Six of the subjects in the cranberry group took Advil or aspirin regularly. No other over the counter drugs were taken regularly by subjects in the cranberry group. Eight subjects in the cranberry group took some form of dietary supplement regularly. Participants were asked to refrain from using antioxidant supplements for 2 weeks prior to and during the study. Four subjects were taking a multivitamin daily, two were taking vitamin C, and two were taking vitamin E. Fish oil, vitamin A, calcium, and folic acid were taken by one or more subjects.

The food frequency questionnaires indicated that the majority of patients in the study did not eat adequate amounts of fruits and vegetables (Table 12). Only one subject in the cranberry group consumed at least 3 servings of fruit daily and only four subjects consumed 3 or more servings of vegetables daily. Only one subject in the cranberry group consumed 3 servings of fruits and 3 servings of vegetables per day.

Placebo Group

There were six females and seven males in the placebo group (Table11). Subjects ranged from age 20 to 67. The mean age of subjects in the placebo

group was 50 ± 58 years. Nine of the subjects in the placebo group were over the age of 50. Four of the subjects had initial blood glucose levels greater than 7.0 mmol/L. The remaining nine subjects had normal blood glucose levels initially. Six of the subjects had been diagnosed with DM less that 2 years prior to participation in the study (range 4 to 18 months), five had been diagnosed 2 to 5 years prior (range 28 to 60 months), and two subjects had been diagnosed more than 5 years prior to the study (range 132 to 210 months). The mean number of months since diagnosis was 50 months. Three of the subjects in the placebo group exercised less than twice per week, and nine of the subjects exercised more than twice per week, with three of the nine exercising 5 or more days per week.

Five of the subjects in the placebo group were not taking prescription medication during the study. Only three subjects in the placebo group were taking antihypertensive medications and only two were on cholesterol lowering drugs. Four of the subjects in the placebo group took Tylenol or aspirin regularly, and one of these subjects took Benadryl regularly. No other over-the-counter drugs were taken regularly by subjects in the placebo group. Nine of the subjects in the placebo group were taking one or more dietary supplements. Prior to the study, six subjects were taking a multivitamin, four were taking calcium, two were taking vitamin E, and others were taking gincoba, chromium, glucosamine, or fish oil supplements. During the study these subjects were required to refrain from taking vitamin E and chromium.

Results from the food frequency questionnaires (Table 13) indicated that three subjects in the placebo group consumed 3 or more servings of fruit daily and four subjects consumed 3 or more servings of vegetables per day. Two of these subjects consumed 3 servings of fruit and 3 servings of vegetables per day.

BLOOD ANALYSES

Fasting Blood Glucose

There were no statistically significant differences in blood glucose levels between the cranberry group and the placebo group when subjects were grouped simply by treatment or gender. When subjects were grouped by initial blood glucose levels, differences were seen between subjects in the cranberry group with initial blood glucose levels greater than 7.0 mmol/L compared to patients in the placebo group with initial blood glucose levels less than 7.0 mmol/L. There were no significant differences when comparing the cranberry group with initial blood glucose levels less than 7.0 mmol/L to the placebo group with initial blood glucose levels less than 7.0 mmol/L, or when comparing the cranberry group with initial blood glucose levels greater than 7.0 mmol/L to the placebo group with initial blood glucose levels greater than 7.0 mmol/L.

When subjects were grouped by years since diagnosis of DM, there was not a significant decrease in blood glucose levels from week 0 to week 12 (Table

14), but blood glucose levels were significantly lower (p = 0.036) in the cranberry group than the placebo group for patients who had been diagnosed with DM more than 5 years prior to participating in the study. There were no significant differences in blood glucose levels in patients who had been diagnosed with DM less than 2 years, or 2 to 5 years prior to participation in the study.

Table 14: Mean Fasting Glucose Levels by Years since Diagnosis[a]

Treatment	Years Since Diagnosis	N	Time (Weeks)		
			0	6	12
Cranberry	0 - 2	6	6.6 ± 1.9	6.6± 1.7	6.3 ± 0.9
Placebo	0 - 2	2	6.1 ± 0.8	7.1 ± 1.9	6.4 ± 0.7
Cranberry	2 - 5	6	6.9 ± 0.4	7.3 ± 0.7	6.6 ± 0.8
Placebo	2 - 5	6	7.5 ± 3.5	7.4 ± 2.9	6.8 ± 2.4
Cranberry	>5	5	7.1 ± 1.7	6.5 ± 1.4	6.6 ± 1.2*
Placebo	>5	2	6.6 ± 1.1	7.4 ± 0.4	8.1 ± 2.0*

[a] mmol/L, 1 reading per person per time
* = Blood glucose significantly lower (p = 0.036) in cranberry group than placebo group.

In patients less than 50 years of age, serum glucose levels decreased significantly (p = 0.030) in the cranberry group compared to the placebo group at week 6 (Table 15). This was not the case at week 12. Serum glucose

levels decreased 7% in the cranberry group and increased 33% in the placebo group at week 6 in subjects less than 50 years of age.

In patients older than 50 years, there were no significant differences between the cranberry group and the placebo group either at week 6 or at week 12. Mean serum glucose level decreased 0.5 mmol/L between week 0 and week 12 in the cranberry group and 0.3 mmol/L in the placebo group.

Table 15: Mean Serum Glucose Levels by Age Group[a]

Treatment	Age Group	N	Time (weeks)		
			0	6	12
Cranberry	<50	6	7.0 ± 1.8	6.5 ± 1.5* **	6.9 ± 1.1
Placebo	<50	4	7.6 ± 3.8	9.1 ± 2.8* **	8 .3± 1.8
Cranberry	>50	8	6.8 ± 1.7	6.7 ± 1.5	6.3 ± 1.5
Placebo	>50	9	6.4 ± 1.2	6.4 ± 1.2	6.1 ± 1.2

[a] mmol/L, 1 reading per person per time
* Significant difference (p = 0.030) in blood glucose levels from week 0 to week 6

Glycosylated Hemoglobin

There were no significant differences in HbA_{1C} levels when subjects were grouped by treatment, gender, age group, or initial blood glucose levels. Mean HbA_{1C} decreased slightly in women in both the cranberry group and the placebo group from week 0 to week 6 (Table 16). Both groups increased from

week 6 to week 12. In males, mean HbA_{1C} decreased in the cranberry group between week 0 and week 6 and returned to the week 0 value at week 12. In the placebo group, the mean was the same at week 6 as at week 0, but increased from week 6 to week 12. From week 0 to week 12 there was a difference bordering on significance ($p = 0.060$) for males between the cranberry group and the placebo group.

Table 16: Mean HbA_{1C} Levels by Gender[a]

Treatment	Gender	N	Time (weeks)		
			0	6	12
Cranberry	F	6	6.2% ± 1.0	6.1% ± 1.0	6.4% ± 1.0
Placebo	F	6	6.5% ± 1.5	6.3% ± 1.3	6.5% ± 1.1
Cranberry	M	8	6.5% ± 1.5	6.3% ± 1.3	6.5% ± 1.1*
Placebo	M	7	7.0% ± 1.4	7.0% ± 1.2	7.4% ± 1.5*

[a] 1 reading per person per time
No significant differences between cranberry and placebo when subjects grouped by gender.
* Difference bordering on significance ($p = 0.060$) for males at week 12 between treatments.

When subjects were grouped by years since diagnosis (Table 17), HbA_{1C} levels decreased in the cranberry group from week 0 to week 6 (-0.3), and increased in the placebo group (+0.2) in subjects who had been diagnosed with DM more than 5 years prior to the study. HbA_{1C} levels decreased significantly ($p = 0.031$) in the cranberry group compared to the placebo group at week 6 in subjects diagnosed with DM more than 5 years before the study.

Both the cranberry group and the placebo group either had returned to week 0 values or had a slight increase in mean HbA_{1C} levels at week 12 regardless of time since diagnosis.

Table 17: Mean HbA_{1C} Levels by Years since Diagnosis[a]

Treatment	Years Since Diagnosis	N	Time (Weeks)		
			0	6	12
Cranberry	0 - 2	6	6.2% ± 0.8	6.1% ± 0.8	6.2% ± 0.6
Placebo	0 - 2	2	5.9% ± 0.3	6.0% ± 0.2	6.2% ± 0.3
Cranberry	2 - 5	6	6.3% ± 0.7	6.5% ± 0.9	6.7% ± 0.8
Placebo	2 - 5	6	6.9% ± 1.9	6.8% ± 1.7	7.1% ± 1.8
Cranberry	>5	5	6.6% ± 1.8	6.3% ± 1.4*	6.6% ± 1.2
Placebo	>5	2	7.2% ± 0.2	7.4% ± 0.6*	7.8% ± 1.3

[a] 1 reading per person per time
* Significant decrease ($p = 0.031$) in HbA_{1C} levels in cranberry group compared to placebo group.

Fructosamine Levels

There were no statistically significant differences in fructosamine levels from week 0 to week 12 between the cranberry group and the placebo group when data was analyzed for treatment effects, gender effects, time since diagnosis, or initial blood glucose levels, but subject age was influential.

Fructosamine levels improved significantly (p = 0.027) in the cranberry group compared to the placebo group in patients less than the age of 50 from week 0 to week 6, but increased in the cranberry group from week 6 to week 12 (Table 18). There was not a statistically significant difference in fructosamine levels from week 0 to week 12 in either the group under the age of 50 or the group over the age of 50.

Table 18: Mean Fructosamine Levels by Age Group[a]

Treatment	Age Group	N	Time (weeks)		
			0	6	12
Cranberry	<50	6	2.06 ± 0.81	2.05 ± 0.36*	1.96 ± 0.73
Placebo	<50	4	1.43 ± 0.25	2.41 ± 0.47*	1.82 ± 0.65
Cranberry	>50	8	2.09 ± 0.37	1.90 ± 0.36	2.13 ± 0.31
Placebo	>50	9	1.99 ± 0.29	1.90 ± 0.41	2.07 ± 0.29

[a] mmol/L, 2 readings per person per time
* Significantly (p = 0.027) lower in cranberry group compared to placebo group at week 6. No significant differences from week 0 to week 12.

Aldose Reductase Activity

GC analysis of serum sorbitol measured sorbitol in only 6 serum samples, and all were in the week 0 blood draw. Each sample was measured using 2 duplicates and 2 replicates of each duplicate. Results were not consistent since none of the results indicated that there was sorbitol in both duplicates and both replicates. Table 19 has detail of results of sorbitol analysis.

Table 19: Aldose Reductase Activity Results[a]

SUB	TRT	Sorbitol											
		Draw 1				Draw 2				Draw 3			
		D1		D2		D1		D2		D1		D2	
		R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
CR04	Cranberry	X	–	X	–	–	–	–	–	–	–	–	–
CR05	Cranberry	X	X	–	–	–	–	–	–	–	–	–	–
CR06	Placebo	X	–	–	–	–	–	–	–	–	–	–	–
CR08	Placebo	–	–	X	–	–	–	–	–	–	–	–	–
CR18	Placebo	X	–	–	–	–	–	–	–	–	–	–	–
CR21	Placebo	X	–	–	–	–	–	–	–	–	–	–	–

[a] X Indicates detectable sorbitol TRT Indicates Treatment SUB Indicates Subject
D Indicates Duplicate R Indicates Replicate
– Indicates no sorbitol detected

Serum Insulin

There were no statistically significant differences in serum insulin levels between the cranberry group and the placebo group when subjects were grouped by treatment, gender, initial blood glucose level, or age group. There were statistically significant differences in serum insulin levels when patients were grouped by time since diagnosis of DM.

When subjects were grouped by time since diagnosis of DM, there was an overall statistically significant difference ($p = 0.015$) in serum insulin levels between the treatment group and the placebo group at week 12 (Table 20). At

week 12, serum insulin levels in patients in the placebo group with diabetes more than 5 years were significantly higher than in any other group. In the group that had DM greater than 5 years, serum insulin levels decreased significantly ($p = 0.002$) in the cranberry group compared to the placebo group.

Table 20: Mean Insulin Levels by Years since Diagnosis[a]

Treatment	Years Since Diagnosis	N	Time (Weeks)		
			0	6	12
Cranberry	0 - 2	6	97 ± 40	104 ± 63	96 ± 53
Placebo	0 - 2	2	165 ± 129	270 ± 270	120 ± 65
Cranberry	2 - 5	6	72 ± 0	81 ± 55	63 ± 38
Placebo	2 - 5	6	114 ± 96	110 ± 91	73 ± 37
Cranberry	>5	5	61 ± 33	54 ± 19	51 ± 18*
Placebo	>5	2	120 ± 25	153 ± 21	324 ± 339* **

[a] pmol/L, 1 reading per person per time
Overall significant decrease ($p = 0.015$) in cranberry group compared to placebo group.
* Cranberry group decreased significantly ($p = 0.002$) compared to placebo group
** Significantly higher than any other group.

Lipid Levels

There were no statistically significant differences in serum triglycerides, total cholesterol, LDL, HDL or percent HDL between the cranberry group and the placebo group when patients were grouped by treatment, gender, initial blood

glucose, or age group. There were significant differences in lipid levels between the cranberry group and the placebo group when subjects were grouped by time since diagnosis. Table 21 lists mean lipid levels by treatment.

Table 21: Mean Lipid Levels[a]

Treatment	Lipid	N	Time (weeks)		
			0	6	12
Cranberry	Triglycerides	14	1 ± 1	2 ± 1	1 ± 1
Placebo	Triglycerides	13	2 ± 1	2 ± 1	2 ± 1
Cranberry	Cholesterol	14	5 ± 1	5 ± 1	5 ± 1
Placebo	Cholesterol	13	5 ± 1	5 ± 1	5 ± 1
Cranberry	LDL	14	4 ± 1	3 ± 1	3 ± 1
Placebo	LDL	13	3 ± 1	3± 1	3 ± 1
Cranberry	HDL	14	1 ± 0	1 ± 0	1 ± 0
Placebo	HDL	13	1 ± 1	1 ± 0	1 ± 0

[a] mmol/L, 1 reading per person per time
No significant differences between cranberry and placebo groups.

Triglycerides

Triglycerides were significantly lower ($p = 0.011$) in the cranberry group than the placebo when subjects were grouped by years since diagnosis of DM (Table 22). Triglyceride levels were highest in the placebo group with DM

more than 5 years. Among patients with DM more than 5 years, triglyceride levels decreased significantly (p = 0.002) from week 0 to week 12 in the cranberry group compared to the placebo group. Triglyceride levels decreased 16% in the cranberry group and increased 152% in the placebo group. In the group with DM more than 5 years, triglyceride values decreased significantly (p = 0.013) in the cranberry group compared to the placebo group.

Table 22: Mean Triglyceride Levels by Years since Diagnosis[a]

Treatment	Years Since Diagnosis	N	Time (Weeks)		
			0	6	12
Cranberry	0 - 2	6	1 ± 1	2 ± 1	1 ± 1
Placebo	0 - 2	2	2 ± 1	2 ± 2	2 ± 1
Cranberry	2 - 5	6	1 ± 1	1 ± 1	1± 1
Placebo	2 - 5	6	2 ± 1	1 ± 1	1 ± 1
Cranberry	>5	5	2 ± 1	2 ± 2	1± 1*
Placebo	>5	2	1. ± 0	2 ± 0	3 ± 0* **

[a] mmol/L, 1 reading per person per time
Overall cranberry group decreased significantly (p = 0.011) compared to the placebo group from week 0 to week 12.
* Decreased significantly (p = 0.002) in the cranberry group compared to the placebo group.
** Significantly higher than any other group.

Total Cholesterol

Total cholesterol levels decreased significantly (p = 0.034) from week 0 to week 12 in the cranberry group compared to the placebo group when subjects were grouped by years since diagnosis of DM (Table 23). Subjects in the placebo group that had been diagnosed with DM over 5 years ago had significantly higher cholesterol levels than any other group at week 12. In subjects with DM over 5 years, cholesterol levels improved significantly (p = 0.007) in the cranberry group compared to the placebo group.

Table 23: Mean Total Cholesterol Levels by Years since Diagnosis[a]

Treatment	Years Since Diagnosis	N	Time (Weeks)		
			0	6	12
Cranberry	0 - 2	6	5 ± 1	5 ± 1	5 ± 1
Placebo	0 - 2	2	5 ± 1	5 ± 1	5 ± 1
Cranberry	2 - 5	6	5 ± 1	5 ± 1	5 ± 1
Placebo	2 - 5	6	6 ± 1	6 ± 1	6 ± 1
Cranberry	>5	5	6 ± 1	5 ± 1	5 ± 1*
Placebo	>5	2	4 ± 1	5 ± 1	6 ± 0* **

[a] mmol/L, 1 reading per person per time
Overall cranberry group significantly (p = 0.034) lower than placebo group.
* Decreased significantly (p = 0.007) in the cranberry group compared to the placebo group.
** Significantly higher than any other group.

Percent HDL Cholesterol

From week 0 to week 12, percent HDL improved significantly ($p = 0.021$) in the cranberry group compared to the placebo group in subjects who had DM more than 5 years (Table 24). There were no statistically significant differences in percent HDL between the cranberry group and the placebo group in patients that had been diagnosed with DM 0 to 2 or 2 to 5 years prior to the study.

Table 24: Mean Percent HDL Levels by Years since Diagnosis[a]

Treatment	Years Since Diagnosis	N	Time (Weeks)		
			0	6	12
Cranberry	0 - 2	6	21.0 ± 5.8	21.1 ± 5.4	22.8 ± 8.1
Placebo	0 - 2	2	23.2 ±10.2	23.4 ± 8.1	23.3 ± 8.3
Cranberry	2 - 5	6	23.6 ± 8.9	26.4 ± 12.1	24.1 ± 10.2
Placebo	2 - 5	6	24.3 ± 9.1	26.3 ± 8.4	25.8 ± 9.4
Cranberry	>5	5	18.8 ± 4.6	24.3 ± 7.7	22.4 ± 6.3*
Placebo	>5	2	24.9 ± 6.9	23.9 ± 9.8	16.8 ± 0.6*

[a] 1 reading per person per time
* Increased significantly ($p = 0.021$) in the cranberry group compared to the placebo group.

Hexanal

The results of analysis of serum hexanal were not sufficient to draw any conclusions. Six subjects in the cranberry group and 2 subjects in the placebo

group had measurable serum hexanal levels at one of the 3 blood draws. None of the subjects had measurable levels of serum hexanal at more than one of the blood draws. Table 25 contains the results of the hexanal analysis.

Table 25: Hexanal Results[a]

Subject	Draw	Treatment	Hexanal (mmol)
CR01	1	Cranberry	1.512
CR02	1	Cranberry	1.720
CR13	2	Cranberry	4.766
CR15	2	Cranberry	4.830
CR16	2	Cranberry	4.797
CR5	3	Cranberry	4.183
CR25	2	Placebo	-0.032
CR21	3	Placebo	2.810

[a] Data insufficient to draw conclusions

DISCUSSION

HUMAN SUBJECTS

Recruiting, Scheduling, and Compliance

The most challenging aspect of this study was working with human subjects. Recruiting, scheduling, and compliance were all important issues in carrying out this investigation. Many of the people who replied to the recruitment ads were not eligible for the study due to medical complications or use of hypoglycemic medications. A large hospital in the area was recruiting for a similar study at the same time, so it was difficult to find eligible subjects. The fact that blood could be drawn only at Cutler Health Center on the University of Maine campus also affected recruiting. Several participants had to travel from rural areas of Maine to participate in the study, while others decided not to participate due to the need to travel. In the end, the number of subjects was three short of the goal of thirty subjects.

Scheduling was also challenging for several reasons. It was necessary to schedule blood draws on days that were convenient for the staff at the phlebotomy lab. Whenever possible several subjects were scheduled to have blood drawn on the same day, so it was necessary to schedule extra staff at the phlebotomy lab. Most of the subjects were employed, so it was necessary to schedule blood draws around their work schedules. The subjects also needed to fast for 12 hours prior to the blood draw, so blood draws were scheduled in the mornings.

Compliance may have been an issue in this study. Subjects were called or emailed periodically to remind them to take the capsules, but there was no method in place to ensure that subjects took the capsules. Hexanal could have been used as a marker of compliance due to its high antioxidant activity; however, the results were not sufficient to draw conclusions. Unused capsules were not returned at the conclusion of the study, which would have been one measure of subject compliance. Several of the subjects had improvements in laboratory values at the week 6 blood draw, but did not sustain the improvements at week 12. This could be due to decreased compliance during the last 6 weeks of the study, or due to other unknown factors.

Subject Demographics

Differences between the subjects on the cranberry treatment and the subjects on the placebo may have affected the results of this study. Subjects were randomly assigned to either the cranberry group or the placebo group, and the demographics of each group were very different. The results might have been different if subjects had been matched between the cranberry group and the placebo group instead of randomly assigned to a group. If the two groups had been more similar, it is likely we would have seen more significant results.

Patients in the placebo group were likely healthier overall than patients in the cranberry group. Overall the patients in the placebo group were younger and had been diagnosed with DM more recently than subjects in the cranberry group. The mean age of the placebo group was 4 years younger than the cranberry group, but the number of subjects over the age of 50 was comparable in both groups (8 cranberry, 9 placebo). The mean number of months since diagnosis was 23 months less in the placebo group than in the cranberry group. There were six subjects in each group that had been diagnosed with DM less than 2 years prior to participation in the study, but the cranberry group had five subjects who had DM for more than 5 years, compared to only two in the placebo group. Since DM is a progressive disease, it is likely that patients who have had DM longer will have more complications from the disease, and they may be less healthy. The subjects who have had DM for a shorter time are likely to have lower values on laboratory analyses than subjects who have had the disease longer, and lab values are more likely to be stable throughout the study.

There were fewer subjects in the placebo group with elevated blood glucose levels initially than in the cranberry group. Six subjects in the cranberry group had elevated blood glucose levels at week 0 compared to four in the placebo group. It is likely that cranberry supplementation will have more of an effect for patients with elevated blood glucose levels, but because a larger number

of subjects in the placebo group had normal blood glucose levels, statistical significance may be difficult to see.

There were fewer subjects in the placebo group taking prescription medications. Eight subjects in the cranberry group were being treated for high blood pressure compared to three in the placebo group. Four subjects in the cranberry group were taking medication for high cholesterol compared to two in the placebo group. This may be due to the increase risk of macrovascular complications associated with DM. The higher number of subjects with hypertension and hyperlipidemia in the cranberry group may have been related to the number of subjects who had DM for a longer period.

Intake of fruits and vegetables was poor in both groups. One subject in the cranberry group and two in the placebo group consumed 3 servings of fruits and 3 servings of vegetables per day. One subject in the cranberry group compared to three in the placebo group consumed 3 servings of fruit per day, and four subjects in each group consumed 3 servings of vegetables per day. Serving sizes on the food frequency questionnaire were larger than those used in diabetic carbohydrate counting and the Food Guide Pyramid, so use of a different measure may have resulted in improved fruit and vegetable intakes.

Three subjects in the cranberry group consumed cranberry juice occasionally, compared to seven in the placebo group. There was little consumption of red colored fruits and vegetables in either group. The red fruits and vegetables have anthocyanin and flavonoid contents similar to cranberries. Subjects that normally have a high intake of cranberry juice and other fruits and vegetables that contain anthocyanins and flavonoids may have seen less benefit in this study due to the fact that they were already consuming a beneficial diet.

A very diverse group of subjects participated in this study. The wide range of initial blood glucose values and levels of control of the disease may have affected the ability to see statistical significance when analyzing data. If all subjects had elevated blood glucose levels at the beginning of the study, the effect of cranberry supplementation may have been more significant.

INDICATORS OF CONTROL OF DIABETES MELLITUS

Aldose Reductase Activity

The method used to analyze sorbitol in serum samples as an indicator of aldose reductase activity was not sufficiently sensitive. It is likely that more subjects had sorbitol present in their serum than was measured. All samples that had measurable sorbitol were in the week 0 blood draw, and results were not consistent between duplicates and replicates. It is not likely that sorbitol decreased in the placebo group from week 0 to week 12, especially in subjects whose blood glucose levels and HbA_{1C} levels increased. Some

chromatograms showed small peaks at the sorbitol retention time, but the peaks were too small to measure. Use of a different method or refinement of the current method would have resulted in more precise measurement of sorbitol in the serum samples. High concentrations of sorbitol are not normally present in the serum, so it is not surprising that the results were insufficient. If red blood cells had been used to analyze sorbitol instead of serum, the results would likely have been more significant.

Glucose, HbA1C, Fructosamine, and Insulin Levels

In most of the analyses performed as indicators of DM control, significant differences were seen only when data was grouped by length of time since diagnosis of DM and only in the group that had the disease the longest. More pronounced results are likely to be seen with cranberry supplementation in individuals who have had the disease the longest because they may have the higher glucose levels that result in more damage from hyperglycemia. The higher glucose levels would lead to protein glycosylation.

Grouped by Treatment and Gender

There were no significant differences in blood glucose, HbA_{1C}, fructosamine, or insulin levels when subjects were grouped simply by treatment or when they were grouped by gender. This is likely due to the fact that many of the

subjects already had good control of their DM at the beginning of the study. Seventeen subjects (63%) had normal blood glucose levels initially. When glucose levels are in the normal range, it is less likely that an improvement will be seen. If the initial screening process had excluded subjects with normal fasting blood glucose levels, it is likely that cranberry supplementation would have shown more benefit overall.

As discussed earlier, protein glycoslylation occurs in relation to the level of hyperglycemia, so subjects with good control of DM have less glycosylation of protein. This would affect results of the HbA_{1C} and fructosamine analyses. Only two subjects in the cranberry group and three in the placebo group had HbA_{1C} levels greater than 7% initially. One in each group had HbA_{1C} levels greater than 9%. With subjects with well controlled DM, there is little opportunity for improvement in laboratory values.

DM may affect insulin levels in two ways. As discussed earlier, type 2 DM may be due to decreased pancreatic production of insulin, decreased insulin sensitivity in the cells of the body, or a combination of both problems. In subjects with decreased insulin production, serum insulin levels decline as the disease progresses. In subjects with decreased insulin sensitivity, insulin levels increase as the disease progresses. Most subjects in this study had normal insulin levels throughout the study. Only two subjects had elevated insulin levels at week 0, four had elevated insulin levels at week 6, and only

one subject had elevated insulin levels at week 12. The high number of subjects with normal insulin levels suggests that there would be little improvement in insulin levels especially when subjects are grouped by treatment or by treatment and gender.

The diversity of the subjects is also a factor when analyzing data. The age range of subjects and the variability of control of glucose levels decreased likelihood of seeing significant differences when subjects were grouped simply by treatment or by treatment and gender.

Grouped by Years since Diagnosis

Most statistical significance in data analysis was seen when subjects were grouped by time since diagnosis. Blood glucose levels and insulin levels were significantly higher at week 12 in subjects in the placebo group who had DM more than 5 years. This is to be expected because DM is a progressive disease. The longer a person has DM, the higher the degree of insulin insensitivity. As insulin sensitivity decreases, the body must produce more insulin to control blood glucose levels. Eventually the pancreas is unable to produce enough insulin to control glucose levels, and oral hypoglycemic drugs or exogenous insulin becomes necessary.

When subjects were grouped by years since diagnosis of DM, HbA_{1C} levels decreased significantly in the cranberry group compared to the placebo group

at week 6 in the group that had DM the longest. Mean HbA_{1C} levels were highest in the placebo group that had DM more than 5 years. The longer a person has DM, the more likely that elevated glucose levels will cause protein glycosylation and the subsequent damage. This is the group that will benefit most from inhibition of aldose reductase and non-enzymatic protein glycosylation.

The fact that HbA_{1C} levels decreased at week 6 and then increased at week 12 may have been due to decreased compliance in the last 6 weeks of the study or it may have been due to a degradation of anthocyanins in the cranberry powder as the study progressed. It is not known how the processing of the cranberry powder used in this study affected the anthocyanins and flavonoids. It would have been beneficial to measure the anthocyanin and flavonoid content of the cranberry powder at the beginning of the study and again at the end of the study. This would be an indication of whether the changes seen from week 6 to week 12 were due to degradation of anthocyanins and flavonoids.

As with other indicators of severity and control of DM, significant differences in insulin levels were found only when subjects were grouped by time since diagnosis of DM. There was an overall significant difference in insulin levels, with subjects in the placebo group that had DM the longest having significantly higher insulin levels than any other group. This is expected due

to the increase in insulin sensitivity as the disease progresses. It should be noted that there was a wide range of insulin levels and the standard deviations were extremely high. A few subjects had highly elevated insulin levels.

Another important factor in analyzing data when subjects were grouped by years since diagnosis is that there were only two subjects in the placebo group with DM more than 5 years. One of the subjects in this group had near normal glucose and HbA_{1C} levels initially (5.8 and 7.3 respectively) but had large increases from week 0 to week 6 (glucose 5.8 to 7.1 and HbA_{1C} 7.3 to 7.8), and from week 6 to week 12 (glucose 7.1 to 9.5 and HbA_{1C} 7.8 to 8.7). The other subject in this group had initial glucose and HbA_{1C} values in the desirable range and decreased by week 12. The small number of subjects in this group and the diversity in the control of the disease may have influenced results of the study.

Grouped by Initial Glucose Levels

When subjects were grouped by initial glucose levels, differences were seen in glucose levels at week 12. Glucose levels were significantly lower in cranberry subjects with initial glucose levels greater than 7.0 mmol/L, than in the placebo group with initial glucose levels less than 7.0 mmol/L. This is likely due to the fact that subjects with elevated glucose levels are more likely to see improvement in these values, while subjects with normal glucose levels remain stable.

Grouped by Age

One might think that blood glucose, HbA_{1C}, fructosamine and insulin levels would be higher in older subjects, but that was not the case in this group of subjects. Mean glucose levels were not significantly higher in the group of subjects older than 50 years. This could be due to the fact that only three subjects over the age of 50 had DM for more than 5 years, and five subjects under the age of 50 had DM for more than 5 years. Results of this study indicated that age did not influence the effect of cranberry supplementation on glucose levels, but time since diagnosis did. This is likely due to the fact that the subjects who had DM the longest were not necessarily the oldest participants.

LIPID LEVELS

Hexanal

Headspace analysis of hexanal concentration of serum samples was used to measure lipid oxidation and antioxidant status with cranberry supplementation. The method was not effective in this study. It is possible that if there had been another standard at a lower concentration (3.125 μM), more subjects would have had detectable amounts of hexanal measured in their serum. Due to the poor outcomes of this analysis, the effect of cranberry supplementation on oxidant status and lipid oxidation is unknown.

Triglycerides, Total Cholesterol, LDL, HDL and Percent HDL

Significant differences were seen in lipid levels only when subjects were grouped by time since diagnosis of DM, and then only in triglyceride levels, total cholesterol, and percent HDL. In all cases the differences were seen in the group who had DM for more than 5 years. Subjects in the placebo group who had the disease more than 5 years had higher lipid levels than any of the other groups. Mean triglyceride and total cholesterol levels were stable from week 0 to week 12 in all groups except those with DM more than 5 years. In this group the mean values decreased for subjects on the cranberry treatment and increased for subjects on the placebo treatment.

The changes in triglyceride levels are likely due to the correlation between hyperglycemia and elevated triglycerides. As blood glucose levels increase, triglyceride levels increase proportionately. As discussed previously, one of the two subjects in the placebo group with DM for more than 5 years had significant increases in glucose levels during the 12 week study. It is likely that the hyperglycemia resulted in the elevated triglyceride levels.

Changes in percent HDL were likely a result of changes in total cholesterol levels. Percent HDL is calculated by dividing HDL by total cholesterol and multiplying by 100, so a change in total cholesterol will impact percent HDL independent of changes in HDL levels.

PREVIOUS WORK

There have not been any previous in vivo studies investigating cranberry supplementation to decrease the side effects of DM in humans. The results of this study correspond with research previously done in rats and in vitro, investigating the effect of supplementation of anthocyanins and flavonoids on diabetes. Jankowiski et al. (45) investigated the effects of anthocyanin pigments from red wine on blood and urine glucose levels in 80 rats. There was a decrease in glucose concentrations with supplementation of anthocyanins. In the current study, there was a decrease in blood glucose levels in patients on the cranberry treatment who had DM the longest and who had elevated blood glucose levels initially.

Keenoy and De Leeuw (44) investigated the effect of a flavonoid preparation on protein glycosylation and antioxidant status in twenty-eight subjects with type 1 DM in a three month study. There was a significant decrease in HbA_{1C} levels with flavonoid treatment. In the current study, HbA_{1C} levels decreased in the group of subjects who had DM over 5 years and in the group with elevated glucose levels initially. Daflon 500, the flavonoid preparation used in the Keenoy and Le Leeuw study, contains a much higher concentration of flavonoids than cranberry juice. Due to the lower concentration of flavonoids in cranberry juice, a higher dosage would likely be needed to see the same effects. If a higher dosage of cranberry juice had been used in the current

study, this might have resulted in significant differences overall between the cranberry group and the placebo group.

Hamada et al. (40) investigated the effect of epalrestat, a known aldose reductase inhibitor, in subjects with type 2 DM who had been taking the drug for 2 months. There were decreases in the end products of protein glycosylation (sorbitol, fructose, triosephosphats, TBARS and 3-DG levels), but no changes in plasma glucose or HbA_{1C} levels. In the current study, there were decreases in glucose and HbA_{1C} levels in subgroups of the subjects, but end products of protein glycosylation were not measured, and analysis of sorbitol was unsuccessful.

Pedersen et al. (52) conducted a study comparing the antioxidant capacity of consumption of cranberry and blueberry juice in the plasma of nine female volunteers. Results indicated that consumption of blueberry juice did not significantly increase plasma concentrations of total phenols or vitamin C as cranberries did, though blueberries have a higher concentration of phenolic substances than do cranberries. Cranberry juice is fortified with vitamin C and the blueberry juice used in this study was not. Researchers question how readily the phenols were absorbed in the gut. Consumption of cranberry juice did lead to significant increases in total phenols and a 30% increase in serum vitamin C concentration. It is possible that increases of total phenols in serum following cranberry juice consumption was primarily due to the increased

vitamin C levels. If anthocyanins are not readily absorbed, then it is possible that the dosage used in the Cranberry Diabetes Study was insufficient to see significant results. It is likely that a higher intake of cranberry juice would have resulted in more significant differences.

The form of cranberry juice may also have been a factor in the results of this study. The processing of the Decas cranberry powder may have decreased the anthocyanin content of the product. Liquid cranberry juice was used in Pederson's study, which may have contained more anthocyanins and flavonoids than the powder that had additional processing. The heat used in processing the cranberry juice into powder may have caused degradation of the anthocyanins and other flavonoids in the juice. This would decrease the antioxidant activity of the powdered juice.

A study conducted by Vincent et al. (38) investigated the ability of vitamin C to inhibit aldose reductase in human erythrocytes. Results indicated that ascorbic acid decreased intracellular sorbitol concentrations by 25 to 45 %. Vitamin C may be beneficial for people with diabetes, and most commercial cranberry juice cocktail has high levels of vitamin C. Many of the subjects in the placebo group did not consume fruits and vegetables high in vitamin C. Subjects who did not consume adequate vitamin C might have benefited from the small amount of vitamin C in the placebo, which would have masked the effects seen for patients on the cranberry treatment.

CONCLUSIONS

The results of this study suggest that cranberry supplementation may be beneficial for patients with type 2 DM who have poor control of glucose levels, but further research must be conducted. The subjects in the cranberry group were very different than in the placebo group. Differences in age, control of DM, and time since diagnosis between the placebo group and the cranberry group likely affected the results of this study. The number of subjects with normal blood glucose and HbA_{1C} levels was likely also a factor in the results of this study.

The form of cranberry juice may have affected the results of this study as well. It is unknown how the bioavailability of the spray dried cranberry powder used in the study compares to commercial cranberry juice cocktail. The processing method used for the Decas cranberry powder may have affected the anthocyanin and flavonoid content of the product. It is possible that using liquid cranberry juice or raw cranberries might have produced different results. The anthocyanins in the cranberry powder may also have degraded throughout the course of the study. This would explain the decreases in laboratory values at week 6 and the increases at week 12.

There is a need for further research in this area. Future research should focus on subjects with hyperglycemia, because these are the subjects that are likely to have more of the side effects that are associated with DM. It is also

important to investigate the correct dose of cranberry juice. In this study all subjects received the same dose regardless of body weight, but people with a larger body mass would need a higher dosage than smaller individuals to see effects of cranberry supplementation. Investigation of how supplementation with whole cranberries or cranberry juice differs from supplementation with juice or powders is also important because processing may affect the anthocyanins found in cranberries.

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APPENDICES

APPENDIX: A

Consent Form

Information About Study:

I am invited to participate in a research study entitled "Can Cranberry Supplementation Reduce Risks for Diabetes?" conducted by Dr. Mary Ellen Camire and Belinda Chambers at the University of Maine Department of Food Science and Human Nutrition. This preliminary study is designed to investigate the potential health benefits of cranberry juice for people with Type 2 diabetes. The study is one in which there will be two treatment groups. One group will be taking cranberry capsules and one group will be taking a placebo or control. Only the primary investigator will know who is taking the cranberry capsules and who is taking the placebo. This study is sponsored by the Cranberry Institute and the Wisconsin Cranberry Board.

Procedure:

I have been asked to consume six capsules each day for 12 weeks. I will need to take two capsules in the morning, two capsules midday and two capsules with my evening meal. Each capsule contains 0.123 grams of sugar. The gelatin capsules contain either spray dried cranberry juice powder made with magnesium hydroxide, (from Decas Cranberry Products Inc.) or a placebo containing cellulose, ascorbic acid, fructose, sucrose magnesium hydroxide and food coloring. The capsules are made from gelatin; I do not have a dietary aversion to eating gelatin. If I have difficulty swallowing the capsules, I can open the capsules, dissolve the powder in water, and drink the mixture, or I can mix

the powder with yogurt or another food and eat it that way. As part of the study I will be asked to complete a health history and a food frequency questionnaire. This questionnaire will contain questions such as "When were you diagnosed with diabetes?" and "Did (do) your siblings have Type 2 diabetes?" and "How many servings of fruits and vegetables do you eat each day?" I should discontinue use of all vitamin supplements(vitamin E, vitamin C, chromium, etc.) except a multivitamin, at least two weeks prior to the start of the study. I should not change my normal consumption of blueberries, cranberries and grapes during the course of study. A sample of my blood will be drawn at the beginning of the study and again at the sixth week and the twelfth week of the study.

I am at least 18 years old, I am not pregnant, and I am not taking medication for diabetes, autoimmune, heart, liver or kidney disease. I have Type 2 diabetes and am controlling my blood sugar with diet alone. My participation in the study is voluntary. Either myself or the primary investigator may terminate the study at any time. The supplements that will be given to me are food products. The blood collection process is no different from one I would experience in a doctor's office or a hospital laboratory.

Risks & Benefits:

There may be some minor bruising from the blood drawing procedure, but the risk is no different from any other blood drawing procedure. I will be given $150 for my participation in the study, $50 for each blood drawing procedure. I may receive copies of my lab results if I so desire. I may also have some immediate

health benefits such as decreased oxidation of LDL cholesterol, decreased retinopathy, nephropathy and neuropathy.

Confidentiality:

All information and data that are collected in this research project will be kept confidential and locked in the investigator's office. A number code will be used on all records and results to ensure confidentiality. Once the final manuscript has been published, all confidential records will be destroyed. All records and personal information about my involvement in this project will be kept confidential, and my name will in no way be associated with the results of this project. The results of this project will be released in summary form only.

Contact Information:

If I have any questions I can reach Dr. Mary Ellen Camire or Belinda Chambers at (207) 581-3581 at or by email at camire@maine.edu or belinda.chambers@umit.maine.edu

Print

Name:_____Signature:_____

Date: _____

APPENDIX B

Health History Questionnaire

Number Identifier: _____
Date_____
Age: _____

1. At what age were you diagnosed with Type 2 diabetes? _____

2. How long have you had Type 2 Diabetes? _____Years_____Months

3. Are you taking medication for your diabetes? Yes_____
   No_____

4. Are you taking any prescription medications? Yes_____No_____
   If yes, what are you taking?

5. Are you taking any over the counter medications? Yes
   _____No_____
   If yes, what are you taking?

6. Are you taking any dietary supplements? Yes_____No_____
   If yes please list:

7. How often do you exercise?

8. What types of exercise do you perform?

9. During the next 12 weeks, do you plan to begin an exercise program or increase the amount of exercise you do?
   Yes_____No_____

10. Do any of your family members have diabetes?

Mother	Yes_____	No_____
Father	Yes_____	No_____
Grandmother	Yes_____	No_____
Grandfather	Yes_____	No_____
Siblings	Yes_____	No_____

APPENDIX C

Food Frequency Questionnaire

1. How many ½ cup servings of fruit do you eat each day? _____each week? _____
2. How many ½ cup servings of vegetables do you eat each day? _____each week? _____

3. Do you drink alcohol? Yes_____No_____
   If yes, how many servings of alcohol do you drink daily, weekly monthly or yearly?
   Place a number in the column to indicate how often you drink the alcoholic beverages below, or put a check in the last column if you never drink the alcoholic beverages.

	Serving Size	Daily	Weekly	Monthly	Yearly	Never
Beer	12 ounces					
Wine	8 ounces					
Hard Liquor	1 ounce					

4. How many times each day, week, month or year do you eat the following fruits?
   Place a number in the correct column to indicate how often you eat the listed fruits. If you do not eat the fruit place a check mark in the never column.

Fruit	Serving Size	Daily	Weekly	Monthly	Yearly	Never
Apples	1 apple					
Apricots	3 whole fresh, 1 cup dried					
Avocado	1 whole					
Bananas	1 whole					
Blackberries	1 cup					
Blueberries	1 cup					
Cantaloup						
Cherries	10 cherries					
Cranberry juice	1 cup					
Dates	10 dates					

Fruit	Serving Size	Daily	Weekly	Monthly	Yearly	Never
Grapefruit	½ grapefruit 1 cup juice					
Grapes	10 grapes					
Lemonade	1 cup					
Oranges	1 whole 1 cup juice					
Papayas	1 cup					
Peaches	1 whole					
Pears	1 whole					
Pineapple	1 cup					
Plums	1 whole					
Prunes	5 large 1 cup juice					
Raisins	1 cup					
Raspberries	1 cup					
Rhubarb	1 cup					
Strawberries	1 cup					
Tangerine	1 whole					
Watermelon	1 cup					
Other_____						
Other_____						
Other_____						

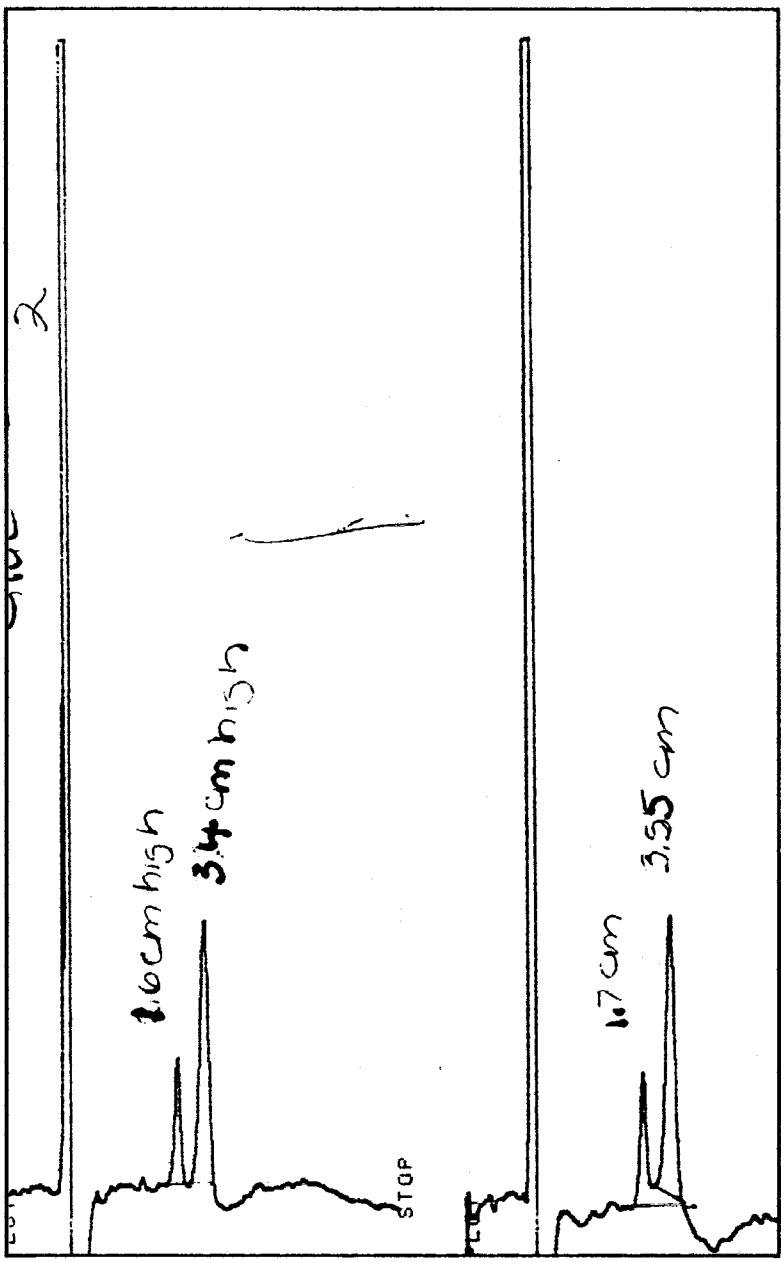
5. How many times each day, week, month, year do you eat the following vegetables?

   Place a number in the correct column to indicate how often you eat the listed vegetables. If you do not eat the vegetable place a check mark in the never column.

Vegetable	Serving Size	Daily	Weekly	Monthly	Yearly	Never
Asparagus	4 spears					
Bean sprouts	1 cup					
Beets	1 cup					
Broccoli	1 cup					
Cabbage	1 cup					
Carrots	1 cup					
Cauliflower	1 cup					
Celery	1 stalk					
Cucumber	1 cup					
Eggplant						
Green beans	1 cup					
Lettuce	1 cup					
Mushrooms	1 cup					
Onion	1 cup					
Parsnips	1 cup					
Radishes	4 radishes					
Sauerkraut	1 cup					
Spinach	1 cup					
Sweet peppers	1 pod					
Other_____						
Other_____						
Other_____						

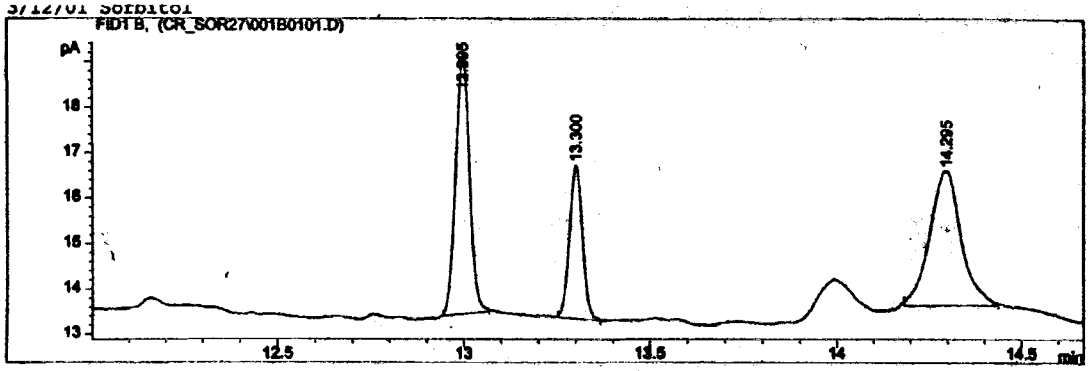
APPENDIX D

Figure D-1: HPLC Chromatogram



APPENDIX E

Figure E-1: Sorbitol Chromatogram



BIOGRAPHY OF THE AUTHOR

Belinda Chambers was born in Colorado Springs, Colorado on September 10, 1969. She was raised in Colorado Springs, Colorado and graduated from Dougherty High School in 1987. She attended Denver Technical College and graduated in 1992 with an Associates degree in Sports Medicine. She attended Colorado State University and graduated in 2000 with a Bachelor's degree in Human Nutrition. She moved to Maine and entered the Food Science and Human Nutrition graduate program at The University of Maine in the summer of 2000.

After receiving her degree, Belinda will be returning to Colorado to pursue a career as a Registered Dietitian. Belinda is a candidate for the Master of Science degree in Food Science and Human Nutrition from The University of Maine in May, 2002.