Quercetin and Prostate Cancer:

A Case-Control Study

By, Anna Dorothea Forester

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Anna Forester

has been approved

Mentor/Advisor

Member

Member

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ABSTRACT:

Background: Quercetin, an abundant flavonoid in the diet, has been shown to have several effects on prostate cancer cell lines, where quercetin can decrease colony formation and increase apoptosis. Quercetin appears to have an impact on numerous markers for prostate cancer.

Objectives: 1) The purpose of this study is to evaluate the effects of dietary intake of quercetin as measured by the National Cancer Institute's Diet History Questionnaire (DHQ) and risk of prostate cancer. 2) A secondary objective is to evaluate the association between dietary intakes of quercetin, and the presence of inflammation in prostate tissue.

Setting and Subjects: In this case-control study; subjects include all men referred to the Portland VA Medical Center (PVAMC) urology clinic for a prostate biopsy, and Prostate Specific Antigen normal controls(PSA<4 ng/mL) receiving care through PVAMC primary care. Men completed a detailed food frequency questionnaire that also captured lifestyle and tumor characteristics.

Methods: Quercetin was added to the DHQ software by this researcher. Dietary intake of quercetin was divided into quartiles and odds of prostate cancer (as compared to clinic controls and to biopsy negative controls separately) were determined using unconditional logistic regression. The association between dietary intake of quercetin and the presence of inflammation was determined using binomial logistic regression.

Results: None of the regression analyses reached statistical significance.

INTRODUCTION:

Prostate cancer can be defined as cancer that forms in the epithelium of the prostate; a gland in the male reproductive system found below the bladder and in front of the rectum. According to the National Institutes of Health, the estimated number of new prostate cancer cases for the year 2008 is 186,320 and 28,660 deaths from prostate cancer are predicted for this year (1).

Prostate cancer is the second leading cause of cancer mortality in men living in the United States and the most diagnosed cancer type among US males. Approximately one in six American men will be diagnosed with prostate cancer in his lifetime (2). The estimated lifetime risk is 17.6% for Caucasians and 20.6% for African-Americans, with a lifetime death risk of 2.8% and 4.7% respectively (3).

A diagnosis of prostate cancer can be sought following the abnormal results of a digital rectal examination and elevated serum concentration of prostate specific antigen (PSA), or transrectal ultrasound. Each of these methods has their strengths and weaknesses. Diagnosis most frequently occurs during population screening, in which at risk asymptomatic men are screened, or opportunistic screening, which occurs on an individual basis (4).

The etiology behind prostate cancer is not fully understood, but steroid hormones, specifically androgens are a significant risk factor. Additional risk factors for prostate cancer include: diet, obesity, health screening history, age, race, family history and the presence of specific genetic polymorphisms (2). The relative risk for prostate cancer also has considerable geographic variability. Evidence points to this variability being more related to environment and

lifestyle patterns, than genetics. Men who move from a country with a low risk of prostate cancer, to a country with a higher risk, tend to have a relative risk that is comparable to the country to which they moved (5). Due to the geographic discrepancies in prostate cancer rates, preventive lifestyle changes including nutrition related alterations are likely key issues in prostate cancer prevention (6).

CARCINOGENESIS AND CHEMOPREVENTION

Chemoprevention can be defined as the use of substances, whether natural or synthetic, to block, reverse, or retard the process of carcinogenesis (7). Carcinogenesis consists of three major steps, initiation, promotion, and progression. Initiation is an irreversible, short step, while promotion is a long term process that involves chronic exposure to a tumor promoter. Progression refers to advancement in aggressiveness and spread to other organs. Promotion and also progression are ideal targets for interventions (7). The goal of primary chemoprevention is to decrease the incidence of a given cancer, thus reducing both treatment-related side effects and mortality (3).

Prostate cancer is an ideal target for chemoprevention due to its long latency, tumor marker availability, and identifiable preneoplastic lesions (6). The potential benefits of quercetin are enhanced by the low likelihood of side effects of quercetin consumption and supplementation. It is widely available in the food supply and is already part of our dietary pattern.

EPIDEMIOLOGICAL EVIDENCE

Evaluations of diet and prostate cancer have found an alteration in prostate cancer risk associated with intake of different food components. The World Cancer Research Fund conducted a 6 year evidence review of 7,000 research papers to find conclusive evidence about the prevention of a variety of cancers. They examined the evidence from the standpoint of dietary components that increased or decreased risk, and categorized the findings into convincing evidence, probable evidence, limited-suggestive, limited-no conclusion, and a substantial effect on risk being unlikely (8). The findings regarding prostate cancer is summarized in Table 1. Findings for fruits and vegetables have been somewhat consistent, leading investigators to consider various functional compounds that may explain these findings. However, many individual nutrients have failed to garner consistent results; this may be due to the presence of other compounds in fruits and vegetables that have not been researched thoroughly, such as quercetin, and other flavonoids.

Table 1- Evidence relating Diet with Prostate Cancer

Evidence	Decreases Risk	Increases Risk
Convincing	None Identified	None Identified
Probable	Foods Containing Lycopene Foods Containing Selenium Selenium	Diets High in Calcium
Limited-Suggestive	Pulses (Legumes) Foods Containing Vitamin E Alpha-Tocopherol	Processed Meat Milk and Dairy Products
Limited-No Conclusion	Cereals (grains) and their products; dietary fiber; potatoes; non-starchy vegetables; fruits; meat; poultry; fish; eggs; total fat; plant oils; sugar (sucrose); sugary foods and drinks; coffee; tea; alcohol; carbohydrate; protein; vitamin A; retinol; thiamin; riboflavin; niacin; vitamin C; vitamin D; gamma-tocopherol; vitamin supplements; multivitamins; iron; phosphorous; zinc; other carotenoids; physical activity; energy expenditure; vegetarian diets; seventh-day Adventist diets; body fatness; abdominal fatness; birth weight; energy intake	
Substantial Effect on Risk Unlikely	Beta-carotene	

This table represents the evidence as it exists, if there is a food or food item missing from the table, it is not that the item does not possess beneficial properties, it is that there has not been enough research for inclusion into this table. Flavanoids are a category of bioactive dietary components whose relation to prostate cancer risk remains inadequately explored.

QUERCETIN

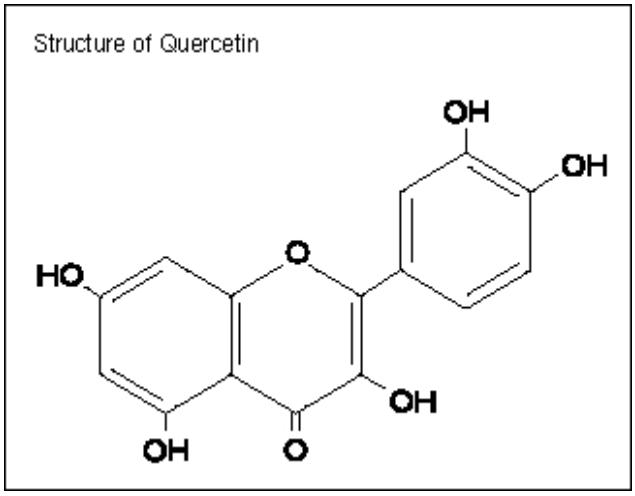

Fig 1- Structure of Quercetin-*Image from http://www.medizin-forum.de/prostatitis/quercetin-d.html*

The flavonol quercetin is currently being examined by several groups as a possible chemopreventive agent. A flavonol is an active plant compound with nutritional benefits. Among the benefits of quercetin are the ability to act as an anti-inflammatory, antioxidant and

phytoestrogen (9). Quercetin is widely distributed in the food supply. In the US diet, the most abundant sources of quercetin are found in foods such as apples, onions, berries, tea, and alcohol. Quercetin is readily and rapidly absorbed into the body as a variety of conjugates. The exact method of absorption is uncertain, however, numerous studies have found a rise in plasma quercetin directly after eating an item containing the flavanol, and animal studies suggest that absorption may be enhanced by the simultaneous ingestion of lipids (10). The peak plasma concentration of quercetin appears to occur at 7 hours post prandially, and tapers off by 26 hours (9, 11).

Quercetin is considered to be "sparingly soluble and chemically unstable in aqueous intestinal fluids" (12). The solubility of quercetin is important to note for several reasons. First, there may be an interaction between dietary constituents simultaneously consumed with quercetin in the diet. Second, the solubility of an antioxidant determines the site of action and storage in the body. Water soluble antioxidants perform their functions in the cytoplasm of cells, whereas fat soluble antioxidants tend to function within the membranes of cells. In vitro research has focused on many aspects of the relationship between quercetin and prostate cancer, including antioxidant activity, altered cell cycle, and altered gene expression.

IN VITRO-GENERAL

The examination of quercetin and prostate cancer risk has focused mainly on in vitro studies of human prostate cancer cell lines and there has been little work looking at how

quercetin is metabolized and used in free living human subjects. Results of a thorough literature review examining the evidence for quercetin and prostate cancer as key search words can be found in appendix A.

Currently there is no recommended therapeutic dose that men are advised to consume, nor is there a therapeutic plasma or cell concentration that provides positive benefits. Due to the poorly understood nature of the action of quercetin there is little consensus in the literature, and each research article seems to be examining a different potential mechanism creating little repetition in the data.

Examinations of the effect of quercetin on markers of cell proliferation and apoptosis have been conducted in LNCaP an androgen dependent cell line, DU-145 and PC-3 androgen independent cell lines. There are some general conclusions that may be draw from in vitro studies findings. Quercetin administration has been consistently shown to inhibit cancer cell proliferation in PC-3, DU-145, and LNCaP cell lines, and these effects tend to occur in a dose dependent manner (13-20) Following direct application of quercetin, PC-3 cell colony formation is suppressed by 40% ($p<0.001$) for 25µM quercetin and 69% ($p<0.0001$) for 50µM treated cells. Colony formation in DU-145 is suppressed by 35% ($p<0.001$) for 25µM and 40% ($p<0.001$) for 50µM concentrations. However, lower concentrations had no effect on these types of cells and none of the quercetin concentrations had an effect on the less aggressive LNCaP cells (21).

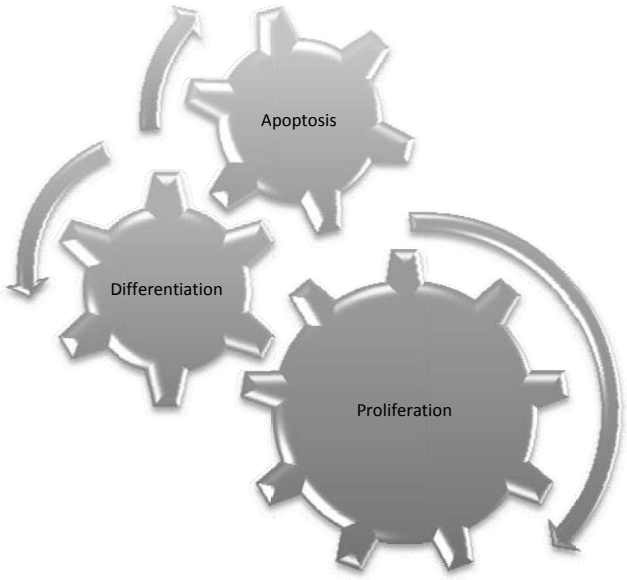

Figure 2- Cellular Processes

Cellular processes occur in three phases (fig 2). Proliferation is when cells increase in numbers. In cancer, the process of proliferation is often up regulated. Differentiation is when cells become specialized to perform tasks. In cancer, cells are often de-differentiated. Apoptosis, or programmed cellular death, is an important component to prostate, as well as other cancer types, because impaired apoptosis can result in tumor growth. Quercetin has been seen to have an effect on cell apoptosis in vitro. The percentage of apoptotic events was increased by 43±1.47 in LNCaP and 32.5±1.42 in PC-3 cells in 100 µM quercetin treated cell lines (22). PC-3 cell lines, that were quercetin treated were seen to have a decrease in Bcl-2 and Bcl-x expression and increased the level of capsase-3. Bcl-2 and Bcl-x are considered to be anti-

apoptotic genes and capsase-3 is a protease which plays a role in apoptosis. A concomitant increase in cells in the Sub G phase was observed in quercetin treated cells and an increase in the G2M phase was also seen (22). A change in these cell cycles may allow for DNA repair, as well as inhibit the proliferation of damaged cells.

QUERCETIN-BIOLOGIC MECHANISMS FOR CHEMOPREVENTION

Quercetin has demonstrated the ability to function as a phytoestrogen, an antioxidant, and an anti-inflammatory. These potential mechanisms may explain the in vitro findings, as well as a possible chemopreventive action.

Phytoestrogen Activity

The role of androgens in prostate carcinogenesis is controversial, and thus prevention via phytoestrogen intake is also controversial. It is thought that the activity of the androgen receptor is beneficial in preserving normal prostate function. However, androgens, such as testosterone and dihydrotestosterone are known to stimulate prostate cells to grow (8), thus they can be considered tumor promoters. The manner in which they achieve this and the role they play in carcinogenesis are current research topics that are still being refined. It appears that androgen dependent prostate cancer cell lines, the LNCaP type, respond to androgens in a biphasic manner. Low doses can stimulate proliferation, whereas a high dose can arrest cancer

cells (24). It also appears that "most treatment-naïve prostate cancers are androgen-dependent, meaning that they respond to androgen-ablation therapy. However, these tumors eventually become androgen-independent and grow despite androgen ablation" (25). This androgen independence is seen in recurrent cancer following deprivation or ablation of testosterone.

Prostate cancer growth is androgen dependent in its early stages, through the activation of the Androgen Receptor (AR). In vitro, androgen dependent cancer cell lines are considered to be more invasive than non-androgen dependent cell lines (26). Thus, an androgen deficiency can been seen as partially protective for prostate cancer, however low levels of testosterone have also been shown to be associated with more aggressive cancers (27). Due to the association of prostate cancer to testosterone and other androgens, castration, either chemical or surgical is often seen as a means of treatment. Additionally, it is thought that over time, prostate cancer becomes androgen independent, and these treatments will be ineffective for later forms of prostate cancer (27).

In addition to the debate regarding androgen ablation as a treatment modality, the role that estrogen plays in prostate cancer is also being investigated in the literature. The assumption that lower testosterone levels are associated with decreased risk of prostate cancer has led some to examine the negative feedback role of estrogen on the hypothalamic-pituitary-gonad axis (fig 3).

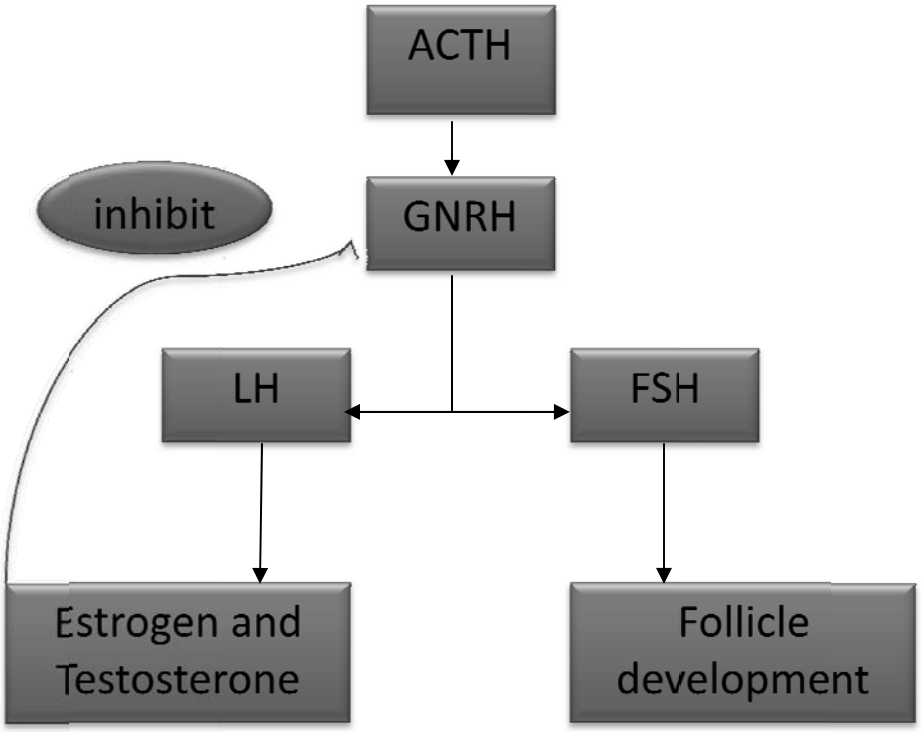

Figure 3-The Hypothalamic-Pituitary-Gonad Axis

Estrogen exerts a negative feedback on GNRH, and could lead to a reduction in testosterone production through a reduction in LH synthesis. The role of estrogen further complicates the association between testosterone and prostate cancer. Estrogens are involved in local cell signaling, including proliferation, much like testosterone, and they also play a role in inflammation (28). There are dual receptors for estrogen within the prostate gland, the estrogen receptor α and the estrogen receptor β. The estrogen receptor α promotes aberrant proliferation, inflammation, and cancer. The estrogen receptor β promotes hypertrophy, hyperplasia, and possibly inflammation and cancer (28).

A phytoestrogen is a plant compound that exerts estrogen like effects. There is research that indicates that a diet high in phytoestrogens can create a variation in the estrogen receptor β and may reduce risk of prostate cancer (29). In addition to the previous hypothesis, there is evidence that a high dose of isoflavones, a type of phytoestrogen may interfere with the hypothalamic-pituitary-gonad axis, in a similar manner to estrogen, and thus may reduce LH secretion and the formation of testosterone (30).

The association between quercetin and androgen receptors in LNCaP cells has been a popular hypothesis. LNCaP cells are androgen dependent cancer cells and are less aggressive than androgen independent PC-3 and DU-145 cells. When the regulation of AR receptors was examined in LNCaP cells, several studies have found a down regulation of AR with quercetin administration (31-34). In addition to inhibiting proliferation as seen in PC-3 and DU-145 cells, quercetin may decrease the androgen receptor expression, thus lowering prostate cancer risk.

Antioxidant Activity

The antioxidant activity of quercetin is another possible mechanism of action for this flavonol. The overproduction of reactive oxygen species (ROS) or free radicals has been implicated in carcinogenesis of the prostate. "Oxidative stress is defined as a state in which the level of toxic reactive oxygen intermediates overcomes the endogenous antioxidant defenses of the host (35)." ROS can cause damage to molecules in the body, such as: DNA, lipid membranes, and proteins (36-37). ROS are free radicals, meaning that they have unpaired electrons, and

they contain oxygen. They are generated due to exposure to pro-oxidative substances and function as part of the defense system of the host (38). The function of antioxidants is to control or eliminate free radicals.

Oxidation involves the addition or withdrawal of energy by oxygen from reduced carbon-based molecules, leading to the formation of free radicals. Several oxidants and free-radical generators are tumor promoters (7). Antioxidants can help prevent tumorgenesis through the inhibition of cell proliferation and transformation seen in oxidative stress (38). The elimination of oxidative stress can have two effects, one is to reduce the numbers of oxidative species produced, and the other is to increase the amount of antioxidants in the host (35).

The function of antioxidants is to control or eliminate free radicals. Different nutrients function as antioxidants in different cellular compartments, based on their solubility. Quercetin, which can be considered as water soluble, would be expected to function in water containing parts of the human body, such as the blood and cytoplasm. The fact that quercetin absorption is enhanced by the ingestion of fatty acids and emulsifiers indicates that it is may be able to also function as a scavenger of lipid ROS.

Anti-inflammatory Activity

Prostaglandin biosynthesis has been implicated in the process of inflammation and also plays an important role in the pathogenesis of malignancy (7). Chronic inflammation can lead to an increase in cell proliferation, and a decrease in apoptosis, both of these processes may be implicated in tumorgenesis. Cyclooxygenase (COX) catalyzes an important step in the conversion of arachidonic acid to prostaglandins, which are mediators of inflammation. The inducible COX-2 enzyme, a peroxidase, mediates the inflammatory response and plays a role in cell proliferation, and tumor invasion (7). Genes responsible for COX-2 expression are deregulated in some tumor types. Quercetin was found to significantly inhibit COX-2 expression with IC50 values between 5 and 10μM in colorectal cells (39).

Hydrogen peroxide, an oxidant, may be a link to inflammation, as it activates matrix prometalloproteinase-2 (pro-MMP-2), an enzyme implicated in the malignant progression of many tumor types. MMP-2 enhances tumorgenesis through the induction of the membrane type 1-matrix metalloproteinase (MT1-MMP) expression, and increases invasion and migration of HT 1080 human fibro sarcoma cells. Upon in vitro treatment of PC-3 prostate cancer lines with quercetin, MMP-2 and MMP-9 proteins were significantly decreased in a dose dependent manner. Pro-MMP-9 was significantly increased at 100μM (13). Thus, it appears that the anti-inflammatory effects of quercetin may be related to its function as an antioxidant.

To date, two large epidemiological case control studies have included the analysis of quercetin and prostate cancer, though neither study was designed with prostate cancer as a primary endpoint or quercetin as the primary exposure variable (40-41). One examined many different phytochemicals, and the other different flavonols and chronic disease, including prostate cancer. The prior found a significant reduction in prostate cancer risk associated with quercetin, OR= 0.64; 95% CI (0.44, 0.92) when a model was used that adjusted for age, education, body mass index, cigarette smoking status, and total energy intake. When the model was further adjusted to include total vegetable intake, quercetin was not associated with a significant risk reduction, OR= 0.73; 95% CI (0.49, 1.09) (40). The second epidemiological study found a significant risk reduction for overall cancer incidence associated with higher intakes of quercetin RR=0.77; 95% CI (0.65, 0.92), p for trend=.01. When prostate cancer was specifically examined, there was no significant reduction in risk associated with higher quartiles of consumption, RR=0.76; 95% CI (0.40, 1.42) (41).

QUERCETIN KINETICS

Epidemiological studies cannot control for the relative bioavailability of quercetin. Although in vitro studies have had strong positive results when examining the effects of quercetin treatment on prostate cancer cell lines the doses of quercetin used may not be able to accumulate in human tissues resulting in the lack of strong epidemiological findings. The most

important determinants of bioavailability are the chemical structure of the aglycone (the non sugar component of a glycoside) and the type of glycoside. Polyphenols are rarely present in plants as aglycones, and are usually bound to different sugars. Quercetin is usually present as a glycoside. Quercetin glycosides from onions have a better absorption rate than pure aglycone (42). Prior to absorption from the gastrointestinal tract, the glycosidic linkages are cleaved by enzymes originating from the small intestine or the colon (43). Quercetin is taken up in the intestine and then metabolized by phase II enzymes in the intestine and liver to methoxy, glucuronic acid and sulfate conjugates (44).

There has been numerous research attempts aimed at determining the bioavailability of dietary quercetin. Most of these focus on the amount of plasma quercetin recorded from time 0 to 48 hours, since the half life of quercetin is approximately 21-25 hours (9, 11, 44-46). These studies aim to determine the rise in plasma quercetin following the ingestion of a known amount. These studies do not attempt to determine the amount of baseline quercetin in free living subjects, nor do they examine the plasma quercetin for people who are consuming a variety of quercetin from different dietary sources. These studies do show that quercetin is rapidly absorbed into the blood stream, peaking at approximately 0.7 h (9, 42), and that the amount of quercetin absorbed varies among subjects (45-47). Previous research has also demonstrated that long term supplementation with quercetin raises plasma concentrations in human subjects; however, this study did not use a control group (45).

An interesting study was conducted on male Wistar Rats, in which their jejunal sacs were incubated in vitro with whole red wine and de-alcoholised wine whose phenolic content was chemically identical. Higher amounts of quercetin were found in the mucosal tissue in the

group that received whole wine, suggesting that alcohol can help increase quercetin bioavailability and uptake (47). This hypothesis, however, has not been explored in vivo or in human subjects.

STATE OF THE EVIDENCE

The state of the evidence for quercetin as a chemopreventive agent is incomplete. There is evidence that baseline quercetin is increased by an increased regular consumption of quercetin containing foods. There is evidence that in vitro quercetin has effects as a chemopreventive agent, including inhibition of cell proliferation and induction of apoptosis in cancer cell lines. There is limited epidemiological evidence about the effect of quercetin intake and risk of prostate cancer. The determination of the impact of plasma quercetin concentration on risk of prostate cancer is an important piece of information that is missing from the current quercetin and prostate cancer research. Consistent findings of an association between quercetin consumption and relative risk of prostate cancer are one key component of elucidating the relationship.

SPECIFIC AIMS OF CURRENT STUDY

- Primary Aim # 1: To evaluate the association between consumption of quercetin as measured by a food frequency questionnaire and risk of prostate cancer compared to biopsy negative controls and PSA normal clinic controls.
    - Hypotheses for Primary Aim #1: Higher dietary intake of quercetin is associated with a reduction in prostate cancer risk.

- Primary Aim # 2: To evaluate the association between quercetin intake and inflammation of prostatic tissues.
    - Hypotheses for Primary Aim #2: Higher dietary intake of quercetin is associated with a lower likelihood of inflammation.

MATERIALS AND METHODS:

SUBJECTS

The present project is a secondary analysis of the Diet and Prostate Cancer Risk (DPC) study. Eligible subjects included all men in the data set for the "Diet and Prostate Cancer Risk" protocol developed by Dr. Jackilen Shannon. This includes all men referred to the Portland VA Medical Center urology clinic for a prostate biopsy, due to an elevated, >4 ng/mL PSA or abnormal clinical findings. All patients provided written informed consent according to both the PVAMC and Oregon Health & Science University (OHSU) Institutional Review Boards' requirements. The original protocol, consent forms, and Health Insurance Portability and Accountability Act (HIPAA) authorization forms were reviewed and approved by the PVAMC and OHSU Institutional Review Boards.

Subject recruitment and response rate has been previously described (48), briefly, of the 408 potential clinic controls that were successfully contacted, 236 (57.8 percent) agreed to participate and completed the Diet History Questionnaire (DHQ). 3 participants were excluded from the final analyses as it was determined that they were being treated for a prostate condition, and 3 participants did not fully complete the DHQ.

Biopsy-negative controls and prostate cancer cases were identified among men referred to the PVAMC urology clinic for a prostate biopsy. 494 men agreed to participate in the study and complete the DHQ. Of 291 patients with negative biopsy results, 36 were diagnosed

with prostatic intraepithelial neoplasia (PIN) and were excluded from these analyses. We excluded 59 patients who did not get a biopsy, as we had insufficient information on their cases. Eleven men failed to fully complete the dietary questionnaire (48).

After the diet study was explained to these men via telephone, and verbal consent was given, they scheduled a time to come into the VA or CTRC facility to complete a short baseline questionnaire, the FFQ interview, and to provide a blood specimen, prior to their biopsy. Men that were unable to travel prior to their surgery came in one hour early for their biopsy, so that data could be collected at that time. The interview was completed by a trained research dietitian. Additional data, such as patient and tumor characteristics, were taken from medical records and recorded on the baseline questionnaire form. In subjects who were selected to undergo a prostate cancer biopsy, but whose results were negative for prostate cancer, the presence of inflammation of the prostate tissue was noted in their urology notes during biopsy slide analysis and placed in their medical records.

DIETARY INTAKE

Dietary intake was assessed using a modified version of the National Cancer Institute's Diet History Questionnaire (DHQ) (49). The NCI DHQ was modified to collect baseline information on age, education, marital status, race/ethnicity, smoking status, and alcohol use, as well as to accommodate it being administered by an interviewer instead of self administered. Further modification of the DHQ was performed to capture more detailed information on fish

consumption, garlic, and supplement use. The questionnaire was examined for stray marks and incomplete marks prior to being scanned by a computer. All questionnaires remained at the CRC for scanning and nutrient calculations. Dietcalc© software, which was developed for use with this instrument was used to quantify nutrient levels in the diet of subjects. This software employs a method which accounts for gender, portion size, and mean nutrient content of the food. For the purpose of this study, all of the fruit and vegetable portion sizes have been converted to medium. Most participants reported consuming medium portions, and it was difficult for them to estimate serving sizes for fruits and vegetables.

ADDING QUERCETIN TO THE DHQ

The addition of quercetin to the nutrient analysis software, Dietcalc®, was completed using a database developed by the USDA entitled Flavonoid Content of Selected Foods (50). The Dietcalc® software allows for the conversion of food values to nutrient data. The addition of quercetin to the DHQ required some averaging and expert opinion work. Food items from the DHQ were examined for correspondence with the USDA data.

When a food item from the DHQ had more than one corresponding value for quercetin from the USDA, the quercetin was taken as a straight average of all the possible foods. For example: apples with the skin have a quercetin content of 4.42 mg per 100 gm edible portion, whereas without the skin, they value is 1.50 mg per 100 gm, so the value for apples in the DHQ

was calculated to be 2.96. This type of averaging was performed for 24 separate food items, with various modulations and special considerations taken into account for each food item.

Some food items, such as onions, had several values for different varieties and cooking methods. For onions, these values were typically in the 14-19 mg of quercetin per 100 gm edible portion, with the exception of white raw onions, which had a value of 5.19, this was more than two standard deviations from the average, which was 14.398, so the average was recalculated without the value for raw white onions, and was determined to be 16.7 mg/100gm.

Some items in the USDA were not those that are typically eaten in the US, or were not referring to those intended by the DHQ, and these were excluded from calculations and analysis. For example, frozen, but unprepared vegetables were not included, since they are not typically consumed in their frozen state, although canned, fresh, frozen and prepared, and cooked were. An example of this type of calculation is green beans from the DHQ, the USDA values used include canned, frozen cooked, and raw values for green snap peas, as wells as the value for raw yellow snap peas. Unprepared frozen green snap peas were excluded from the mean value calculation, and other cooking techniques for yellow peas were not available. For pickled fruit and vegetables, cucumbers were used exclusively, since it was likely to be the main food item consumed in this category. Pickles have a relatively low quercetin content, the inclusion of items higher in quercetin may have falsely elevate the amount of quercetin calculated for this food item.

An example of excluding sub categories of foods that are not typically eaten in the United States can be made from spinach and other greens as queried by the DHQ. This average

value was calculated using raw spinach, and cooked turnip greens, raw water cress, cooked Kale, and canned Kale. Several of the more obscure greens were excluded, such as: lovage leaves, perilla leaves, fennel leaves, crown daisy, garden cress, chicory, Chinese kale, sweet potato leaves, and water spinach.

The DHQ item "other vegetables" was calculated as an average of all of the vegetables that had not been previously accounted for in other single food items. Included in this category was: beets, celeriac, cucumber, gourd, endive, kohlrabi, mushroom, parsnip, and radish. The average of these food items was 0.19667 mg per 100 gm edible portion of quercetin. Fennel was excluded from this averaging due to its abnormally high quercetin content of 48.8 mg. It was thought that it would falsely elevate the quercetin value for other vegetables and not be reflective of the quercetin consumed for this food item.

Several of the DHQ items can be considered mixed dishes such as pizza, salads, Mexican mixtures, dessert items and soup. The NCI was contacted regarding their methodology for determining the constituents of these types of food items. The NCI uses standard recipes based on US dietary data collected from the 1994-96 US Department of Agriculture's Continuing Survey of Food Intake by Individuals (CSFII). For the purpose of this study *the Joy of Cooking* was used as the standard recipe for mixed food items. Some foods had multiple recipes, such as chili, and for these the recipes which had classic in the title were used.

All ingredients in the recipe were converted into grams for the entire recipe so that the most accurate total weight of the recipe could be obtained. This was accomplished by converting the ingredient amount as written, into grams using USDA data from the nutrient

analysis library to determine the weight of ingredients. The recipe components with known quercetin content had their quercetin calculated to reflect the grams corresponding to their ingredient weight in the recipe. The milligrams of quercetin per 100 grams of the recipe were determined, using the total weight of the recipe and the total quercetin content of the recipe, divided into 100 gram portions.

When calculating the quercetin for mixed items, several considerations had to be made. When the item was a cooked item, the value for quercetin that reflected a cooked product, such as onions, was used whenever possible. If a recipe called for one of two possible choices, those two choices were averaged for quercetin content. For example, under fruit pies, an average of all of the fruits from the USDA was used (16 fruits total), excluding those foods considered to be rarely eaten in the United States, such as: lingonberry, bog whortleberry, and elderberry. Any item with unknown quercetin content was determined to be 0 for calculations. One exception to this was that orange juice from USDA data was extrapolated to include whole oranges.

Several food items from the DHQ which required a recipe could mean multiple possible recipes with unique and varied quercetin contents, and the potential recipes for these dishes was averaged in manner similar to mono-ingredient DHQ item numbers. For example: the DHQ item cream based soup was determined using a mean average of cream of mushroom and cream of broccoli soups. Bean based soup was determined to be an average of the recipes for Lentil Soup and US Senate Bean Soup. Pizza was an average for a tomato sauce based basic cheese pizza, with and without meat.

Some recipe items had other recipe embedded within them. Tacos were used as the standard recipe for the DHQ item Mexican Mixtures. Contained within the recipe for tacos is the ingredient salsa. In order to determine the quercetin content of Mexican mixtures, the quercetin content of salsa was first determined, then the recipe for tacos was calculated using the same techniques described previously.

Some questions in the DHQ refer to a cluster of foods, with similar properties, such as beef stew, pot pie and other mixtures, or chicken mixtures. Since grains, meat and dairy have no known quercetin value; these mixtures all used the recipe for beef stew as their reference, since it was a mixture of meat and vegetables, in proportions that were likely to be similar to others. For items that used pasta, the cooked weight of pasta was determined to be three times the dry weight (50). This was then combined with the weight of spaghetti sauce, from the USDA database. For dried fruit, the percentage of water was determined using USDA information and this value was used to determine the conversion of fresh weight into dry weight for dried fruit.

The quercetin content of a total of 61 food items was determined and added to the DHQ nutrient analysis software. The entire spreadsheet of calculated quercetin amounts and the corresponding foods from the DHQ can be found in appendix B. A simple sample calculation for lentil soup, one component of the DHQ category for bean based soup can be found in appendix C.

ANALYTIC METHODS

Statistical analysis was performed using SPSS version 15.0 software (51).

The primary endpoint is prostate cancer, with inflammation as a secondary endpoint. Prostate cancer risk was determined as compared to both clinic normal controls, as well as biopsy negative controls. A multivariate logistic regression was performed to assess the influence of diet on risk of prostate cancer, examining dietary intake of quercetin as the primary predictor variable. Initial descriptive analyses were conducted to evaluate the distribution of the primary predictor variables and other covariates.

Intake of quercetin, as well as other dietary components was categorized into quartiles based upon the distribution of each variable within the clinic control group. Odds ratios and 95% confidence interval for each quartile were estimated as compared to the lowest quartile using unconditional logistic regression. Potential confounding variables, including age, race (collapsed into a binary variable), alcohol intake, total energy intake, dietary fat intake, smoking, other dietary carotenoids and other risk factors for prostate cancer were added independently to the univariate model. Variables that changed the odds ratio of the primary predictor variable by +/- 10% were considered confounders. The -2 log likelihood ratio statistic was used to compare the model with and without the potential confounder. The confounder was determined to contribute significantly to the model if the model was found to have a more significant likelihood ratio test.

Correlations were investigated and variables were considered for inclusion in the final model only as appropriate. Potential interactions between quercetin intake and alcohol or other antioxidant nutrients were also evaluated.

Sample size calculations were done by Dr. Jackilen Shannon prior to approval of the DPC protocol, #722. The ratio of cases to controls has been used to determine the power of the existing study to identify significant associations between dietary variables and risk of prostate cancer. Assuming 150 cases, with 1.5 controls per case, and an exposure variable of 0.25, since the data will be divided into quartiles, the study has the power to detect odds ratios as close to 1.0 as 0.42 and 2.0.

Between groups differences in demographics, other risk factors, and dietary intake were calculated using ANOVA procedures (tables 2 and 3). For differences that were found to be significant, an age adjusted multivariate regression was performed to see if the difference was a significant independent predictor in case versus biopsy negative or clinic normal control groups. Variables were included as potential confounders in the multivariate analysis if the p value from ANOVA for baseline characteristics and dietary components between groups was less than p=0.10. Additionally, BMI, although not significant was included as a potential confounder.

Once a final model was developed, stratification by potential interaction terms was assessed. A base model that was adjusted for age, and then additionally adjusted for race, and then finally the full model, were all tested with the interaction terms of lycopene intake and alcohol intake. A separate comparison of high grade prostate cancer, as determined by a Gleason score > 6, and low grade prostate cancer, Gleason = 6 was performed, using the same

methods delineated previously. Comparison was divided into high and low grade cancer cases against clinic normal controls, and high and low grade cancer case against biopsy negative controls. No stratifications were performed in this model.

The presence of prostatic inflammation in biopsy negative subjects was then examined as the outcome variable. Inflammation was assessed using binomial logistic regression, using a model identical to the model for prostate cancer, with the same stratification methods.

Correlations between quercetin, lycopene, and total servings of tomato products were assessed using Pearson's correlation. Total servings of vegetables and quercetin were also assessed using Pearson's correlation.

RESULTS

Differences between the three subject groups by select demographic markers were examined in table 2. The p value is for ANOVA procedures. The only significant differences between the three groups were average age, p=0.001, and education between cancer cases and biopsy negative controls, p=0.007.

Table 2- Characteristics of Cases and Controls from the DPC study

Characteristic	Cancer Cases (N=143)	Biopsy Negative Controls (N=256)	Clinic Normal Controls (N=236)	P value
	N ± SD	N ± SD	N ± SD	
Average Age	65.56 ± 7.04	63.00 ± 6.50	64.34 ± 6.97	0.001
Average BMI	28.9 ± 5.61	29.48 ± 4.86	30.08 ± 6.90	0.16
Race	N (%)	N (%)	N (%)	
White	130 (91%)	234 (91%)	222 (94%)	0.423
African American	9 (6%)	8 (3%)	6 (3%)	
Hispanic	3 (2%)	5 (2%)	2 (1%)	
Native American	1 (1%)	7 (3%)	5 (2%)	
Asian	0 (0%)	1 (0%)	1 (0%)	
Other	0 (0%)	1 (0%)	0 (0%)	
Marital Status				
Single	9 (6%)	18 (7%)	30 (13%)	0.157
Married/live in partner	91 (64%)	164 (64%)	140 (59%)	
Widowed	6 (4%)	8 (3%)	3 (1%)	
Divorced	15 (10%)	53 (21%)	47 (20%)	
Education[a]				
<9 years	10 (7%)	11 (4%)	10 (4%)	0.007
10-12 years	48 (34%)	50 (20%)	61 (26%)	
Tech College/Some College	43 (30%)	91 (36%)	85 (36%)	
College Graduate	32 (22%)	76 (30%)	68 (29%)	
Other	3 (2%)	13 (5%)	6 (3%)	
Smoking Status				
Former/Current	113 (79%)	172 (67%)	187 (79%)	0.096
Never	22 (15%)	59 (23%)	43 (18%)	
Income				
<15,000	23 (16%)	39 (15%)	43 (18%)	0.383
16-25,000	24 (17%)	35 (14%)	37 (16%)	
26-35,000	23 (16%)	30 (12%)	24 (10%)	
36-50,000	16 (11%)	36 (14%)	36 (15%)	
51-75,000	7 (5%)	23 (9%)	31 (13%)	
>75,000	8 (6%)	19 (7%)	14 (6%)	
Don't Know/Refuse to Answer	34 (24%)	58 (23%)	41 (17%)	
NSAID				
Yes	31%	34%	39%	0.586
No	61%	56%	59%	

a= significant difference between case and biopsy negative controls

A comparison of quercetin intake in milligrams among the three subject groups reveals no statistically significant differences in consumption, p=0.625 (fig 4).

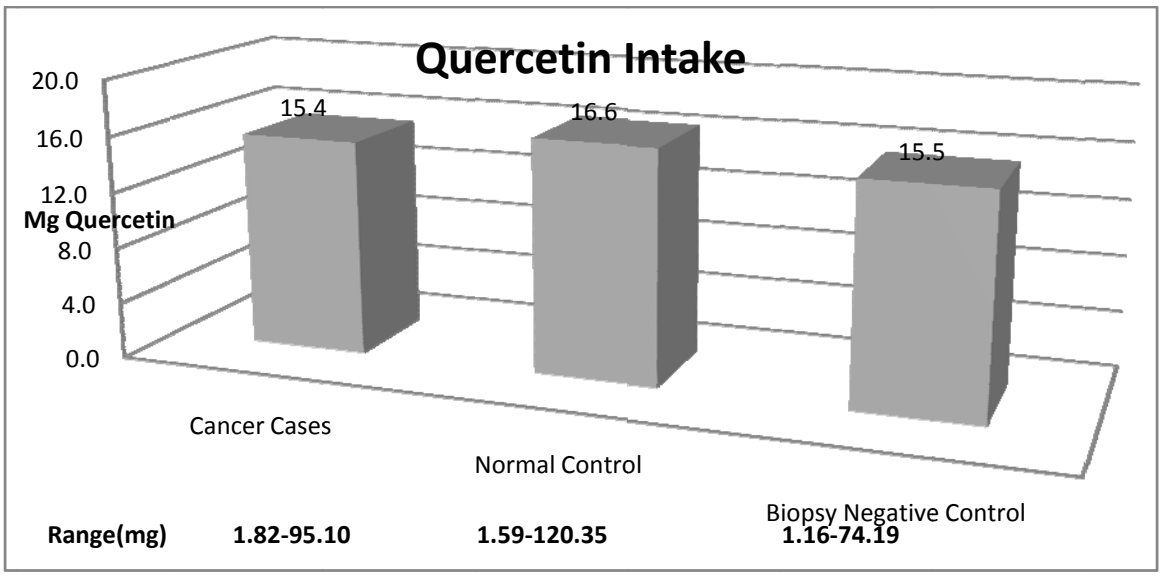

Figure 4- A Comparison of Quercetin Intake among Subject Groups

Average nutrient intake and standard deviations were calculated for all three subject groups. An ANOVA comparison for differences in consumption of selected nutrients between groups was performed and the p value for differences between the groups can be found in Table 3.

Table 3-Intake of Selected Food and Nutrients

Nutritional Variable	**Cancer Cases**	**Biopsy Negative Controls**	**Control**	**P value**
Quercetin (mg)	15.40 ± 14.83	15.54 ± 13.11	16.58 ± 13.91	0.625
Total Selenium (mcg)	119.97 ± 47.53	124.96 ± 51.74	127.37 ± 43.47	0.344
Total B12 (mcg)[c]	7.15 ± 3.55	6.84 ± 3.52	7.87 ± 3.92	0.007
Total Folate (mcg)[b]	400.09 ± 163.74	408.44 ± 158.83	441.66 ± 143.18	0.015
Fruit servings per day	2.48 ± 2.63	2.29 ± 1.58	2.61 ± 1.82	0.174
Vegetable svgs per day	3.75 ± 1.97	3.70 ± 1.87	3.84 ± 1.72	0.704
Ounces of Meat per day	4.93 ± 2.56	4.91 ± 2.66	5.36 ± 2.64	0.125
Total Vitamin D (mcg)	6.49 ± 3.82	6.10± 4.37	6.83 ± 3.54	0.122
Total Vitamin E (mg)	10.07 ± 4.83	10.32 ± 5.47	10.18 ± 4.57	0.884
Total Lycopene (mcg)	6981.94 ± 10671.45	6242.12 ± 8255.94	6436.81± 7241.92	0.704
Total Zinc (mg)	18.17 ± 8.56	17.68 ± 8.83	19.21 ± 8.37	0.136
Total Fat (g)	99.29 ± 46.63	102.72 ± 50.74	99.90 ± 45.08	0.727
Total Energy (kcal)	2421.82 ± 858.99	2404.87 ± 1004.27	2335.02 ± 891.71	0.662
Alcohol (g)[a]	6.94 ± 13.18	13.97 ± 34.27	8.75 ± 18.94	0.013
Vitamin C (mg)	126.32 ± 116.33	124.36 ± 80.51	143.31 ± 87.42	0.054
Tomato Servings	0.51 ±0.68	0.44 ± 0.49	0.45 ± 0.43	0.475
Total Dietary Fiber (g)	21.341 ± 8.88	21.52 ± 8.47	21.68 ± 6.65	0.919
Number of Dairy svgs	1.87 ± 1.42	1.91 ± 1.72	1.92 ± 1.35	0.961

a= significant difference between case and biopsy negative controls. b= significant difference between case and clinic normal controls. c=significant difference between biopsy negative controls and clinic normal controls

When comparing across the three subject groups: significant differences ($p \leq 0.05$) were found for alcohol consumption in grams between case and biopsy negative controls, $p=0.013$, vitamin B12 between the two control groups, $p=0.007$, and folate between case and clinic normal controls, $p=0.015$. Differences from tables 2 and 3 that failed to reach significance, but were considered to be significant enough to include as possible covariates in the models ($p \leq 1.00$) included: smoking status $p=0.096$, and vitamin C $p=0.054$.

Correlations were examined. Quercetin was found to be significantly correlated with number of vegetable servings (table 4). This indicated that the inclusion of both of these variables in the final model may not be warranted since they could be explaining the same variation, and it will thus reduce the significance of quercetin.

Table 4-Correlation Between Servings of Vegetables and Quercetin

	Number of Vegetable Servings	Quercetin
Number of vegetable Servings		
Correlation Coefficient	1	0.34
Significance	.	<.001
Quercetin		
Correlation Coefficient	0.34	1
Significance	<.001	.

Lycopene was considered to be a possible interaction factor with quercetin as well (Table 5). Both lycopene and quercetin are found in tomato products, and both exhibit antioxidant behavior.

Table 5-Correlation Between Servings of Tomatoes, Quercetin, and Lycopene

	Number of Tomato Servings	Lycopene	Quercetin
Number of Tomato Servings			
Correlation Coefficient	1	0.94	0.40
Significance	.	<.001	<.001
Lycopene			
Correlation Coefficient	0.94	1	0.35
Significance	<.001	.	<.001
Quercetin			
Correlation Coefficient	0.40	0.35	1
Significance	<.001	<.001	.

Despite the correlation between lycopene and quercetin, an exclusion of lycopene may not be warranted, since there could be a synergistic relationship between the two nutrients.

The odds ratios (OR) for all logistic regression models will be presented as highest quartile of quercetin intake/lowest quartile of quercetin intake. The most basic, age adjusted model for quercetin and prostate cancer can be found in table 6. There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. clinic normal controls, OR= 0.70, 95% CI (0.42, 1.17). There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. biopsy negative controls, OR= 1.00, 95% CI (0.56, 1.79).

Table 6-Age Adjusted Logistic Regression Model of Quercetin

	Prostate Cancer Versus Clinic Normal Controls	Prostate Cancer Versus Biopsy Negative Controls
Variable	**OR (95% CI)**	**OR (95% CI)**
Quercetin Quartile 1	Referent	Referent
Quercetin Quartile 2	0.87 (.52, 1.45)	0.92 (0.52, 1.62)
Quercetin Quartile 3	0.61 (.37, 1.02)	1.00 (0.56, 1.79)
Quercetin Quartile 4	0.70 (.42, 1.17)	1.00 (0.56, 1.79)
Age	0.97 (0.94, 1.00)	1.06 (1.03, 1.09)

When race was included in the model (table 7), there was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. clinic normal controls, OR= 0.71, 95% CI (0.39, 1.30). There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. biopsy negative controls, OR= 1.06, 95% CI (0.59, 1.89).

Table 7-Age and Race Adjusted Logistic Regression Model of Quercetin

	Prostate Cancer Versus Clinic Normal Controls	Prostate Cancer Versus Biopsy Negative Controls
Variable	**OR (95% CI)**	**OR (95% CI)**
Quercetin Quartile 1	Referent	Referent
Quercetin Quartile 2	0.80 (.44, 1.46)	0.96 (0.54, 1.71)
Quercetin Quartile 3	0.60 (.33, 1.08)	1.03 (0.57, 1.82)
Quercetin Quartile 4	0.71 (.39, 1.30)	1.06 (0.59, 1.89)
Age	1.03 (1.00, 1.06)	1.06 (1.03, 1.09)
Race	2.51 (1.02, 6.16)	1.64 (0.73, 3.67)

The full model, which includes adjustments for age, race, and folate intake, is shown in table 8. There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. clinic normal controls OR= 1.17, 95% CI (0.60, 2.30). There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. biopsy negative controls, OR= 1.24, 95% CI (0.64, 1.09).

Table 8-Multivariate Model Predicting the Likelihood of Prostate Cancer

	Prostate Cancer Versus Clinic Normal Controls	Prostate Cancer Versus Biopsy Negative Controls
Variable	**OR (95% CI)**	**OR (95% CI)**
Quercetin Quartile 1	Referent	Referent
Quercetin Quartile 2	1.13 (0.59, 2.14)	1.20 (0.65, 2.21)
Quercetin Quartile 3	0.81 (0.43, 1.53)	1.19 (0.64, 2.23)
Quercetin Quartile 4	1.17 (0.60, 2.31)	1.24 (0.64, 2.42)
Age	1.02 (0.99, 1.05)	1.06 (1.03, 1.09)
Race (white/non-white)	0	1.53 (0.65, 3.46)
Folate Quartile 1	Referent	Referent
Folate Quartile 2	0.39 (0.21, 0.73)	0.72 (0.39, 1.32)
Folate Quartile 3	0.42 (0.21, 0 .81)	0.46 (0.24, 0.86)
Folate Quartile 4	0.37 (1.90, 0.71)	1.00 (0.52, 1.94)

Testing for interactions found quercetin and lycopene, quercetin and smoking status, and quercetin and alcohol consumption to have significant interactions. Once the variables were entered into multinomial logistic regression they were determined to not be significant in relation to the complete model, due to a decrease in the p value of the -2 log likelihood statistic and were not included in the final analysis.

Alcohol and lycopene were still considered variables of interest, due to significant between group differences, as well as alcohol being a significant source of quercetin. It was thought that there may be an interaction between the alcohol content of the diet with quercetin, and further examination was warranted.

The full multivariate model was stratified by alcohol intake in grams (Table 9). There was no statistically significant association between quercetin intake and risk of prostate cancer for the lowest half of alcohol consumers for cancer cases vs. clinic normal controls OR=0.93, 95% CI (0.38, 2.29). There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. biopsy negative controls, OR= 0.87, 95% CI (0.34, 2.21). Nor was a statistically significant association seen for the highest half of consumers for cancer cases vs. clinic normal controls OR= 1.63, 95% CI (0.56, 4.77). There was no statistically significant association between quercetin intake and risk of prostate cancer risk cancer cases vs. biopsy negative controls, OR= 1.81, 95% CI (0.66, 4.98).

Although not statistically significant, an interesting pattern emerges where quercetin seems to have protective effect for the lower half of alcohol consumers, and an increased risk associated with quercetin intake with the higher half of alcohol intake.

Table 9-The Likelihood of Prostate Cancer, stratified by alcohol use

Zero to low alcohol consumption

	Prostate Cancer Versus Clinic Normal Controls	Prostate Cancer Versus Biopsy Negative Controls
Variable	**OR (95% CI)**	**OR (95% CI)**
Quercetin Quartile 1	Referrent	Referrent
Quercetin Quartile 2	0.72 (0.31, 1.72)	0.88 (0.37, 2.11)
Quercetin Quartile 3	0.65 (0.28, 1.52)	0.85 (0.36, 2.02)
Quercetin Quartile 4	0.93 (0.38, 2.29)	0.87 (0.34, 2.21)
Age	1.03 (0.99, 1.08)	1.08 (1.03, 1.13)
Race (white/non-white)	1.94 (0.52, 7.27)	2.70 (0.26, 12.39)
Folate Quartile 1	Referrent	Referrent
Folate Quartile 2	0.32 (0.14, 0.74)	0.59 (0.26, 1.36)
Folate Quartile 3	0.40 (0.16, 0.97)	0.43 (0.18, 1.01)
Folate Quartile 4	0.28 (0.11, 0.71)	1.88 (0.64, 5.51)

Moderate to High Alcohol Consumption

	Prostate Cancer Versus Clinic Normal Controls	Prostate Cancer Versus Biopsy Negative Controls
Variable	**OR (95% CI)**	**OR (95% CI)**
Quercetin Quartile 1	Referrent	Referrent
Quercetin Quartile 2	1.94 (0.71, 5.31)	1.88 (0.75, 4.69)
Quercetin Quartile 3	1.06 (0.39, 2.89)	1.92 (0.74, 4.98)
Quercetin Quartile 4	1.63 (0.56, 4.77)	1.81 (0.66, 4.98)
Age	1.01 (0.97, 1.06)	1.04 (1.00, 1.09)
Race (white/non-white)	2.83 (0.77, 10.41)	1.29 (0.46, 3.57)
Folate Quartile 1	Referrent	Referrent
Folate Quartile 2	0.48 (0.18, 1.28)	0.90 (0.36, 2.22)
Folate Quartile 3	0.43 (1.51, 1.20)	0.47 (0.18, 1.21)
Folate Quartile 4	0.48 (0.18, 1.29)	0.82 (0.33, 2.01)

When the full model was stratified by high and low lycopene intakes, an interesting pattern emerged (Table 10). It appears that low lycopene intake, coupled with higher folate intake has a significant reduction of risk associated with it. The physiology supporting this finding is nonexistent, and it may be an artifact of the analysis. The OR for quercetin failed to

reach statistical significance when stratified by lycopene intake. The lower 50th percentile of lycopene intake had no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. clinic normal controls OR= 1.35, 95% CI (0.48, 3.79). There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. biopsy negative controls, OR= 1.07, 95% CI (0.39, 2.93). The higher 50th percentile of lycopene intake had a non-statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. clinic normal controls, OR= 0.60, 95% CI (0.18, 1.96). There was no statistically significant association between quercetin intake and risk of prostate cancer for cancer cases vs. biopsy negative controls, OR= 1.58, 95% CI (0.54, 4.63).

Table 10-The Likelihood of Prostate Cancer, stratified by Lycopene

Zero to low lycopene intake

	Prostate Cancer Versus Clinic Normal Controls		Prostate Cancer Versus Biopsy Negative Controls	
Variable	**OR (95% CI)**		**OR (95% CI)**	
Quercetin Quartile 1	Referrent		Referrent	
Quercetin Quartile 2	1.39	0.60-3.26	1.34	0.60-3.01
Quercetin Quartile 3	1.06	0.45-2.50	0.80	0.36-1.79
Quercetin Quartile 4	1.35	0.48-3.79	1.07	0.39-2.93
Age	1.01	0.97-1.06	1.06	1.02-1.11
Race (white/non-white)	1.66	0.30-9.14	0.71	0.18-2.80
Folate Quartile 1	Referrent		Referrent	
Folate Quartile 2	0.36	0.16-0.83	0.54	0.25-1.17
Folate Quartile 3	0.26	0.10-0.67	0.42	0.17-1.02
Folate Quartile 4	0.12	0.04-0.40	0.53	0.19-1.90

Moderate to High Lycopene Intake

	Prostate Cancer Versus Clinic Normal Controls		Prostate Cancer Versus Biopsy Negative Controls	
Variable	**OR (95% CI)**		**OR (95% CI)**	
Quercetin Quartile 1	Referrent		Referrent	
Quercetin Quartile 2	0.56	0.17-1.82	1.31	0.45-3.82
Quercetin Quartile 3	0.37	0.11-1.22	1.93	0.63-5.94
Quercetin Quartile 4	0.60	0.18-1.96	1.58	0.54-4.63
Age	1.03	0.98-1.08	1.05	1.00-1.10
Race (white/non-white)	2.97	0.97-9.06	2.79	0.86-9.09
Folate Quartile 1	Referrent		Referrent	
Folate Quartile 2	0.72	0.23-2.27	1.23	0.39-3.90
Folate Quartile 3	1.13	0.34-3.68	0.58	0.19-1.82
Folate Quartile 4	1.02	0.33-3.20	1.31	0.42-4.05

The Gleason score first appeared in the literature in 1974, and was developed by Donald Gleason as a method to identify patients at immediate risk who might benefit from pre-treatment from those who are not (52). The Gleason score is a method of histological grading for degree of malignancy and was developed to aid in the correlation of clinical staging with survival. The Gleason score is based on over-all pattern of growth of the prostate tumor through examination at low magnification. There are two patterns of growth per tumor that are identified, and these are assigned corresponding digits, these are then combined with each other to produce the Gleason score, which can range from 2-10 (52). For each individual score (1-5) the characteristics (table 11) can be summarized as:

Table 11- The Gleason Score	
Grade	Description
1	The cancerous prostate closely resembles normal prostate tissue. The glands are small, well-formed, and closely packed.
2	The tissue still has well-formed glands, but they are larger and have more tissue between them.
3	The tissue still has recognizable glands, but the cells are darker. At high magnification, some of these cells have left the glands and are beginning to invade the surrounding tissue.
4	The tissue has few recognizable glands. Many cells are invading the surrounding tissue.
5	The tissue does not have recognizable glands. There are often just sheets of cells throughout the surrounding tissue.

A Gleason score of 2 is associated with the best prognosis, and a Gleason score of 10, the worst.

The Gleason scores of the cancer cases were used as an outcome variable, in Table 12 and Table 13, to see if quercetin had a different effect on risk of low grade as opposed to high

grade prostate cancer. In Table 12, high and low grade cancer cases were compared to biopsy negative controls. There was no statistically significant association between quercetin intake and risk of prostate cancer for high-grade prostate cancer vs. biopsy negative controls. OR= 1.67, 95% CI (0.66, 4.22). There was no statistically significant association between quercetin intake and risk of prostate cancer for low-grade prostate cancer vs. biopsy negative controls, OR= 1.08, 95% CI (0.50, 2.33).

Table 12-Cancer Cases vs. Biopsy Negative Controls-Stratification by Gleason Score

	Low-grade Prostate Cancer (Gleason=6) vs. Biopsy negative controls		**High-grade Prostate Cancer (Gleason>6) vs. Biopsy negative controls**	
	OR	**95% CI**	**OR**	**95% CI**
Quercetin Quartiles				
1	1	Referent	1	Referent
2	1.01	0.43-2.37	1.46	0.63-4.22
3	0.82	3.62-1.85	1.65	0.71-3.83
4	1.08	0.50-2.33	1.67	0.66-4.22

*model includes; race, age, and folate intake

In Table 13, high and low grade prostate cancer cases were compared to clinic normal controls. There was no statistically significant association between quercetin intake and risk of prostate cancer for high-grade prostate cancer vs. clinic normal controls. OR= 0.88, 95% CI (0.38, 2.04). There was no statistically significant association between quercetin intake and risk of

prostate cancer for low-grade prostate cancer vs. clinic normal controls, OR= 1.59, 95% CI (0.63, 3.98).

Table 13-Cancer Cases vs. Clinic Normal Controls-Stratification by Gleason Score

	Low-grade Prostate Cancer (Gleason=6) vs. Clinic normal controls		High-grade Prostate Cancer (Gleason>6) vs. Clinic normal controls	
	OR	**95% CI**	**OR**	**95% CI**
Quercetin Quartiles				
1	1	Referent	1	Referent
2	0.98	0.48-2.16	1.49	0.63-3.53
3	0.51	0.22-1.16	1.12	0.48-2.62
4	0.88	0.38-2.04	1.59	0.63-3.98

*model includes; race, age, and folate intake

INFLAMMATION

Inflammation data for the biopsy negative controls were used to compare the presence and absence of inflammation among this subset of subjects. Table 14 includes the simple, age adjusted model for quercetin with the presence of inflammation as the endpoint. There was no statistically significant association between quercetin intake and presence of inflammation in biopsy negative controls OR= 1.01, 95% CI (0.49, 2.04).

Table 14-Age Adjusted Model Predicting the Likelihood of Inflammation among Biopsy negative controls

Inflammation/ no inflammation (n=119/124)

Variable	OR	(95% CI)
Quercetin Quartile 1	1	Referent
Quercetin Quartile 2	0.78	0.39-1.57
Quercetin Quartile 3	1.23	0.60-2.51
Quercetin Quartile 4	1.01	0.49-2.04
Age	1.04	1.00-1.08

When the model was further adjusted for race, Table 15, there was no statistically significant association between quercetin intake and presence of inflammation in biopsy negative controls OR= 0.99, 95% CI (0.47, 1.98).

Table 15-Age and Race Adjusted Model Predicting the Likelihood of Inflammation among Biopsy negative Controls

Inflammation/ no inflammation (n=119/124)

Variable	OR	(95% CI)
Quercetin Quartile 1	1	Referent
Quercetin Quartile 2	0.74	0.36-1.51
Quercetin Quartile 3	1.25	0.61-2.56
Quercetin Quartile 4	0.97	0.47-1.98
Age	1.04	1.00-1.09
Race (white/non-white)	0.98	0.33-2.85

Examination of the full model, as seen in Table 16, shows that there was no statistically significant association between quercetin intake and presence of inflammation in biopsy negative controls OR=0 .95, 95% CI (0.41, 2.00).

Table 16-Multivariate Model Predicting the Likelihood of Inflammation among Biopsy Negative Controls

Inflammation/ no inflammation (n=119/124)

Variable	OR		(95% CI)
Quercetin Quartile 1		1	Referent
Quercetin Quartile 2	0.72		0.33-1.59
Quercetin Quartile 3	1.24		0.57-2.69
Quercetin Quartile 4	0.95		0.41-2.20
Age	1.04		1.00-1.09
Race (white/non-white)	0.97		0.33-2.86
Folate Quartile 1		1	Referent
Folate Quartile 2	0.93		0.43-2.01
Folate Quartile 3	1.12		0.51-2.48
Folate Quartile 4	0.90		0.38-2.16

Using inflammation as an endpoint, stratification of results by alcohol intake was performed. Table 17 shows the simple age adjusted model, stratified by alcohol as a binary variable, with the lowest 50% of consumers characterized as zero to low intake, and the highest 50% of consumers characterized as moderate to high intake. There was no statistically significant association between quercetin intake and presence of inflammation in biopsy negative controls for the subjects who consumed less alcohol OR= 1.67, 95% CI (0.57, 4.94), nor for the subjects who consumed more alcohol OR= 0.65, 95% CI (0.25, 1.74).

Table 17-Multivariate Model Predicting the Likelihood Inflammation, stratified by alcohol use

Zero to low alcohol consumption (n=105)

Inflammation/ no inflammation (n=53/52)

Variable	**OR**	**(95% CI)**
Quercetin Quartile 1	1	Referent
Quercetin Quartile 2	0.79	0.28-2.25
Quercetin Quartile 3	0.71	0.25-2.07
Quercetin Quartile 4	1.67	0.57-4.94
Age	1.00	0.94-1.07

Moderate to High Alcohol Consumption (n=138)

Inflammation/ no inflammation (n=66/72)

Variable	**OR**	**(95% CI)**
Quercetin Quartile 1	1	Referent
Quercetin Quartile 2	0.72	0.27-1.88
Quercetin Quartile 3	2.07	0.76-5.67
Quercetin Quartile 4	0.65	0.25-1.74
Age	1.09	1.03-1.15

When the full model was used to assess the impact of alcohol consumption (Table 18) as it interacts with quercetin and affects inflammation, there was no statistically significant

association between quercetin intake and presence of inflammation in biopsy negative controls for the subjects who consumed less alcohol OR= 1.21, 95% CI (0.32, 4.54). Additionally, there was no statistically significant association between quercetin intake and presence of inflammation risk for biopsy negative controls for the subjects who consumed more alcohol OR= 0.90, 95% CI (0.28, 2.86).

Table 18-Multivariate Model Predicting the Likelihood Inflammation, stratified by alcohol use

Zero to low alcohol consumption (n=105)

Inflammation/ no inflammation (n=53/52)

Variable	**OR**	**(95% CI)**
Quercetin Quartile 1	1	Referent
Quercetin Quartile 2	0.57	0.17-1.97
Quercetin Quartile 3	0.73	0.23-2.32
Quercetin Quartile 4	1.21	0.33-4.54
Age	1.012	0.95-1.08
Race (white/non-white)	1.86	0.16-22.36
Folate Quartile 1	1	Referent
Folate Quartile 2	0.83	0.27-2.59
Folate Quartile 3	1.31	0.39-4.39
Folate Quartile 4	2.44	0.44-13.55

Moderate to High Alcohol Consumption (n=138)

Inflammation/ no inflammation (n=66/72)

Quercetin Quartile 1	1	Referent
Quercetin Quartile 2	0.83	0.28-2.43
Quercetin Quartile 3	3.01	0.95-9.57
Quercetin Quartile 4	0.90	0.28-2.86
Age	1.08	1.02-1.15
Race (white/non-white)	0.84	0.23-3.06
Folate Quartile 1	1	Referent
Folate Quartile 2	1.33	0.43-4.08
Folate Quartile 3	0.83	0.27-2.53
Folate Quartile 4	0.49	0.15-1.55

DISCUSSION

Although there were no significant findings of an association between quercetin intake and prostate cancer nor was there an association between quercetin intake and inflammation in the current study, more research should still be conducted to examine a potential relationship. There were several factors which may have limited the ability of the present study to identify an association.

PROSTATE SPECIFIC ANTIGEN

Subjects were identified as normal controls, cases and biopsy negative controls based, in part on their PSA. Prostate cancer is often detected using prostate specific antigen (PSA) as a marker. PSA is produced by the prostate in both healthy and unhealthy men. The PSA is a blood test that if abnormal leads to biopsy. However, PSA is not entirely able to separate men who have prostate cancer, from those who do not (53). Some men with normal, less than 4 ng/mL have microscopic evidence of prostate cancer (54). Additionally, most screening guidelines do not recommend PSA screening in men with limited life expectancies because of potential harm associated with the screening process, as well as the fact that there is no evidence that PSA screening reduces prostate cancer mortality (55). Quercetin was not found to inhibit PSA production, in vitro using PC-3 cell lines when inhibition of PSA was defined as greater than 50%

blocking of DHT-induced PSA production (56). Thus, it is not known whether high quercetin would lower PSA in humans and bias our selection process of scoring by PSA.

One study was found to examine intensive lifestyle change in free living subjects on PSA. After one year of following a vegan diet, supplemented with soy, fish oil, vitamin E, selenium, vitamin C, moderate aerobic exercise, stress management techniques, and participation in a one hour a week support group, PSA levels dropped by .25 ng/mL from 6.23 to 5.98, whereas control group participants had a PSA score that was raised from 6.36 to 6.74 (57). Although the results of this study are statistically significant, it is not clear whether they are clinically significant. This is a major lifestyle change with little actual payoff, the PSA is still over 4 ng/mL. Based on this limited data, it seems unlikely that selecting subjects based on high PSA excludes those with a healthy lifestyle and high quercetin intake.

GLEASON SCORE

Subjects with prostate cancer were stratified by Gleason score in some analysis. Although the Gleason score can be beneficial in identifying patients who are at high risk and may benefit from more aggressive treatments, there are limitations to the utility of the Gleason score. One such consideration is the finding that, since its inception Gleason grading and scoring is largely subjective and the range of Gleason scores diagnosed has narrowed to where almost all patients today present with a Gleason score of 6, 7, or 8. This may in part be due to the subjectivity of the score, coupled to the fact that the medical expert responsible for

assigning the score are not all equally skilled in determining Gleason grades (58). Using Gleason scores may not have a wide enough variation to be able to detect an association between Gleason score and quercetin.

In addition to the use of the Gleason score, tumor staging is an important piece of information that was not used by the current project. Tumor staging refers to the determination of the spread and tissue involvement if the cancer. There are four stages I, II, III, and IV and there are several classification schemes that can be used to delineate stage. Stage I are the least advanced of the stages and these are accompanied by the best prognosis. The American Joint Commission on Cancer is a proponent of the TNM staging system. The T stands for the extent of the tumor, N is the extent of spread to the lymph nodes, and M is the presence of metastasis (59).

QUERCETIN CONTENT OF FOODS

Of primary significance is the limited information about which foods contain quercetin, and the amount of quercetin contained in these foods. Much of the preliminary research looking into the quercetin content of foods was performed in Europe, and looks at foods that are not typical to the US diet, such as regional berries and vegetables. Additionally, there are contradictory findings contained within the USDA data that is available regarding food that one would expect to have a similar level. In some foods the raw had higher quercetin content than the cooked, whereas others had a reversed relationship. There may be wide discrepancies in

the protocols used to identify and quantify the quercetin of these foods. Additionally, with such a wide variety of numbers, averages taken for use in the FFQ may have smoothed over some significant differences among individual diets.

One example of a limitation on the available information, and the ensuing extrapolation performed is within the category of legumes. The only information regarding legumes in the USDA information was regarding fava beans. A comparison between fava beans to pinto beans and lentils was made regarding percent calories from carbohydrate, fat and protein. It was determined that they were at least somewhat similar in macronutrient composition, with less than a 10% difference in macronutrient distributions. The quercetin content of fava beans was then used for all beans, which may not have been entirely accurate. Several food items with more similar macronutrient content had differing values for quercetin, such as green and red peppers.

Another possible limitation is that no information was available regarding grains and nuts, other than buckwheat flour, which is not considered a staple of the North American diet. Due to the lack of information regarding other foods groups, the quercetin intake was limited to fruits, vegetables, and some beverages. The men that consumed large amounts of grains or nuts, but low levels of fruits and vegetables, may have had higher intakes of quercetin than what was calculated due to this lack of information.

Recipe standardization was another potential reason for a lack of significant differences. Standard recipes were used for many mixed dishes, such as: stew, chili, burritos, and soup. Given that much of the quercetin in the US diet comes from onions, and tomatoes, there is a

potential for over reporting of quercetin consumption for men who do not like these foods and do not include them in their cooking, since they were often an ingredient on standardized recipes. Use of these recipes may also smooth over the inclusion of some ethnic and regional variations in meal preparation. For example, fresh dill has the potential to be a significant source of quercetin, although it is not typically consumed in large quantities. Hot peppers are also quite high in quercetin, if a subject particularly liked spicy foods, and thus added hot peppers, chili powder, or hot sauce to a large amount of food, it would not be detected using the DHQ.

Additionally, the use of a straight mean calculation when there was more than one corresponding food value from the USDA may have limited the current study's ability to detect quercetin intake. People do not eat all of the subcategories of food equally, and it may not have been reflective of true intake to use a non-weighted mean score.

The DHQ is a standard, widely used food frequency questionnaire that is capable of detecting intake of several macronutrients, micronutrients, and food components. The DHQ, is not specifically designed to determine phytochemicals content of foods. Because of this, some foods that contain quercetin were not queried, and some food groups that have a wide variation in quercetin content, such as berries, were collapsed into a mean average. The DHQ allows the participants to select from small, medium, and large serving sizes of fruits and vegetables. However, the participants of this study were typically answering medium, so it was decided to collapse all fruit and vegetable servings into the medium size, this may have also reduced the accuracy of measuring quercetin intake in the present study.

The above statements speak to the relative equality of quercetin consumption across all three subject groupings. There may have been significant differences in quercetin consumption between groups if a more specific tool was used to measure intake. The lack of significance of the OR of quercetin in relation to cancer status, including high and low grade cancer, as well as inflammation, may be in part due to there being no difference in quercetin intake as measured by the DHQ.

LIMITATIONS OF THE DIET HISTORY QUESTIONNAIRE

The DHQ is a lengthy document, and it typically takes 45 minute to one hour to administer the form. It is possible that subjects lost interest, or there was a selection bias in that only subjects who were feeling well enough, had enough time, and had sufficient energy to complete the DHQ were included in this study.

The reliance of self reported daily intake without a serum index to validate dietary intake can be considered questionable in this case. Although the food frequency questionnaire itself was validated, and FFQs are commonly used in epidemiological studies, serum quercetin would have strengthened the findings. Unfortunately, analysis of serum quercetin is still in its infancy, and a reliable protocol for use in this study was not produced. An analysis of serum quercetin in this study may not have been reliable due to a lack of fasting or non-fasting

requirements of the subjects. Given the short half life of quercetin, it may not have been a reliable estimate of typical quercetin consumption, and may have captured the amount of quercetin eaten in the prior meal.

USDA FLAVONOID CONTENT OF SELECTED FOODS

The USDA Database for the flavonoid content of selected foods, 2003 was created through a collaborative effort between the USDA and the Epidemiology Group, Jean Mayer USDA Human Nutrition Research Center on Aging, Frances Stern Nutrition Center, Tufts University School of Nutrition Science & Policy, and Tufts New England Medical Center, Boston, MA; the Bell Institute of Health and Nutrition, General Mills, Minneapolis, MN; and Unilever Bestfoods, North America, Englewood Cliffs, NJ. It was conducted in two phases, the first, was a literature search, and the second involved direct testing of 60 food items for flavonoid content. The second part of the process is ongoing. For analysis from the literature review to be accepted, "good separation from HPLC analysis" (50) was used as criteria, along with a 5 point quality index. The use of HPLC analysis typically converts quercetin from the glycoside form to the aglycone form, and that is what was tested for and reported on (50). The values for food were reported in gm per 100 grams of the edible portion. Beverages were reported as served, with respect to their specific gravities. The wide variation in data reported in the USDA report could be due to different lab techniques, as well as regional differences in the stress applied to

the plant, since plants produce flavanoids in response to stress. This makes the determination of differences between different types of foods in the same species difficult.

STRENGTHS

There were several key areas of strength with the current study. The case-control study design was an appropriate method for examining a rare disease, such as prostate cancer. The same registered dietitian conducted all of the FFQ interviews, and thus there was not a study bias in recording foods appropriately, or in translating verbal statements onto the FFQ sheet. Due to the fact that all interviews were conducted by a registered dietitian, the subjects were able to ask questions and get clarification, as opposed to self-administered questionnaires, where they may have left a confusing question blank, or answered it inappropriately. Similarly, all biopsy appointments were conducted by the same urologist, thus limiting bias during the appointment. All men were receiving primary care through the Portland VA medical center, thus limiting some potential geographic variation, and selecting a homogenous sample.

HOW THE CURRENT STUDY FITS INTO THE KNOWLEDGE BASE

An interesting finding of the present study is the correlation of lycopene, tomato servings consumed, and quercetin. The three variables are correlated, and one possible future hypothesis is that part of the difference between the significance of servings of tomato products, as opposed to lycopene supplementation, could be due to the presence of quercetin in tomatoes. Although lycopene did not meet the criteria of an interaction term, and stratified analysis did not yield a significant interaction with quercetin.

There is still much work to be done in examining the relationship between nutrition and prostate cancer. One powerful antioxidant that has received a lot of attention as of late is lycopene. Lycopene is a fat soluble carotenoid that is present in high concentrations on tomato products. One major function of lycopene is as a scavenger of reactive oxygen species, and it is the most prominent tissue carotenoid in the prostate (60).

Due to the nature of previous trials and research, it is difficult to ascertain whether the positive influence of diet and prostate cancer can be reduced down to the presence of a single nutrient, or a combination thereof. Additionally, there are numerous flavanoids and other bioactive components of food that have the potential to work synergistically with the nutrient in question. Leading to the question: is it the specific antioxidant or the food that contains the antioxidant?

Numerous studies have found a link between lycopene and decreased risk of prostate cancer. Tomatoes account for 85% of the consumption of lycopene in the American Diet,

making them the clear primary source of lycopene, but tomatoes also contain many other nutrients, including additional antioxidants, such as: quercetin, polyphenols, ascorbic acid, and α-tocopherol. In vitro studies that examined the effect of pure lycopene have shown positive antioxidant effects, but in vivo effects of pure lycopene is not supported in the literature, and most animal and human studies have looked at tomatoes and tomato products, which are also high in quercetin.

SUMMARY AND CONCLUSIONS

Thus far, examinations of quercetin and prostate cancer risk have focused mainly on in vitro studies of human prostate cancer cell lines and there has been little work looking at how quercetin is metabolized and used in free living human subjects. The literature is relatively consistent that there is an effect of quercetin administration and the attendant up regulation and down regulation of specific genes that control cell cycle, tumor suppression, and oncogenesis from in vitro studies. Currently there is no recommended chemopreventive dose that men are advised to consume, nor is there a plasma or cell concentration that provides positive benefits. There have been different findings of appropriate dose requirements for the modulation of different genes, receptor modulations, and protein expressions; and it has not been determined that therapeutic doses of quercetin are able to accumulate in the human prostate.

To date, there have been two epidemiological studies that examine the association between quercetin and prostate cancer risk, and no studies that examine quercetin kinetics in free living subjects consuming a mixed diet that have been published. The current study adds a piece of important information to the limited body of literature, since it is the first to attempt to examine quercetin as a primary predictor variable and prostate cancer as the primary outcome. The lack of statistically significant results from this study does not necessarily mean that there is not an association between quercetin intake and prostate cancer or inflammation. The lack of statistically significant results highlights the holes that need to be filled regarding the present

knowledge base of what foods have quercetin and the lack of a validated FFQ to examine quercetin intake.

Neither the hypothesis of the primary aim, that higher dietary intake of quercetin is associated with a reduction in prostate cancer risk, nor the hypothesis of the secondary aim, that higher dietary intake of quercetin is associated with a lower likelihood of inflammation were accepted. Future directions in quercetin research should include the development of a specific FFQ, continued testing of foods for quercetin content, reliable methodology for determining plasma quercetin, and more information regarding quercetin kinetics and absorption.

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APPENDIX A

Evidence Table for Quercetin and Prostate Cancer

Study Identification	Participants	Interventions	Outcomes
Nair H., Rao K., Aalinkeel R., Mahajan S., Chawda R., Scwartz S. (2004). Inhibition of Prostate Cancer Cell Colony Formation by the Flavonoid Quercetin Correlates with Modulation of Specific Regulatory Genes. *Clinical and Diagnostic Laboratory Immunology, Jan*, 63-69. RCT conducted in vitro,	PC-3, DU-145, LNCaP cells from the American Type Culture Collection.	Gene Arrays: Gene expression was analyzed using GEArray kits. Two arrays, one for quercetin treated and one for untreated samples were conducted simultaneously. Genes were hybridized using cDNA probes, washed and exposed to Kodak Biomac MS film, the autoradiograph was analyzed with gene tool software. PCR: relative abundance of each mRNA species was assessed by 5' fluorogenic nuclease assay to perform real-time quantitative PCR. Relative expression was calculated by the comparative Cτ method. Quercetin treatment consisted of 0 µM, 1.6µM, 3.1µM, 6.2µM, 12.5µM, 25µM and 50µM concentrations for colony formation. PC-3 cells were treated with 25µM to test for the modulation of the expression of cell cycle genes, upregulation, and downregulation.	PC-3 cell colony formation was suppressed to 114±20 ($p<0.001$) for 25µM quercetin and 59±13 ($p<0.0001$) for 50µM from 190±14 for untreated. Untreated DU-145 cells produced 99±5, and these were suppressed to 64±11 ($p<0.001$ for 25µM and 59±13 for 50µM concentrations. Lower concentration shad no effect on these types of cells and none of the quercetin concentrations had an effect on the less aggressive LNCaP cells. Cell cycle genes: Quercetin inhibited CCND1, CCND2, CCND3, CCNE1, CCNE2, CDK2, CDK4, E2F2, E2F3, CDK8, CDC7L1. PCNA, CCNF, CDC2, CDC16. 9 different tumor suppressant genes were up regulated by quercetin by more than 50%, these are:

			CBP, PTEN, MSH2p21, ciP1, p300, VHS, BRCA1, NF2,TSC-1, TGFβR1, ALK-5. Quercetin induced TGFβR2 , p53, Rb, p57Kip2, and TSC-2. Quercetin downregulated by 61% or more: akt-1, erb-2, bcr, c-myc.
McCann S., Ambrosone C., Moysich K., Brasure J., Marshall J., et al. (2005). Intakes of Selected Nutrients, Foods, and Phytochemicals and Prostate Cancer Risk in Western New York. *Nutrition and Cancer, 53*(1), 33-41. Case Control population based, Men with and without prostate cancer were administered a questionnaire.	433 subjects with prostate cancer, 538 without. Recruited from hospitals for case and DMV records and Health Care Finance Administration lists for controls	Questionnaire: medical and lifestyle factors, sociodemographic characteristics, height, weight, family history of prostate cancer were administered by trained nurses. Diet measured for 2 year period using food frequency questionnaire. USDA composition tables for selected foods were used to calculate nutrient intake.	Model one and two adjusted for age, education, BMI, cigarette smoking status, and total energy intake, the second model further adjusted for total vegetable intake. In regards to quercetin, there were significant risk reductions using model one, OR = 0.66; 95% CI = 0.47-0.94, but these results were attenuated using model two, OR =0.73; 95% CI = 0.49-1.09.
Paliwal S., Sundaram J., Mitragotri S. (2005). Induction of Cancer-Specific Cytotoxicity Towards Human Prostate and Skin Cells Using Quercetin ad	DU145 prostate cells, nonmalignant skin and cancer cells from a catalog, all cells grown on	These cells were applied with and without ultrasound prior to Quercetin exposure for 60 seconds, 0-50μM of quercetin was applied to wells with cells that were analyzed for viability after 48 hours. Malignant and	In the absence of ultrasound, quercetin had no significant impact on prostate cancer or normal cells as well as in the presence of

Ultrasound. *British Journal of Cancer, 92*, 499-502. RCT in vitro	monolayers.	nonmalignant cells were also treated with ultrasound and quercetin and then their hsp was analyzed using Western blots after 48 hours.	ultrasound.
Vijayababu M., Arunkumar A., Kanagaraj P., & Arunakaran J. (2006). Effects of Quercetin on Insulin-Like Grpwth Factors (IGFs) and their Binding Protein-3 (IGFBP-3) Secretion and Induction of Apoptosis in Human Prostate Cancer Cells. *Journal of Carcinogenesis, 5:10*. RCT in vitro using androgen independent prostatic carcinoma PC-3 cell line	PC-3 cell line purchased from the National Center for Cell Science, in Pune India, control and quercetin treated with 50µM and 100µM concentrations	Cell proliferation was assessed by Thanidine incorporation method. IGF-I, -II and IGFBP-3 secretion in conditioned media of control and quercetin treated PC-3 cells were quantitated immunoradiometrically.Bcl-2, Bcl-x, Bax, and Capsase-3 were analyzed using a Western Blot, a Beckman Vantage flow cytometer was used to assess cell cycle, DNA was extracted and quantified using UV-visible spectroscopy and electrophosphoresed, DNA strand breaks in apoptotic cells were measured using TUNEL.	There was a 50% growth inhibition with 100µM concentration of quercetin for PC-3 cells. IGFBP-3 and IGF-II secretion was significantly reduced for all control conditions, (25, 50, 75, and 100µM) at both 24 and 48 hours. IGF-I was reduced at 24 hours for 25 and 50 µM and 25 µM at 48 hours, but was not detectable for the other conditions. Quercetin treatment created a decrease in Bcl-2 and Bcl-x expression and increased the level of capsase-3. A concomitant increase in cells in the Sub G phase was observed in quercetin treated cells and an increase in the G2M phase was also seen. TUNEL showed that at 25 and 50 µM concentrations of quercetin, the percentage of apoptotic cells

			increased up to 10 fold.
Maggiolini M., Vivacqua A., Carpino A., Bonofiglio D., Fasanella G., Salerno M., Picard D., & Ando S. (2002). The Mutant Androgen Receptor T877A Mediates the Proliferative but Not the Cytotoxic Dose-Dependent Effects of Genistein and Quercetin on Human LNCaP Prostate Cancer Cells. *Molecular Pharmacology, 62(5),* 1027-1035. RCT in vitro	Human prostate cancer cells, LNCaP	LNCaP cells were cultured, fixed, amplified, and evaluated using a semiquantitative RT-PCR. They were then grown and exposed to ligands for 24 hours before lysis. ATP bioluminescence assay was used to evaluate cell proliferation.	Quercetin activates the AR Mutant T877A expressed in LNCaP cells, induces nuclear localization of AR in LNCaP cells, modulates the mRNA of AR, PSA, and PAP, upregulates AR expression, displays a biphasic effect on proliferation of LNCaP cells
Knekt P., Kumpulainen J., Jarvinen R., Rissanen H., Heliovaara M., Reunanen A., Hakulinen T., & Aromaa A. Flavonoid Intake and Risk of Chronic Diseases. *American Journal of Clinical Nutrition, 76*, 560-568. Observational study	10,054 men and women from Finland from population.	Multiphasic screening examination s in a mobile clinic. Food habits for one year prior to interview, residence, occupation, smoking, disease symptoms, medication use, height, weight and BMI at baseline were all assessed. Cancer was determined through the Finnish Cancer registry, 1093 new cancer cases were noted during a maximal follow up of 30 years. Cox proportional hazards model was used to estimate the	Total incidence of cancer was lower at higher quercetin intakes RR:0.77, 95% CI=0.65-0.92,

		strength of the association between flavanoids and disease risk.	
Yuan H., Pan Y., & Young C. (2004). Overexpression of C-Jun Induced by Quercetin and Resverol Inhibits the Expression and Function of the Androgen Receptor in Human Prostate Cancer Cell Lines. *Cancer Letters, 213,* 155-163. RCT in vitro	LNCaP Cells from the American Type Culture Collection that were treated with 0, 50 or 100 µM of quercetin	LNCaP cells were treated with 0, 50 or 100 µM of quercetin. They were then put through a Western Blot Analysis. Cell extracts were also prepared for luciferase assays. Additionally DNA binding was assessed through autoradiography	Quercetin inhibited the AR promoter. By deviations of 40% at 50µM and 35% at 100µM from 100% at control conditions. Quercetin caused a 2 fold induction of c-Jun at 50µM and a 7 fold induction at 100µM. p-c-Jun was induced 7 fold at 50µM and 35 fold at 100 µM
Yuan H., Gong A., Young C. (2005). Involvement of Transcription Factor Sp1 in Quercetin-Mediated Inhibitory Effect on the Androgen Receptor in Human Prostate Cancer Cells. *Carcinogenesis, 26(4),* 793-801. RCT using in vitro	Human prostate cancer cell lines, LNCaP and PC-3 obtained form the American Type Culture Collection	Transient transfection and luciferase reporter gene assays, western blot analysis, nuclear extracts, EMSA, Coimmunoprecipitation, and purification of GST fusion and GST pull down assays were all performed with cells treated with 100µM of quercetin	Sp1 regulated AR protein is repressed by quercetin, transactivation function of AR is inhibited by quercetin, Sp1 protein level is not decreased in the presence of quercetin, and quercetin repressed AR hyperphosphorylation.
Knowles L., Zigrossi D., Tauber R., Hightower C., & Milner J. (2000). Flavonoids Suppress	Human PC-3 cells purchased from the American Type	PC-3 control and case cells were plated and the controls were treated with DMSO. Cell proliferation was measured using a RPMI 1640	Treatment with 100µM of quercetin caused a 75% growth inhibition; 50µM caused 55% inhibition,

Androgen-Independent Human Prostate Tumor Proliferation. *Nutrition and Cancer, 38*, 116-122. RCT, in vitro	Culture Collection	suspension and proliferation was computed using a growth inhibition equation. Apoptosis and cell cycle analysis was conducted using a Coulter XL-MCl tabletop cytometer	25µM, 35% inhibition of PC-3 cell proliferation from control values. No change in cell cycle distribution was discovered for cells treated with quercetin alone, DNA fragmentation was not induced after exposure to 100µM of quercetin.
Ma Z., Huynh T., Ng C., Do P., Nguyen T, & Hyunh, H. (2004). Reduction of CWR-22 Prostate Tumor Xenograft Growth by Combnined Tamoxifen-Quercetin Treatment is Associated with Inhibition of Angiogenesis and Cellular Proliferation. *International Journal of Oncology, 24*, 1297-1304. In vitro RCT, animal study	Male SCID mice of 9-10 weeks old,	gavaged with 50, 100, and 200 mg/kg of quercetin for 28 days to determine optimal dose, Mice were then gavaged with 200 mg/kg a day to determine treatment effects. Tumor incidence was recorded daily, using a digital caliper, and the tumor volume was recorded using a formula. Fixed tumor tissues were subjected to immunohistological study and microvessel density was calculated. A western Blot was carried out to determine changes in expression levels of cell-cycle regulated proteins. VEGF levels in CWR22 prostate tumors were determined using semi-quantitative RT-PCR. Total RNA was extracted and quantitated using a spectrophotometer. The amplified DNA sequence was electrophoresed and VEGF	Quercetin at 200 mg/kg significantly reduced tumor weight (p=.031). Treatment with quercetin caused the first palpable tumor to be discovered at 12 days, as compared to 9 for the control. Final tumor volume was reduced by 51.1% and tumor weight decreased by 18.9% (p<.01) Ki-67 indices were reduced by 66.0% (p<.01) the total amount of cdcd-2 was not changed, but there were differences in the phosphorylation pattern, a band with slower electrophoretic mobility was noted in quercetin treated cells. Vessel density and size was not

		variants to each band were calculated.	changed from control to treatment with quercetin alone, and therefore there were no VEGF expression assays for quercetin alone.
Zand R., Jenkins D., Brown T., Diamandis E. (2002). Flavonoids can Block PSA Production by Breast and Prostate Cancer Cell Lines. *Clinca Chimica Acta, 317,* 17-26. RCT, in vitro	PC-3(AR)2 cells from the American Type Culture Collection.	PC-3(AR)2 were grown and were incubated with 10^-5 and 10^-8 mol/l of flavanoids, controls were incubated with nilutamide at 10^-7 mol/l. PSA production was then tested for dose-response activity, estradiol was tested. PSA was quantified using an ELISA-type immunofluorometris procedure.	Inhibition of PSA was defined as greater than 50% blocking of DHT-induced PSA production. Quercetin was not found to inhibit PSA production.
Nakanoma T., Ueno M., Iida M., Hirata R., & Degucji N. (2001). Effects of Quercetin on the Heat-Induced Cytotoxicity of Prostate Cancer Cells. *International Journal of Urology, 8*, 623-630. In vitro RCT study design.	PC-3, LNCaP, and JCA-1 cancer cell lines.	Quercetin, heat, and heat plus quercetin were evaluated for their effects. Quercetin was used in a 50µmol/L concentration. A MTT reduction assay was used to evaluate cytotoxicity. Cell proliferation was monitored through counting cell numbers on a hemocytometer after trypsinization. Alterations in cell cycles were evaluated through a flow cytometer. An apoptosis detection kit was used to determine the extent of apoptosis after treatment.	Treatment with quercetin alone was found to inhibit the growth of JCA-1 and LNCaP cells at a concentration of 12.5µmol/L and PC-3 at 50µmol/L. At 50µmol/L quercetin alone was found to reduce the number of surviving JCA-1 and LNCaP cells at 24 hours and at 48 hours the number of surviving PC-3 cells was reduced. Quercetin also enhanced the heat induced cytotoxicity against JCA-1 and

			LNCaP, but not PC-3 lines. Quercetin decreased the number of S phase cells in JCA-1 (50.9%-30.2%) and LNCaP (25.8%-10.6%) but not in PC-3. Quercetin resulted in a decrease of hsp70-positive cells in the JCA-1 line (98%-85%) as well as in LNCaP (94%-74%). Quercetin produced an increase in subG1 cells JCA-1 (1%-11%_ and LNCaP (3%-21%) there was no significant change in PC-3 lines. The number of late apoptotic cells increased in response to quercetin in the JCA-1 line (3.1%-10.9%) and the number of early apoptotic cells in the LNCaP line increased (1.1%-3.9%), but there was no effect on PC-3 cells.
Shenouda N., Zhou C., Browning J., Ansell P., Sakla M., Lubahn D., & Macdonald R. (2004). Phytoestrogens in Common Herbs Regulate Prostate Cancer Cell Growth	PC-3 and LNCaP cells were obtained from the ATCC	A total cellular protein concentration was determined following DHT treatment to form a growth inhibition curve; Total cellular protein concentration was measured. Flow cytometry was used to examine cell cycle kinetics. Apoptosis was	Quercetin led to an eventual reduction in cellular concentration of proteins to 0%of controls with a 100µM dose. For LNCaP and a reduction to 20% for PC-3 cells. IC50 was determined to be 50 for pc-3 and 25 for

in Vitro. *Nutrition and Cancer, 46*, 200-208. RCT, in vitro		detected using an APO-Direct apoptosis kit and analyzed using flow cytometry. DNA fragmentation was evaluated using a UV transilluminator. Western blots were performed.	LNCaP cells. A time course study of growth inhibition revealed that for PC-3 there was a 49.5± 2.3 at day 1, a 50.1± 1.0 at day 2, and a 50.5±1.2 at day 3 reductions. In LNCaP there was a 48.6±1.2 reduction at day 1, a 49.7±3.1 reduction at day 2 and a 50.6±3.5 reduction at day 3. Cell cycle changes include a 62.6±1.9 change for $G_{1,}$ a 8.0±0.6 change in S, and a 19.6±0.8 change in G_2M for PC-3 lines. A 66.5±1.5 change in G_1, a 6.3±0.6 change in S, and a 27.3±1.8 change in G_2M was observed in LNCaP cells. The percentage of apoptotic events was increased by 43±1.47 in LNCaP and 32.5±1.42 in PC-3 cells. The counts per minute in the control cells were 2350, with quercetin they were 475.
Krazeisen A., Breitling R., Moller G., & Adamski J. (2001). Phytoestrogens Inhibit Human 17β-	17β-HSD 5	An enzyme reaction was used to examine the conversion of androstenedione to testosterone as well as androstanediol to	The IC_{50} for androstenedione to testosterone was 9µM. For androstanediol to androtestosterone

Hydroxysteroid Dehydrogenase Type 5. *Mollecular and Cellular Endocrinology, 171,* 151-162. RCT, in vitro, human		androtestosterone and IC_{50} values of phytoestrogens was obtained	was 5µM
Vijayababu M., Arunkumar A., Kanagaraj P., VenkataramanP., Krishnamoorthy G., & Arunakaran J. (2006). Quercetin Downregulates Matrix Metalloproteinases 2 and 9 Proteins Expression in Prostate Cancer Cells (PC-3). *Mollecular and Cellular Biochemistry, 287*, 109-116. in vitro	PC-3 cells	Cells were treated with 0, 25, 50, 74 and 100µM of quercetin. Cell proliferation was assessed using thymidine incorporation method, and the samples were counted using 1409 Wallac DSA liquid scintillation counter. Antigens were detected using western blot assays.	PC-3 cells showed a significant decrease in thyamidine uptake, 3-4 fold. Time response data show a 50% growth inhibition at 100µM for 24 hours. MMP-2 and MMP-9 proteins were significantly decreased in PC-3 cells in a dose dependent manner. Pro-MMP-9 was significantly increased at 100µM. Quercetin decreased the activities of these proteins and an increase in pro-MMP-9 at 100µM
Kachadourian R., Day B. (2006). Flavonoid-Induced Glutathione Depletion: Potential Implications for Cancer Treatment. *Free Radical Biology & Medicine, 41*, 65-	PC-3 cells, A549 and HL-60 cells	10mM stock solution of quercetin was used. Mitochondria were isolated and a variety of tests were performed. Intracellular levels of GSH were determined, extracellular and mitochondrial levels of GSH were determined.	Intracellular levels of GSH in A549cells were undetectable 2 hours after treatment with quercetin and were 51.0±0.7 % as compared to control 4 hours after treatment. The percent

76. In vitro		MRP1 and cytochrome c were detected using western blots. Percentage LDH was calculated and flow cytometry was performed.	concentration of intracellular GSH was 36, compared to 25 in controls and the percent concentration of LDH was 175 compared to 75 in A549 cells. The percent of intracellular GSH was 94 compared to 50 and LDH was 157 compared to 50 in HL-60 cells. The percent of intracellular GSH was 95, compared to 25, and LDH was 80 compared to 50 in PC-3 cells. Although additional tests were run using a variety of flavanoids, results for quercetin specifically were not given.
Vijayababu M., Kanagaraj P., Arunkumar A., Iiangovan R., Aruldhas M., Arunakaran J. (2005). Quercetin-Induced Grpwth Inhibition and cell death in Prostatic Carcinoma Cells (PC-3) are Associated with Increase in p21 and Hypophosphorylated Retinoblastoma Proteind Expression.	PC-3 cells	Treated with 50 and 100µM concentrations of quercetin for 24 hours. Cell proliferation was assessed by thymidine incorporation. Quantitation of cell cycle distribution was performed using flowcytometry analysis. Western blots were run	PC-3 cells showed a significant decrease in thymidine uptake that was magnified by duration and concentration of dose. Western blot analysis showed that quercetin induced the expression of p21/Cip1 and increased the expression of Cdc2/Cdk-1, cyclin B1 and produced no change in cyclin A protein. There was an

Journal of Cancer research and Clinical Oncology, 131, 765-771. In vitro, RCT			increase in hypophosphorylated levels of pRb in a dose dependent manner, but there was no change in the pRb2/p130 protein levels. The level of Bax increased markedly in a dose dependent manner. The level of expression. The level of expression of Bcl-2 and Bcl-X_L was significantly decreased. After 24 hours the amount of apoptotic cells increased 10 fold for both treatment concentrations
Huynh H., Nguyen T., Chan E., & Tran E. (2003). Inhibition of ErbB-2 and ErbB-3 Expression by Quercetin Prevents Transforming Growth Factor Alpha (TGF-α)-and Epidermal Growth Factor (EGF)-Induced Human PC-3 Prostate Cancer cell Proliferation. *International Journal of Oncology, 23*, 821-829.	PC-3, LNCaP cells from the American Type Culture Collection	Cell number and thyamidine incorporation were examined; a western blot was performed to determine the effects of quercetin on ErbB-3, ErbB-2, PI-3K, and Ras/Raf/MAPK pathways. Quercetin was given at concentrations of 0, 14.5, 29, and 59μM/ml	Quercetin significantly inhibited both PC-3 and LnCap cell numbers, as early as 24 hours post treatment; a dose dependent reduction in DNA synthesis in PC-3 cells was also noted 24 hr after treatment, the magnitude of growth inhibition appeared to be greater for the faster growing PC-3 cells than the LnCap cells. ErbB-3, ErbB-2 levels were both reduced by quercetin

in vitro RCT			treatment. C-Raf-1 was not affected by quercetin; phosphorylated MEK2 was increased three fold by quercetin treatment. P85 subunit and Akt-1 did not change. At 29µM quercetin blocked EGF- and TGF-α-induced PC-3 cells.
Morris J., Pramanik R., Zhang X., Carey A., Ragavan N., Martin F., & Muir G. (2006). Selenium or Quercetin-Induced Retardation of DNA Synthesis in Primary Prostate Cancer Cells Occurs in the Presence of a Concomitant Reduction in Androgen-receptor Activity. (2006). *Cancer Letters, 239*, 111-122. In vitro, RCT	Primary prostate epithelial cells isolated from tissue following surgical resection and LNCaP cells.	Cells were stained for BrdU to examine DNA synthesis. A luciferase assay kit was used to determine luciferase activities normalized by protein concentration. Protein bands were visualized following an immunoblot analysis of AR expression. Cells were incubated at different concentrations of quercetin prior to all of these treatments.	Clear dose related reductions in the proportion of FITC-stained nuclei to PI stained nuclei were observed. A dose related reduction in %BrdU incorporation was observed (43.9 ±6.9%). Ar-reporter gene activity was reduced by almost 50%
Brusselmans K., Vrolix R., Verhoeven G., & Swinnen J. (2005). Induction of Cancer Cell Apoptosis by Flavanoids is Associated with	Human LNCaP from ATCC, and MDA-MB-231 cells	Cultured with quercetin. Transfection of LNCaP cells with RNA, Acetate incorporation assay and TLC analysis was conducted. FAS activity, an immunoblot assay, a proliferation cytotoxicity assay, and	Quercetin had a dose dependent inhibition of lipogenesis in LNCaP cell lines, there was a decrease in lipid synthesis, there was a similar effect in MDA-MB-231 cells. 100µM

Their Ability to Inhibit Fatty Acid Synthase Activity. *The Journal of Biological Chemistry, 7*, 5636-5645. in vitro RCT		Hoechst staining was conducted.	caused a 19% reduction in FAS activity, but western blot revealed that FAS protein levels were not influenced, the synthesis of phospholipids was affected. There was a dose response relationship observed with proliferation. Cell death was indued from approx 3% stained to 17% stained in MDA-MB-231, and 7-27% stained in LNCaP in 24 hours.
Kobayashi T., Nakata T., & Kuzumaki T. (2002). Effect of Flavonoids on Cell Cycle Progression in Prostate Cancer Cells. *Cancer Letters, 176*, 17-23. in vitro	Human LNCaP prostate carcinoma cells	Quercetin treated10, 20, 30 µM and untreated control cells were analyzed using flow cytometry, immunoblot assays for total protein, p21, Cyclin-B-associated and cdc2-associated histone H1 kinase activities. Isolation of nuclei was assessed using gel retardation.	Quercetin did not significantly affect cyclin B expression, quercetin did not induce p21, nor did it alter the amounts of p27

APPENDIX B

Food ID	Name	Food energy - kcal	Mg of Quercetin in 100 gm edible portion
49	Beef stews/pot pies/mixtures	510.82	0.794
52	Beef, gr, meatballs/loaf/mixtures	303.32	0.794
78	Chicken, mixtures	397.53	0.794
92	Soups, broth w ndles/rice	155.7	0
93	Soups, w veggies	186.63	0.14
94	Soups, bean-type	203.7	1.19445
95	Soups, creamed	201.85	0.77313
113	Pies, fruit	338.77	1.955
117	Crisps/cobblers	370.77	1.8238
137	Pasta, meatless red sauce	232.22	0.2275
138	Pasta, meat/fish sauce	378.45	0.2275
139	Lasagna, rav, shells, etc	517.45	0.2275
141	Pasta salad	273.01	1.006
142	Pizza, with meat	471.7	0.06759
143	Pizza, without meat	386.35	0.08412
145	Mexican mixtures, all	401.19	2.4325
146	Oranges, tangelo etc	58.49	0.19
147	Grapefruit, all	41.53	0.5
148	Apples	77.96	2.96
149	Applesauce/ckd apples	102.97	2
150	Pears	95.41	0.42

151	Peaches/nectarines/plums	54.72	0.4
157	Strawberries	21.36	0.545
158	Grapes, all	73.49	2.317
159	Dried fruit, no apricots	81.1	27.499
160	Apricots, dried	40.44	18.68
161	Fruit salads/other fruits	53.25	2.406
162	Orange/grpfrt jce, all	99.43	0.08
163	Other juice	126.29	3.716
164	Tomato/veg juice, all	45.48	1.46
165	Beans, NFA	148.1	0.55
166	Beans, fat added	222.44	0.55
167	Chili	323.54	1.8609
168	Potatoes, white, NFA	114.57	0.01
169	Potatoes, fried	265.83	0.01
170	Potato salads	248.73	0.15959
172	Lettuce, NFA	4.82	1.87
173	Pickled veg/frt	14.53	0.04
174	Raw spinach/greens	5.02	4.325
175	Ckd spinach/greens, NFA	30.81	4.5
176	Broccoli, NFA	24.63	2.135
177	Carrots, NFA	12.98	0.035
178	Tomatoes, raw	9.32	0.895
179	Tomato salsa	12.22	4.03957
180	Tomato catsup	18.16	0.86

181	String beans, NFA	31.36	2.125
182	Cabbage/sauerkraut	23.32	0.0975
183	Coleslaw	129.9	0.0609
184	Peas, NFA	64.2	0.12
186	Caulifl/Br Spr, NFA	10.64	0.165
187	Peppers, NFA	6.31	0.325
188	Peppers, hot	20.11	19.415
189	Onions, NFA	7.11	16.7
191	Veg med, NFA	63.55	0.05167
192	Other vegetables	12.93	0.19667
239	Jams, jelly, reg	38.64	0.58
240	Coffee, reg, no cr/sug	10.39	0.05
242	Tea, reg, no cr/sug	5.38	2.02
243	Tea, decaf, no cr/sug	4.51	2.805
251	Beer	217.91	0.05
252	Wine	176.72	0.3

APPENDIX C

Sample Calculation for Bean Based Soup

Recipe-Lentil Soup from *The Joy of Cooking*

Ingredient	**Weight of Ingredient (grams)**	**Quercetin (mg)per 100 grams**	**Quercetin (mg) in recipe**
Carrot	183	0.07	0.1281
Celery	120	3.5	4.2
Onion	150	19.36	29.04
Tomato	396.893	4.12	16.3494
Lentils	990	0.55	5.445
Water	2267.96	0	0
Total Weight of Recipe	**4107.853**	Total Quercetin in Recipe	**55.1625**
Milligrams quercetin per 100 grams of recipe	**1.343**		