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The association between urinary phytoestrogen levels and prostate-specific antigen levels in men aged 40 and over in the US population

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Abstract

The association between urinary phytoestrogen levels and prostate-specific antigen levels in men aged 40 and over in the US population

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Primary Objective: To determine if there is an association or correlation between phytoestrogenic levels in men aged 40 and older and their PSA levels.

Secondary Objective: To confirm the findings of previous studies that suggest a positive association between phytoestrogens and PSA levels in men.

Research Design and Methods: The sample analyzed is available in the 2009-2010 National Health and Nutrition Examination Survey (NHANES) dataset and includes 676 male subjects age 40 and over after excluding those with missing data for phytoestrogens. Serum PSA concentration was considered the dependent and urinary phytoestrogen levels were consider independent variables. Six phytoestrogens were analyzed. These included daidzein, equol, genistein, enterodiol, enterolactone, and O-Desmethylangolensin (O-DMA). Possible confounders included race/ethnicity, smoking status, age, educational level, poverty-income ratio (PIR), HDL, LDL, and total cholesterol, and triglycerides. These variables were confounders because each has been correlated and/or associated with increased prostate cancer risk and can possibly be associated with phytoestrogen levels.

Results: No statistically significant associations between phytoestrogen levels and PSA were found.

Conclusions: In a small sample of the US adult male population aged 40 and over, it appears that phytoestrogenic levels have no association with PSA levels.

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Introduction

An inverse association between the concentration of phytoestrogen and prostate-specific antigen (PSA) levels among men aged 40 and over has been suggested in various studies. That is, the greater the concentration of serum and, by extension, urinary phytoestrogen, the lower the PSA level. Because of the strong correlation between elevated PSA levels and elevated prostate cancer risk, PSA levels can be viewed as a proxy for the cancer risk.

The link between androgenic stimulation and the severity of prostate cancer has been well documented. This was first shown through the landmark studies of Huggins and Hodges in 1939. They demonstrated prostate cancer regression by withdrawing androgenic stimulation. Further work has shown that estrogen treatment of men with metastatic prostate cancer causes regression in disease by reducing androgenic stimulation. Phytoestrogen may mimic the behavior of estrogen and lower androgen stimulation.

The clinical observation of Huggins and Hodges first demonstrated that prostate cancer is a hormone-regulated disease and that hormone manipulation can treat the disease. Carcinogenesis is a process over time and promoted by androgenic stimulation. There is laboratory, epidemiologic and clinical data to suggest that prolonged androgenic stimulation of the prostate can cause prostate cancer. A number of laboratory animal studies have administered an initiator followed by androgen therapy resulting in a portion of the laboratory animals developing

prostate cancer.[1,2] Several case control and cohort studies suggest that men with higher serum testosterone levels over time are at higher risk for prostate cancer diagnosis. Two large prospective randomized placebo controlled clinical trials have demonstrated that reducing androgenic stimulation of the prostate decreases the tendency of prostate cells to become cancerous.[3]

The hypothesis of this paper is that high serum phytoestrogen levels may correlate with lower androgenic stimulation and lower PSA levels. Higher phytoestrogen levels and lower androgenic stimulation of the prostate in turn may translate into lower risk of prostate cancer.

PSA level has been shown to be a rough proxy for prostate cancer risk. Several previous epidemiologic studies suggest that those who have higher phytoestrogen levels may have lower risk of prostate cancer.[4,5] Direct assessment of prostate cancer risk is beyond the scope of this study. A positive finding will merit further assessment and could lead to a recommendation that men increase dietary phytoestrogen intake to reduce risk of prostate cancer.

In the US, prostate cancer is the most commonly diagnosed visceral cancer and 2nd leading cause of cancer death in American men.[6] In fact, prostate cancer is only surpassed by skin cancer as the most frequently diagnosed cancer in American men.[7] Prostate cancer represents approximately 14.4% of all visceral cancers diagnosed in men each year.[8] The American Cancer Society estimates

that 233,000 men will be diagnosed with prostate cancer and 29,840 will die of the disease this year.[6] About 1 in 7 American men will be diagnosed with prostate cancer in their lifetimes and about 1 in 36 shall die from the disease.[7]

Literature Review

Prostate Cancer Risk Factors

Risk factors for prostate cancer include age, race/ethnicity, family history, history of physical activity, obesity, diet and smoking.

Prostate cancer is largely a disease of older men, with a median age of diagnosis of 66 and approximately 6 out of 10 cases occurring among men 65 and older.

Race is generally considered to be a sociopolitical categorization and loosely correlates with area of geographic origin. Prostate cancer is most prevalent in Black African-American and Afro-Caribbean men. In 2012 African-Americans have had an age-adjusted incidence that is 1.59 times that of white Americans and age-adjusted mortality rates 2.39 times higher than whites.[8]

Family history is also a risk factor. Studies suggest that two 1st degree relatives increase risk of diagnosis by a factor of 5 and 3 or more 1st degree relatives increases risk by 11 times.[9]

Factors such as, smoking, exercise, weight, and diet have been shown to influence the progression and severity of the cancer.

The association between smoking and prostate cancer is complicated. In a study involving 5366 prostate cancer patients, Kenfield et al found that smokers had an increased mortality rate from prostate cancer compared to nonsmokers (HR = 1.61; 95% CI =).[10] A second study initially showed no statistically significant correlation between smoking and prostate cancer diagnosis but with refinement of the data, heavier smokers were found to have increased risk of prostate cancer compared to light smokers (cigarettes per day or years: RR = 1.22; 95% CI = 1.01, 1.46; pack years of smoking: RR = 1.11; 95% CI = 1.01, 1.22) and current smokers had an increased risk of fatal prostate cancer compared to nonsmokers (RR = 1.14; 95% CI = 1.06, 1.19).[11] The heaviest smokers were 1.24-1.3 times more likely to die from cancer than nonsmokers. The correlation between smoking and prostate cancer that appeared after stratification appeared so strong that even subjects who qualified as past smokers had elevated risk (RR = 1.09; 95% CI = 1.02, 1.16) when compared to nonsmokers. In a large meta-analysis of case-control and prospective studies published from 1966 to 2000, smoking appeared not to be a risk factor for prostate cancer diagnosis but was a risk factor for death from prostate cancer.[12]

In regards to exercise, physical activity apparently has a correlation with the cancer's aggressiveness. Studies suggest that men who are physically active tend to have less aggressive prostate cancer. This may be a healthy volunteer effect in which those concerned about health exercise are more likely to be screened for prostate cancer. Screening does increase the risk of prostate

cancer diagnosis and specifically the diagnosis of less aggressive more indolent cancer. In a prospective cohort study, two cohorts of PCa patients from Seattle and the state of Connecticut were observed for 15 years (1986-2001). The Seattle area utilized PSA screening far more intensively than Connecticut in the early PSA era (1987-90). The data showed that the Seattle cohort had significantly greater incidences of radical prostatectomy and radiotherapy than their Connecticut counterparts (OR = 5.2, 95% C.I. = 3.22-8.42)[13], clearly supporting the notion of PSA screening increasing PCa diagnosis risk. In the Health Professionals Follow-up Study, a cohort of 47,620 US health professionals was observed from 1986 to 2000. Although there was no significant association found between incidence and exercise, there was a lower risk for advanced prostate cancer and death from prostate cancer among men aged 65 or older who had significant exercise history (RR = .33, 95% C.I. = .17-.62 for advanced disease) and (RR = .26, 95% C.I. = .11-.66 for death from prostate cancer).[14]

Dietary intake and dietary components such as fat, cholesterol, etc. have been shown to be associated with PCa risk. There is data that suggests that a diet rich in beans, lentils, peas, raisins, dates, and other dried fruits were associated with a significant reduction in the risk of prostate cancer development.[15] In regards to dietary components, there is evidence suggesting that fat is linked to PCa risk. In one study, higher fat intake was associated with an increased PCa risk (RR = 1.79, C.I. = 1.04–3.07; p =.06) after adjustment for age and energy intake.[16] In a

cross-sectional study involving 950 Japanese men, it was found that among those who did not take statins, subjects with triglyceride levels greater or equal to 150 mg/dL had a 66% increased risk of prostate cancer diagnosis compared those with lesser levels (95% C.I. 1.21–2.29, p = .002).[17] Furthermore, the researchers discovered a direct correlation with increasing triglyceride levels and cancer risk.

Another dietary component, cholesterol, may also be correlated with PCa risk. In a study conducted by Murtola and associates, 3 prostate cell lines were cultured; normal epithelial prostate cells, prostate cancer cells, and immortalized prostate cells. LDL cholesterol administration resulted in an increase in the number of cancer cells and a slight decrease in the number of normal epithelial prostate cells.[18] In a separate study, prostate cancer risk, total serum cholesterol, and HDL cholesterol were examined and it was shown that high total cholesterol levels (i.e. levels greater than or equal to 240 mg/dl as opposed to levels lower than 200) were associated with an increased overall risk (HR = 1.22, 95% C.I. 1.03-1.44, p = .01) as well as an increased risk for advanced prostate cancer (HR = 1.85, 95% C.I. 1.13-3.03, p = .05) while higher HDL cholesterol was associated with a decreased PCa risk.[19] In a study by Mondul et al, spurred by the observed decrease in cancer risk among users of statins, which is associated with a cholesterol reduction, researchers concluded that subjects with lower cholesterol levels (<200 mg/dl) were less likely to develop advanced cancer than those with

high cholesterol (greater or equal to 240 mg/dl), with a RR = .68 (95% C.I. .4 – 1.18, p = .12).[20]

Prostate Cancer as an Androgen Driven Disease

There is strong indication that PCa is an androgen driven disease. Huggins and Hodges' research produced the earliest evidence demonstrating that prostate cancer is an androgen sensitive disease. In one study, eight prostate cancer patients were castrated and underwent measurement for acid phosphatase levels before and after the castration. A higher level of acid phosphatase correlates with prostate cancer progression. After the castration, drastic drops in acid phosphatase activity were observed, which is correlated with PCa regression.[21]

In a later study, Huggins' and Hodges produced further support for the link between androgenic stimulation and PCa progression. In this study, patients, following castration, were injected daily for 2 weeks with 25 mg of testosterone and displayed an increase in acid phosphatase activity by 3-5 times the previous amount.[21]

Although the exact role of androgens in the development of prostate cancer is currently unknown, there is much evidence suggesting it is a prostate cancer promoter. This is demonstrated in a lab study in which injection of cancer initiator

followed by prolonged androgenic stimulation resulted in development of PCa and tumor progression in the lab rats.[22]

Prostate cells are so dependent on androgen, in fact, that some studies suggest that some prostate cells produce some androgens themselves to supplement the levels already available. A study performed by Dillard and colleagues demonstrated that undifferentiated prostate cancers could produce androgen from cholesterol, revealing a possible mechanism behind the cholesterol-PCa risk association.[23]

Additional evidence that androgenic stimulation is linked to prostate risk is provided by a case control study done by Hyde and colleagues. They show a positive association between serum free testosterone levels and incident prostate cancer such that every standard deviation increase in the free testosterone level correlated with a 9% increase in prostate cancer risk (95% C.I, 1-1.18). Those who developed prostate cancer had a slightly higher mean free testosterone level of 290 pmol/L (+/- 96 as S.D. for both groups), significantly greater than 277 pmol/L for those who did not develop it ($p = .043$).[24]

Today we know that the prostate is an androgen dependent organ and prostate cancer is an androgen dependent disease. Cell biologists have demonstrated the presence of the androgen receptor on the outer surface of benign prostate cells and prostate cancer cells.

Androgen physiology and observations involving 5-alpha reductase and its inhibition also support the theory that prolonged androgenic stimulation causes prostate cancer and prolonged reduced androgenic stimulation prevents prostate cancer.

In normal physiology, the enzyme 5-alpha reductase converts testosterone to dihydrotestosterone (DHT). This enzyme is found in high levels within prostate cells. DHT is far more potent an androgen than testosterone. It is 8 to 10 times greater affinity for androgen receptors than testosterone and 15-30 times more than the affinity of other androgens.[25] 5-alpha reductase amplifies androgenic stimulation of the prostate.

Some epidemiologic study supports the hypothesis that prolonged androgenic stimulation causes prostate cancer. In a population study conducted by Ross and colleagues. Japanese subjects were used as the reference population for comparison with black and white subjects. The study's results showed that blacks had 31% higher values of 3α, 17β androstanediol glucuronide and 50% higher values of androsterone glucuronide than Japanese subjects, while white subjects had 25% and 41% higher values, respectively.[26] These substances are indices of 5-alpha reductase activity and the figures correlate with the PCa risk of blacks which is higher than whites, which in turn is higher than Japanese. Further support for a disparity in DHT levels between Asians and other races correlating

with PCa risk can be found in data from another population study conducted by Lookingbill et al. comparing a Chinese population to a Caucasian population.[27]

Additional evidence supporting the plausibility of DHT's integral role in androgen's influence on PCa risk and development can be found in the Prostate Cancer Prevention Trial, a long term prospective placebo controlled randomized study involving more than 18,000 men median age 62 at the start of the trial. Men were given the 5-alpha reductase inhibitor finasteride or a placebo in blinded fashion and screened annually for prostate cancer. The study ultimately found that finasteride caused a 25% reduction in prostate cancer risk with about seven years of follow-up. This study shows that reducing androgenic stimulation within the prostate by decreasing conversion of testosterone to the more potent DHT reduced the conversion of normal prostate cells to cancerous cells. The fact that 5-alpha reductase~~s~~ activity correlates quite closely with the PCa risks gives strong support to the idea of 5-alpha reductase playing an integral role in increased androgen stimulation correlating with PCa risk. These findings were replicated in the REDUCE trial using the 5-alpha reductase inhibitor dutasteride.[28]

The hypothesis is that long-term androgen exposure causes prostate cancer and evidence does exist that supports the claim. Studies taking long term stimulation into account support the hypothesis. In 2003, Parsons and colleagues conducted a prospective cohort study that measured hormonal levels of 794 members of the

Baltimore Longitudinal Study of Aging from 1968-'98. The study's results show that long term elevated free testosterone levels greater than 5.7 ng/mL were associated with an increased PCa risk (RR = 2.59, 95% C.I. = 1.28-5.29, p = .03).[29] In yet another long-term study, researchers matched 222 cases with 390 controls from the Physicians' Health Study and utilized hormonal and sex hormone binding globulin (SHBG) measurements spanning 10 years to show a strong correlation between increased PCa risk and increased free testosterone after adjusting testosterone and SHBG levels simultaneously ($OR_{quartile\ 2}$ = 1.41, $_3$ = 1.98, $_4$ = 2.60, 95% CI = 1.34–5.02; p = .004).[30] Evidence such as this make for a strong argument for a correlation between long-term androgen stimulation and increased PCa.

Well-designed studies do exist,that suggest no significant association between serum androgen concentration or androgenic stimulation and prostate carcinogenesis. Such is the meta-analysis done by Roddam et al.[31] Rhoden and Morgentaler found that the use of testosterone replacement therapy (TRT), for 1 year in hypogonadal men did not result in an increase in prostate cancer risk.[32] The results should be taken with some skepticism as there are significant limitations. The studies included in the meta-analysis, had hormonal concentrations measured only once per subject and there was no one standard method of measurement among them. Another important limitation the meta-analysis shared with the TRT study is relatively short-term one year of androgen exposure.

PSA and Prostate Cancer Risk

PSA concentration is the focus of this research because of its correlation with prostate cancer risk.

Chu and colleagues discovered the prostate-specific antigen in 1979.[33] It is synthesized in the prostate and leaks into the bloodstream. PSA secretion and serum PSA level increases with androgenic stimulation. PSA levels also increase with the size of the prostate. Increases in PSA can also indicate the presence of prostate cancer.

Risk of PCA increases with increasing serum PSA. Granted PSA has moderate sensitivity and is not specific, but a higher proportion of the population with serum PSA level between 4 and 10 has prostate cancer, compared to the population with PSA 3 to 4. A higher proportion of those with PSA 3 to 4 have PCa compared to the population with PSA levels less than 3. (Ref Thompson PCPT paper which showed this)

It can be logically assumed that if both elevated androgen and PSA levels are significantly associated with elevated PCa risk, there is great probability that elevated androgen is strongly correlated with increased PSA levels as well. This is suggested by one study, in which PSA and testosterone readings obtained

from 8794 subjects revealed a positive correlation between androgen level and PSA..[34] Further support for the link between high PSA and androgen levels comes from a report by Suzuki. Of 420 subjects with PSA levels < 10 ng/mL, cases had testosterone levels significantly greater than the controls (p=.0198).[34]

Evidence does exist that appears to contradict the link between testosterone and PSA. There are studies suggesting that low testosterone levels are correlated with more aggressive prostate cancers (cancers with Gleason scores equal to or above 8).[35] Data from these studies, however, are not a sufficient. For one, in many of the studies that do support a correlation between androgenic stimulation and PSA, the reported measurements show strong statistical significance. Also, several of the clinical studies have produced virtually identical conclusions and many of the epidemiological studies have produced similar ORs or RRs indicating they estimate a similar sized effect.

Literature on Phytoestrogens and Prostate Cancer Risk

With the previous arguments and evidence supporting a possible correlation between androgen levels and prostate cancer in mind, now we may focus on the possibility of an association between phytoestrogen and prostate cancer.

If there is a correlation the implication is that increased phytoestrogen intake might be advocated as part of a healthy diet and lifestyle geared towards

reducing the risk of prostate cancer if phytoestrogens are associated with lower PSA and lower risk of prostate cancer.

Phytoestrogens, as its name implies, are plant-derived compounds that mimics the sex hormone estrogen due to its structural similarities. The most common source of phytoestrogen in the diet is soy. Estrogen is structurally similar to androgens. Because its administration lowers androgen production, estrogen is commonly used to reduce androgen level in the treatment of prostate cancer.

Estrogens' major effect on prostate cancer is results from its interactions with the hypothalamus. When the hypothalamus senses high levels of sex hormone (androgen or estrogen) it decreases GnRH secretion, which leads to a decrease in FSH secretion from the pituitary. This in turn leads to a decrease in androgen secretion from the testes. A possible secondary mechanism beyond estrogen's interaction with the pituitary-hypothalamic axis is that estrogen may be able to bind to androgen receptors and, thus, may act by blocking androgen from the androgen receptor.

This study focuses on urinary phytoestrogenic levels in lieu of serum levels. There is high correlation between the two levels and urinary measurements are often favored since serum levels are harder to obtain.

Research into the possibility of a link between the 2 variables (phytoestrogen levels and PSA levels) has resulted in quite varying results. On one hand, there seems to be a great amount of evidence pointing to the possibility of an inverse association between the phytoestrogen levels and prostate cancer risk and/or PSA. In a study among a cohort of Japanese men, it was again found that phytoestrogen appears to have a protective effect against prostate cancer. Phytoestrogens genistein, daidzein, and equol had ORs of 0.38 (95% C.I., .13-1.13), .41 (.15-1.11), and .34 (.11-1.1) respectively,[36] in addition to possibly affecting one's risk for cancer development, phytoestrogens apparently have significance on PSA measurement even after PCa development.

In a clinical study with 29 prostate cancer patients conducted in 2003 suggested that phytoestrogens significantly decrease serum PSA level. In the study, controls were fed a diet consisting of regular wheat bread while those in the experimental group were given bread consisting of soy grits. After the trial, those in the experimental group experience on average a 12.7% decrease in their PSA concentration while the control group experienced a 40% increase ($p = .02$).[37] It should be noted that a compound may be chemopreventative and not necessarily chemotherapeutic. The 5-alpha reductase are such. There are good studies to show that phytoestrogens are not a treatment for prostate cancer.

On the other hand, there is some data suggesting that phytoestrogen levels do not reduce risk of PCa. In a meta-analysis performed by Roddam, sex hormone data from 18 prospective cohort studies suggest that serum estradiol concentrations have no effect on one's risk of developing prostate cancer.[38] This result was reproduced in the prospective study conducted by Gann et al.[10]

More relevant to the research question at hand, there is data challenging the existence of an association between phytoestrogen and PSA levels. One study suggested that a diet rich in phytoestrogens had no effect on any PSA levels, free or total.[39]

Such variance in conclusions regarding phytoestrogen's association with PSA levels and PCa risk stresses the fact that more research in this particular field is needed. This study will address this concern as well as possible confounding that may arise due to the association high phytoestrogenic intake has with healthy dieting.

Methods

Hypothesis: The hypothesis of this paper is that high serum phytoestrogen levels may correlate with lower androgenic stimulation and lower PSA levels.

Sample and Study Population: The study design for this investigation is an observational, cross-sectional study, utilizing data from the National Health and Nutrition Examination Survey (NHANES). The Centers for Disease Control and Prevention conducts NHANES yearly as a means to provide accurate, representative insight into the health conditions and state of the US population. To achieve the production of this diagram, NHANES utilizes complex sampling to ensure its subjects are chosen randomly and to accurately represent US demographics. In addition, to achieve that representation it combines interviews/questionnaires with physical and laboratory exams. Data for the study was comprised from the 2009-2010 NHANES dataset. The sample analyzed resulted from restricting the study's focus to males aged 40 and over and, subsequently, excluding any subjects without phytoestrogenic readings.

Exclusions: The first exclusion was of all female participants, which resulted in the overall number of participants dropping from 10537 to 5225. The next exclusion was of any participant younger than 40 years, since NHANES does not have PSA data for men in that category, limiting the total number of participants from 5225 to 2026. The 3rd exclusion removed any participants with missing data for any of the 6 phytoestrogens under analysis, further decreasing the number of participants from 2026 to 676.

Study Measures

Independent Variables: The primary independent variables under investigation were continuous. They were the urinary levels of phytoestrogens daidzein (URXDAZ), equol (URXEQU), genistein (URXGEN), enterodiol (URXETD), enterolactone (URXETL), and O-Desmethylangolensin or o-DMA (URXDMA), all in ng/dl. All independent variables are continuous.

Dependent Variable: Prostate specific antigen (PSA), a biomarker that may indicate prostate state, diagnose prostate cancer in benign conditions, and indicate the progression of prostate cancer. It was measured in ng/mL.

Covariates: Covariates included: HDL cholesterol, LDL cholesterol, total cholesterol and triglyceride levels, all in mg/dl. Five Ethnic/racial groups were included: non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other races including multi-racial; age and PIR (poverty-income ratio), a measure of the economic status of the participant, and the level of education a participant reached.

Statistical Analysis: An analysis of correlations was performed between PSA and the phytoestrogens, between PSA and the covariates, between each of the phytoestrogens, and between each of the covariates. Bivariate analysis was then conducted to determine whether an empirical relationship between PSA and each of the phytoestrogens existed. In addition, a second set of bivariate analysis was conducted to determine whether any association existed between PSA and each of the covariates. All continuous variables were categorized for crosstab analysis in both sets and significance was determined from the Pearson Chi-Square p-values. All covariates that were found to have a p-value < .35 in the

bivariate analysis (i.e. age, HDL, total cholesterol, and race/ethnicity) and significant correlation with PSA were entered into multivariate linear regression models. Multivariable linear regression was performed in over 30 scenarios and the 6 models produced and containing a single phytoestrogen were selected for examination and discussion in the study. In addition, the data was analyzed using SAS version 9.2 and SPSS Statistics 21. Specifically, SPSS complex sampling program was utilized with the 2-year MEC examination weights for the participants provided by NHANES (wtmecyr2) and the dataset was sorted by strata (smdvstra) and subsets via primary sampling units (smdvpsu).

Results

The average phytoestrogen levels among US males were as follows: equol: 36.8611 ng/dl, genistein: 199.5041 ng/dl, o-DMA: 70.8237 ng/dl, enterodiol: 218.6476, enterolactone: 1101.0899 ng/dl, daizdein: 70.8237 ng/dl. The average PSA level for US males was 1.6519 ng/dl. Bivariate and multivariable analysis via linear regression revealed that no significant association exists between PSA levels and any of the phytoestrogenic levels, even after controlling for any of the covariates. There were also no significant association between PSA and any of the covariates. Highly significant correlations existed between phytoestrogens genistein and daizden (R^2 = .935, p < .000), O-DMA (R^2 = .177, p < .000), and equol (R^2 = .197, p < .000), enterolactone and enterodiol (R^2 = .423, p < .000), equol and daizden (R^2 = .17, p < .000) and O-DMA (R^2 = .158, p < .000), and daizden and O-DMA (R^2 = .377, p < .000). Some highly significant correlations between the covariates include: HDL and triglycerides (R^2 = -.147, p = .007), LDL (.307, p < .000), and total cholesterol (R^2 = .474, p < .000), triglycerides and total cholesterol (R^2 = .457, p < .000), and LDL and total cholesterol (R^2 = .775, p < .000). The only variables significantly correlated with PSA were HDL (R^2 = .118, p = .002), age (R^2 = .212, p < .000), race/ethnicity (R^2 = .079, p = .04), and total cholesterol (R^2 = .076, p < .049). In regards to bivariate and multivariable, even after controlling for the covariates, none of the phytoestrogens displayed significant associations with PSA.

Discussion

It appears that the amount of phytoestrogenic consumption, reflected by urinary concentrations, had no appreciable effect on the PSA levels for subjects in the study, in the unadjusted analysis and even after adjusting for the covariates, refuting the hypothesis, due to the lack of association. There also appears to be no association between total cholesterol and LDL, contrary to previous studies.

The study has several strengths and several limitations. Strengths include the analysis of associations as well as correlations between the dependent variable and independent variables and covariates, but also the independent variables themselves as well as between the independent variables and the covariates. This thorough approach was utilized to ensure the measures of association used were valid and accurate by allowing comparison of results obtained with facts already established in scientific literature. For example, the high correlation between LDL and total cholesterol (R^2 = .766) and the high correlations between several of the phytoestrogens reflect the close association between the variables in biology that have already been established. Another strength of this study was its thoroughness in methodology as seen from the analysis of correlation combined with bivariate and multivariable analysis to attempt to provide a clear picture of any possible association. The fact that collinearity was carefully accounted for by creation and testing of numerous models in the linear regression to further aid in the attempt to create an accurate picture of the possible associations is also a strength in this study. Limitationw of this study,

however, were significant. The main limitation was a result of the study's short duration coupled with significant amounts of missing data. Although it can be argued, that 2 years can suffice as a "long-term" period for phytoestrogen exposure, it appears highly plausible that a longer time-span was necessary for this study to have truly provided an accurate assessment of association between phytoestrogen exposure and PSA levels, due solely to so much missing data from the NHANES dataset. Much of the missing data resulted in a significant amount of participants to be excluded from analysis. For example, it is established that African-American males have a significantly greater risk for PCa. However, in the analysis, race is weakly associated with PSA levels, suggesting selection bias in the study. Further support for selection bias in the study includes the fact that there was collinearity between covariates that typically show no correlation with one another. Age, for example, correlated strongly with race/ethnicity (R^2 = .132, p = .001). Large amounts of missing values in the triglyceride categories, some of the phytoestrogens, and LDL also resulted in much exclusion and undoubtedly may have dampened the associations these variables have with PSA as independent variables and covariates.

Moving forward, one could make a major contribution to the scientific field of PCa by examination and improvement upon this study. By performing a large long term prospective cohort, case-control, or an interventional randomized prospective clinical trial of increased dietary phytoestrogen versus a normal diet.,.. The issue of the missing data would definitely need addressing for further

improvement as well as selection of a wider pool of participants to account for missing data while maintaining representative quality.

The results of this study in the face of such strong evidence[4,5] supporting the hypothesis in addition to the biological basis and possible mechanism laid out behind phytoestrogen's activity only goes to further indicate that more research is necessary.

References

1. Brawley, O.W.; Ford, L.G.; Thompson, I; Perlman, J.A.; Kramer, B.S. 5-Alpha-reductase inhibition and prostate cancer prevention. Cancer Epidemiol Biomarkers Prev. 1994 Mar; 3:177–82.

2. Noble, R. L. The development of prostatic adenocarcinoma in NB rats following prolonged sex hormone administration. Cancer Res.,1125-1139, 1959

3. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, Feng Z, Parnes HL, Coltman Jr CA 2006 Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst 98:529–534

4. Ozasa K, Nakao M, Watanabe Y, Hayashi K, Miki T, Mikami K, Mori M, Sakauchi F, Washio M, Ito Y, Suzuki K, Wakai K, Tamakoshi A, for the JACC Study Group. “Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men”. Cancer Sci. 2004; 95: 65–71.

5. Dalais FS, Meliala A, Wattanapenpaiboon N, Frydenberg M, Suter DA, Thomson WK, Wahlqvist ML. Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. Urology 2004; 64: 510–5.

6. Cancer Facts and Figures 2012. Atlanta, GA: American Cancer Society, 2012. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-031941.pdf.

7. Cancer Facts and Figures 2014. Atlanta, GA: American Cancer Society, 2014.

http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf.

8. SEER Stat Fact Sheets: Prostate Cancer. Washington D.C.: National Cancer Institute, 2013. http://seer.cancer.gov/statfacts/html/prost.html

9. Steinberg, G. D., Carter, B. S., Beaty, T. H., Childs, B. and Walsh, P. C. Family history and the risk of prostate cancer. The Prostate (1990), 17: 337–347. doi: 10.1002/pros.2990170409.

10. Kenfield, S.A.; Stampfer, M.J.; Chan, J.M.; Giovannucci, E. "Smoking and Prostate Cancer Survival and Recurrence" JAMA. 2011; 305(24): 2548-2555. doi:10.1001/jama.2011.879.

11. Huncharek, M; Haddock, K.S.; Reid, R; Kupelnick, B. "Smoking as a Risk Factor for Prostate Cancer: A Meta-Analysis of 24 Prospective Cohort Studies" Am J Public Health. 2010 April; 100(4): 693–701. doi: 0.2105/AJPH.2008.150508 PMCID: PMC2836346

12. Hickey K, Do KA, Green A. Smoking and prostate cancer. Epidemiol Rev 2001;23:115–25.

13. Lu-Yao G, Albertsen PC, Stanford JL, et al. Screening, treatment, and prostate cancer mortality in the Seattle area and Connecticut: fifteen-year follow-up. Journal of Gen. Intern Med. 2008; 23: 1809–1814.

14. Giovannucci EL, Liu Y, Leitzmann MF, et al: A prospective study of physical activity and incident and fatal prostate cancer. Arch Intern Med 2005 165:1005-1010.

15. Mills PK, Beeson WL, Phillips RL, Fraser GE (1989) Cohort study of diet,

lifestyle, and prostate cancer in Adventist men. Cancer 64: 598–604.

16. Hsing AW, et al. Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study. Cancer Res. 1990;50(21):6836–40.
17. Hayashi N., Matsushima M., Yamamoto T., Sasaki H., Takahashi H. & Egawa S. (2012) The impact of hypertriglyceridemia on prostate cancer development in patients aged >/=60 years. BJU International 109, 515–519.
18. Murtola TJ, Syvälä H, Pennanen P, Bläuer M, Solakivi T, et al. The Importance of LDL and Cholesterol Metabolism for Prostate Epithelial Cell Growth. PLoS ONE 2012; 7(6): e39445. doi:10.1371/journal.pone.0039445
19. Mondul AM, Weinstein SJ, Virtamo J, Albanes D: Serum total and HDL cholesterol and risk of prostate cancer. Canc Causes Contr 2011, 22:1545-1552.
20. Mondul AM, Clipp SL, Helzlsouer KJ, Platz EA. Association between plasma total cholesterol concentration and incident prostate cancer in the CLUE II cohort. Cancer Causes Control 2010; 21:61-8.
21. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1941;1:293-297
22. Bosland MC, Mahmoud AM. Hormones and prostate carcinogenesis: Androgens and estrogens. J Carcinog 2011;10:33
23. Dillard PR, Lin MF, Khan SA. Androgen-independent prostate cancer cells

acquire the complete steroidogenic potential of synthesizing testosterone from cholesterol. Mol Cell Endocrinol 2008;295:115–20.

24. Hyde, Zoë; Flicker, Leon; , McCaul, Kieran A; Almeida, Osvaldo P; Hankey, Graeme J; S. A. Paul Chubb, Bu B. “Associations between testosterone levels and incident prostate, lung, and colorectal cancer. A population-based study”. Yeap Cancer Epidemiol Biomarkers Prev. 2012 August; 21(8): 1319–1329. Published online 2012 July 24. doi: 10.1158/1055-9965.EPI-12-0129

25. Hemat, R.A.S. Principles of Orthomolecularism (New York: Orotext, 2004), 426.

26. Ross RK, Bernstein LA, Lobo RA, et al. 5-Alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. Lancet 1992;339:887–9.

27. Lookingbill, D. P., Demers, L. M., Wang, C., Leung, A., Rittmaster, R.S., & Santen, R.J. “Clinical and biochemical parameters of androgen action in normal healthy caucasian versus Chinese subjects”. J. Clinical Endocninol. Metabolism 1991; 72:122-1248.

28. Andriole GL, Bostwick DG, Brawley OW, et al. REDUCE Study Group. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 2010;362(13):1192-1202.

29. Parsons JK, Carter HB, Platz EA, Wright EJ, Landis P, et al. Serum

testosterone and the risk of prostate cancer: potential implications for testosterone therapy. Cancer Epidemiol Biomarkers Prev. 2005;14:2257-60. doi: 10.1158/1055-9965.EPI-04-0715

30. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. “Prospective study of sex hormone levels and risk of prostate cancer”. J Natl Cancer Inst. 1996; 88:1118–1126.

31. Roddam A, Allen N, Appleby P and Key T. “Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies”. Journal of the National Cancer Institute, 2008; 100: 170–183. (doi:10.1093/jnci/ djm323)

32. Rhoden, E.L.; Morgentaler, A. "Testosterone replacement therapy in hypogonadal men at high risk for prostate cancer: results of 1 year of treatment in men with prostatic intraepithelial neoplasia". J Urol. 2003 Dec.; 170(6.1): 2348–2351 doi:10.1097/01.ju.0000091104.71869.8e

33. The Man Behind the PSA Test. Buffalo, NY: Roswell Park Cancer Institute. https://www.roswellpark.org/cancer/prostate/about/history-psa.

34 Bankhead, Charles. “AUA: Testosterone and PSA Intertwined in Prostate Cancer Risk.” MedPage Today. http://www.medpagetoday.com/MeetingCoverage/AUA/5774. (Last modified May 25, 2007)

35. Hoffman M.A., DeWolf W.C., Morgentaler A. “Is low serum free testosterone a marker for high grade prostate cancer?” Journal of Urology, 2000 March; 163(3):824-827.

36. Ozasa K, Nakao M, Watanabe Y, Hayashi K, Miki T, Mikami K, Mori M, Sakauchi F, Washio M, Ito Y, Suzuki K, Wakai K, Tamakoshi A, for the JACC Study Group. Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men. Cancer Sci. 2004; 95: 65–71.

37. Dalais FS, Meliala A, Wattanapenpaiboon N, Frydenberg M, Suter DA, et al. (2004) Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. Urology 64: 510–515.

38. Roddam AW, Allen NE, Appleby P, Key TJ. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst. 2008;14:170–183. doi: 10.1093/jnci/djm323.

39. Jenkins DJ, Kendall CW, D'Costa MA, Jackson CJ, Vidgen E, Singer W, Silverman JA, Koumbridis G, Honey J, Rao AV, Fleshner N, Klotz L. Soy consumption and phytoestrogens: effect on serum prostate specific antigen when blood lipids and oxidized low-density lipoprotein are reduced in hyperlipidemic men. J Urol. 2003; 169: 507–511.