1-1-2011

The Effect of Phytoestrogen Chemoprevention of Prostate Cancer

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This is to certify that the doctoral dissertation by

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has been found to be complete and satisfactory in all respects,
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2013

Abstract

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By

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MPH (Honors), American Public University, 2011

BS, Excelsior College, 2000

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

Walden University

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Abstract

Prostate cancer (CaP) remains the most commonly diagnosed cancer and second leading cause of cancer mortality among men in several ethnic groups in the United States. Lower CaP incidence among Asian men has been attributed to increased intake of soy derived phytoestrogens (SDPs); however, its association has not been extensively explored in U.S. men. The purpose of this study was to determine the effect size of serum prostate specific antigen (sPSA) and serum estradiol (sE2) following dietary SDP intervention. The study was based on an original conceptual model that aims to avert early prostate tissue damage through identification of critical prevention endpoints. Research questions examined the correlation between dietary SDPs and sPSA and sE2 levels. This quantitative meta-analysis study used data abstracted from 8 randomized controlled trials yielding 530 participants ages 50-85. Outcome specific meta-analysis using the random effect model adjusted for heterogeneity and determined cumulative effect size that favored intervention. Odds ratios established a positive correlation between intake of dietary SDP and detection of serum SDP (sSDP) among treated groups. Positive correlations between both dietary and sSDP with sE2 levels, and inverse correlations between both dietary and sSDP with sPSA levels, were indicated among treated compared to placebo groups. Hedges' *g*, correlation, and standardized mean difference statistics confirmed analyses. Implications for positive social change include developing professional dietary standards to use SDPs for CaP chemoprevention among U.S. and other men, as well as a medical option for treatment of CaP. Further research exploring mechanisms of SDP action on hormones may be beneficial to men at risk for CaP and individuals at risk for other cancers linked to changes in hormonal levels.

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Dedication

This work is dedicated to those who have faced the challenges of CaP diagnosis. It is also a dedication to those who continue the exploration to find new therapies that could help improve health outcomes. It is the hope, that the information in this work, which is a representation of commitment, focus and dedication will help in the process to uncover new strategies for the prevention or therapy of CaP. It is truly a blessing to have been able to complete this work guided by scholars that care deeply about CaP and other cancers and the need for valuable prevention strategies.

Acknowledgments

I am tremendously grateful and profoundly appreciative to my dissertation committee members including chairperson and content expert Dr. Mary Lou Gutierrez, methodology expert Dr. Xianbin Li, and the URR faculty member Dr. Michael Dunn. Also, thanks to members of the University's tutoring staff who have all contributed in assisting to guide and direct this truly wonderful scholarly endeavor. Thanks to family, relatives and friends that have always provided encouragement and support throughout this process. I am certainly indebted for all the thoughtfulness extended and hope that the results of this work will help to alleviate the suffering endured by those affected by this disease. In addition, there is hope that the findings could possibly aid in defining both preventative and therapeutic models to address issues relating to this disease and other cancers. I hope that these findings might also open diverse avenues for further research into other cancers that remain difficult to treat and affect the lives of millions globally.

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Chapter 1: Introduction to the Study

Background of the Study

Cancer is now the leading cause of mortality worldwide, with more than 7.6 million cases (World Health Organization [WHO], 2012). Prostate cancer (CaP) among men in Western countries (Guy et al., 2008; Hill & Doyon, 2006; Jemal et al., 2007) is significantly higher in incidence rate than the rate observed among men living in Asian countries (Adlercreutz, Yamada, Wahala, & Watanabe, 1999; Marks et al., 2004; Miller, 1988; Parkin, Pisani, & Ferlay, 1999). The observed lower incidence rate of CaP has been associated with higher intake of soy derived phytoestrogens (SDPs) in Asian countries (Adlercreutz et al., 1999). Longer life expectancy and improved screening methods are likely to increase the number of CaP cases within the global population (White et al., 2004; White et al., 2010), and as many as 10%-15% of adult men could be affected (Bosland, 2000; Qin, Wang, Kaneko, Hoshi, & Sato, 2003; Singh, Matanhelia, & Martin, 2008). Environmental causes have been implicated with mortality, while ethnic differences and consumption of soy as a dietary staple have been credited for some lower observed disease incidence (Adams, Chen, Newton, Potter, & Lampe, 2004; Hsing & Devesa, 2001).

CaP is the most common noncutaneous cancer among males (Jemal et al., 2007; Sharma et al., 2009). The need for available chemoprevention and other therapies has generated tremendous interest within public health and medical communities (National Cancer Institute [NCI], 2012). While recent reports indicate a decline in some cancers, the incidence of CaP remains greater than 10% (American Cancer Society [ACS], 2012).

CaP is also the second leading cause of cancer mortality among men in the USA (ACS, 2012; Centers for Disease Control and Prevention [CDC], 2012). The ACS estimated that in the year 2012, there were approximately 241,740 new cases and 28,170 deaths from CaP (ACS, 2012). Estimates of new cases for the year 2013 were slightly lower (238,590 new cases) with an expected mortality of 29,720 (NCI, 2013). At diagnosis, CaP disproportionately affects African American (AA) men in both incidence and mortality compared to Whites (ACS, 2012; CDC, 2012). Age is not alterable and is an integral factor in the etiology of the disease (ACS, 2012; CDC, 2012). According to the CDC (2012), AAs are 1.86 times more likely to develop CaP before the age of 65 (103.1/100,000), compared to European Americans (55.3/100,000). Similarly, AAs are 1.42 times more likely to be deceased at age 65 and older (1,137.8/100,000) compared to European Americans (800.7/100,000).

Cancer mortality potentially continues to increase worldwide with the potential to affect more than 13.1 million individuals by the year 2030 (WHO, 2012). Age is an extremely significant factor in the development of all cancers. In particular a greater than 80% of CaP cases are diagnosed after 60 years of age (ACS, 2012; CDC, 2012).There is some concern that men in Asian countries could also be affected in their older decades despite the high level of soy intake in the diet, therefore adding urgency to this dilemma and disease burden (Thapa & Ghosh, 2012). Improved therapies and prevention strategies are therefore urgently needed. These strategies may help alleviate the increased disease burden experienced among various populations. Many lives are affected and myriads of

negative health issues accompany present therapies. More effective treatment strategies including chemoprevention would be beneficial.

Although dietary intake of soy has increased in the U.S. over the past decades, evidence of quantity and frequency of consumption, and the group within the population that accounts for the highest dietary intake, remains uninvestigated. The appropriate dietary quantity and intake duration of soy or its extracted phytonutrients in relation to CaP chemoprevention are yet to be established. Randomized clinical trials have been designed to qualify intervention employing soy derived phytoestrogens (SDPs), but their examination is limited to studies which in most cases fail to yield significant and generalizable results (Venkitaraman et al., 2008). Epidemiological research has shown that there is 40 times greater consumption of soy in the Asian diet than consumed in the Western diet (Sharma et al., 2009). Understanding the effect that SDPs could have preventing CaP within the context of duration and concentration and more directly through serum levels of PSA and estradiol (E2) following dietary intake of SDPs is therefore prudent. Recent estimates indicate that CaP is now present among younger aged men within the population (ACS, 2012). This earlier onset of CaP incidence among younger aged men is further evidence of the need for novel preventive strategies or curative therapies.

Phytoestrogens

According to Ganry (2004), Morrissey et al. (2004), and Napora et al. (2011) SDPs, plant derived nonsteroidal compounds, are known to possess weak estrogenic properties. Food sources rich in these compounds include legumes and soy, with the latter

supplying an abundance of the SDPs in this study (CDC, 2012; Napora et al., 2011). Figure 1 provides information on both natural and synthetic SDPss and the end-product following ingestion and biochemical activity.

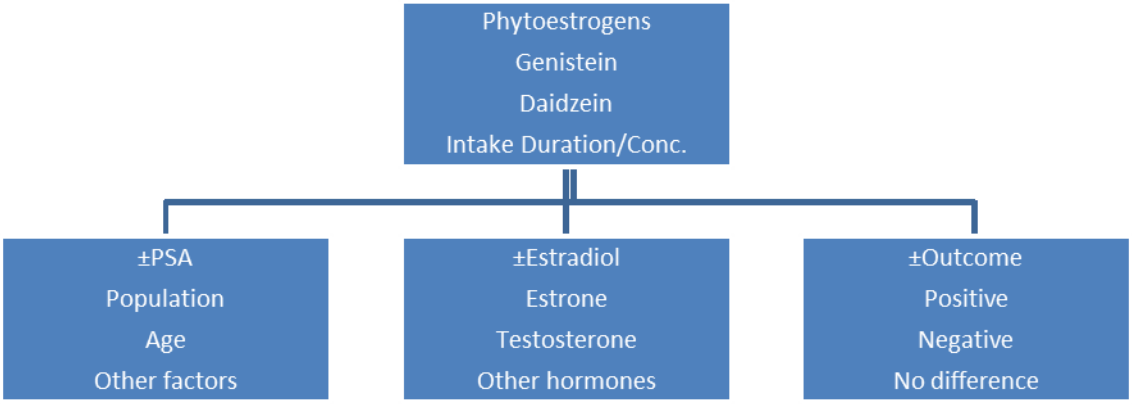

Figure 1. Major phytoestrogens found in soy and the influence on PSA and estradiol.

In addition to their chemoprotective properties, SDPss have the capacity to protect the prostate gland from tumor progression, and are important as anti-inflammatory compounds (Napora et al., 2011). SDPs are comprised of lignans, flavonoids, coumestans and isoflavones with the latter predominantly derived from soy (CDC, 2012; Strauss et al., 1998). Among isoflavones found in soy are genistein, glycitein and daidzein (Strauss et al., 1998), of which genistein and daidzein are the focus of this study. Genistein's utilization is immediate within the body, while daidzein undergoes metabolism by bacteria in the gut (CDC, 2012). Daidzein's convertion to O-desmethylangolensin, then equol and is the available estrogenic product (CDC, 2012).

There are three main types of SDPss. Broad similarity exists between estrogens, isoflavones, and lignans, which comprise this group (BCERT, 2007). Lignans provide a major source of the consumed SDPs in the American based diet (BCERT, 2007). They

appear to have an inverse association with cardiovascular health (BCERT, 2007). Cancers have also been associated with some SDPs (BCERT, 2007). The established knowledge is that Asian based diets have a higher proportion of SDPs that correlate with a lower CaP incidence (Hsing & Devesa, 2001). An increase in the dietary levels of SDPs among men in the Western population could potentially provide beneficial chemoprotection. However, while genistein and daidzein are the major isoflavones found in soy, lignans make up the major polyphenols found in cereals, grains, some fruits, and vegetables. However, like daidzein, these lignans undergo conversion to other compounds by microbes found in the intestines, and newly formed compounds provide protection from some types of disease (Hsing & Devesa, 2001).

Equol fermentation produces daidzein, while enterolactone and entereodiol are products of fermented lignans (Linus Pauling Research Institute, 2010). Compounds derived from lignans are possibly lacking in carcinogenic activity. However, lignan derived compounds are considered SDPs and when converted by bacteria can exert mild estrogenic effects (Linus Pauling Research Institute, 2010). Similar to SDPs, equol has also demonstrated cytotoxic activity against CaP cell lines, as does its lignan derived compound (Venkitaraman et al., 2008). Lignans exert their effect on cells including those within the prostate. This process could result in unwanted cellular differentiation and formation of precancerous lesions due to hypertrophy or hyperplasia (Venkitaraman et al., 2008).

Prostate Cancer and Phytoestrogens

The reciprocal phytoprotection associated with an increase in incidence of CaP appears to be lacking in the US male population (CDC, 2010) and may simply be a reflection of the minimal quantity of SDPs consumed within the Western diet. Greater understanding of this association needs assessment. It is necessary to understand what factors are integral to any inferred chemoprotection to be able to understand the true effect of consumption within the target population. This encourages incorporation into the Western diet associated CaP etiological and subsequent preemptive model. This has resulted in the need to understand the association and to determine the exact effect size (ES) of intervention.

CaP is considered hormonally controlled (Sekine et al., 2007; Ho, 2004). Predominantly, female hormones, E2 and estrone are the two major estrogen hormones secreted by the ovaries (NCI, 2012). The use of estrogens for CaP therapy has produced some positive results; however, concerns related to increase in hormone refractory cancer are valid. Hormonal therapy benefits include blocking of tumor growth, decrease of PSA levels and ability to use as adjunct therapy at any time other treatment modalities. The benefits of estrogen therapy have accompanying side effects. These side effects include impotence, weight gain and fatigue among others. Studies have shown that the delivery of estrogen through E2 skin patches can be effective in CaP therapy. Benefits also occur without some of the prior mentioned side effects such as impotence and weight gain (Strax, 2005). CaP is the most invasive tumor and ranks only behind lung cancer in mortality incidence among men (Agarwal, 2000; Bektic et al., 2003). Treatment of CaP

has focused on employing androgen deprivation therapy (ADT; Smith, 2007; Shahani et al., 2008) or orchidectomy (Singh et al., 2008). Increasing therapeutic use of ADT has been reported (Shahani et al., 2008; Singh et al., 2008; Smith, 2007). While these intervention processes have realized some benefits, some have also resulted in the development of unwanted events, including toxic conditions that can persist for extended periods (Lycette et al., 2006). Mitigating these unwanted events through new therapeutic agents including an increase in SDP intake. An increase in SDP intake is necessary, as there is some evidence of intake in the Western diet. However, this intake remains minimal in relation to that consumed in Asian diets.

Employing ADT, as previously indicated, has been associated with adverse health conditions including osteoporosis, hot flashes, and changes in cognition (Basaria et al., 2006; Cherrier et al., 2003). ADT therapy has been associated with the development of diabetes and other health conditions, which can influence other unwanted yet serious cardiovascular conditions (Napora, Short, Muller, et al., 2011). In fact, evidence implicates estrogen receptors (Härkönen & Mäkelä, 2004), as favorable to the development and progression of CaP. This conflicting report kindles the need to understand the context of ES. It also provides an opportunity to assess the true effect of studied SDPs in reducing or preventing CaP develo,pment or progression. Numerous epidemiological studies and in vitro experiments have demonstrated the chemoprevention benefits from SDPs (Kumar et al., 2011). It might therefore be plausible to assert that the chemoprotective influence of SDPs is the ability to regulate important receptor mechanisms. This subsequently results in a decrease in cancer development and

incidence. In fact, research by (Bektic et al., 2003), infers that genistein has an effect on ERβ and consequently a direct relationship on the down-regulation of those androgen receptors. ERβ has demonstrated its influence in tumor growth and proliferation (Härkönen & Mäkelä, 2004).

Estrogens have remained important in the treatment of androgen-dependent CaP (Pendleton et al., 2008). The difficulty of treating CaP including hormone refractory disease continues to influence the search for novel agents for optimum therapy (Pendleton et al., 2008). Hormone refractory disease does not respond effectively to presently employed drugs (Cho, Di Blasio, Rhee, & Kattan, 2003). The thought is that greater than 20% of men who have been treated for androgen-dependent CaP will experience disease recurrence (Cho, Di Blasio, Rhee, & Kattan, 2003). This portends poor prognosis and decreased survivability. Present reports have indicated a greater survival rate of CaP diagnosed individuals because of the improved treatment methods and earlier diagnosis (ACS, 2012). Different levels of survival are reportedly associated with tumor stage with better outcomes when diagnosed as a localized tumor with almost 100% survival (ACS, 2012). Distant metastases on the contrary are associated with approximately 29% survival (ACS, 2012). Treating metastatic and recurrent disease is therefore a health issue of paramount importance.

Estrogens, Estradiol and CaP

Estrogens, also known as oestrogens, are primarily female hormones. These estrogens have important value in areas including hormone replacement therapy (CDC, 2012). They influence gene regulation because of their ability to diffuse across cell

membranes (CDC, 2012). Hormones that occur naturally in women include estrone (E1), estriol (E3), and 17β–estradiol (E2) with E2 observed as the predominant form (Ockrim, Lalani, Kakkar, & Abel, 2005). Although primarily synthesized in the ovaries of women, the discovery of estrogen receptors ER-alpha and ER-beta in the male prostate gland has increased some evidence of its value in the development of CaP (Ockrim et al., 2005).

E2 is produced by the male reproductive system and is derived from testosterone (Ockrim et al., 2005). The connection between its serum concentration and SDP concentration might provide insight into the etiology of CaP. In fact, the androgen receptor appears to be the important target during prevention and treatment (Bonkhoff & Berges, 2008). Estrogen concentration is greatest in the male reproductive tract (Lombardi et al., 2001) resulting in the existing correlation between androgens and CaP proliferation (Morrissey et al., 2004). This understanding has resulted in reports suggestive of estrogen's role in CaP etiology including a metabolic pathway association resulting in an associated genetic link (Bosland, 2005).

The discovery of estrogen receptors, including possibly close correlation between a specific genetic polymorphism on ER-α and its link to the development of CaP, demonstrates a close relationship between estrogen and CaP (Bonkhoff & Berges, 2008; Bosland, 2005). In addition, the relationship between ER-β and CaP development is expressed through its down regulation in more malignant disease (Bosland, 2005) and during the progression of hormone-refractory disease (Bonkhoff & Berges, 2008). In addition, this ER-β relationship is observed in normal prostate tissue, benign prostatic

hyperplasia (BPH), and CaP, providing an opportunity for chemoprevention (Bonkhoff & Berges, 2008).

Risk of Developing CaP

While some cancers have experienced decline over the past decades, CaP continues to show an increase in incidence (NCI, 2012). In 2011, US estimates of CaP were approximately 241,740 new cases, with an expected mortality of 28,170 (NCI, 2012). Those numbers are representative of the upsurge in CaP incidence from 2007 when 223,307 cases were diagnosed in the US (CDC, 2012). However, compared to the estimated number of new cancer deaths that would occur based on 2007 data, the actual number of deaths (28,170) represent a slight decrease from 29,093 cases (CDC, 2012). A decrease in mortality from cancer is associated with an increase in early detection.

As mentioned earlier, the risk of developing cancer increases with age. Figure 2 illustrates the percent of U.S. men who developed CaP over various decades starting at age 30 through 70. At age 30, men have a relatively low risk of developing CaP with 0.01% by the time they reach age 40, 0.32% at age 50 and 2.49% at age 60. However, the percentage of men currently age 40 and older who will develop CaP after 30 years increases proportionately to 8.30% of men by age 40, 14.40% by age 50, and 16.11% by age 60. At age 70, men have the same risk (8.50%) of developing CaP after 10 years than men currently aged 40 after 30 years.

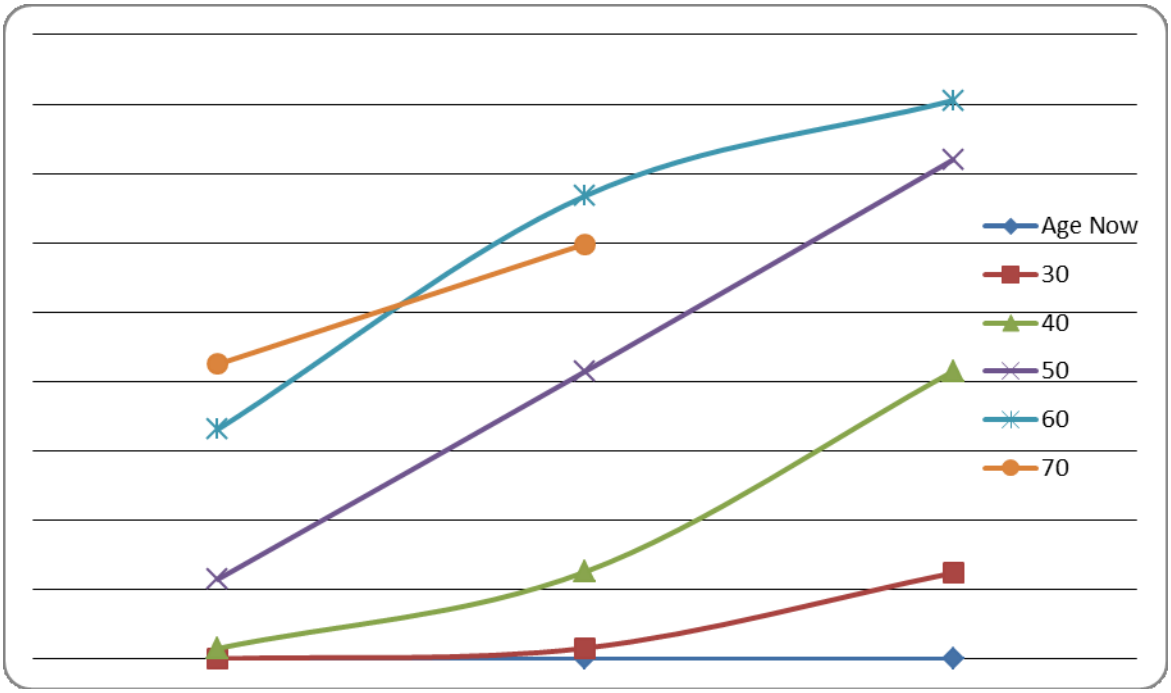

Figure 2. Likelihood (%) of Developing CaP Over 3 Decades Based on Current Age, 2005–2007. *Source:* Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlader N, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Cronin K, Chen HS, Feuer EJ, Stinchcomb DG, Edwards BK (eds). *SEER Cancer Statistics Review, 1975–2007,* National Cancer Institute. Bethesda, MD, based on November 2009 SEER data.

Problem Statement

The disease burden of CaP among Western men is significantly greater than that observed among men in Asia, where soy is a dietary staple. While there has been extensive epidemiological research evidence supportive of the anticancer value of soy compounds among the Asian population, recent inclusion of SDPs in Western diet has not yet shown a noticeable reduction in disease incidence and outcome. Epidemiological evidence indicates soy based diets containing SDPs provide valuable chemoprotection. The threshold level of concentration and duration of soy based dietary intake contributing

to chemoprotection represents invaluable knowledge. Intake duration and concentration could help address the risk of CaP among high risk minority and other populations. This study addresses the problem of recurrence and identification of potential failure for any successful therapeutic intervention. Potentially, this research will provide evidence for further studies that could decrease CaP incidence and influence broad social change through improved health status and overall quality of life (QOL).

Purpose of the Study

The purpose of this study was to determine the ES of serum PSA and E2 levels following dietary genistein and diadzein intervention. Serum levels of PSA and E2 are important predictors of therapeutic response and disease outcome. Studying the response of PSA and E2 in American men and disease outcome has not previously occurred in that cohort. Guided by the principles of primary and secondary prevention and an original model of cancer prevention, the dual prevention intervention model, the study sought to answer whether intake duration and concentration of SDPs directly influence magnitude and direction of CaP response. Information for analysis in this study came from selected published randomized controlled trials (RCTs) of SDP intervention designed to prevent or delay recently diagnosed CaP, increasing PSA and hormone refractory CaP in men between 50–85 years old.

Theoretical Base: Dual Prevention Intervention Model

The theoretical base for this study has roots in one of the recognized multistep models of development of neoplasia or tissue growth arising from the biological sciences. The putative multistep pathway model (Giovannucci, 1999) provides evidence for the

indication of chemoprevention through SDPs. Giovanucci's model was structured on the premise that intake levels of SDPs are associated with the lower incidence of CaP observed among men in Asian countries. CaP carcinogenesis through the presence of increasing PSA is one of the mechanisms depicted in this model as early evidence of damage to the normal prostate tissue resulting from genetic mutation and cellular damage. Biological models do not focus on the principles of primary cancer prevention and control and the approach to disease prevention has unfortunately reflected this premise in that screening for CaP is a secondary prevention effort aimed at detecting increasing PSA. At the same no new biomarkers to detect changes in the normal prostate tissue have been discovered or suitable for use at a population level.

The strategy of the dual prevention intervention model (DPIM) is to present an original contribution to the field of public health that aims to avert early prostate tissue damage (Michelin & Gutierrez, 2013). Figure 3 depicts the premise of the DPIM conceptual model, which posits that the development of CaP and other cancers result from continued interaction to environmental and biological exposures. Over time, these exposures result in cellular changes from stress and resulting mutations that may occur with prolongued exposure. Beyond the multistep causative mechanisms, the DPIM has identified critical points at which a public health primary prevention approach through phytochemical intervention and a potential therapeutic intervention through secondary prevention can be implemented in high risk populations such as African Americans. The primary prevention component of the DPIM recognizes that other theoretical models proposed in the literature focus on the identification of mitigating factors that result in

cellular biological changes. These activities lead to the development and progression of CaP. Preventing or delaying cellular biological changes that result in CaP development and progression is the focus of the DPIM.

The goal of this research is to prevent, delay, or reverse disease through dietary or medicinal addition of SDPs to avert development and progression of prostate tumor. Mitigating factors such as age, inflammation, change in hormonal status and genetic mutations is of valuable importance to this process. While several studies have provided scientific support for events that describe such association (Wang, Clubbs, & Bomser, 2006), the true ES of SDP intervention on individuals at various stage, grade or type of CaP has not been completely analyzed and presented in the present literature. While the model by (Giovannucci, 1999) provided for later androgen deprivation therapy (ADT) and other chemotherapeutic agents for treatment, the DPIM focuses on the identification of a critical point for primary prevention intervention. The broader scope of the DPIM is shown through this meta-analysis that showed the true ES of SDP intervention. The research revealed the much desired importance of secondary prevention using SDPs inclusive of E2 therapy. The study result showed that decrease in serum PSA concentration was a desired outcome from SDP intervention.

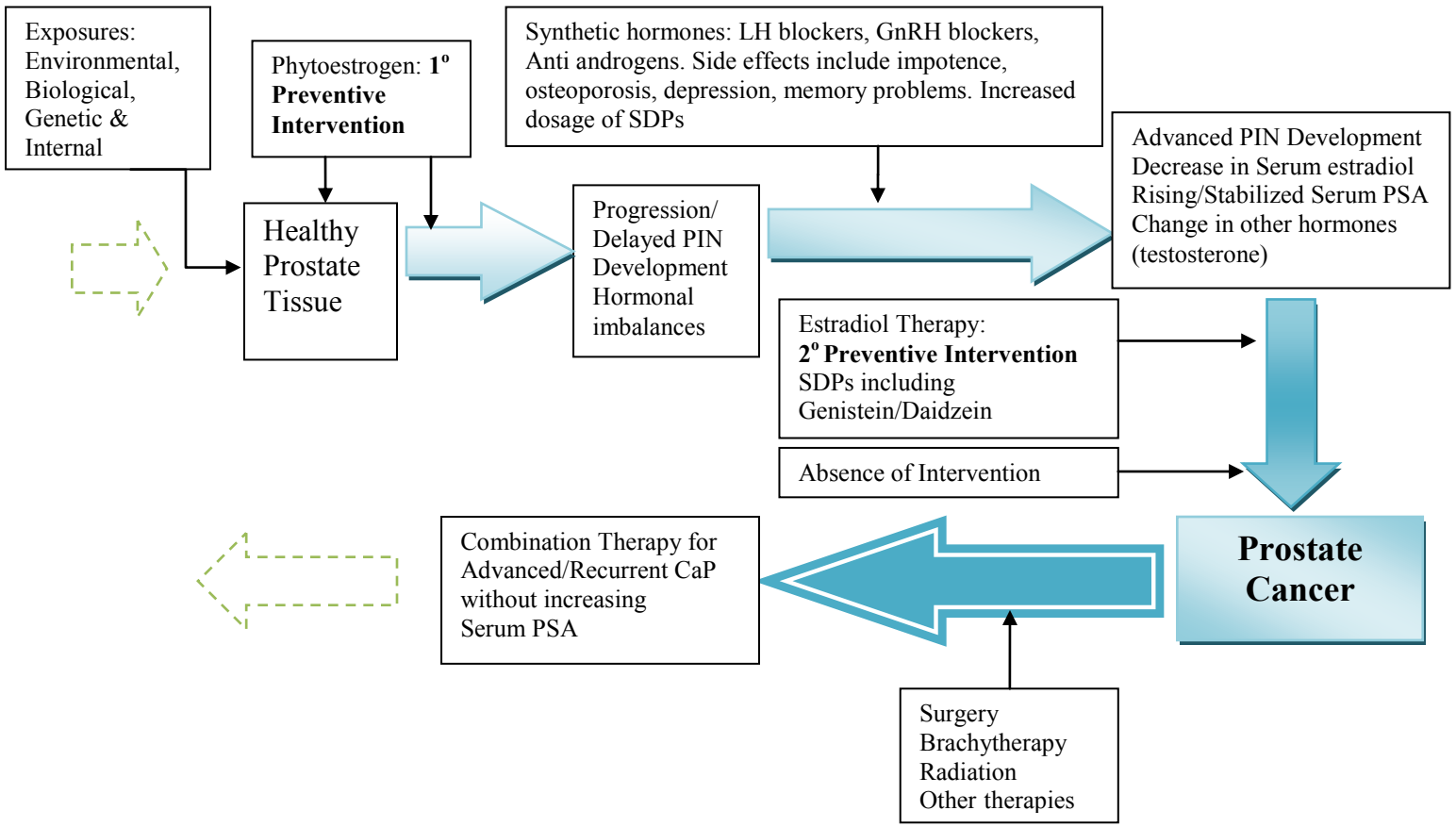

Figure 3. Dual Prevention Intervention Model (DPIM). Michelin & Gutierrez, 2013, n.p.

Nature of the Study

The study was a quantitative analysis of data from published randomized-controlled trials (RCTs) demonstrating the chemoprevention ability of soy and its derived SDPs. The population sampled included men ages 50-85 in the United States. The studies were analyzed employing meta-analysis. The independent variables were dietary quantities of the SDPs genistein and daidzein. The dependent variables included serum levels of E2 and PSA. I sought to determine the ES in SDP dose-response intervention in selected male populations during the therapeutic application for recently diagnosed and recurrent CaP, and the contribution of duration and concentration in determining the size and direction of action. The principles of primary and secondary prevention and the original conceptualization of the DPIM of cancer prevention guided this research study. I sought to answer whether intake duration and concentration of SDPs directly influence magnitude and direction of serum PSA and E2 during various conditions of CaP disease. I conducted a statistical analysis employing CMA specialized software to determine the ES using Odds Ratio statistic and the REM. Odds Ratios were calculated through 2x2 contingency tables. Confirmatory analysis included Cohen's *d*, *Q* statistic, and mean standardized differences. Heterogeneity was measured using *g* statistic. (More details provided in Chapter 3).

Research Questions and Hypotheses

The ingestion, duration, and concentration of SDPs are responsible for the associated lower incidence of CaP and other prostate diseases observed among Asian males (Dalais et al., 2004). Inversely, its decreased intake among men in Western

countries appears to be associated with a higher incidence of CaP and other prostate diseases. Answering the research questions provided some perspective on the effects of different independent variables on dependent variables and subsequently on disease outcome among men in Western countries. I sought to answer the following questions:

Research Question 1: Is there a correlation between dietary SDPs and serum SDP levels?

Null Hypothesis H1o: There is no correlation between dietary SDP levels and serum SDP levels.

Alternative Hypothesis $H1_A$: There is a correlation between dietary SDP levels and serum SDP levels.

Research Question 2: Is there a correlation between dietary SDP levels and serum PSA levels?

Null Hypothesis H2o: There is no correlation between dietary SDP levels and serum PSA levels.

Alternative Hypothesis $H2_A$: There is a correlation between dietary SDP levels and serum PSA levels.

Research Question 3: Is there a correlation between dietary SDP levels and serum E2 levels?

Null Hypothesis H3o: There is no correlation between dietary SDP levels and serum E2 levels.

Alternative Hypothesis $H3_A$: There is a correlation between dietary SDP levels and serum E2 levels.

Research Question 4: Is there a correlation between serum SDP levels and serum PSA and serum E2 levels?

Null Hypothesis $H4_0$: There is no correlation between serum SDP levels and serum PSA levels.

Alternative Hypothesis $H4_A$: There is a correlation between serum SDP levels and serum PSA levels.

Research Question 5: Is there a correlation between serum SDP levels and serum E2 levels?

Null Hypothesis$H5_o$*:* There is no correlation between serum SDP levels and serum etradiol levels.

Alternative Hypothesis$H5_A$*:* There is a correlation between serum SDP levels and serum E2 levels.

Definition of Terms

Androgen deprivation therapy (ADT): Treatment that is used to block or suppress the production or action of the male hormones known as androgens. This is achieved by either removal of the male testicles, or blockage with female hormones or drugs called antiandrogens. This type of therapy is also referred to as androgen ablation or androgen suppression (NCI, 2012).

Benign prostatic hyperplasia/Benign Prostatic Hypertrophy (BPH): A non-cancerous condition which involves an excess growth of prostate tissue that pushes against the urethra and the bladder resulting in a blocking of normal urine flow (NCI, 2012).

Cancer recurrence: Cancer which has returned after a period of time in which the cancer was unable to be detected. This cancer might return to the same place as the previous tumor but could also return to a different region of the body (NCI, 2012).

Chemopreventive: The use of drugs, vitamins, and other compounds to try and reduce the risk of development, recurrence or progression of cancer (NCI, 2012).

Chemoprotective: The quality displayed by some drugs used for treating cancer. The chemoprotective agent protects the healthy tissue from the toxic effects of drugs used during cancer therapy (NCI, 2012).

Chemotherapy: The killing of cancer cells using drugs (NCI, 2012).

Diagnosis: "the process responsible for identification of a disease such as cancer from its sign and symptoms" (NCI, 2012).

Daidzein: An isoflavone fond as a biochemical component of soy (NCI, 2012).

Disease incidence: The number of new cancer cases over a specific period of time which is usually recognized as one year.

Distant metastasis: This is a reference to cancer that has spread from the primary tumor to distant organs or lymph nodes. Also related to as distant cancer.

Digital Rectal Examination (DRE): A procedure in which a trained medical practitioner performs a digital examination of the prostate employing appropriate and established medical practice (NCI, 2012).

Double-blind: A clinical trial in which the medical staff, the patient, and the people who analyze, the results, do not know the specific type of treatment the patient receives until the trial has been completed (NCI, 2012).

Isoflavones: Nonsteroidal plant derived phytoestrogen compounds which can bind the estrogenic receptors and can affect the normal estrogen regulation process (NCI, 2012).

Effect size: This is considered a numerical way of assessing the strength or magnitude of a reported relationship even it is seen as casual or otherwise (Villanova University, 1984).

Epidemiology: According to the WHO (2012), epidemiology is the study of the distribution and determinants of health-related states or events which includes diseases and the use of epidemiological practices to control these diseases and other health conditions.

Estrogens: Any of several major female sex hormones produced primarily by the ovarian follicles of female mammals, and capable of inducing estrus (oestrus), developing and maintaining secondary female sex characteristics, and preparing the uterus for reception of a fertilized egg. It is used especially in the synthetic form as a component of oral contraceptives in certain treatments and other forms of therapy (NCI, 2012).

Etiology: "the origin or cause of disease" (NCI, 2012).

Fixed Effects: The assumption that there is only one true effect size (Wiley/ITMA, 2009).

Genistein: An isoflavone found as a biochemical component of soy (NCI, 2012).

Gleason Score: According to the (NCI, 2012), Gleason score is the system presently employed to grade CaP tissue in context to how it appears when viewed

microscopically. The Gleason score ranges from 2 through 10 and is a reflection of the likeliness of the tumor spreading to adjacent tissues. Lower Gleason score indicates a decreased likelihood of the tumor spreading while a higher Gleason score is indicative of tumor with greater likelihood of spreading. It also demonstrates a higher variability from normal prostate tissue.

Hormone Refractory Disease/Hormone Resistant Disease (HRD): A disease condition that does not respond to hormone therapy (NCI, 2012).

Morbidity: Relates to a disease or incidence of disease within a population. It also refers to the adverse effects resulting from a treatment (NCI, 2012).

Mortality: A reference to the death rate which is also the number of deaths in a particular group over a specific period of time. Mortality might also be reported for people with a particular disease, live in one area of the country, and who belong to a certain age, gender, and ethnicity (NCI, 2012).

Outcome: "an effect that is measurable or a specific result. Examples of outcomes include decreased pain, reduced tumor size, and the improvement of disease" (NCI, 2012).

Phytoestrogens: Morrissey et al. (2004) indicated that phytoestrogens are considered nonsteroidal compounds derived from plant sources and which have estrogenic activity.

Prognosis: This is the likely outcome or course of a disease but can also be defined as the chance of recovery from a disease or recurrence of the disease (NCI, 2012).

Prostate Specific Antigen (PSA): A protein substance that is produced by the prostate gland and when found in the blood at a higher level than considered normal might be an indication of CaP, BPH, inflammation or infection (NCI, 2012).

Prostatic Intraepethelial Neoplasia (PIN): Noncancerous growth of the cells lining the internal and external surfaces of the prostate gland. Having high-grade prostatic intraepithelial neoplasia may increase the risk of developing CaP. (NCI, 2012).

Prostatitis: Considered inflammation of the prostate gland (NCI, 2012).

Radical prostatectomy: A surgical procedure that removes all of the prostate gland and some of the surrounding tissues (NCI, 2012).

Radiotherapy: Also called irradiation or radiation therapy. Involves the use of various high-energy radiation sources including x-rays, gamma rays, neutrons, protons and other sources to destroy cancerous cells and shrink tumor. Radiation can be from an internal source placed near the cancer cells or from an external source where beams were directed against the cancer cells. In systemic radiotherapy, the source is radiolabeled monoclonal antibodies that reach tissues after traveling through the blood (NCI, 2012).

Random Effects: The assumption that the true effect could vary from one study to the next (Wiley/ITMA, 2009).

Randomized Clinical Trial: A study in which the participants are assigned by chance to separate groups that compare different treatments; neither the researchers nor the participants can choose which group. Using chance to assign people to groups' means that the groups will be similar and that the treatments they receive can be compared

objectively. At the time of the trial, it is not known which treatment is best. It is the patient's choice to be in a randomized trial (NCI, 2012).

Receptors: Structure on cell surface or inside cell that selectively receives and binds a specific substance (MedicineNet, Inc., 2012).

TRAMP mice: Transgenic adenocarcinoma of the mouse prostate (Hurwitz, Foster, Allison, Greenberg, & Kwon, 2001).

Assumptions

This study is based on several major assumptions. The first assumption is that the lower incidence of CaP observed among Asian men is attributable to the greater intake of dietary soy derived SDPs/isoflavones namely genistein and daidzein (Willis & Wians, 2003). The second assumption implies that the resulting metabolic changes from SDP intake produce bioactive compounds linked to this lower incidence of prostate diseases observed within the Asian male population (Bektic et al., 2005; Kumar et al., 2011). The third assumption was that that an increase intake of soy derived dietary isoflavones among men in Western countries would result in a decrease in CaP incidence. The fourth assumption was that the beneficial changes in CaP are associated with serum, a decrease in serum PSA level, and an increase in serum E2 level. The fifth assumption was that a reduction in disease incidence would result in beneficial health outcomes within the western male population and reduce the disease and economic burden associated with disease treatment and management. The sixth assumption was a reduction or elimination of potentially unnecessary screenings, treatment, and a reduction of valuable quality of life issues that are associated with care and management of the disease. The seventh

assumption was that the employment of meta-analysis will mitigate differences in and between studies and provide statistical relevant ES information. The eighth assumption is that if findings demonstrate a positive correlation to E2 and inverse relationship to PSA response then further exploration of therapeutic or chemoprevention explored for other hormonal driven cancers including breast and ovarian cancers. The ninth assumption was that the variables had the potential to influence the outcomes and subsequent data presented in the analysis. Finally, evidence from this meta-analysis examination used to establish recommended daily dietary value for these studied SDPs.

Limitations

The following limitations were anticipated. This meta-analysis used data of prior research from selected published RCTs. These output data included the use of already established methods of data collection. The strategies employed in those prior analyses and the evidence presented from those studies is not amenable. These data are an accumulation of output data from other studies that included subjects who are different demographically, in age, in ethnicity, and socioeconomic status, possibly with numerous different health backgrounds. There is also the issue of reliability of the information source since it is difficult to monitor if diets consumed consisted of the soy products that are associated with the studied chemo-protective activity. Limitations also included the inability of the researcher to define criteria for selection as these were already determined by the RCT process. An important limitation relates to the coding, or data extrapolation process, as this process requires proper monitoring to ensure correct information retrieval, then the effect size values regarding the treatment outcomes would be incorrect.

Another limitations might also be present in the selection process used to recruit subjects for the different studies. Several conditions including age, health history and weight of participants were possibly important confounders and therefore, could have a negative influence on the produced output data.

Delimitations

The following were delimitations in this study. The role of environmental, genetics, and other exposure factors can be important delimiting factors. Factors contributing to the initial onset of prostate disease are observed through prostate intraepithelial neoplasia (PIN) development following extensive exposure. Delimitations might also be evident in the method of analysis been employed to determine the true effect of duration on the chemo preventive role of SDPs.

Significance of the Study

The dynamics of the interaction between phytoetsrogens, tumor development, proliferation, prevention, and even recurrence are valuable to greater understanding of implementation of workable intervention strategies. It is through understanding of the ES from research evidence that strategies can be developed, novel therapies implemented and, further research pursued that could help improve health outcomes. Information might also be important in its role of decreasing CaP disease incidence in populations that are considered at greater disease risk. There is not only an economic burden but also the social and psychological experiences of those affected by CaP (Turner et al., 2009). An event that can reduce the impact of social and other burden on the individual, the family and the wider supportive family of social network, would be valuable. Therefore,

addressing issues of quality of life (QOL), physical and mental strain, and improving the broader outlook associated with survivability and improved health outcomes (Sharpley, Bitsika, & Christie, 2011; Turner et al., 2009). Understanding how CaP impacts those affected and those in the broader community through greater awareness are also important concepts that the study addressed. With CaP considered a disease that will increase as the male population gets older and there is a greater possibility, of the incidence of CaP, than any intervention that could reduce such incidence including the tremendous economic and health burden that is associated would be of tremendous social value. In the social context, there would also be a greater desire to have soy products become a dietary staple. This could change the present outlook of the disease within the more at-risk communities and importantly among the populations now experiencing higher incidence.

This study is significant in that it presents an original contribution to the field of public health that aims to avert early prostate tissue damage through identification of a critical point at which a primary prevention is incorporated. The goal of this research and the DPIM (Michelin & Gutierrez, 2013) is to prevent, delay, or reverse disease through addition of SDPs either dietary or medicinally to avert progressive development of prostate tumor. While several studies had provided scientific support for the events describing such association, in this study, I analyzed relevant literature and determined the true ES of SDP intervention on individuals with various stage, grade or type of CaP. Through the broader scope of the DPIM, the meta-analysis of the true ES revealed the

much more important secondary preventive role of SDPs through E2 therapy compared to expected decreases in PSA as the desired outcome.

Summary and Transition

Chapter 1 provided an overview of CaP in the US population. There is need for examination of the shortfalls regarding the present therapeutic practices. How these reflect the need for novel therapies for improved health outcomes. Some relevant discourse is presented based on the studies employed in this meta-analysis and how this present a worthy context to the research focus regarding the employment of various soy-based products for CaP therapy or prevention. This is important not only in context to complementary and adjuvant therapy but as the defining therapeutic intervention process. Chapter 2 will be devoted to the review of supportive literature that will offer scientific insight and knowledge base. This information will span a vast array of material incorporating theoretical and experimental discourse and providing even greater understanding and relevance to the various aspects of the disease process. The study discusses potential benefits of SDPs for therapeutic intervention in combating the incidence of CaP. Chapter 3 is a detailed presentation of the research methodology which relates to ES, and importantly how this is demonstrated employing either an open- or fixed-effect model analysis. However, in this study, I used the REM for ES analysis. REM represents a more precise evaluation of the aim and objectives of this study. The assumptions will support or offer a clear relationship between SDPs and their value in helping to reduce CaP. The debilitating effects of CaP remain in ethnic populations still

affected by higher disease incidence. These populations continue to experience greater disease burden than the male population in Asian countries.

Chapter 2: Literature Review

Overview

The purpose of this study was to determine through OR meta-analysis the ES of genistein and daidzein on PSA and E2 serum levels. The magnitude and direction of that response to dietary and then serum genistein and daidzein levels will also be determined. The influence on both PSA and E2 serum levels could be valuable in determining treatment outcomes in diagnosed CaP cases, rising PSA, and HRCaP. The effect of dietary and serum SDPs on PSA and E2 serum levels has not previously undergone analysis in this cohort of American men. The chapter has several sections. These include the literature search strategy, background of prostate and hormone refractory cancer, epidemiology and pathophysiology of CaP, theoretical base and theoretical applications of CaP and SDPs, E2, and PSA. Literature on differing views and methodology related to RCTs involving chemoprevention intervention is also included. There is also a concluding summary of the chapter and transition to Chapter 3.

Literature Search Strategy

Conducting a review of pertinent literature involved extensive search of Science Direct, SciVerse, PubMed, and Walden University's online library. Search terms used included *phytoestrogens and cancer therapy, phytoestrogens and prostate cancer, genistein* and *cancer therapy*, and several combinations of terms that included either *prostate cancer, genistein, daidzein,* and/ or *phytoestrogens*. The articles selected for analysis in this study were selected following a search of Science Direct, SciVerse and PubMed using a collection of terms that included *phytoestrogens* and *prostate cancer*

therapy. The selected studies also had to include the terms of *randomized-controlled double-blinded clinical trials*, which employed either *genistein* and/or *daidzein* as included elements of the study process.

Prostate and Hormone Refractory Cancer

CaP including HRCaP (Crawford, 1992; Sadar, Hussain, & Bruchovsky, 1999) follows surgical castration or disease not responding to androgen deprivation therapy (ADT; Napora, et al., 2011; Sharifi, Gulley, & Dahut, 2005). It accounts for over 10% of cancer cases, and is expected to fail all present therapies. The subsequent recurrence is associated with poor health outcomes as a consequence of this difficulty to treat the resulting disease (Singh, Matanhelia, & Martin, 2008).

While results might be somewhat unpredictable, evidence suggests that use of secondary hormonal therapy could provide some health benefit (Pendleton et al., 2008; Ryan & Small, 2003). This perspective can be relevant when appreciating the dietary intake of SDPs. Intake of SDP is estimated to be 60 times greater within the Asian population than among Western men (Qin et al., 2003; White et al., 2004). Several researchers, including Adams et al. (2004), Boyle, Maisonneuve, and Napalkov, (1995), and Hsing and Devesa, (2001), have also acknowledged that there are health benefits that can be attributed to the intake of SDPs. These include a difference in the incidence of prostate diaseases observed among Asian men who have immigrated to Wetsern countries (Adams et al., 2004; Boyle, Maisonneuve, & Napalkov, 1995; and Hsing & Devesa, 2001). Some context to potential health benefits of SDPs also relates to the

observations made regarding the detection of isoflavones in the serum of newly born babies (Adlercreutz, Yamada, Wahala, & Watanabe, 1999).

This implied evidence of chemoprotection provides the opportunity for complementary and alternative modalities (CAM) to be implemented as intervention or proposed treatment strategies (White et al., 2004). The inclusion of SDPs and other SDPs into the Western diet has not seen similar lowering of CaP incidence. This observation suggests that CaP and other prostate diseases are still been defined by a higher incidence rate among Western men. Globally, the most prevalent cancers include prostate, lung, colorectal, stomach, and breast cancer (WHO, 2012). Significantly, in regions surveyed by the WHO, the report implies that the highest incidence occurs in Europe and the Americas (WHO, 2012). However, the lowest observed incidence is in Southeast Asian countries where SDPs are an important dietary staple (WHO, 2012).

Hormonally influenced cancers include CaP, ovarian, and breast cancers. These cancers affect millions globally. The concerns regarding response to present therapies have resulted in the need for complementary modalities such as herbal and enzyme therapies, hydrotherapy, and the use of soy-derived components including the isoflavones genistein and daidzein (Auerbach, 2006). These have shown the potential to elicit positive therapeutic response (Auerbach, 2006). The lack of information regarding the etiology of HRCaP from initially-treated cancer that had responded to therapy, has also been demonstrated through *in vitro* analysis and expression of the androgen receptor Ki67, receptor BCAR and decreased staining of *5α-reductase*, aromatase and estrogen receptor β (Celhay, Yacoub, Irani, et al., 2010). Evidence suggested that during relapse

there appeared to be a lower expression of estrogen receptor α which was also inversely proportional to the proliferation of tumor cells (Celhay, Yacoub, Irani, et al., 2010). Therefore, progression from a disease that initially responded to hormone therapy and subsequently fails to respond presents an important point regarding the issue of recurrence and tumor progression (Celhay, Yacoub, Irani, et al., 2010).

The concern of finding an appropriate model for research is also still an issue. Hypothetical models such as the oxidative stress model by Thapa and Ghosh (2006), and the putative multistep model by Giovannucci (1999), which is the model followed by this research, provide some context to the development of the disease and opportunity for therapy and chemoprevention. However, the benefit of the mammalian model is tied to the relationship between species and is suggestive of some parallels in the etiology of the disease. Certain phases of the mammalian model have shortfalls, which underscores the complex development and progression associated with CaP. Antioxidants are found predominantly in fruits and vegetables and also viewed as important anticancer agents. This antioxidant involvement in reducing the inflammatory process is among the important benefits and anticancer activity of soy derived SDPs (Borek, 2005).

These sections provide an attempt to understand these associations with CaP etiology and how these complex events can be understood within the context of SDP intervention. Understanding the complex CaP models that provide additional background regarding the multifaceted development of the disease and including the significant role of estrogen in its etiology is also valuable. Problems relating to disease recurrence following ADT and surgical castration provide some insight into the complex

management problems. Poor outcomes appeared to be associated with a decreased expression of the estrogen receptor ∞ and what is considered high proliferation index (Celhay, Yacoub, Irani, et al., 2010). With CaP considered a disease, where the risk factor and incidence increases with age, then understanding the value of SDPs as a preventive or curative option, will be important. In fact, there appears to be some credence afforded to this thought, with the evidence suggesting that men who had developed a westernized lifestyle following migration and included lifestyle changes subsequently also had an increased risk of CaP development (Haenszel & Kurihara, 1968; Staszewski & Haenszel, 1965; Wang, Clubbs, & Bomser, 2006).

Evidence presented through findings from *in vitro* studies (Wang, Clubbs, & Bomser, 2006), also indicates the important role of genistein in the signal regulation of estrogen. Genistein is one of the isoflavones that is of interest to this study. Human studies have also demonstrated the chemoprotective value of SDPs with focus placed on the soy derived isoflavones genistein and diadzein that are soy derived. The effect of these compounds in alleviating the disease burden in the population is a focus of this dissertation and will be analyzed employing meta-analysis of data from representative selected randomized trials that will reveal the true ES of these SDPs on CaP within the population. The result of either being preventative or curative in relation to CaP will be defined following analysis of intake duration. This will demonstrate their ability to significantly assist in reducing the present burden in prostate disease now experienced among males in Western countries when compared to that experienced among males in Asian countries.

Epidemiological data appear to indicate the presence of SDPs in neonates following birth from mothers known to have consumed high quantities of SDP in the diets (Adlercreutz, Yamada, Wahala, & Watanabe, 1998). Presentation has been made on the available understood natural history of CaP, including the screening methods and subsequent tests that are employed in establishing a positive diagnosis. Present theories that have been established in relation to hormone refractory disease, the success and failure of presently available drugs for disease treatment, and the continuing trend of employing SDPs, and significantly those associated with soy products which have experienced a continuing upsurge in use over the past decades.

CDC (2012) data indicate a trend that acknowledges a statistical decrease in disease incidence within the US population. However, trends appear to be different from those provided by the World Health Organization (WHO). Reports indicate an increase in cancers and globally cancer related mortality is predominantly from five cancers including CaP (WHO, 2012). However, while significant decrease in cancer incidence is represented by a total 1.6% among the US population, data indicate only minimal reduction in CaP (CDC, 2012). Disproportionately, the incidence rates remain higher among minority populations, and African American men record the lowest annual decrease of 1.7% between the years 1998 to 2008 (CDC, 2012). There is need to appreciate the value of these WHO supported reports. WHO (2012) also detailed the significance of CaP in various geographical regions, with greater incidence in the developed economies (WHO, 2012).

Improved medical therapies and improved diagnostic tools have not resulted in a significant decrease in incidence among CaP and other prostate maladies experienced by men in Western countries. Evidence from several epidemiological studies have also shown an increase in CaP cases among men in Western countries with annual incidence and mortality rates greater than 200,000 and over 20,000 respectively (Jemal et al., 2009; White et al., 2010). Hormones are known to influence the growth and proliferation of prostate tumor cells (Bonkhoff & Berges, 2009; Harkonen & Makela, 2004; Lombardi et al., 2001; Qin et al., 2003; Singh et al., 2008; Weihua et al., 2002). However, hormone independent CaP accounts for a vast number of disease recurrences, even in men who have undergone radical prostatectomy/surgical castration (Chang, Cheng, Huang et al., 2009). There is also evidence that PSA levels can rise independent of prostate disease (Ito, Yamamoto, Ohi, et al., 2003), which can also complicate any true dependence on PSA levels as a reliable indicator of either disease progression or limitation.

This disease is not only difficult to treat, but does not respond to several of the present therapeutic agents and is marked by poor prognosis and disease outcomes. A beneficial role of dietary SDPs has been proposed for hormonally dependent diseases (Strauss et al., 1998). The work by Hamilton-Reeves, Rebello, Thomas, Slaton, and Kurzer (2007) implies that intake and duration of soy components possesses protective capability for men with high risk of developing CaP. This protection was due to a lowering of serum hormones including E2 from ingested soy proteins and therefore seen as beneficial to the deterrence of CaP (Hamilton-Reeves et al., 2007), probably through estrogenic and antiestrogenic effects, and enzymatic and other nonhormonal factors

considered important to the actions (Strauss et al., 1998). In Asian men where soy based diet is a staple, there appears to be a lower incidence of CaP and various prostate diseases. Understanding the strength of the association between SDPs and preventing CaP is important to initiating a prevention program.

Previous *in vitro* work by Fotsis et al. (1995), Onozawa et al. (1998), and Shao et al. (1998) has demonstrated the PSA lowering ability of this SDP. A study by (Sharma et al., 2009) also appeared to corroborate phytoprotective ability through improved quality of life (QOL) indicators for cognitive and erectile dysfunction issues. Others were not improved in androgen deprivation therapy (ADT) treated subjects (Sharma et al., 2009). The (ACS, 2012; CDC, 2012) implied that these problems can be attributed, to the extensive utilization and quite often, unnecessary use of medical treatment for prostate diseases. There is also significant disease recurrence following radical prostatectomy, hormone treatment, radiotherapy, chemotherapy and other adjuvant therapies (ACS, 2012; CDC, 2012). Importantly, and certainly invaluable to the foundation of this study is the fact that epidemiological evidence points to no distinguishable tumor present in male chimpanzees that primarily consumed plant based diet containing lignans and SDPs (Fuhrman, 2012; Musey et al., 1995). These compounds are attributed to the almost nonexistent cancer incidence and burden in these mammals (Fuhrman, 2012; Musey et al., 1995). While early diagnosis offers tremendous opportunity for effective treatment and $\geq$ 99% survival, the lack of a reliable therapy and a method for early diagnosis, presents some concern. The slow growth of some tumors also allow for what is considered surveillance or watchful waiting (WebMD, 2012). A scheduled surveillance

allows for implementation of the most suitable therapy (WebMD, 2012). In fact, uncertainty remains regarding the appropriate time for androgen therapy, and concerns regarding associated side effects from testosterone suppression include osteoporosis and reduced QOL (Vaishampayan et al., 2007). QOL certainly becomes a major issue during CaP therapy. In fact, individuals who are diagnosed with metastatic disease where survival is highly reduced remain a group that needs access to more effective therapy. Treatment should be geared to increasing survival and improving QOL. There is also need to understand the antioxidant capacity SDPs (DiSilvestro et al., 2006), though there is already established support of antioxidant relationship to cancer prevention (Borek, 2005; Collins, 2005; Czernichow, Thapa & Ghosh, 2012; Galan, & Hercberg, 2005 Jayaprakash & Marshall, 2011). Earlier prevention could avert more complicated progressive disease conditions.

Reliability and sensitivity concerns also plague the PSA test, which is still employed as a primary diagnostic tool (ACS, 2012; CDC, 2012). The test is also important in the follow-up process for disease response during therapy, and as the precursor process to the digital rectal examination (DRE) and other diagnostic tools (ACS, 2012; CDC, 2012). Consequently, a reduction in PSA level in response to SDP intake, while valuable for disease response, can demonstrate even more value if the statistical analysis corroborate and complement survival and better health outcomes. The association between SDP intake and decreased PSA is also important in future analysis regarding lower incidence rate in those high-risk populations. Also important is recognition that the reliability and specificity of the PSA test still seem inadequate, since

the present cut-off value of 4ng/ml can be unreliable in confirming CaP. Lowering the value to 2.5/3.0 ng/ml, especially in younger males has therefore, been proposed (WebMD, 2012). Some beneficial association is evident regarding the influence of SDPs on PSA during less advanced disease, although demonstrating diminishing correlation (White et al., 2010).

Another concern relates to prostate diseases affecting younger men and not only men in the later decades, where mortality might be attributable to other diseases (CDC, 2012). Again, the need for more reliable therapeutic and preventative interventions should therefore be paramount. Disease recurrence is also anticipated among five percent of all those affected by CaP (ACS, 2012). With age considered possibly the greatest risk factor, prevention is certainly a prudent strategy.

BPH, introduced earlier, does not necessarily imply progression of CaP. However, an association has been established and, prevention of BPH through SDP intake might significantly reduce this problem including the potential for further disease initiation (Lee, Gomez, Chang, et al., 2003). Both BPH and CaP have been acknowledged as having lower incidence among Asian men, among whom ADPs is a dietary staple. It is this protective role that appears to be important in the lower incidence of prostate diseases such as BPH and CaP among Asian men. It is important to determine the consumption practices of those products that can benefit the health of men in Western countries.

CaP risk is still evident even after orchiectomy or androgen ablation to surgically inoperable cancer as development of resistance usually occurs within months or a few

years (Singh et al., 2008). This development of androgen-insensitive tumors usually has poor prognosis and decreased survivability (Singh et al., 2008). CaP is a very complex disease and management with the aim of effecting long term curative outcome, is still a very difficult situation. There is still uncertainty about the etiology of CaP although evidence points to continuous mutation of abnormal cells that subsequently proliferates and metastasizes (Mayo Clinic, 2011). The potential for SDPs in a preventative context therefore, appears quite prudent. Information from several randomized-controlled studies implies further benefits through the reduction of PSA than other biochemical responses. Estrogen therapy remains widely employed and continues to be associated with concerns, including possible involvement in influencing CaP growth and proliferation (Bonkhoff & Berges, 2009; Celhay et al., 2010). SDPs therefore, appear to be multifaceted in their ability to provide chemoprotection to prostate gland cells (Guy et al., 2012; Hsing, Tsao, & Devesa, 2000). The added biochemical characteristics of SDPs as an antioxidant might certainly be important in the displayed chemoprotective ability (Borek, 2005). Among biological concepts that have been studied are modifications on the androgen receptor (AR) gene through mutation or amplifying activities, and phosphorylation of AR protein (Celhay et al., 2010).

Epidemiology of Prostate Cancer

While 1 in 6 males are diagnosed with CaP, approximately 2.5 million that have been affected are among present CaP survivors in the USA (ACS, 2012). In 2012, mortality from CaP is estimated at 28,170, with nearly 241,740 males among those newly diagnosed (ACS, 2012). In fact, CaP accounts for the second highest cancer incidence

rate among US males and was responsible for over 33,720 deaths in 2011 (ACS, 2011). Although there is a decrease in incidence, there remain the need for improved therapies and effective prevention strategies. This is especially significant since the disease appears to affect at an average age of 67 (ACS, 2012).

While the disease has a higher incidence in males in Western countries, compared to those in the Asian male population, pathological data indicates similar incidence of latent disease, in approximately 80% of males ≥ 80 years old (Kumar & Anderson, 2002). This lower incidence mentioned among males in the Asian population has been associated with the potential protective activity of SDPs consumed in the diet of Asian males (Kumar & Anderson, 2002). Figure 2 (page 11) provides context to the relationship of incidence over time. Animal and *in vitro* studies have also shown that there is significant anticancer activity from SDPs such as genistein and daidzein and that these isoflavones may be responsible for the lower incidence of CaP and another condition BPH observed among Asian males (CDC, 2010).

There is also concern that estrogens that were once effective in treating CaP, might also be contributory to the development and progression of the disease (Bonkhoff & Berges, 2009). Importantly, while this form of therapy appeared to be effective against androgen-dependent disease, the potential risk of been associated with malignancy is certainly disconcerting (Bosland, 2005). According to (Bosland, 2005) many theories have explained this association including evidence of various genetic polymorphism in particular codon regions of the ER-α gene that either increases the risk or development of CaP. While ER-α has demonstrated this association of CaP development ER-β has

demonstrated tumor suppression activity (Bosland, 2005; Giton, de la Taille, Allory, et al., 2008). Age as indicated earlier is a significant predisposing factor for CaP. CaP appears to be most likely initiated in gland cells and is referred to as adenocarcinomas (ACS, 2012). These are the cells that are responsible for the production of the fluid that improves the environmental conditions for sperm cells (ACS, 2012). Reports indicate that CaPs can experience either slow or rapid growth, and evidence exists of the cancerous tumors in older males but mortality been attributed to another disease condition (ACS, 2012).

The development of CaP can also be attributed to PIN lesions considered a precancerous event and can affect males even in the early decades of life (ACS, 2012; Weihua, Warner, & Gustafsson, 2002). Microscopic analysis of biopsied PIN cells and determination of the level of their abnormality can be helpful in determining the chances of developing CaP (ACS, 2012). There is also a greater chance of CaP occurring when a high grade abnormality is assessed during PIN analysis (ACS, 2012). The presence of high grade PIN on biopsy also reflects an increased possibility of (20% - 30%) greater association with CaP development, and that there are cancerous lesions in other parts of the body (ACS, 2012). Other important findings that may be indicated through the biopsy process include atypical small acinar proliferation (ASAP) and proliferative inflammatory atrophy (*PIA*) (ACS, 2012). While there is some uncertainty regarding *PIA*, it is still thought of as a precursory event, while *ASAP* appears to indicate a need for follow-up biopsy (ACS, 2012). This seem even more compelling when evidence indicate

the presence of ER-β receptors in normal, hyperplasic and prostate tumor cells, but a decreased expression in CaP (Weihua, Warner, & Gustafsson, 2002).

Prostatic Specific Antigen

PSA is a protein and falls in the group of serine protease, and is produced by prostate tumors (Adams, Chen, Newton, Potter, & Lampe, 2011). Prostate tumors have the ability to destroy protective barriers and allow PSA to spill into the blood. This allows for serum PSA concentration to be employed as a useful tumor marker (Adams, Chen, Newton, Potter, & Lampe, 2011). However, while the concentration of serum PSA appears to be proportional to the tumor volume, it is also not always elevated during CaP and other situations such as BPH, inflammation and other prostate conditions are responsible (Adams, Chen, Newton, Potter, & Lampe, 2011). With increased use of PSA as the screening method, there has been the detection of several asymptomatic cases among tested populations, which could certainly account for a higher described western incidence (Kumar & Anderson, 2002; Bonkhoff & Berges, 2009).

Consequently, the lack of available testing in some populations might also account for undiagnosed cases which could increase the present number. Prevention strategies might certainly be the model responsible for reducing the high incidence in Western countries, and inclusion of dietary SDPs appears a potentially viable option. This research aims to identify through meta-analysis of the data from selected randomized controlled trials the dynamics that is evident through analysis and determination of the ES of SDPs intervention. This thought is certainly shared by several investigators (Landstrom et al., 1998; Mentor-Marcell, Lamartiniere, Eltoun, Greenberg, & Elgavish,

2001; Morrissey et al., 2004), in reference to the influence of genistein to decrease tumor cells in mice models. Inducing apoptosis *in vitro* by the SDP resveratrol in selected prostate carcinoma cell lines also demonstrated evidence of SDPs on prostate adenocarcinoma cell growth (Mentor-Marcell Lamartiniere, Eltoun, Greenberg, & Elgavish, 2001). The true effect of SDPs will therefore be unearthed from this intensive research process. The prostate gland located at the neck of the male urethra and bladder. It is approximately the size of a walnut in younger adults but can be larger in older males (ACS, 2012). Figure 4 below shows a healthy prostate with a walnut representation of its normal size. Tumors and other infections of the prostate result in an enlarged prostate. There is a variation in the size of the prostate and it is usually larger as the man ages.

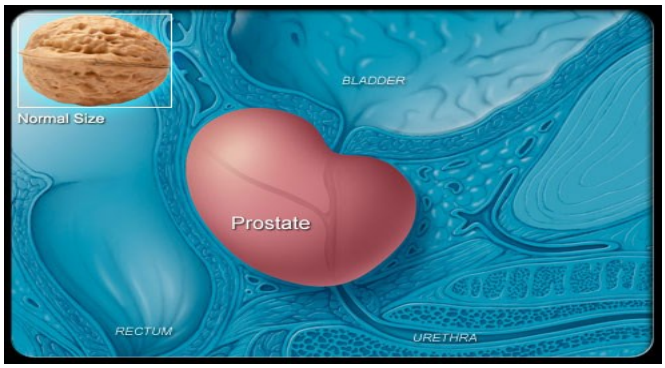

Figure 4. Healthy Prostate, Nearby Organs and Internal Structures. Source: The Prostate, National Cancer Institute, 2012.

In Figure 5 a representation of the prostate gland affected with a developed tumor demonstrates the resulting enlargement. The enlarged prostate gland results in secondary health issues as it causes constriction of the urethra. Constriction of the urethra is associated with problem urinating and other medical issues.

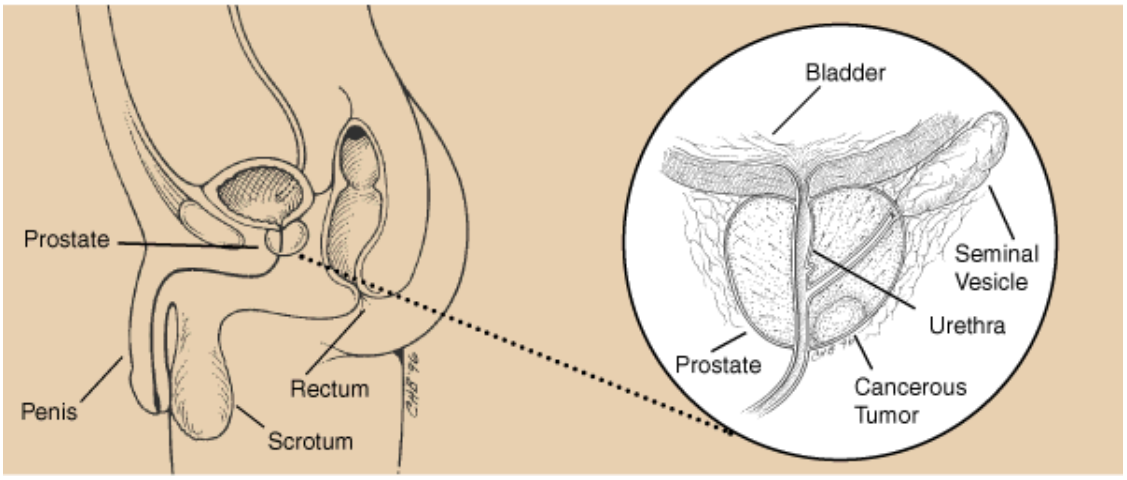

Figure 5. Prostate Gland with Identifiable Cancerous Tumor. Source: What is prostate cancer? ACS, 2012.

In some contexts, it is considered both muscular and glandular with ducts that open into the prostatic urethra and consist of a central and two adjacent lobes (NCI, 2012). The prostate gland enlarges with age and this can result in constriction of the urethra and subsequent difficulty in the passage of urine (NCI, 2012). The processes that encourage growth, differentiation, and functionality of the prostate gland while largely dependent on the male hormone androgens, relies in some significant context to the action presence of estrogens (Harkonen & Makela, 2004). The enzyme 5-alpha-reductase acts on the male hormone testosterone an androgen produced in the testicles converting it to dihydrotestosterone (DHT) which subsequently influences growth of the prostate (ACS, 2012). The presence of male hormones during the adult life allows the prostate to maintain its size (ACS, 2012), but several factors can influence changes that result in unfavorable health conditions. Problems involving the prostate appear to begin during later decades in life, although earlier problems can affect passage of urine through the

urethra (NCI, 2012). Problems that can occur over time include infection (prostatitis), benign prostatic hyperplasia (BPH) and CaP (ACS, 2012; NCI, 2010).

Screening Methods

CaP screening methods have afforded some amount of earlier opportunity for active surveillance and early treatment. However, several concerns have arisen from the use of the PSA test which is employed for many years as a major determinant for initiating further screening, determining response to various therapies and to predict the outcome from treatment. The PSA screening also takes into account the importance of values as they relate to percent- free PSA, PSA velocity, PSA density and age-specific PSA ranges (ACS, 2012). Complementary modalities including digital rectal examination (DRE), prostate ultrasound (transrectal ultrasound), and prostate biopsy have been used as independent screening tools (WebMD, 2010). However, these treatments have not shown improved survivability and health outcomes (WebMD, 2012). Benefits of earlier diagnosis and detection of latent disease achieved through development of improved screening techniques (WebMD, 2010). Benefits we can appreciate, although accompanied by uncertainties including a high incidence of false positive results (ACS, 2012). This can result in the unnecessary use of other procedures and high economic costs (ACS, 2012).

PSA Testing and Importance

Approved by the US Food and Drug Administration (USFDA), the PSA test looks at the level of serum PSA. The value ≥ 4ng/ml warrants further testing (NCI, 2012). Other testing includes prostate biopsy for tissue analysis or monitoring of PSA velocity

(NCI, 2012). These tests reflect changes in PSA levels over specified times (NCI, 2012). Elevated PSA levels over time and a positive biopsy might be indicative of CaP (NCI, 2012). Cells are biopsied, and a Gleason score from 2-10 is assigned following analysis (NCI, 2012). Some individuals have shown no increased health benefits from PSA analysis (ACS, 2012). There is some controversy about the value of PSA analysis as a valuable screening tool (ACS, 2012). Confirmation is evident with an observed mortality rate of 3% within a population with a 9% diagnosed incidence rate (ACS, 2012). Presently, there is about 2% - 2.5% mortality rate that occurs among the 18% diagnosed following PSA screening (ACS, 2012). Mortality rate is within the context of reliability regarding PSA values. Increasing PSA might not be significant of CaP, but from prostatitis and BPH as indicated earlier. PSA is specific for its association with prostate activity and is not itself specific for the association with CaP (Kumar & Anderson, 2002).

However, a rising PSA following surgical castration and following radiation is certainly cause for concern and can be extremely problematic (Vaishamapayan et al., 2007). Values that are provided through this screening method are therefore, indicative of the possible risk of CaP with that risk increasing to ≥ 90% when the PSA level is > 20ng/ml if the considered level of 2.5ng/ml is considered normal for males between the ages of 40 – 49 years old (Kumar & Anderson, 2002). Employing the digital rectal examination (DRE) is another screening method that can detect abnormality of the prostate gland (ACS, 2012). Tissue biopsy and microscopic analysis of cells is to confirm the presence of cancer, and to determine the course of action regarding treatment or surveillance (ACS, 2012). Screen due to factors such as age, ethnicity and genetics.

Staging provides a good indication of how far the disease has spread following screening assessment. The method most commonly employed by clinicians is the elaborate TNM classification approved by the American Joint Committee on Cancer (AJCC) which assesses the disease centered on the progression of the primary tumor (T), any evidence of spread to nearby lymph nodes (N), and evidence of distant metastasis (M) (ACS, 2012).

Tumor Grading

An important part of diagnosing CaP is the staging process. Information on staging comes from an understanding of the disease process. The disease process is defined by a system that provides assessment based on the tumor containment or spread to other tissues (ACS, 2012, NCI, 2012). Tumor spread and containment are important in determining treatment options and other factors such as response to therapy and subsequent outcome (ACS, 2012). Grading of tumors is an important part of the therapeutic process. Grading helps to define the disease state and provides important information on the present status of the disease (ACS, 2012). Both Gleason grade and tumor node metastasis (TNM) staging are valuable screening methods, that are associated with CaP diagnosis and are available to guide treatment modalities and the potential for disease progression or containment (ACS, 2012). They are also extremely focused, directed and elaborate scientifically based assessment of the disease process, and is therefore invaluable in any therapeutic decision and follow-up care or employed curative strategies. Below is the ACS (2012) outline of the application of the Gleason grade, Stage

and Score in defining what approaches including chemotherapy, surgery, hormone therapy and others might be best suitable for intervention and follow-up strategies.

Gleason Grading

Important to the understanding of tumors is what is considered the 'Gleason Grade'. The 'Gleason' grading system classifies tumors based on a staging scale of 1 – 5. This staging scale is an indication of the disease at the time of diagnosis (ACS, 2012). Grade 1 tumors have cells with the appearance of normal prostate cells (ACS, 2012). Cells considered grade 5 on the tumor grading scale are abnormal and poorly differentiated (ACS, 2012). These cells have now infiltrated the prostate and are observable throughout that gland (ACS, 2012). Cells categorized between grades 2 – 4, demonstrate varying increasing ranges of infiltration of tumor cells within prostate tissue (ACS, 2012).

Important to understanding the state of the prostate tumor is through the Gleason score. Gleason score is a complementary tool within the assessment process. Most CaP tumors have mixed cell types with different tumor grades, and the Gleason score is an additional assessment criteria (ACS, 2012). The Gleason score provides a better rational analysis of the state of the tumor (ACS, 2012). Tumors are assigned two numbers which helps to identify the designated dominant and minor tumors (ACS, 2012). Addition of the numbers assigned to each tumor grade then provides a better assessment and subsequent description of the present tumor through this assigned score (ACS, 2012). Therefore, a dominant grade 2 tumor and a minor tumor grade 4 would classify as six on the Gleason scoring scale (ACS, 2012). Appreciation of the value of the Gleason score comes with the

understanding that scores range from 2 – 10 with a higher value representing tumors that have a greater differentiation from normal prostate cells and those that are likely to be aggressive in their ability to spread (ACS, 2012).

The preceding discussion, therefore represents a generalized outlook that a Gleason score between 2 – 4 or Grade 1 cancer is the representation of cancer that can be considered low grade and consists of cells that are highly differentiated (ACS, 2012; NCI, 2012). A Gleason score between 5-7 or grade 2 cancer, represents a cancer that consist of cells that are moderately differentiated (ACS, 2012; NCI, 2012). A score of 8-10 on the Gleason scale, represents a Grade 3 cancer, consist of poorly differentiated cells. The grade 3 tumor represents tumors considered high grade (ACS, 2012; NCI, 2012). While poorly differentiated cells are possibly a definitive representation of high grade tumor that needs aggressive treatment the problem concerned those cells which are moderately differentiated as there appears to be no significant model of determining the progression of these cells, and this is apparently a significant volume of the cancer grades identified during diagnosis (ACS, 2012; NCI, 2012).

While grading provides invaluable information on proliferation of tumor cells within the prostate tissue, another important tool within the broad diagnostic process, is that considered staging. Here, is another tool which while complex in its assessment provides even greater capability and context to the diagnosis process, in its ability to providing a more thorough understanding of the disease in relation to the spreading of the cancer outside of the defined gland (ACS, 2012). There are two recognized staging

methods used during CaP staging. The one employed most during CaP staging which and approved by the AJCC (ACS, 2012) is described. .

The American Joint Committee on Cancer System

A staging system is a standard way for the cancer care team to describe how far a cancer has spread (ACS, 2012). The most widely used staging system for CaP is the American Joint Committee on Cancer (AJCC) TNM system (ACS, 2012). The ACS document indicates that there are five important areas regarding the TNM system (ACS, 2012). These are important determining factors for staging of the disease and certainly could be important for influencing and employed treatment strategy. In this AJCC staging system there are several important concerns. The concerns include the degree of the primary tumor (T category), the presence or absence of tumor metastasizing and if lymph nodes (N category) that are near the tumor are affected (ACS, 2012). These are the components of the TNM system. The PSA level at diagnosis, the Gleason score following surgery or when biopsied are also important in the diagnostic process (ACS, 2012).

Pathophysiology of CaP

While the development of CaP in humans is still defined by the difficulty in establishing its true etiology, evidence suggests some similarity between the disease and that described in male canine (Waters et al., 1998). According to this model, several defining similarities exist between both species including the development of PINs, the late onset of the disease, and the variation exhibited morphologically and genetically in observed prostatic lesions (Waters et al., 1998). The canine model certainly appears to explain several components of the disease. Among areas that are defined through this

similarity and support the use of the canine model are progression of the disease, ability to define predisposing factors and testing of therapeutic agents (Waters et al., 1998). The issue of PIN progression to tumor growth and subsequently metastasis of the disease is therefore important (Waters et al., 1998).

However, while the above is indicative of the positive value of other mammals in understanding CaP tumor, (Maini et al., 1997) there is an indication of limitations including reduced spontaneity, and the fact that canines are more difficult to manipulate genetically. Employment in this type of research could also be potentially expensive. With problem depending on mammalian models to understand CaP development, using transgenic mice have been valuable. The transgenic mice allows for understanding the importance of neuroendocrine cells in CaP development. Neuroendocrine cells are rare within the prostate. However, they contribute significantly to the development of very aggressive prostate adenocarcinoma that is also observed in humans (di Sant' Agnese, 1998). The transgenic mice model is also of tremendous value in attempting to understand the issue of CaP development without androgen stimulation. , although preceded by the presence of PIN and a rapidly metastasizing tumor (Garabedian et al., 1998).

CaP is still a very difficult disease to understand and transgenic mice model provides a valuable opportunity to study the neuroendocrine disease development and the complex role of neuroendocrine cell type in disease etiology (Garabedian et al., 1998). Transgenic mouse models' do provide some insight into possible development of CaP, but the value xenographs, representative of human CaP lines such as LNCaP and CWR-

22 have in some context seen some difficulty because of species differences (Stearns et al., 1988). However, their use has provided important context to the issue of chemoprevention and drug development (Stearns et al., 1998). What appears to be of interest regarding the use of mammalian models to understand CaP development and progression is the combination of many interacting factors and ones that illustrate a complex disease. These complex associations are also likely to be integral in not only development of the disease but the progression, metastasis and the complex nature of recurrence.

Biology of the Prostate

The prostate gland is about walnut size and located at the neck of the male's urethra and bladder. The gland is both muscular and glandular and has ducts that opens into the prostatic urethra; it has a central, right, and left side adjacent lobe (ACS, 2012). While several important functions are attributable to the prostate gland, with age there are important changes that can lead to conditions including benign prostatic hyperplasia (BPH) and CaP (ACS, 2012). With age increase, the prostate undergoes growth changes including a doubling in size during puberty (ACS, 2012). However, while the prostate continues to grow throughout the lifespan of men, enlargement can occur and result in problems, especially after the age of 60 (ACS, 2012). In fact, greater than 50% of BPH cases occur after that age and increasing age poses a greater risk (ACS, 2012). BPH is associated with thickened bladder wall, irritation, frequent urination, and urgency even when there is only a small amount of urine. Although BPH is a natural part of the aging

process, a decrease in the ratio of testosterone to estrogen hormonal levels can result in increased prostate growth (ACS, 2012). Estrogen is predominantly a female hormone.

A second theory implicates the continued production of dihydrotestosterone (DHT) a substance derived from testosterone and which controls prostate cell growth (ACS, 2012). The continued production of DHT and its storage in the prostate, even with the reduced production of an age decreasing testosterone, results in continued cellular growth (ACS, 2012). A third theory emphasizes the concept of cellular instructions from earlier initiated instructions for cellular growth. Cells that have early growth initiated instructions, could themselves resume growth or provide growth instructions to other cells. Instructions could involve greater sensitivity to hormones that could then influence growth (NKUDIC, 2010). This theoretical model could explain since cellular activity is continually under biological influence. The DPIM also addresses chemoprevention under these conditions and therefore provides the best model for this study.

Staging

Staging is an important part of diagnosis and the therapeutic process. There is also a greater understanding of the disease and certainly an opportunity for discourse between health care professional and individual. There are two staging methods associated with CaP diagnosis (ACS, 2012). The clinical stage provides an opportunity for the physician to have the best estimation of the present disease status (ACS, 2012). Several areas including findings from laboratory tests, prostate biopsy, and physical examination including the DRE, and imaging studies are very valuable (ACS, 2012). The second stage or pathologic stage, when analysis of extracted tissue is completed (ACS, 2012). Staging

types can result in differences in assessing cancers for staging since tissue removal and assessment could indicate a change in location of cancer cells (ACS, 2012). Staging also provides a realistic assessment of the cancer, as it provides a more detailed assessment of the disease process (ACS, 2012). Pathologic staging is dependent on analysis of the removed tissue, then it might be appreciated as advantageous in employing radical prostatectomy than the modalities of watchful waiting (expectant management) or radiation therapy' (ACS, 2012).

Staging employs various categorical assessments in determination of CaP disease. However, the category denoted as T1 is not among those used when defining pathologic staging of the disease (ACS, 2012). The staging categories presented provides a brief description of this process (ACS, 2012). There are four (4) categories for clinically describing the local extent of a prostate tumor, ranging from T1 to T4 with most consisting of subcategories (ACS, 2012). The N categories describe whether the cancer has spread to nearby (regional) lymph nodes (ACS, 2012).The M categories describe whether the cancer has spread to distant parts of the body with the most common sites of CaP spread been bones and distant lymph nodes (ACS, 2012). The lungs and liver are other tissues, which CaP can affect (ACS, 2012).

Estradiol

The secretion of E2 is from ovaries in females. E2 is an estrogen hormone. However, males secrete smaller quantities between 5-40 pg/ml. Studies have also shown the effectiveness of E2 for cancer chemoprevention.

Stage Grouping

Stage grouping follows a determination of the T, N, and M categories (ACS, 2012). Stage grouping is the cumulative assessment of CaP staging, Gleason scoring and prostate-specific antigen (PSA) concentration (ACS, 2012). If Gleason, score or PSA concentration is absent then staging relies on the T, N, and M categories (ACS, 2012; NCI, 2012). The use of Roman numerals provides an indication of how advanced the when diagnosed. Cancers that are least advanced is represented by the numeral I and IV and provides a representation of the most advanced cancer (ACS, 2012). This representation through numeric values help to determine what options are available for treatment and the prognosis for survival outlook (ACS, 2012). A second staging model the Whitmore–Jewett system has staging algorithm based on A, B, C and D staging. Less use is made of the Jewett system in the clinical setting (ACS, 2012). However, if used during the screening process, effort should be to provide comparative assessment based on the more widely employed TNM system (ACS, 2012).

Dual Prevention Integrated Model

The study initially considered the putative multistep cellular biochemical pathway model by Giovanni (1999). While this theoretical concept defines a biochemical pathway to CaP etiology and provides some constructs for the theoretical basis of this study, it does not address the primary and secondary basis for chemoprevention within the public health framework. The DPIM (Michelin & Gutierrez, 2013) presented in Chapter 1 is an original framework addressing the conceptualized primary and secondary strategies needed to address the process of CaP and other cancers. The DPIM is structured on the

premise that dietary intake levels of SDPs, in particular genistein and daidzein, are associated with the prevention of cellular damage responsible for the lower incidence of CaP observed among males in Asian countries. Theoretical models proposed in the literature have identified mitigating factors that result in cellular biological activities leading to development and progression of CaP. The goal of these models and of this research is the elucidation of factors which if manipulated could reverse or prevent CaP disease by addition of specialized isoflavones within dietary or medicinal contexts able to avert progressive development of prostate tumors. Among the mitigating factors, age, inflammation, change in hormonal status and genetic mutations are key. While several studies have provided scientific support for the events describing such association at the cellular level, a primary prevention approach has not been taken into consideration. Therefore, in addition to the conceptual approach, meta-analysis of the relevant literature and determination of the true ES of SDP intervention on individuals with various stage, grade or type of CaP remains an important gap in the literature.

The model defined by Michelin and Gutierrez (2013) guides the conceptualization of this study providing a framework for initiating dietary SDPs as a chemoprevention strategy. The early evidence of damage to the normal prostate tissue resulting in genetic mutation and cellular damage (Giovannucci, 1999) provides an early opportunity for this chemopreventive access. In contrast to the development of neoplasia as decribed in the model by Giovanucci (1999), the DPIM focuses on preemptive chemoprevention through SDPs to prevent and reduce early prostate tissue damage (Michelin & Gutierrez, 2013). Averting further tissue damage by implementing DPIM strategies is the primary

prevention function. Other therapies including Androgen Deprivation Therapy (ADT) and other chemotherapeutic agents might provide some positive response (Giovannucci, 1999). SDPs can also function where ADT is required and have shown to be successful. The proposed study also addresses the problem of recurrence and identification of potential failure for any successful therapeutic intervention. Through the DPIM the study demonstrates the opportunity for SDP chemoprevention. There is also the potential for immunotherapeutic exploration. The model certainly accounts for that prospect through the indication of antibody intervention. Other models include the role of oxidative stress (Thapa & Ghosh, 2006) and environmental factors in the disease process. The context of the seminal role of the proposed study allows for greater application of the Michelin and Gutierrez (2013) model at it sought to determine the ES of SDP intervention and the potential for chemopreventive treatment.

Additional research has also demonstrated the value of antioxidant enzymes (Jung et al., 1996) during *in vitro* antitumor analysis. Genistein is also a valuable inhibitor of CaP cell proliferation through activity that affects the EGFR-Akt/p70S6K pathway and by down regulating important androgen receptors (Oh, et al., 2010). Genistein's use supports the hypothesis that values plant derived compounds as effective biological antitumor agents. This evidence is provided through several *in vitro* studies that have implied an inverse activity in endocrine-resistant tumor cells (Lian et al., 2003), such as apoptosis through the activity of a flavonoid derivative (Chan et al., 2000; Patra et al., 2011), and down regulation of PSA (Han et al., 2007). Hormone refractive CaP following radical prostatectomy (surgical castration) poses tremendous challenges even with the

best medical therapies and is associated with poor survival outcomes (Hess-Wilson & Knudsen, 2006). Meta-analysis of selected randomized controlled trials involving CaP patients between 50 and 70 years old allowed better understand of the defining principles that allow SDPs such as genistein and daidzein to valuable for chemoprevention. Even within the context, that CaP could also be hormone driven through the intricate associations of estrogens and testosterone (Maskarinec et al., 2006).

Difficulty in treating hormone refractory CaP (Celhay et al., 2010) has resulted in the employment of combinations of presently used drugs such as paclitaxel (taxol) (Ping, Hour, Ling, & Yu, 2008). Other combinations include employing several plant based compounds including antioxidants, epigallocatechin gallate and genistein (Ping, Hour, Ling, & Yu, 2008). Studies employing TRAMP mice at different stages of the developmental process throughout life showed the latter establishing an approximate 50% reduction in the development of poorly differentiated prostate tumors when compared to controls (Wang, Eltoum, & Lamartiniere, 2007). These studies are supportive of evidence from epidemiological data implicating the presence of soy in Asian diets as the rationale for this lower CaP incidence.

Estrogens and Cancer Etiology

There is valuable information that provides support to the role of estrogen in cancer etiology (Ganry, 2005). The inverse relationship between estrogens and cancers is continually reported (Kumar et al., 2011). However, greater role for SDPs is necessary due to the increase in adverse event become associated with exposure to animal derived estrogens (Colli & Colli, 2005). Plant derived SDPs include genistein and daidzein two

major phytochemicals, which are the focus of this study. They have become associated with chemoprotection and the beneficial association in decreasing CaP incidence seen in some populations (Dalais et al., 2004). The ES of such biochemical activity is the focus of this intensive discourse. It will provide a better evaluation of the true relationship between the effect of SDPs and response in PSA and E2 levels. These are valuable markers for cancer progression or therapeutic response. Estrogen is important to many biological events including the early initiation of prostate development; however, as indicated there is also some supportive evidence of its role in CaP development (Ganry, 2005).

However, this premise is open to some amount of criticism since there is also evidence of CaP recurrence following surgical castration, and therapy employed to disrupt hormonal activity in the prostate gland (Feldman & Feldman, 2001; Hess-Wilson & Knudsen, 2006; Leewansangtong & Soontrapa, 1999). What is apparent is that the models by (Giovannucci, 1999) and (Thapa & Ghosh, 2004), appear to be significantly opposite in the approach to describing CaP development. The model by (Thapa & Ghosh, 2004) links the metabolism of androgen to the development of CaP (Friedman, 2005). Certainly, there is a close association between hormonal influence and early initiation of prostate development. Subsequently, the continuing influence of hormones can be a determining factor in the early development of the disease. Ross et al., (1998) implies, the evidence expounded regarding such link, might lack any realistic context. This argument certainly demonstrates a need for further understanding CaP development. Defining how such biological activity through isoflavones such as genistein can influence disease

prevention or developmental delay is also prudent. A second model associates androgen administration to the development of the disease (Prehn, 1999). Suggestions regarding androgens as an important initiator for CaP development come from earlier evidence regarding a developmental association between the prostate gland and that hormone (Ross et al., 1998). However, questions were again raised regarding the issue of hormone independent CaP, which has allowed for hypothesizing a broader developmental construct for the disease (Ross et al., 1998). Subsequently, a more recent genetically associated model the Estradiol-Dihydrotestosterone (E-D) is now been suggested (Ross et al., 1998). The E-D model is defined through a complex of interactions of specific genes, and provides value to ethnic factors that has been proposed (Ross et al., 1998). This study examines the context of ES based on these factors, since hormonal response is associated with the basis of SDP activity. Here, the levels of PSA and E2 are among the markers that allow for examination of such posited association.

Analysis of SDP intervention data provides some context to the value of soy-based diets and the role of particular isoflavones such as genistein and daidzein in disease prevention/treatment will be central to this research process. The estrogenic activity of SDPs and the influence hormones such as E2 during the therapy was determined from the analysis process. Research has indicated that soy derived SDPs such as those referenced throughout this discourse and especially genistein possess strong antioxidant properties important in the chemoprotective ability. The need to understand the true effect of SDPs on CaP is also within the context of the presentation of some results from studies categorized as inconclusive following data analysis (Nakamura et al., 2011). In a study

employing mice, there is reference made to the progression of tumors to secondary organs and associated with lymph node infiltration (Nakamura, et al., 2011; Napora, et al., 2010). However, (Swami, et al., 2005) present results which are the contrary to those previously reported and mention the inhibitory effect of the isoflavone genistein during *in vitro* analysis on DU 145 human CaP cells.

Estradiol and PSA

Numerous studies allude to the changes that occur over time in relation to the influence of both the disease state and the intended intervention on PSA and E2 levels in the prostate gland. The reference range for E2 among adult males is 10-40 pg/ml (Adams, Chen, Newton, Potter, & Lampe, 2004); Kumar et al. 2007); Miyanaga et al., 2012; Schroeder et al., 2005; & White et al., 2010). There appears to be a certain amount of E2 hormone produced in males. Evidence points to a decrease in E2 during the CaP disease process. Presence of SDPs appears to have a positive relationship with E2 levels in the prostate gland. Therefore, intake of SDPs will have a direct increase in estriadol and decrease disease progression. However, the inverse relationship occurs between SDPs and levels of PSA. PSA is the marker employed to assess either tumor presence, response to therapy, and in some context the evidence of any health outcome. The literature is also marked with uncertainties, a dilemma that still confronts those diagnosed or treated for CaP. Numerous *in vitro* studies have demonstrated a positive chemoprotective role from SDPs on different tumor cell lines (White et al., 2004), but conflicting evidence is presented when human trials have been conducted (Sharma et al., 2009). Some studies reflect positive associations, but the strength of those associations has not undergone

rigorous examination. The magnitude and direction of the association was determined through this dissertation.

Evidence and Interpretation of CaP Development and Treatment

Both evidence and interpretation proposed for the initiation, progression and failure of ADT and other therapies differ. While most theoretical models support the role of several factors including age, genetic mutation, exposure to environmental toxicants and nutrition, as important, the central dogma reflected through those theories seems to focus on the influence of Reactive Oxygen Species (ROS). Different explanations regarding tumor initiation and the subsequent events that are responsible for hormonal and PSA fluctuations have been provided as well. Diet as implied, is among the factors that have an influence on the development and progression of CaP. Such concern is shared by (Marks et al., 2004) when looking at the differences in prostate tissues in males living in both westernized and an Asian locale, but from a similar ethnic background. While the conclusion from this study supports the importance of environmental factors in predisposing to CaP development, those factors such as lower E2 and urinary soy metabolites levels provided important correlational context. In this study, both were in Japanese American males than Non Japanese males, which could be indicative of lower consumption of soy-based products. Loss or absence of phytoprotection attributable to SDPs could certainly be an integral factor in allowing disease progression.

In the theoretical model proposed by Khandrika, Kumar, Khoul, Maroni & Koul, (2009) the damaging implications of ROS on cellular structures such as nrf2 and disruption of specialized nrf2-nRE axis appear more pronounced during a lower

antioxidant environment. Progression and the subsequent detriments of cellular damage from increased ROS also appear to target cells that have a greater antioxidant capacity therefore, exposed to greater damage (Khandrika, Kumar, Khoul, Maroni & Koul, 2009). Similar opportunity for antioxidant damages were observed in an earlier model (Valko, Rhodes, Monkol, Izakovic, & Mazur, 2006). Here, there is cellular damage resulting from ROS, which leads to DNA damages. This subsequently progresses to preneoplastic conditions and could potentially progress irreversibly, leading to a cancerous state (Valko, Rhodes, Monkol, Izakovic, & Mazur, 2006). This model certainly accounts for similar factors such as oxidants and carcinogens that places stress on the homeostatic condition, and subsequently leads to the precancerous state with further potential for the development of cancerous lesions (Khandrika, Kumar, Khoul, Maroni & Koul, 2009). It appears that with the initiation of antioxidant intervention, through SDP inclusion, that cellular damage could be delayed, prevented or reversed (Khandrika, Kumar, Khoul, Maroni & Koul, 2009). Support for this is presented in the research by Borek (2005) when there is mention of antioxidants as potentially useful for preventing certain cancers that are known to be hormonally regulated. The evidence supports that among these are CaP, breast and endometrial cancer. The results from this study could be important in defining how other cancers are approached within the context of prevention, treatment and management of the disease.

This is important especially since breast cancer among females have a 10-20% recurrence following initial treatment, which is associated with a five-year cancer-free status (Borek, 2005). Also important is the recurrence associated with CaP which was

mentioned earlier in this discussion and is considered to be between 18 – 24 months (Reference). While antioxidants such as vitamin E and C are employed to prevent recurrence in many females, a similar trend in use has also been seen in males diagnosed with CaP. Antioxidant activity, which has become associated with SDPs might certainly play an important role in the lower CaP incidence. While these models appear to introduce the variable of SDP within the disease process, post-cellular destruction by factors responsible for initiating CaP development is still evident.

Another important view on possible prevention of CaP development, is posited through the mediation of topoisomerase II activity which is an important enzyme in DNA synthesis (Patra et al., 2011). Here, topoisomerase II∞ is seen as the predominant enzyme in cells that are proliferating and is therefore significantly expressed in tumor cells (Patra et al., 2011; Hsinang et al., 1988; Heck & Barnshaw, 1986).

Literature on Methodology of Studies on SDP and CaP

Meta-analysis was the methodology employed for this study as it allowed for greater understanding of the ES response of SDP treatment on the populations assessed through different studies selected for this research. An appreciation of this study certainly provides the opportunity to conduct what can also be considered ‘quantitative syntheses’, of the data obtained from primary research employing statistical methodology (DeCoster, 2004). In reference to thoughts shared by (Marsh, Johnson & Carey, 2001), employing meta-analysis will also provide a more in depth understanding and explanation of those integral associations between SDPs and the initiating biochemical, environmental and personal factors that result in disease development and progression. This in depth

appreciation of the value of meta-analysis allows for employing it as a tool for statistical evaluation of interventions and the subsequent success that can be attributed to their implementation (Marsh, Johnson & Carey, 2001).

The larger population size made possibly by pooling several studies and analyzed through meta-analysis provides the probability for even greater reliability and validity of the findings. The analysis therefore provides more value to the intervention process and improves its reliability and validity. In medicine meta-analysis seeks to assess intervention in terms of 'treatment effect', and this can sometimes be referenced through statistical measures such as odds ratio (OR), risk ratio (RR), or risk difference (RD) (CMA, 2006). In relation to studies focused on the social sciences, meta-analysis employs the term 'effect size' in reference to 'standardized mean differences and correlations' (CMA, 2006). However, both references can be employed during meta-analysis application and is reflected throughout this study. However, while the use of some statistical tools including Cohen's *d*, multiple regression and ANOVA can be used to generate "treatment effect or [ES]" data. The availability of a professionally designed comprehensive meta-analysis software, allows for even greater specificity, validity and improved reliability of the computed data. Such professionally designed analytical software allows access to all statistical tools in an individual package and employed to generate more statistical reproducible results. While two forms of meta-analyses are available, this study will employ the method designed to analyze quantitative research literature. This study as implied will be a meta-analysis of the effect of soy derived SDP compounds on PSA and E2 levels before and during intervention. How such an

intervention can be quantified as a consequence of the "Effect size or Treatment size" observations will be studied (CMA, 2006). This reference is pertinent since there are both treatment and control groups involved in these studies and the resulting evidence is generated from a contrast between sometimes quite differing interventions (CMA, 2006). While some studies that seek to understand an effect but has no evidence of treatment intervention, this would be defined as a more thorough "effect size" association (CMA, 2006). This study also exemplifies the dual role of different statistical measures (CMA, 2006). Using these tools as part of the methodology process, will allow for closer scrutiny of data output, and the opportunity to understand various context of that data as it relates to the size of the effect when multiple regression and Cohen's d are employed for analysis.

Summary and Transition

This chapter has provided information regarding different aspect of the present state of the research regarding CaP and particularly to that relating to the issue of SDPs, as prudent chemopreventive or therapeutic agents. Other models suggest, there are many factors that contribute to the development and progression of CaP. Significantly, while there is some indication of intervention following cellular damage and possibly where this might be irreversible, none of the models appear to present earlier isoflavone intervention, or an ES response to therapy employing these plant derived compounds. There is similarly a lack of definitive evidence in the context of SDP intervention, through research, that also provides a defining relationship between the effect of SDPs and PSA and E2 levels. Therefore, while the literature points to a decrease in PSA and E2

in pre-cancerous situations, there still needs to be statistically defining correlational assessment since those values might not singularly reflect disease state. Positive treatment response or therapeutic outcomes might also be absent. Meta-analysis was done using selected randomized-controlled trials will be able to provide greater appreciation for the value of SDPs in managing many aspects of CaP development. The next chapter will therefore provide a through outline of the process to be employed in conducting this research. It will outline the value of meta-analysis within public health research and especially in understanding issues relating to disease intervention strategies and response to those interventions.

Chapter 3: Research Method

Introduction

The purpose of this study was to determine the ES, magnitude, and direction of PSA and E2 serum levels following chemoprevention using the SDPs genistein and daidzein. The response of serum levels of PSA and E2 are important markers for therapeutic response and prognosis for different types of CaP disease outcome. Analysis focusing on this cohort of US men is deficient. The REM answered five major theoretical questions relating to the intake of dietary SDPs and subsequently the response of serum SDP, PSA, and E2 levels. Odds ratio (OR) employing the 2x2 contingency table was used to determine the effect of SDP the independent variable on dependent variables serum SDP, PSA and E2 levels. Meta-analysis was used to determine the cumulative ES and magnitude and direction of the independent variable on the various dependent variables. This chapter describes the research design and approach to determine the influence of chemoprevention using dietary SDPs. The effect of the intervention in influencing change in serum levels of PSA and E2 was determined. The published articles used in this meta-analysis comprised of the instrumentation and materials. The data collection process described the initial search strategy and how the literature was sorted prior to selection of those that met the study criteria. Finally, the data analyses section reviewed the research questions and hypotheses and the use of the REM to assess the ES.

Research Design and Approach

Meta-analysis is the approach and design employed in conducting this study. The approach follows that used by many researchers including Ma, Qin, Wang, and Katoh (2008). It is important to appreciate this as a method which provides an opportunity to conduct what can also be considered quantitative syntheses of the data obtained from primary research through the employment of a statistical methodology (DeCoster, 2004). In reference to thoughts shared by Marsh, Johnson, and Carey (2001), employing meta-analysis also provided a more in depth understanding and explanation of those integral associations between SDPs and the initiating biochemical, environmental, and personal factors that result in disease development and progression. The in-depth appreciation for the value of meta-analysis allowed it to be employed for reliable statistical evaluation. These evaluations include examination of interventions and determining if any reported success or failure is attributable to the inclusion or lack of implementation (Marsh, Johnson, & Carey, 2001).

Meta-analysis is beneficial to the research process and demonstrates the ability to sythesize systematically the ES findings among several studies (Kraemer, 1983). This provided a broader platform on which to evaluate the effects of a particular intervention (Kraemer, 1983). The value of meta-analysis to combine several studies therefore allowed for the cumulative determination of correlation or association between variables. This occurred in this study as treatment and responses were analyzed on the effect seen within broader population. Variables were coded and the true effect size of the intervention was determined through the indicated analytical applications. Those included

OR, Hedges's *g*, and the RMD that demonstrated the association between test variables and response outcomes. Meta-analysis allowed for analysis of a larger population. This analysis provided increased probability of greater reliability and validity to be expressed by the meta-analysis findings. In medicine meta-analysis seeks to assess intervention in terms of treatment effect. Treatment effect can be acknowledged through statistical measures such as odds ratio (OR), risk ratio (RR), or risk difference (RD; CMA, 2006).

In relation to studies focused on the social sciences, the term *effect size*, is applicable in meta-analysis (CMA, 2006). Another term that is applicable is the *standardized mean differences and correlations* (CMA, 2006). However, both references could be employed during meta-analysis application and is reflected throughout this study. In addition, while the use of some statistical tools including Cohen's *d*, Hedges' *g,* Odds ratio (OR), multiple regression and ANOVA can be used to generate treatment effect size data, through the availability of the professionally designed CMA software, allowing for greater specificity of the computed data. Inclusion of all separately indicated ES tools provided ready availability and access to all reliable statistical tools.

While two forms of meta-analyses are available, this study employed that defined to analyze quantitative literature research. This study as implied studied the effect of SDPs on PSA and E2 levels before and after intervention and assessed its effect relative to the effect size or treatment size observed (CMA, 2006). This reference is pertinent since there are both treatment and control groups involved in this study and the resulting evidence is generated from a contrast between intervention and control/placebo (CMA, 2006). In studies that sought to understand an effect but with no evidence of treatment

intervention, defining those would be done through an "effect size" association. This study exemplified the dual role of both statistical measures (CMA, 2006). Using these tools as part of the methodology process allowed for closer scrutiny of data output and the opportunity to understand various context of that data as it relates to the size of the effect when multiple regression and Cohen's *d* are utilized within the analysis process.

Setting and Sample

The sample for this study was obtained from eight RCT studies, yielding 527 participants. One advantage of meta-analysis is to provide a larger population on which to determine the effect of the intervention, a valuable tool for ES measurement (Wilson, 2010). Determination of the magnitude and direction of the intervention is an important facet of any study (Wilson, 2010). SDPs were the independent variables, and manipulated relative to population size, age of participants and the duration of the study. Meta-analysis use of REM, and Hedges' *g* statistic are among those that can mitigate against any error or confounding in and between studies. Similarly, the dependent variables, which included PSA and E2 serum concentration, considered population size as an important factor. Other variables including age, duration and volume of SDPs and other study factors that could influence results were controlled through meta-analysis.

Instrumentation and Materials

The analytical method followed in designing this study to investigate the dose-response effect of SDP intervention on PSA and E2 levels is illustrated in Figure 1 (Chapter 1, page 4). The characterization of the studies followed the pattern outlined below. Measures available included study name, study author, length of the treatment

(days), number of subjects involved, the type of intervention, cancer status of the subjects, age of the subjects, indices employed in generating scores for the DV that were assessed (i.e PSA value before and following treatment with either Genistein, Daidzein or both Genistein and Daidzein), the score associated with the calculated response (+/-), and the outcome experienced following the employed intervention. The weighted mean difference of PSA and E2 levels was determined at 95% CI for the treatment initiated in each RCT and presented in a tabular format. A forest plot of the differences in PSA and E2 values resulting from SDP intervention produced the overall mean and OR of the effect of the intervention.

Variables

The independent variable in this study consisted of the various quantities of the SDPs genistein and daidzein. The dependent variables in this study included dietary and serum E2 and PSA levels following treatment employing genistein and daidzein.

Several tools used in the statistical analyses included the prior indicated 'effect size' statistics such as OR, CMD, correlation, and Hedges' *g*. These are all available in the CMA (statistical software) (MetaStat, 2012). They ensure the study's reliability and validity of the resulting data (Campbell, French, & Gendreau, 2009). These instruments and materials were associated with data collection and were integral to the meta-analysis employed in this study to determine the ES. These determined the association between the studied intervention and the experimental groups regarding SDP intake and PSA and E2 dose-response.

Data Collection and Statistical Analyses

The data were retrieved from eight published randomized-controlled trials (RCTs). These studies had employed both treated and control designed experiments. There are two methods of conducting meta-analysis namely (a) fixed and (b) random effects models (Borenstein, Hedges, & Rothstein, 2007). In this research, the REM will be employed as it appears more suitable for determining relationships sought including the important process of ES (Borenstein, Hedges, & Rothstein, 2007). As different RCTs have been employed factors such as age and intake duration not accounted for in the studies, could result in different ES results (Borenstein, Hedges, & Rothstein, 2007). The REM also allows for diminishing the random error that can be present within individual studies, through the expansion of the sample size resulting from the use of different studies (Borenstein, Hedges, & Rothstein, 2007). In fact, employing the REM in this research could seem more prudent since the fixed effect model (FEM) assumes a similar ES from all the studies which in fact might certainly not be affirmed and would be remedied through use of the REM (Borenstein, Hedges, & Rothstein, 2007). The principle therefore in employing the REM was based on the premise that the mean of the different effects is a better representation of the true effect of the intended intervention (Borenstein, Hedges, & Rothstein, 2007).

The meta-analysis process consisted of screening several hundred articles looking at the effect of SDPs using randomized controlled trials. Studies that employed *in vitro* and other study methods provided reference information. Presented below (Figure 6) is

an outline of the research process. The research process included the complete process of selecting and eliminating appropriate RCTs.

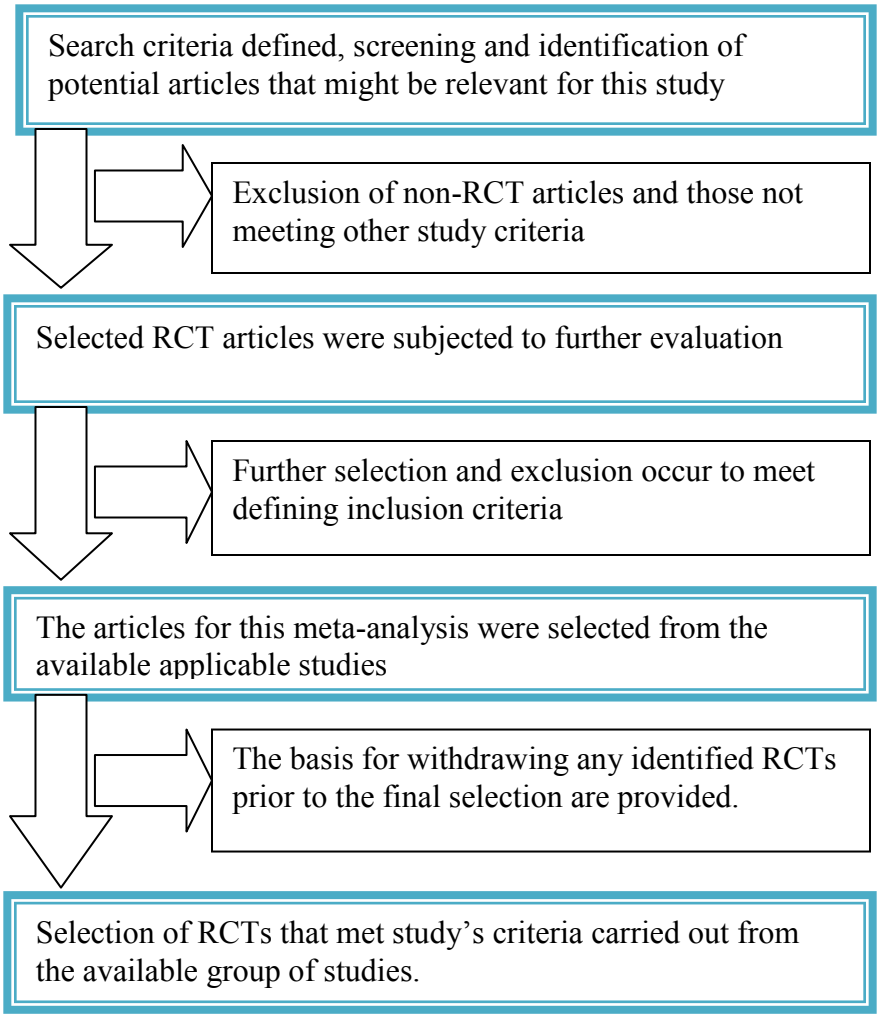

Figure 6. Selection Process of RCTs included in Meta-Analysis. Source: Michelin, 2012.

Research Questions and Hypotheses

The ingestion, duration and concentration of SDPs are responsible for the associated lower incidence of CaP, and other prostate diseases observed among Asian males. Inversely, its decreased intake among males in Western countries appears to be associated with a higher incidence of CaP and other prostate diseases. A number of questions therefore, need to be answered that may provide some perspective on the effects of different independent variables on dependent variables and subsequently on disease outcome among males in Wetsern countries. The proposed study seeks to answer the following questions:

Research Question 1: Is there a correlation between dietary SDPs and serum SDP levels?

Null Hypothesis H1o: There is no correlation between dietary SDP levels and serum SDP levels.

Alternative Hypothesis $H1_A$: There is a correlation between dietary SDP levels and serum SDP levels.

Statistical Plan: IV=Dietary SDPs; DV=Serum SDP; Statistical Test=$\theta_i = \mu_i + \zeta_i$

Research Question 2: Is there a correlation between dietary SDP levels and serum PSA levels?

Null Hypothesis H2o: There is no correlation between dietary SDP levels and serum PSA levels.

Alternative Hypothesis $H2_A$: There is a correlation between dietary SDP levels and serum PSA levels.

Statistical Plan: IV=Dietary SDPs; DV=Serum PSA; Statistical Test=$\theta_i = \mu_i + \zeta_i$

Research Question 3: Is there a correlation between dietary SDP levels and serum E2 levels?

Null Hypothesis H3o: There is no correlation between dietary SDP levels and serum E2 levels.

Alternative Hypothesis $H3_A$: There is a correlation between dietary SDP levels and serum E2 levels.

Statistical Plan: IV=Dietary SDPs; DV=Serum E2;

Statistical Test=$T_i = \theta_i + \varepsilon_i$

Research Question 4: Is there a correlation between serum SDP levels and serum PSA levels?

Null Hypothesis $H4_0$: There is no correlation between serum SDP levels and serum PSA levels.

Alternative Hypothesis $H4_A$: There is a correlation between serum SDP levels and serum PSA levels.

Statistical Test: IV=Serum SDPs; DV=Serum PSA;

Statistical Test= $T_i = \theta_i + \varepsilon_i$

Research Question 5: Is there a correlation between serum SDP levels and serum E2 levels?

Null Hypothesis H5A: There is no correlation between serum SDP levels and serum E2 levels.

Alternative Hypothesis: There is a correlation between serum SDP levels and serum E2 levels.

Statistical Test: IV = Serum SDPs; DV=Serum E2;

Statistical Test = $T_i = \theta_i + \varepsilon_i$

Random Effects Model

The graphical outline below (Figure 7) is an indication of how various factors influence the mean effect in the REM. This provides an ability to generate even more reliable data.

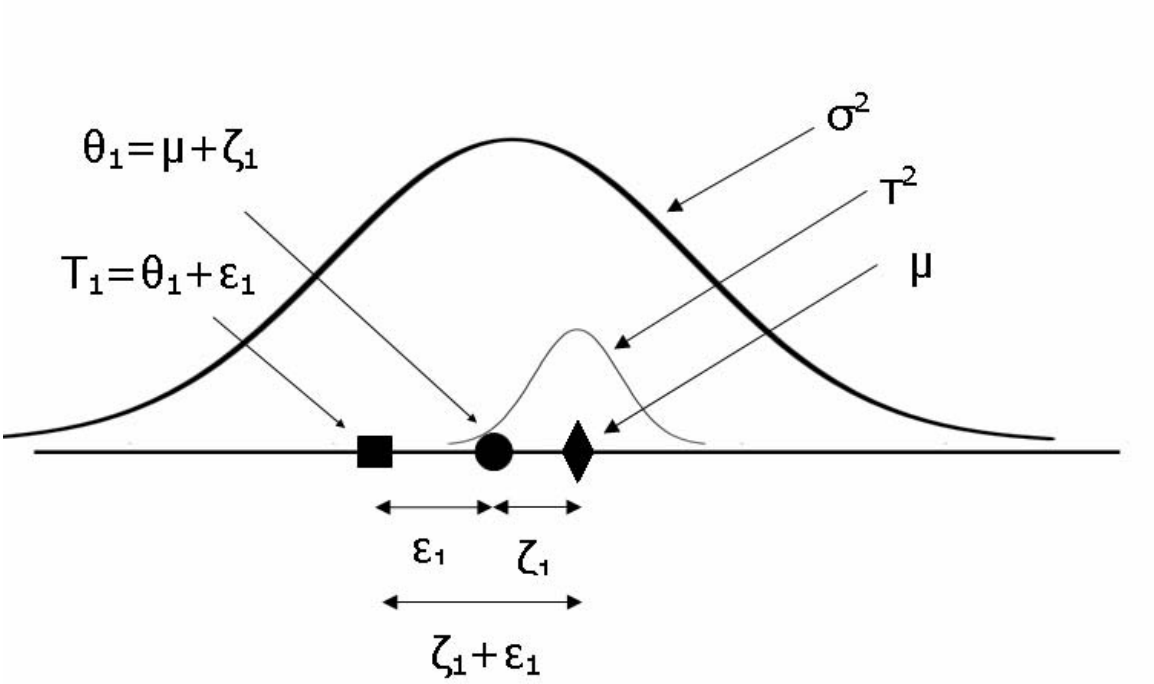

Figure 7. Random Effects Model. The observed effect T1 (▄) is sampled from a distribution with true effect θ_1 (●), and variance σ^2. This true effect θ_1, in turn, is sampled from a distribution with mean μ (♦)and variance τ^2. Source: Borenstein, M., Hedges, L., Higgins, J., & Rothstein, H. (2009). Meta analysis, Fixed-Effects vs Random-Effects. *Introduction to Meta-analysis*. John Wiley & Sons, Ltd. "with permission"

The following formula provides some context regarding the calculations as they

effect, μ = mean of all true effects, ε_1= within-study error, ζ_1 = between-study error

(Borenstein, Hedges, & Rothstein, 2007). An observed effect, the following formula is applicable and will be useful in arriving at this statistical measure:

$T_i = \theta_i + \varepsilon_i = \mu_i + \zeta_i + \varepsilon_i$ (Borenstein, Hedges, & Rothstein, 2007)

Subsequently, employing and calculating the Q statistics will provide evidence of the total variance between studies. Q is represented through the following equation $Q = \sum_{i=1}^{M} W_i(\hat{\theta}_{FE} - \hat{\theta}_i)^2$ (Bowden, Tierney, Copas, & Burdett, 2011).

These formulas will be important in ensuring detail statistical analysis. These formulas also help to eliminate any errors 'in studies and between studies' (Borenstein, Hedges, & Rothstein, 2007). Formulas reduce the potential for confounding which could influence the degree of reliability and validity within the output data. These conditions will be evaluated in subjects diagnosed with and had been treated for CaP at various stages of the disease. The REM is therefore the most suitable method that will be employed in this study, which will allow understanding the true effect of the separate interventions, subsequently combined and as they would be defined throughout a more expansive population.

Consumption of soy based products at different levels and concentrations have been used to determine any relationship between and chemoprotective capacity relating to both newly diagnosed and hormone resistant CaP individuals. The use of meta-analysis for this project provided an opportunity to employ different statistical tools to assess the ES of the treatment or examined intervention. It is the prudent tool to use in this type of study as it allows for the analysis of a greater volume of subjects among several studies, which are then analyzed simultaneously. The benefit of this analytical tool is in the

aggregate data obtained which represents the magnitude and direction of the intervention effects throughout all the applied studies (Wilson, 2010). This aggregate will consequently provide better research data on the effect of the intervention (Wilson, 2010).

Utilizing meta-analysis allows for a detailed assessment of the influence of the independent variable (IV) SDPs on the dependent variables (DV) which includes PSA and E2. Age and other exposure factors were controlled during the analysis process and therefore the data output was not affected or influenced by those factors (Wilson, 2010). ES was determined using OR and other applicable statistics, including the standardized mean difference (SMD), correlation coefficient (CC), and Hedges' *g* (Wilson, 2010). It was necessary that these standardized indexes be compared across the various studies. They represented both magnitude and direction of the relationship investigated in the studies analyzed and will be independent of sample sizes (Wilson, 2010). The meta-analysis software was able to compute ES through input of the means and standard deviation data (Borenstein et al., 2007). However, this ability to use the software interchangeably, can allow for individual usage while examining the relationship between SDP intake and serum PSA and E2 levels (Neill, 2006). The use of the SMD for ES calculation was obtained by dividing Differences in Score by the SD of applicable scores (Neill, 2006). A presentation of the number of studies and effects used to generate the meta-analysis and subsequently the ES and the CI will assist in valuing the reliability and consistency presented through the ES mean (Neill, 2006).

Analysis using the CMA software provided greater statistical reliability and validity to the presented data. The result is the production of high quality reliable and valid data. Presentation of the research design and methodology is further in this chapter.

Effect size computation formula:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{s_{pooled}}$$

where

$$s_{pooled} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

There are also other statistical programs such as SPSS and SAS that could effectively compute this study's data output. However, the CMA software presented earlier in this discussion, provides extensive analytical scope and was employed to conduct all analysis. As an analytical tool it has all the required data applications that can account for each measure of effect being determined (Becker, 1999). This is in fact similar to calculating the degree of association between the independent variables and the dependent variables (Becker, 1999). The ability to compare analysis using different methods to achieve the goals can provide greater validity and reliability to the generated results.

The statistical REM presents an opportunity to have a correlational deduction of the ES of an individual intervention (Becker, 1999; Wilson, 2010). This study employed OR using REM to define the correlation between variables. Other ES statistical methods used included Hedges' *g*, correlation *r*, and SMD (Konstantopolous, 2006). These methods were applicable for this study as the selected RCTs are defined through

treatment and control effect in different groups within the studied populations. Both statistics are included among several accounted for in the CMA software. The formula used for calculating SMD is

$$SMD = \frac{\text{Difference in mean outcome between groups}}{\text{Standard deviation of outcome among participants}}$$ (Cochranes Handbook, 2010).

The SMD formula is also a representation of the expression of the size of the intervention effect in each study relative to any variability that has been observed in a particular study (Cochranes Handbook, 2010). In that context and in relation to ANOVA, Eta squared ((h^2) which represents total variance of an effect will be determined employing the formula $\eta^2 = SS_{effect} / SS_{total}$ (Neill, 2006). In this formula SS_{effect} = ratio of the effect variance and SS_{total} = total variance (Neill, 2006). However, the number of independent variables accounted as well as the number and size of the effects (Becker, 1999) are influenced by η_p^2. Variation in the size of the different SS_{effect} will also influence the interaction effect result (Becker, 1999). Therefore, by using the above method expressed earlier relating to variance exclusion by (Bowden, Tierney, Copas, & Burdett, 2011) formula and the access to the professionally designed CMA statistical software then the results should be able to demonstrate validity and reliability.

Internal and External Validity

Meta-analysis supplements considerably more validity and reliability to the data. This supplementation has resulted in an increased use of meta-analysis especially in the fields of medicine and public health where the effect of an intervention is the pertinent response that is being sought. While there are weaknesses to the application of meta-

analysis, the process can be considered valid and reliable and therefore, will prove a valuable tool in this analytical process. Added validity and reliability will also be derived from employing applications such as multiple regression to conduct aspects of the data analysis. Adding to the validity and reliability of the data, will be the coding and the scale used in conducting this research process. The continuous scale will be employed for this process, since there will be an opportunity to consider the difference in means between a treatment and a control group (Cochrane Collaboration, 2002). The value of having the presence of associated data relating to sample size (s), mean (m) and standard deviation data (SD) available from the research studies can also be appreciated as implied by (Cochrane Collaboration, 2002), as invaluable to the employment of the continuous meta-analysis scale. While there might be immediate access to obtaining the standard deviation from some studies it can easily be obtained from statistical manipulation of other important variables such as the standard errors, confidence intervals, t-statistics, and p-values (Cochrane Collaboration, 2002). Use of the professionally developed CMA (MetaStat, 2007), software will be applied throughout this research.

Summary and Transition

This chapter presented important aspects of the research including the design of the research process. Also addressed were the method of meta-analysis employed for this study and the appropriateness of this method for this process. Chapter 4 will focus on data presentation, interpretation of the results of the findings from the meta-analysis, while Chapter 5 will focus on discussing those findings and making recommendations based on those results. The conclusions that will be presented in Chapter 5, are those that

relate to the value of SDPs in alleviating the burden of CaP among the studied population. Hopefully, the findings will generate invaluable recommendations that can influence ‘positive social change’ within the broader community and even globally.

Chapter 4: Study Findings

The purpose of this study was to determine the cumulative effect of genistein and daidzein intervention on serum PSA and serum E2 levels in American men. This is a cohort in which such analysis had not previously occurred. Guided by the principles of primary and secondary prevention and the newly conceptualized model of cancer prevention (DPIM), I sought to answer whether intake duration and concentration of SDPs directly influence magnitude and direction of CaP response. A selected group of eight RCTs of SDP CaP chemoprevention provided the data used in this study. These studies represented interventions designed to prevent or delay progression of recently diagnosed CaP disease or hormone-refractory CaP in men between 50–85 years old. This chapter includes the characteristics of the randomized clinical trials, and the description and tabulations of the hypotheses tested.

Characteristics of Randomized Clinical Trials

Information presented in this study includes data from eight (8) selected published RCTs of men who have undergone SDP chemoprevention for newly diagnosed CaP, hormone-refractory CaP and those at high risk for the disease following pathological analysis. Studies had pre- and posttest information on at least one of the dependent variables, PSA and E2, in order to be included. The studies selected were among some of the most recent studies examining the association between SDPs and CaP chemoprevention and the correlation to serum PSA and serum E2 levels. The studies were published in peer-reviewed journals and dated from 2004 – 2012. Seven (7) trials were conducted in the USA and one was conducted in research centers across Japan. The

sample yielded 530 male participants ranging between the ages of 50-85 years who matched selection criteria and completed baseline study requirements. The study accounted for a decline in participants during follow-up and the analysis process.

The sample represents those that completed the clinical trial as both experimental group and controls. Participants represented several ethnic groups including European Americans, African Americans, Japanese Americans, Japanese, and Hispanics. However, European Americans dominate the study samples and the group for which this analysis needed to best address ES, African Americans, was not highly represented. Men with a history of CaP with increasing PSA levels, and subjects with CaP diagnosis and with 3 – 6 months prior to surgical intervention, those monitored through active surveillance, and HRC subjects have been included in this study. All groups have contributed to the intervention and placebo groups that are the foundation of the study's conceptualization in relation to chemoprevention from SDPs. One RCT study reported an average age of 49.8 years old (White et al., 2010). Inclusion of this study should not significantly influence data analysis. All studies reported data collection at baseline and 3 month intervals. Incomplete study data due to non-compliance with study protocol following selection and study commencement affected some study results. The lowest number of participants in one study was 28 and the largest 158.

Table 1 provides a description of information abstracted from the eight studies that were used to conduct the meta-analysis. Populations represented included those who responded either positively or negatively within treatment group where intervention was conducted. The odds of benefiting from intervention over placebo was then analyzed. The

table was generated using meta-analysis and the REM statistics. This provided evidence that contrasted the odds between the intervention and placebo groups in relation to administering or lack of intervention. Studies 4 – 6 provided information on E2 response to SDPs. Six (6) studies 1-3 and 6-8 provided evidence for PSA response to SDPs. The odds of benefiting from SDP intervention in the Adams et al. (2004) study are 0.576 times more likely than the placebo. Benefit from SDP intervention is also seen in the studies by Carmody et al. (2008), Schroeder et al. (2005), and White et al. (2010) (*OR*=0.510, *OR*=0.667, and *OR*=0.700, respectively). However, the benefit of SDP intervention is not seen in the Dalais et al. (2004), Hamilton-Reeves et al. (2004), and Kumar et al. (2007) studies; the odds of SDP intervention influencing E2 levels are not significantly different from those of the placebo group. These statistics are consistent with *OR* analysis carried out using 2x2 contingency tables for hypotheses testing to examine intervention or placebo on treatment and control groups. These findings using SDPs as independent varialbes and PSA or E2 as dependent variables were determined using both meta-analysis and 2x2 tables and both methods yielded statistical significance.

Table 1

Events among Treatment and Placebo Groups to Determine OR and other Statistics by PSA or E2 Outcome

Study Authors	PSA Outcome				E2 Outcome			
	Positive Exposure Treatment	Total n Exposed	Positive Exposure Placebo	Total n Placebo	Positive Exposure Treatment	Total n Exposed	Positive Exposure Placebo	Total n Placebo
Adams et al., 2004	34	17	47	14	-	-	-	-
Carmody et al., 2008	10	17	5	14	-	-	-	-
Dalais et al., 2004	7	8	8	8	-	-	-	-
Hamilton-Reeves, 2007	-	-	-	-	4	12	0	17
Kumar et al., 2007	-	-	-	-	3	22	1	27
Miyanaga et al., 2011	3	75	2	78	14	33	16	47
Schroder et al., 2005	3	17	4	16	2	24	3	25
White et al., 2010	14	24	8	25	18	25	7	25

Among the studies selected for this meta-analysis, experimental participants included those who had shown an increase in PSA levels following radical prostatectomy or radiotherapy, participants who had recently undergone prostate biopsy and histological analysis of the biopsied tissues, and men who were recently diagnosed through prostate biopsy and histological analysis, above normal serum PSA levels in defined age groups, pathology with CaP based on a defined inclusion Gleason Score and active surveillance. These criteria have been important in contributing to the foundation of the study's conceptualization in relation to CaP chemoprevention from SDPs.

Statistical Meta-Analyses

The use of the REM in determining the ES and consequently the magnitude and direction of the intervention accommodate all the in-study and between study concerns. The REM accounts for any variance or bias in or between studies during the analysis process. Bias was observed in the participant selection process through either low or non-inclusion of some minority groups, especially African Americans who experience a higher CaP incidence and disease burden (ACS, 2012).

The data analysis tool employed for conducting all aspects of this study was meta-analysis using OR statistic and following the constructs of the REM. This analysis was able to address all univariate, multivariate and ES analysis. ES is important in determining the magnitude and direction of the intervention. Two of the studies accounted for more than two experimental groups and were therefore able to contribute more value to the computed overall cumulative effect of genistein and daidzein on PSA. Three (3) studies reported on E2 values pre- and postintervention and therefore were used

to determine the effect of dietary and serum *isoflavones* levels on serum E2 levels. Seven (7) studies reported on pre and post-test information on PSA. Results were determined on the intervention effect response using *genistein* and *daidzein.* OR analysis was employed to determine the ES of the treatment intervention with dietary SDPs and to understand the correlation between dietary SDP, serum SDP levels, and serum PSA and E2 levels when compared between treatment and placebo subjects. When using OR for ES determination the results are defined through small = 1.50, medium = 2.50 and large = 4.50 odds ratio that there is some odds that benefits from a treatment intervention might or might not occur (Goldin, 2007). The ES as indicated was determined using the OR process but other logarithmic approaches that corrected for bias in the data output were determined from LogOdds Ratio, Variance, and Standard Error calculations.

Research Questions and Hypotheses

Research questions were answered using REM OR meta-analysis and OR 2x2 contingency table statistics. Both meta-analysis and 2x2 table computation resulted in similar OR statistic results. The cumulative ES value from the meta-analysis provides evidence that favours intervention. Intervention subjects showed that both PSA and E2 responded inversely to SDPs which would represent an inverse relationship. The output data was generated using CMA software for ES and magnitude and direction of individual study effect and for cumulative study determination. Odds ratio (OR) statistic for correlation between independent and dependent variables based on the research questions was performed using a 2x2 contingency table. Other statistics including Hedges' *g*, SMD and correlation were also used for meta-analysis data output (MetaStat,

2008). All the studies selected had relevant data on the included variables. Independent variables genistein and daidzein, and dependent variables PSA/E2 values were abstracted from each individual study.

Odds (OR) of the effect of genistein and daidzein on the level of PSA and E2 as evidence of chemoprevention of CaP are presented through the following research questions, hypothesis and OR calculations. The OR provides information on the odds and not the probability of an event occurrence within the treatment group. However, the Cox Index which is determined through meta-analysis has been acknowledged as providing ES information that is known to be unbiased and also provides the best information on ES using continuous variables (TEA, 2011).

Research Question 1:

Is there a correlation between dietary SDPs and serum SDP levels?

Null Hypothesis H1o: There is no correlation between dietary SDP levels and serum SDP levels.

Alternative Hypothesis $H1_A$: There is a correlation between dietary SDP levels and serum SDP levels.

Statistical Analysis:

As shown in Table 2, the *OR* = 0.5957 implies that the untreated SDP group would be more likely to not experience change in serum SDP levels. Therefore the odds are that dietary SDP levels more likely influence a change in the serum SDP levels within the treatment group. Changes in serum SDP levels are more likely a direct result from the intervention and not a chance occurrence This OR also indicates that at 95% CI

there is a 0.2588 to 1.3712 increase of a positive outcome in the treatment group, although this increase at the 5% level is not statistically significant (MedCalc, 2013). This odds ratio therefore supports rejecting the Null hypothesis.

Table 2

Odds Ratio and 95% C.I. between Dietary SDP and Serum SDP Levels

	SDP Serum Outcome			*OR*	95% *CI*
	Positive	Negative	Odds		
SDP Yes	34	17	34/17		
SDP No	47	14	47/14		
Totals	81	31	(34/17)/(47/17)	0.5957	[0.2588, 1.3712]

Research Question 2:

Is there a correlation between dietary SDPs and serum PSA levels?

Null Hypothesis H2o: There is no correlation between dietary SDP levels and serum PSA levels.

Alternative Hypothesis $H2_A$: There is a correlation between dietary SDP levels and serum PSA levels.

Statistical Analysis:

As shown in Table 3 OR = 0.6667 and implies that the untreated SDP group would be more likely to not experience change in serum PSA levels when compared to the treatment group. Therefore the odds are that dietary SDP levels more likely influence a change in the serum PSA levels within the treatment group. Changes in PSA level are more likely a direct result from the intervention and not a chance occurrence. This OR also indicates that at 95% CI of 0.1013 to 4.3878 there is some effect from treatment

although outside the statistical significance level at 5% (MedCalc, 2013). This odds ratio therefore supports rejecting the Null hypothesis.

Table 3

Odds Ratio and 95% C.I. between Dietary SDP and Serum PSA Levels

	Serum PSA Outcomes			OR	95% CI
	Positive	Negative	Odds		
SDP Yes	22	3	22/3		
SDP No	22	2	22/2		
Totals	44	5	(22/3)/(22/2)	0.6377	[0.1013, 4.3878]

p-value=0.018

Research Question 3:

Is there a correlation between dietary SDPs and serum E2 levels?

Null Hypothesis H3o: There is no correlation between dietary SDP levels and serum E2 levels.

Alternative Hypothesis $H3_A$: There is a correlation between dietary SDP levels and serum E2 levels.

Statistical Analysis:

Table 4 indicates the OR = 0.4259 and implies that the untreated SDP group would be more likely to not experience change in serum E2 levels when compared to the treatment group. Changes in E2 level are more likely a direct result of the SDP intervention than from chance occurence. This OR also indicates that at 95% CI there is a 0.0362 to 5.0055 increase of a positive outcome within the treatment group. This also

indicates a CI within the 5% level and therefore statistically significant (MedCalc, 2013). This odds ratio therefore supports rejecting the Null hypothesis.

Table 4

Odds Ratio and 95% C.I. between Dietary SDP and Serum E2 Levels

	Serum E2 Outcomes			OR	95% CI
	Positive	Negative	Odds		
SDP Yes	23	2	23/2		
SDP No	27	1	27/1		
Totals	50	3	(23/2)/(27/1)	0.4259	[0.0362, 5.0055]

p-value=0.050

Research Question 4:

Is there a correlation between serum SDPs and serum PSA levels?

Null Hypothesis $H4_0$: There is no correlation between serum SDP levels and serum PSA levels.

Alternative Hypothesis $H4_A$: There is a correlation between serum SDP levels and serum PSA levels.

Statistical Analysis:

As shown in Table 5 an OR = 0.7000 implies that the untreated SDP group would be more likely to not experience change in serum PSA levels. Therefore the odds are that serum SDP levels is likely to influence a change in the serum PSA levels within the treatment group. Changes in serum PSA level are more likely a direct result from the intervention and not a chance occurrence. This OR also indicates that at 95% CI there is a 0.2024 to 2.4207 increase of a positive outcome in the treatment group at the 5%

confidence level and therefore not statistically significant (MedCalc, 2013). This odds ratio therefore supports rejecting the Null hypothesis.

Table 5

Odds Ratio and 95% C.I. between Serum SDP and Serum PSA Levels

	Serum PSA Outcomes			OR	95% CI
	Positive	Negative	Odds		
SDP Yes	28	8	28/8		
SDP No	25	5	25/5		
Totals	53	13	(28/8)/(25/5)	0.7000	[0.2024, 2.4207]

p-value=0.018

Research Question 5:

Is there a correlation between serum SDPs and serum E2 levels?

Null Hypothesis $H5_0$: There is no correlation between serum SDP levels and serum E2 levels.

Alternate Hypothesis $H5_A$: There is a correlation between serum SDP levels and serum E2 levels.

Statistical Analysis:

As shown in Table 6 an OR = 0.641 implies that the untreated SDP group would be more likely to not experience change in serum E2 levels when compared to the treatment group. Changes in serum E2 level are more likely a direct result from the intervention and not a chance occurence. The OR also indicates that at 95% CI there is a 0.1042 to 3.9447 increase of a positive outcome within the treatment group. This increase is at the 5% level and is statistically significant (MedCalc, 2013). This OR therefore supports rejecting the Null hypothesis.

Table 6

Odds Ratio and 95% C.I. between Serum SDP and Serum E2 Levels

	Serum E2 Outcomes			OR	95% CI
	Positive	Negative	Odds		
SDP Yes	75	3	75/3		
SDP No	78	2	78/2		
Totals	153	5	(75/3)/(78/2)	0.641	[0.1042, 3.9447]

p-value=0.050

Cumulative Effect of SDP on PSA and Estradiol

Six (6) RCTs were analyzed for consideration of the cumulative response to treatment (Figure 8) from SDPs on serum PSA level. The SDP cumulative ES on sPSA resulted in a positive correlation (OR=0.507, 95% CI [0.288, 0.891], *p*=.018). Another set of five (5) studies were analyzed for consideration of the cumulative response to treatment (Figure 9) from SDPs on sE2 level. The SDP cumulative ES on sE2 also showed a positive correlation (OR=0.366, 95%CI [0.134,1.002], *p*=0.050). The ES of each study's dependent variable is represented by a square (■) where the size of the squares contribute more and length and thickness of lines provide evidence of contribution of each study towards cumulative ES. The diamond (♦) represents the cumulative ES for the set of six (6) studies showing the PSA response and the other set of five (5) studies showing E2 response. Further evidence of these correlations were determined through other ES statistics and Forest Plots also generated by the meta-analysis software. These confirmatory findings will be discussed in Chapter 5. The overall ES favoring the treatment or the placebo group is shown in the Forest Plot for the PSA outcome (Figure 8) and E2 outcome (Figure 9).

Study Authors	Odds Ratio	Lower Limit	Upper Limit	Z-Value	p-Value
Adams	0.424	0.169	1.062	-1.831	0.067
Carmody	0.389	0.090	1.673	-1.269	0.204
Dalais	3.400	0.120	96.700	0.716	0.474
Miyanaga	0.632	0.103	3.890	-0.495	0.620
Schroder	1.524	0.290	8.014	0.497	0.619
White	0.336	0.105	1.081	-1.829	0.067
Cumulative	0.507	0.288	0.891	-2.362	0.018

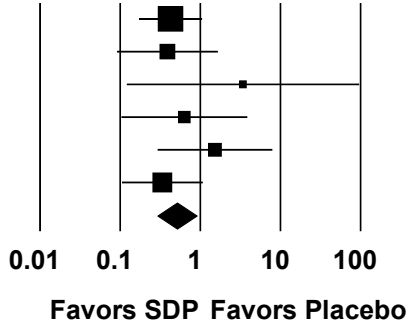

Figure 8. Odds Ratios and 95% CI Effect Size of PSA Response to Phytoestrogen Treatment using REM Meta-Analysis

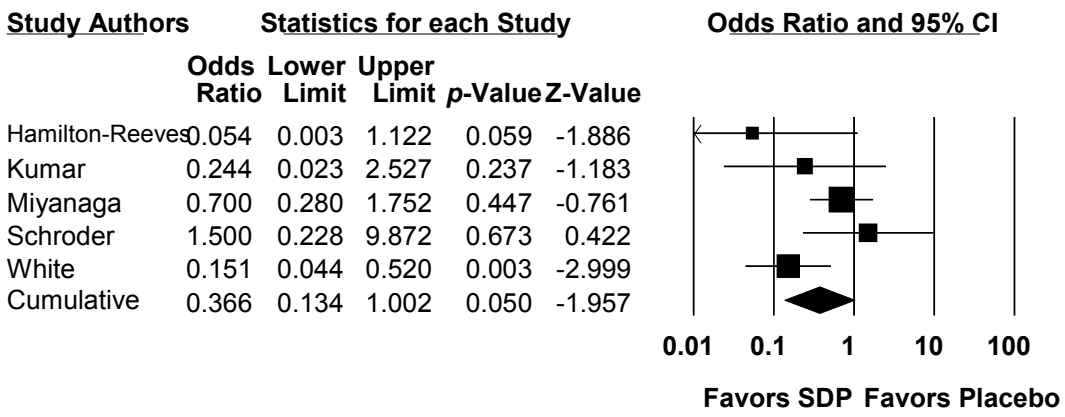

Figure 9. Odds Ratios and 95% CI Effect Size of Estradiol Response to Phytoestrogen Treatment using REM Meta-Analysis

Heterogeneity

The use of heterogeneity provides a measure of inter-study variability, a major consideration for meta-analysis. Heterogeneity helps to measure any inconsistencies that might be present within the results obtained from analysis of the data within the six studies used in each of the meta-analysis (StatsDirect Limited, 2011). The *Heterogeneity statistic* in this study using the REM and odds ratio for ES analysis of PSA response was significant at p=0.019 (Test of Null, 2-Tail). The *Heterogeneity statistic* using the REM to calculate the OR for ES determination of E2 response was significant at p=0.049 (Test of Null, 2-Tail). This heterogeneity function is used to denote the weight of a particular study and I-squared (I^2). I^2 represents the percentage variation across studies due to heterogeneity and not chance (StatsDirect Limited, 2011). Heterogeneity is considered to influence when I^2 reaches 75%.

Heterogeneity is represented statistically by Cochran's Q. Q can be calculated by employing the combined sum of the difference of squared effects of an individual study and the effects which have been pooled across the studies (StatsDirect Limited, 2011). The weight of the studies' effect is also based on the pooling of those studies. Q's distribution is statistically similar to the chi-square distribution with k being the number of studies minus 1 degree of freedom (df). Because of the small amount of studies used in this meta-analysis Q might not be very valuable for testing heterogeneity. Q could under compensate in this study because of the small number of studies, or it could also over-compensate if the meta-analysis consisted of a large number of studies (SDL, 2011). I^2 is therefore the important statistic that helps in the testing of heterogeneity. I^2 indicates the

percentage of variation that is seen across studies and is due to heterogeneity rather than to chance (SDU, 2011). Heterogeneity is also important in that it includes statistical, methodological, and clinical types of heterogeneity. Since heterogeneity relates to those differences or variability that are present among different studies its use provides value to the output data. Key differences that are important between study participants are among those characteristics that account for the clinical heterogeneity and is important to the foundation of this analysis process (Bandolier, 2007). Other key differences between studies are also seen in areas such as the participants, the intervention employed and the observed outcomes (Bandolier, 2007). The *Q* statistic is calculated by employing the combined sum of the difference of squared effects of an individual study and the effects pooled across studies (StatsDirect Limited, 2011). The weight of the effects are mainly based on those pooled studies.

Important differences that existed between study participants included characteristics that account for clinical heterogeneity and are the foundation of this analysis (Bandolier, 2007). Key differences between studies are in areas such as the participants, the intervention employed and the observed outcomes (Bandolier, 2007). These are areas where the use of the random effect model becomes valuable. Other ES calculations were done using different statistical methods including SMD, correlation, Hedges' *g* and risk ratio. The analyses using Hedges' *g* and correlation are presentd as Figures 11 and 12. They provide similarity to the correlation which had been determined by odds ratio statistic.

Summary and Transition

Consistent with the purpose of the study to determine the cumulative effect of genistein and daidzein intervention on serum PSA and serum E2 levels, five research questions were answered using OR and meta-analysis. The first research question sought to determine if the correlation between dietary intake of SDPs translated into equivalent absorption of serum SDPs. The odds ratio indicated a significant ES in the pooled analysis of eight studies, and the null hypothesis was rejected. Overall, there was a correlation between dietary SDP and serum levels of SDP. The second and third questions sought to answer whether dietary SDPs were correlated with serum PSA and E2 levels. Both the second and third null hypotheses were rejected as significant correlations were determined based on OR for PSA at p=0.018, and OR for E2 at p=0.050. The fourth and fifth research questions sought to answser whether serum SDPs were correlated with serum PSA and E2 levels. Findings supported rejecting both null hypotheses. There was a direct correlation betweeen serum SDP levels and response of serum levels of PSA significance p=0.018, and E2 p=0.050.

The correlations were supported by evidence from OR 2x2 contigency table analysis of treated and non-treated individuals and through OR, correlation, Hedges' g and SMD Forest Plot evidence. The cumulative ES supported by all the evidence indicates that there were signifcant correlations between intervention compared to placebo groups. Chapter 5 will provide further discussion on the results of these findings, the implication for positive social change, limitations of the study and an overall interpretation of the results.

Chapter 5: Discussion, Conclusions, and Recommendations

Overview

The purpose of this study was to determine through cumulative evidence the effect of using the SDPs genistein and daidzein on PSA and E2, important markers for early diagnosed CaP and HRC CaP incidence in American men, a cohort where such analysis has not previously occurred. The research process was guided by the principles of primary and secondary prevention and the DPIM (Michelin & Gutierrez, 2013). I sought to answer whether intake of SDPs directly influenced the magnitude and direction of PSA and E2 levels which were important in CaP response to therapy and outcome. Information for this study was obtained from selected published RCTs which used the named SDPs in chemopreventive intervention designed to prevent or delay cancer progression in recently diagnosed men or in men with HRCaP between 50–85 years old.

This study was a quantitative analysis of information from selected published RCTs demonstrating the chemoprevention ability of soy derived SDPs. Analysis of the ES of the intervention in these studies was conducted employing meta-analysis. The independent variables are quintiles of the SDPs genistein and daidzein. The dependent variables included serum levels of E2 and PSA. The study sought to determine the ES in SDP dose-response intervention in the selected male populations during the therapeutic application for recently diagnosed and recurrent CaP, and the contribution of duration and concentration in determining the size and direction of action.

These OR ES statistics provided reliability to the correlations seen in the OR data output. Other statistics that provide value and support the validity and reliability of the

OR data output includes both *Z* and *p* values. Tables 2 through 6 are 2x2 tables that provide correlation of the response between independent and dependent variables. The observed OR is determined at 95% CI for correlation between variables. This provides valuable information on the intervention in relation to the untreated/placebo group. OR = 1 implies no significant difference between treatment and placebo group or no effect from treatment, where OR < 1 implies that the untreated group will be more exposed to experiencing a negative response. An OR > 1 implies that the treated group would more likely benefit from the intervention.

Confirmatory and Transitory Evidence

This study was guided by the principles of primary and secondary prevention following the DPIM (Michelin & Gutierrez, 2013). This model is described in Figure 3 (page 15) and guided the examination of the research questions. These questions related to the chemoprevention of CaP using SDPs. The model showed the potential for therapeutic application. The direct influence of dietary SDPs on serum SDP levels and the strength and direction of treatment on serum PSA and E2 levels was important. Both serum PSA and E2 could influence CaP response and outcome. ES of treatment was determined using OR, SMD, correlation, and Hedges' *g* statistics.

Table 7

REM Statistics for Computing Effect Size and Heterogeneity

Statistical Measures of Effect Size	Meta-Analysis Model	Effect Size value	2 Tail Null Test p-value	*Q*	I^2	Variance
Hedges' *g*	Random Effect(PSA)	-0.368	0.019	3.792	0.000	0.010
Hedges'g	Random Effect(E2)	-0.546	0.049	7.501	46.672	0.072
Correlation	Random Effect(PSA)	-0.184	0.015	3.861	0.000	0.001
Correlation	Random Effect(E2)	-0.280	0.035	8.251	51.519	0.004
SMD	Random Effect(PSA)	-0.375	0.018	3.747	0.000	0.011
SMD	Random Effect(E2)	-0.554	0.050	7.542	46.962	0.080
OR	Random Effect(PSA)	0.507	0.018	3.747	0.000	0.121
OR	Random Effect(E2)	0.366	0.050	7.542	46.962	0.836

There was significant difference between intervention and placebo. This was seen in the OR REM produced forest plots (Figures 8 & 9), which favored intervention over placebo. Other statistical ES tests including SMD, correlation and Hedges' g provided similar results. Forest plots representing Hedges's g are shown on pages 107 and 108, and for correlation on pages 109 and 110. The test for heterogeneity in the study supported the results of ES analysis.

Determining *Q* can compensate for the limitations that could affect the data obtained from the study. Accommodating limitations is possible using meta-analysis (SDL, 2011). I^2 is also an important statistic that helps in the testing of heterogeneity. It

indicates the percentage of variation that is seen across studies and which are due to heterogeneity rather than by chance (SDU, 2011). Importantly, there are different types of heterogeneity. These include statistical, methodological and clinical. Heterogeneity relates to the differences or variability that is present among different studies. In this study, I^2 determined the estimate of effects in this study as represented through the several interventions. During this study, individual investigations sought to determine the effect of an intervention compared to placebo, indicating clinical heterogeneity (Bandolier, 2007). Importantly, several studies employed the research practice of adhering to the intention to treat (ITT) principle. This ITT allowed the researchers to follow the subjects for the length of the research process (Lachin, 2000). There are also important key differences between studies which are related to the participants, the intervention employed and the observed outcomes. Some of these were also observed in this study (Bandolier, 2007). Again, these are areas where the use of the random effect model becomes valuable in helping to resolve issues that could be associated with study differences.

The heterogeneity for studies used to determine the ES of PSA response with OR statistic is represented by Q value=3.747, Q(df) =5 and I^2=0.000, supports the correlational significance determined through the ES analysis. The 2-tail null test for PSA shows a negative Z value of -2.362 and a p=0.018 (Figure 8). Heterogeneity for studies used to determine E2 response is represented by Q value of 7.542 Q(df)=4 and I^2=*46*.962, supports the correlational assessment determined through the ES analysis. The 2-tail null test for E2 shows a negative Z value of -1.957, and a p=0.050 (Figure 9). Some

heterogeneity was observed between studies when E2 response was determined. However, heterogeneity did not appear to influence either the results that were determined during PSA and E2 response to SDP intervention. Since the OR *Z* value -2.204 for PSA response is negative and outside the -1.96 and +1.96 normal distribution with a *p* value 0.028, then these values appear to support a rejection of the null hypothesis. There were no significant differences in the results obtained for PSA and E2 response using other ES statistical analyses. Using SMD to determine ES for E2 response provided an overall *p* value of 0.050 and variance of 0.080 which as indicated is an effect that is within the $p < 0.05$ significance level. A statistical significant *p* value =0.050 indicates the probability that any observed changes could be attributed to the intervention rather than by chance occurrence (Thisted, 2010). Similarly, the overall significance level for E2 determined from Hedges'*g* ES analysis is $p = 0.027$ (Figure 11). This implies a statistically relevant correlation between SDP treated group and placebo groups, but might still not provide sufficient evidence to eliminate some chance occurences (Thisted, 2010).

Overall, the data indicate that there is acceptance that intervention with SDPs for PSA reduction and influencing increases in E2 levels can be important for CaP chemoprevention. Such therapy can be accomodated within the putative model (Giovannucci, 1999) and has the potential for improving health outcomes. The correlation between dietary and serum SDPs, which results in an increase in the latter, appears to have some beneficial role in modulating both serum PSA and E2. With serum E2 maintaining a high level following intervention, the evidence supports its role as an

estrogen replacement for CaP therapy. As a natural alternative source, it could be effective in reducing present CaP incidence, especially in communities where economic status does not allow for available medical interventions.

Interpretation of Key Findings

In this study, eight RCTs were used to calculate the ES of SDP in a chemopreventive role in various conditions where the diagnosis of CaP was concluded. To conduct ES of the intervention, the CMA software was used. The adverse events within both treatment and placebo groups was used in a 2x2 cohort format to calculate OR for ES and magnitude and direction of treatment intervention using meta-analysis. REM was used in this study as it provided a more reliable approach for data analysis than the FEM. REM is able to mitigate differences that are present within and between studies. This accounts for the differences through a broader framework of understanding that studies were done under different situations with different populations. Figures 8, 9, and 10 indicate individual and cumulative ES of treatment intervention. Table 8 reflect the different outcomes relating to PSA and E2 response from SDP intervention. The correlations seen between the DVs and IVs provides evidence of support or rejection of the Null hypothesis for the Alternative hypothesis. As indicated an increase in sSDP, and a subsequent decrease in sPSA and increase in sE2 then the Null Hypothesis is rejected. Other conditions relating to the acceptance or rejection of the Null hypothesis for the Alternative hypothesis are presented in the table.

Table 8

Interpretation of Hypotheses Testing Results Using Random Effect Model Effect Size

Hypothesis Testing	Accept Null (T)	Reject Null (F)	Accept Alternative (T)	Reject Alternative (F)
Dietary SDPs	↑ Serum levels	↓ Serum levels	↓ Serum levels	↓ Serum levels
Serum SDPs	↑ SDP	↑ SDP	↓ SDP	↓ SDP
Serum PSA	↓ PSA	↑ PSA	↑ PSA	↓ PSA
Serum E2	↑ E2	↓ E2	↓ E2	↑ E2

The various relationships indicated above in figure 8 are also a reflection of the effect of dSDP and the serum level response of sSDP, sPSA and sE2. The cumulative response to dSDP intervention is provided in Figures 10 and 11. Figures 10 and 11 are representative Forest Plots that show the strength and direction of the contribution from each study's effect to the overall treatment effect on both PSA and E2. The contributory effect of each study to the cumulative effect is identified through the size of each study's representative square and length and thickness of the effect line. The effect of dSDP and subsequently sSDP appears to be significant and favors the use use of intervention during CaP therapy where PSA and E2 responses are beneficial. The effect response of both PSA and E2 to SDP over the time of the experiment are indicated in the two graphical representations. These results were used to confirm similar correlation obtained from OR analysis. Other ES statistics including Correlation and SMD were done and provided similar positive results favoring SDP intervention. While those ES provided values within

a different statistical context of a 95% CI all appeared to provide similar supportive evidence for rejection or acceptance of the null hypotheses.

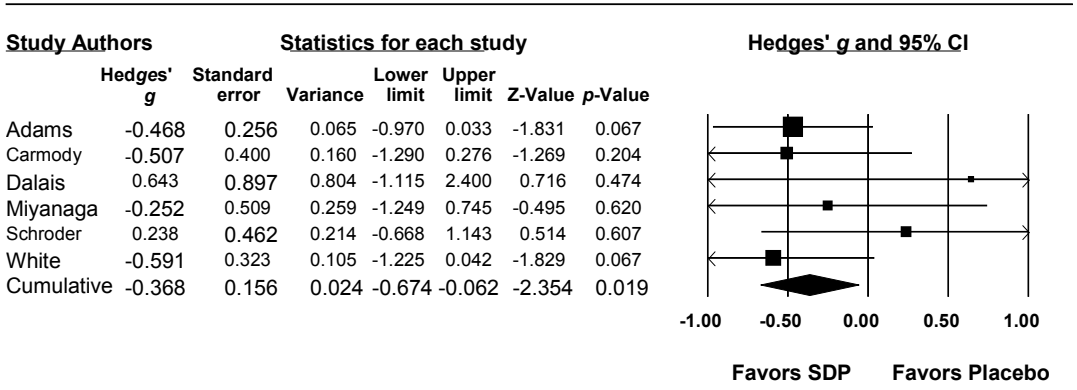

Figure 10. Hedges's g Assessment for Effect Size Statistic for PSA Response to Phytoestrogen Treatment using REM Meta-Analysis

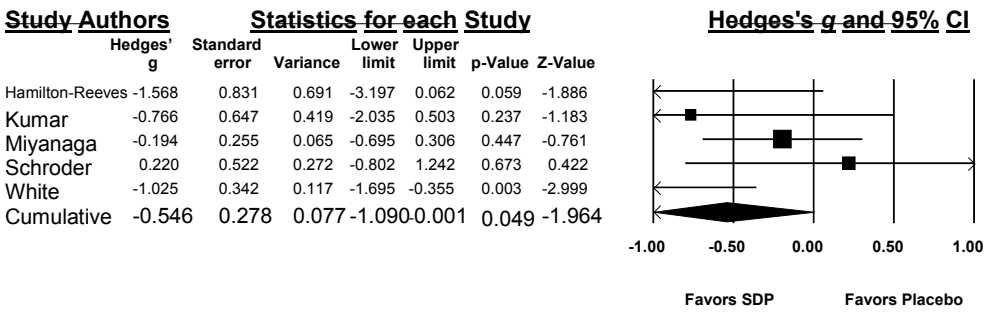

Figure 11. Hedges' *g* Assessment for Effect Size Statistic for Estradiol Response to Phytoestrogen Treatment using REM Meta-Analysis

Hedges' *g* statistic using REM meta-analysis to test for E2 heterogeneity resulted in a *p* value = 0.049 (Figure 11), significant at the 95% CI level. The analysis for E2 response determined that treated subjects had optimum response to SDP significant at the *p*=0.050 level. A similar positive response was determined regarding the PSA response to SDP intervention. Forest plots indicating the cumulative effect of SDP on sPSA and sE2 are provided in Figures 12 and 13 respectively. Correlation for PSA response was determined at the *p*=0.015 level. OR results had also suggested that there were changes in relation to PSA status, which would suggest benefit from the chemoprevention. Statistical significance for OR was determined to be *p*=0.018 as previously reported. While lower PSA levels do not always infer improved health outcome during therapy, reduced levels can be seen as an important marker for overall therapeutic response (NCI, 2012). In fact, some factors including age of subjects, duration and concentration of treatment and size of experimental population could affect OR results. These results appear to support the goal of this study and are within the framework of the DPIM (Michelin & Gutierrez, 2013). The previous evidence may provide for effective implementation of chemoprevention prior to disease development. Findings from the various ES statistic supported rejecting the Null. These findings also indicated the potential ability to reduce PSA levels within the population through introduction of dietary SDP. The benefit of maintaining serum E2 concentrations also appears to be a valuable outcome from SDP chemoprevention.

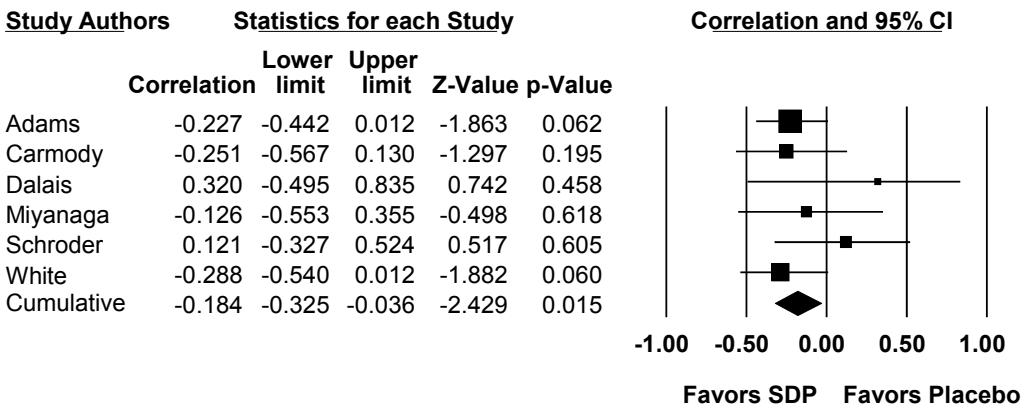

Figure 12. Correlational Assessment for Effect Size Statistic for PSA Response to Phytoestrogen Treatment using REM Meta-Analysis

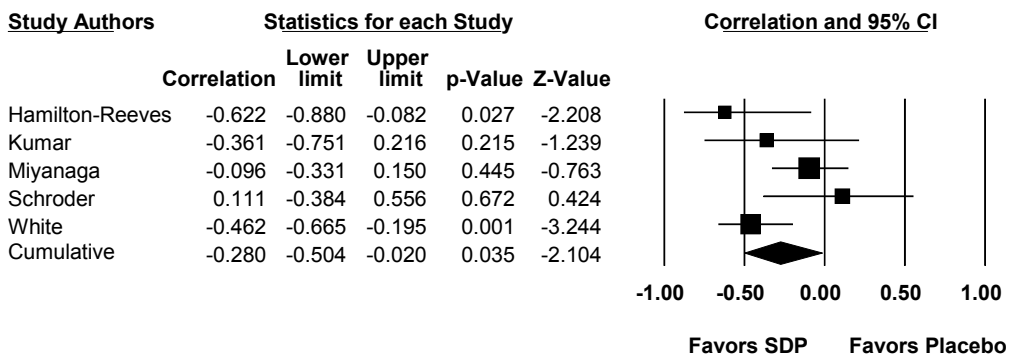

Figure 13. Correlational Assessment for Effect Size Statistic for Estradiol Response to Phytoestrogen Treatment using REM Meta-Analysis

This meta-analysis was conducted using exposure and non-exposure to treatment and placebo groups. The results from the various meta-analysis statistics showed differences in the between studies ES although all observed cumulative effect indicated a rejection of the null hypothesis. The symbol which is representative of the cumulative ES on the confidence level line was evidence of the correlation between SDP intervention and estradiol (E2) and PSA response. This information is presented in Figure 11 with graphical Forest Plot. The individual and cumulative contribution from each study is used in determining ES and magnitude and direction of treatment effect. The studies by (Carmody et al., 2008; Schroder et al., 2006; and White et al., 2010) appear to have significant influence on the overall presented data. The presented data output is also a representation of the effect of each study on the overall ES. This effect is repesented through larger squares and shorter lines that are shown on each line which represents an individual study. There appears to be no significant difference between the effects over time as indicated between the two graphical representations. To confirm results that were obtained from OR analysis, other ES statistics including Hedges' *g*, correlation, and SMD were done. While ES values were represented in different context of a 95% CI it appeared that the overall evidence for rejection or acceptance of the Null hypotheses indicated through were supportive.

Study Limitations

Bias could be attributed to study population selection and size since either could influence data output. Evidence in the various studies show an increase in serum levels from baseline to study completion. These changes are important in the effect analysis that

is computed for independent and dependent variable effect. Implication from this study seem to indicate that the intervention using soy derived SDPs does have some effect on the treatment population. This difference was demonstrated through the analysis data on both treatment and placebo or control groups in this study. However, further research might be needed to determine the true value of genistein and daidzein the test compounds. Within the context of these results and the overall framework of the research model there was evidence that seem to indicate that there is value from the treatment. However, other limitations might have resulted from issues with coding and data transferring for the required meta-analysis. The data reported in the studies might also be subjected to study bias and therefore did not provide the best evidence relating to benefits of therapy when subjected to meta-analysis. While duration as a moderating variable did not appear to have any significant effect on the magnitude and direction of the treatment as seen in Figure 1, possible longer duration might have some effect. Another important context to defining the significant difference between PSA treated and placebo groups is indicated where p= 0. 018 (Figure 8) determined by OR REM statistic, p=0.019 (Figure 10) by Hedges'g REM statistic and p=0.018 determined by SMD REM statistic. These values represent the probability that the response to treatment is different from only chance ocurences. Statistical significance is observed at p values of < 0.01 or $p < 0.001$. Similarly, it is also important to define the significant difference between E2 in treated subjects and in untreated controls. Here, p=0.050 (figure 11) determined by OR REM statistic, p=0.049 (figure 12) determined by Hedges'g REM statistic, and p=0.050, determined by SMD REM statistic. Statistical significance observed at p values of < 0.01

or $p < 0.001$. Statistical significance to SDP intervention was obtained through values for PSA ES response at p=0.018 OR and for E2 ES at p=0.050 OR statistic. Statistical significance at the $p \leq 0.05$ level was therefore indicated from these statistics.

Testing if studies used in the meta-analysis are looking at the same study effect is done by examination of the test for heterogeneity (Higgins, Thompson, Deeks, & Altman, 2003). Heterogeneity is considered significant in determining the quality of this study. Other statistical analyses also reported some important p values. Correlational analyses using meta-analysis for PSA response provided a value p= 0.015 and variance = 0.001 and the cumulative meta-analysis using Hedges'g statistic for heterogeneity among studies for E2 response indicated a p value = 0.050 and variance = 0.072. These findings indicated that there were some differences in response between experimental and placebo groups. The significance of the analysis is supported by the fact that all statistics used in this meta-analysis resulted in significant p values below $p \leq 0.05$.

Effect Size Analyses

ES were calculated adjusting for moderator variables, including median age, duration of intervention, ethnicity of participants, differences between baseline and post-study PSA, baseline and post-study E2, and any correlation between changes following SDP chemoprevention. While there were six studies that provided information on serum PSA levels at baseline and other time points during the study, only five studies provided information that could provide context to be used regarding the influence of SDP on E2 during ES data analysis. However, ES data did not change after input of any of the moderator variables including SDP duration or quantity provided during the experimental

intervention. There were also no significant differences in the data obtained using the FEM and REM (models). The data presented in this discussion were obtained using statistics determined through the REM which was mentioned earlier in Chapter 1 and then discussed extensively in Chapter 3. Meta-analysis used the REM since it offered the opportunity for providing more reliable and valid data.

As a reminder, the purpose of this study was to determine through cumulative evidence the ES of intervention with genistein and daidzein SDPs on serum PSA and E2 levels in males who were experiencing rising PSA levels, undergoing active surveillance following CaP diagnosis, and males recently diagnosed with CaP or with hormone refractory CaP. The study was designed to look at the magnitude and direction of the output ES in response to SDP chemoprevention. The ES observation was made through changes seen primarily among American males, a cohort where such meta-analysis had not previously occurred. The conceptual premise of the study was guided by the principles of primary and secondary prevention adhering to the newly conceptualized DPIM (Michelin & Gutierrez, 2013). The study also sought to answer whether intake duration and concentration of SDPs had a direct influence on the magnitude and direction of E2 and PSA response. The observations from this meta-analysis reflect changes in PSA and E2 levels that occurred as a result of dietary SDP intervention during the duration of the RCTs. Additional observations to correlate these changes in PSA and E2 levels during the course of regular treatment could provide further evidence of correlation with actual disease outcome and whether there is improvement in health status.

Data for this study were obtained from selected published RCTs of SDP intervention designed to prevent or delay hormone-refractory CaP and recently diagnosed CaP in males between 50–85 years old. The REM statistical method was used in the process the data analysis and also provided information on heterogeneity between indivudal study intervention and cumulative meta-analysis study results. It was noticeable that REM did not differ significantly from the FEM statistics. There was an observed difference in the REM and FEM findings comparing treatment and placebo groups in relation to the total study populations. Hedges' *g* and SMD statistics afforded additional context assisted in the determination of ES by providing confirmatory analysis when OR was used for ES statistical analysis. Information obtained from OR statistic was used to determine rejection or acceptance of either the null or the alternative hypothesis. Cumulative OR statistic from the REM analysis is provided in Figure 8 (Page 97).

The SD measure is determined based on similarity to values between -1 to 1. An ES value closer to 1 is a determination that there is a larger difference in effect between treatment and placebo group involved in the study. When ES was determined using Hedges's *g,* analysis of data reporting E2 levels, this showed a positive relationship. The ES of studies by Miyanaga et al. (2011), Kumar et al. (2007), White et al., (2010), Schroder et al., (2005), and Hamilton-Reeves et al. (2007) are represented by g = -0.194, -0.766, -1.025, 0.220, and -1.568, respectively. Here, all values fell below .50 which would imply a SD of one-half (1/2) while 1.0 would be representative of a SD of 1.0 between the two groups. However, the negative values between the groups that reported

predominantly on PSA levels indicate a decisive lower SD between treatment and placebo groups. Heterogeneity testing (using Hedges' *g* REM) indicated a cumulative *Q* value of 3.792 (p=0.019, df (*Q*)=5, and I^2=0.000) and ES of -0.368. The findings from heterogeneity testing implied that there was a 5% chance at 95% CI of a positive effect due to SDP intervention. Also, the effect seen in this study was within that probability of significance determined by the *p*=0.019 value using OR statistic.

Recommendations for Future Research

The DPIM proposed in this study by Michelin and Gutierrez (2013) provides an avenue for exploring early intervention and subsequent therapeutic strategies with disease onset. Prior frameworks including the putative model proposed by Giovannucci, (1999), do not appear to consider early established dietary standards and a public health context is not the conceptualized focus. The DPIM allows for intervention using SDPs at different stages of CaP etiology. Early dietary interventions could prevent or delay the development of PINs. The value of PINs and their importance to CaP etiology was presented earlier in this research. SDP intake prior to PIN development and clinical cancer diagnosis that is accompanied by increasing PSA levels would therefore be prudent. With progression continued inclusion of dietary SDPs and subsequent increase in serum SDP levels could also be suggested. Research is required to determine the optimum serum level at which SDPs afford the best health outcomes. The cumulative OR results do not appear to suggest any significant difference between treated and placebo groups in lowering serum PSA levels. However, there is significance in the levels of E2 that results from intervention. Further research is required to understand in greater detail

the role of E2 in cancer etiology, and how SDPs can positively influence its serum levels. However, based on the results of this research a more prudent approach would be to begin intervention through the development of established dietary levels which appear to have value in observed relationship between SDPs, PSA and E2 levels.

The findings from this study indicate that there is an inverse correlation between serum SDPs and response of serum E2 levels. This could be supportive evidence for the chemopreventive role of dietary isoflavones. The DPIM therefore appears to be better suited for chemoprevention at an earlier stage and potentially be a more effective preventative approach. An indication for SDP intervention which appears to be beneficial based on results from the research is that relating to E2 levels. E2 levels which represent an estrogen which is found in minute quantities within the male can be seen as a health hazard if there is an imbalance and larger than normal quantities are present. Within the articles that focus on E2 levels at pre and post-intervention, there appear to be significant change in E2 serum concentration levels which with ES measure using OR statistic. The evidence of higher E2 levels post-intervention may suggest greater odds for increasing and maintaining higher levels of E2 subsequent to SDP intake and increase in serum levels. E2's role in natural estrogen replacement is therefore a practical application and other studies have shown benefits in treating CaP using E2 therapy (Strax, 2005). It could therefore be implied that if a nutritional standard for E2 is established similar to those for other nutritional supplements then this could be advised especially for males after 50 years old. In relation to the etiological model proposed by Giovannucci, (1999), there are points within that model where supplementation could be administered. Although

phytonutrients are seen as potential supplementation prior to PSA increase and the development of PINs which are important in the diagnosis of CaP, such intervention might act as a suitable prevention modality.

Implications for Social Change

The results appear to support the evidence that factors associated with CaP outcome such as PSA and E2 levels are influenced by isoflavone intervention that might provide an effective chemoprevention approach. The approach to determine the appropriate stage for chemoprevention is therefore the practical social change implication to which these findings should be focused. This study determined that participants undergoing SDP chemoprevention are ten times more likely to have higher levels of serum E2 than the control group. Therefore, social implications for a nutritional standard for E2 can be established similar to those for other nutritional supplements, especially for males 50 years and older. The study also has relevance to members of different ethnic groups who have higher incidence of CaP. Finally, the incidence of CaP continues to be greater in later age decades and the growing aging of the population and increased life expectancy require acceptable alternative prevention methods of prostate abnormalities.

Conclusion

The purpose of this meta-analysis was to determine the cumulative ES of eight randomized clinical trials where the experimental group received SDP as secondary prevention of CaP. From a clinical standpoint, the intervention is successful if the PSA levels are improved. While there has been valuable information gathered from this study, the need for additional research is warranted to conclusively determine SDPs role in

reducing PSA levels. Larger sample size of experimental groups as well as other factors such as duration and concentration of SDPs can be further explored. The results seem to acknowledge the benefits of E2 in cancer prevention and thus more rigorous exploration is recommended. The value as an SDP oral supplement or in hormonal replacement therapy for CaP and other cancers is worth pursuing. Studies that seek to understand the dynamics of a balance between PSA and E2 might also be invaluable to understanding therapeutic and chemo-preventive properties.

More importantly, the results obtained from this analysis indicate the potential benefit of SDPs in the primary prevention of CaP. A new model to illustrate the role of the implementation of earlier intervention was designed. The proposed model was developed as The Dual Prevention Intervention Model, consisting of a dual prevention strategy. The access to intervention in this new model is conceptualized to provide options for SDP chemoprevention. Chemoprevention appears to occur through two defined strategies. First, the critical point at which primary prevention can be targeted through SDP to prevent any cellular damage to prostate tissue, and second, identification of the critical point at which secondary prevention can be targeted. In the present study, RCTs aimed at using SDPs as chemoprevention of further prostate disease or prevention of recurrent disease were assessed. The literature to date had not examined the role of SDP as primary prevention nor as secondary prevention within a public health framework. Although the findings of the correlation between direct intervention with SDPs and serum PSA was not statistically significant, the findings indicate that addressing secondary prevention can result in a change in serum PSA level towards the

desirec clinical direction of a lowered level. Secondly, serum SDP appears to have a direct influence on serum E2 levels which appear to be valuable for CaP outcome. The approach using this model is to provide optimum chemoprevention with the potential for improved health outcomes. The correlation between dietary and serum SDPs and subsequently the effect on both sPSA and sE2 for cancer chemoprevention appears to be supported by ES results. Moderator variables including intervention duration, quantity and concentration of experimental variables, and age did not show any significant effect on ES data. The test for heterogeneity was determined at 95% CI.

The data inidcated that the level of sE2 maintained following SDP intervention, supports SDPs as valuable for estrogen replacement therapy. Estrogen replacement is presently a valuable therapeutic straegy for CaP treatment. As a natural alternative source it could be effective in reducing present incidence of CaP especially in poorer communities. Many underserved communities do not have access to medical care because of the lower socioeconomic status. Acess to early screening, preventive public health methods and subsequent medical intervention is lacking and results in greater disease burden and poorer health outcomes, especially among African Americans who suffer from the greates incidence and mortality from CaP.

References

Adams, K., Chen, C., Newton, K., Potter, J., & Lampe, J. (2004). Soy isoflavones do not modulate prostate-specific antigen concentrations in older men in a randomized controlled trial. *Cancer Epidemiology, Biomarkers & Prevention, 13*(4), 644-648. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15066931

Adlercreutz, H., Markkanen, H., & Watanabe, S. (1993). Plasma concentration of phyto-estrogens in Japanese men. *Lancet, 342*(8881), 1209-1210. doi:10.1016/0140-6736(93)92188-Y

Adlercreutz, H., Yamada, T., Wahala, K., & Watanabe, S. (1999). Maternal and neonatal phytoestrogens in Japanese women during birth. *Am J ObsterGynecol., 180*(3), 737-743. doi:10.1016/S0002-9378(99)70281-4

Agarwal, R. (2000). Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. *Biochem Pharmacol., 60*(8), 1051-1059. doi.org/10.1016/S0006-2952(00)00385-3

American Cancer Society. (2012). What are the key statistics about prostate cancer? Retrieved October 27, 2011, from http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-key-statistics

Auerbach, L. (2006). Complementary and alternative medicine in the treatment of prostate cancer. *The Journal of Men's Health and Gender, 3*(4), 397 – 403. doi.org/10.1016/j.jmhg.2006.10.006

Borek, C. (3005). Antioxidants and the prevention of hormonally regulated cancer. *JMHG*, *2*(3), 346-352. doi.org/10.1016/j.jmhg.2005.06.005

Bosland, M. (2005). The role of estrogens in prostate carcinogenesis; A rationale for chemoprevention. *Rev Urol*., *7*(3), S4 – S-10. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1477605/

Bektic, J., Berger, A., Pfeil, K., Dobler, G., Bartsch, G., & Klocker, H. (2004). Androgen receptor regulation by physiological concentrations of the isoflavonoid genistein in androgen-dependent LNCaP cells is mediated by estrogen receptor β. *European Urology, 45*(2), 245-251. doi.org/10.1016/j.eururo.2003.09.001

Bektic, J., Guggenberger, R., Eder, I., Pelzer, A., Berger, A., Bartsch, G., … , & Klocker, H. (2005). Molecular effects of the isoflavonoid genistein in prostate cancer. *Clinical Prostate Cancer, 4(2),* 124-129. doi.org/10.3816/CGC.2005.n.021

Bosland, M. (2000). The role of steroid hormones in prostate carcinogenesis. J Natl Cancer Inst Monogr., 2000(27), 39-66. Retrieved from http://jncimono.oxfordjournals.org/content/2000/27/39.long

Bosland,M. (2005). The role of estrogens in prostate carcinogenesis: A rationale for cheoprevention. *Reviews in Urology*, *7*(3), S4 – S10. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1477605/

Bonkhoff, H., & Berges, R. (2008). The evolving role of oestrogens and their receptors in the development and progression of prostate cancer. *European Urology*, *55,* 533 – 542. Retrieved from http://jncimono.oxfordjournals.org/content/2000/27/39.long

Borenstein, M., Hedges, L., Higgins, J., & Rothstein, H. (2009). *Meta-analysis: Fixed-effect versus random-effects model. In Introduction to Meta-analysis* (5th ed.). John Wiley & Sons, Ltd.

Brown, M., & Singh, A. (2006). Alkaline phosphatase level increase with initiation of hormone therapy for prostate cancer portends poor prognosis with rapid progression to bone metastases: A case report and review of the literature. *Clinical Genitourinary Cancer, 4*(4), 293-295. doi.org/10.3816/CGC.2006.n.010

Celhay, O., Yacoub, M., Irani, J., Dore, B., Cussenot, O. (2010). Expression of estrogen related proteins in hormone refractory prostate cancer: Association with tumor progression. *The Journal of Urology, 184*(5), 2172 – 2178. doi.org/10.1016/j.juro.2010.06.089

Chan, F., Choi, H., Chen, Z., Chan, P., & Huang, Y. (2000). Induction of apoptosis in prostate cancer cell lines by a flavonoid, baicalin. *Cancer Letters, 160*(2), 219-228. doi.org/10.1016/S0304-3835(00)00591-7

Cho, D., Di Blasio, C., Rhee, A., & Kattan, M. (2003). Prognostic factors for survival in patients with hormone-refractory prostate cancer (HRPC) after initial androgen deprivation therapy (ADT). *Urologic Oncology: Seminars and Original Investigations*, *21(4),* 282-291. doi.org/10.1016/S1078-1439(03)00057-7

Chun, O., Chung, S., & Song, W. (2009). Urinary isoflavones and their metabolites validate the dietary isoflavone intakes in US adults. *Journal of the American Dietetic Association*, 1*09*(2), 245-254. doi.org/10.1016/j.jada.2008.10.055

Colli, J., & Colli, A. (2005). Comparisons of prostate cancer mortality rates with dietary practices in the United States. *Urologic Oncology: Seminars and Original Investigations*, *23*(6), 390-398. doi.org/10.1016/j.urolonc.2005.03.020

Dalais, F.,Meliala, A., Wattanapenaiboon, N., Frydenberg, M., Suter, D., Thompson, K. (2004). Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. *Adult Urology, 64*(3), 510-515. doi.org/10.1016/j.urology.2004.04.009

Dijsselbloem, N., Berghe, W., Naeyer, A., & Haegeman, A. (2004). Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections: Multi-purpose nutraceuticals at the crossroad of hormone replacement, anti-cancer and anti-inflammatory therapy. *Biochemical Pharmacology, 68*(6), 1171-1185. doi.org/10.1016/j.bcp.2004.05.036

Engelhardt, P., & Riedl, C. (2008). Effects of one-year treatment with isoflavone extract from red clover on prostate, liver function, sexual function, and quality of life in men with elevated PSA levels and negative prostate biopsy findings. *Urology 71*(2), 185-190. doi.org/10.1016/j.urology.2007.08.068

Feldman, B., & Feldman, D., (2001). The development of androgen-independent prostate cancer. *National Review of Cancer, 1(1),* 34-45. doi:10.1038/35094009

Ferguson, L. (2002). *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *506–507*, 215-224. doi.org/10.1016/S0027-5107(02)00168-9

Franke, A., Halm, B., & Ashburn, L. (2008). Isoflavones in children and adults consuming soy. *Archives of Biochemistry and Biophysics, 476*(2), 161-170. doi.org/10.1016/j.abb.2008.02.009

Fuhrman, J. (2012). Greens, beans, onions, mushrooms, berries and seeds. Retrieved from http://www.drfuhrman.com/library/gombbs.aspx

Fujita, H., Koshida, K., Keller, E., Takahashi, Y., Yoshimito, T., Namiki, M., et al. (2002). Cyclooxgenase-2 promotes prostate cancer progression. *Prostate*, *53*(3), 232-240. doi: 10.1002/pros.10152

Ganry, O. (2005). Phytoestrogens and prostate cancer risk. *Preventive Medicine*, *41(1),* 1-6. http://www.ncbi.nlm.nih.gov/pubmed/15916986

Giton, F., de la Taille, A., Allory, Y., Galons, H., Vacherot, F., Soyeux, P., et al. (2008). Estrone sulfate (E1S), a prognosis marker for tumor aggressiveness in prostate cancer (PCa). *Journal of Steroid Biochemistry & Molecular Biology*, *109*(1-2), 158-167. doi: 10.1016/j.jsbmb.2007.10.005

Gonzalez, C., Roehl, K., Antenor, J., Blunt, L., Han, M., Catalona, W. (2004). Preoperative PSA level significantly associated with interval to biochemical progression after radical prostatectomy. *Urology, 64*(4), 723-728. doi:10.1016/j.urology.2004.05.019

Guy, L., Vedrine, N., Urpi-Sarda, Gil-Izquierdo, Al-Maharik, N., Boiteux, J., et al. (2008). Orally administered isoflavones are present as glucuronides in the human prostate. *Nutrition and Cancer, 60*(4), 461-468. doi: 10.1080/01635580801911761.

Haenszel, W., & Kurihara, M. (1968). Studies of Japanese migrants: I. Mortality from cancer and other diseases among Japanese in the United States. *Journal of the National Cancer Institute, 40*(1), 43 – 68. http://www.ncbi.nlm.nih.gov/pubmed/5635018

Hamilton-Reeves, J., Rebello, S., Thomas, W., Slaton, J., & Kurzer, M. (2007). Soy protein isolate increases urinary estrogens and the ratio of 2:16∞-hydroxyestrone in men at high risk of prostate cancer. *The Journal of Nutrition*, *137*(10), 2258-2263. http://jn.nutrition.org/content/137/10/2258.long

Härkönen, P., & Mäkelä, S. (2004). Role of estrogens in development of prostate cancer. *Steroid Biochemistry & Molecular Biology, 92*(4), 297 – 305. doi.org/10.1016/j.jsbmb.2004.10.016

Higgins, J., & Thompson, S. (2002). Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine, 21*(11), 1539-1558. doi: 10.1002/sim.1186

Higgins, J., Thompson, S., Deeks, J., & Altman, D. (2003). Measuring inconsistency in meta-analyses. *British Medical Journal, 327*(7414), 557-560. doi: 10.1136/bmj.327.7414.557

Hill, C., & Doyon, F. (2006). The frequency of cancer in France in year 2002, and trends since 1968. *Bulletin of Cancer 93*(1), 7-11. http://www.ncbi.nlm.nih.gov/pubmed/16455500

Ho, S. (2004). Estrogens and anti-estrogens: Key mediators of prostate carcinogenesis and new therapeutic candidates. *Journal of Cellular Biochemistry*, *91*(3), 491-503. doi: 10.1002/jcb.10759

Hsing, A., Tsao, L., & Devesa, L. (2000). International trends and patterns of prostate cancer incidence and mortality. *International Journal of Cancer*, *85*(1), 60-67. doi: 10.1002/(SICI)1097-0215(20000101)85:1<60::AID-IJC11>3.0.CO;2-B

Ito, K., Yamamoto, T., Ohi, M., Takechi, H., Kurokawa, K., Suzuki, K., et al. (2003). Natural history of PSA increase with and withot prostate cancer. *Urology, 62*(1), 64-69. doi:10.1016/S0090-4295(03)00135-3

Jayaprakasha, G., Murthy, K., Etlinger, M., Mantur, S., & Patil, B. (2012). Radical scavenging capacities and inhibition of human prostate (LNCaP) cell proliferation by *Fortunella margarita*. *Food Chemistry*, *131*(1), 184-191. http://dx.doi.org/10.1016/j.foodchem.2011.08.058

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., & Thun, M. (2007). Cancer statistics, 2007. *CA: A Cancer Journal for Clinicians 57*(1), 43-66. doi: 10.3322/canjclin.57.1.43

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J, et al. (2009). Cancer statistics, 2009. *CA: A Cancer Journal for Clinicians 59*(4), 225-249. doi: 10.3322/caac.20006

Jemal, A., Thomas, A., Murray, T., & Thun, M. (2002). Cancer statistics, 2002. *CA: A Cancer Journal for Clinicians, 52*(1), 23-47. doi: 10.3322/canjclin.52.1.23

Jones, G., & Franks, D. (2009). Scholarly writing. In E. Smart, M. Young, & L. Mann (Eds.), *Writing for doctoral studies* (pp. 933-1015). Philadelphia, PA: Sandy Beach Press.

Jung, K., Seidel, B., Rudolph, B., Lein, M., Cronauer, M., Henke, W., et al. (1997). Antioxidant enzymes in malignant prostate cell lines and in primary cultured

prostatic cells. *Free Radical Biology & Medicine, 23*(1), 127-133. doi.org/10.1016/S0891-5849(96)00613-2

Klassen, A., & Platz, E. (2006). What can geography tell us about prostate cancer? *American Journal of Preventive Medicine, 30*(2), S7 – S15. doi.org/10.1016/j.amepre.2005.09.004

Kumar, R., Verma, V., Jain, A., Jain, R., Maikhuri, J., & Gupta, G. (2011). Synergistic chemoprotective mechanisms of dietary phytoestrogens in a select combination against prostate cancer. *The Journal of Nutritional Biochemistry*, *22(8),* 723-731. doi: 10.1016/j.jnutbio.2010.06.003

Landstrom, M., Zhang, J., Hallmans, G., Aman, P., Bergh, A., Damber, J., …, Adlercreutz, H. (1998). Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. *Prostate, 36*(3), 151-161. doi: 10.1002/(SICI)1097-0045(19980801)36:3<151::AID-PROS2>3.0.CO;2-K

Lee, M., Gomez, S., Chang, J., Wey, M., Wang, R., & Hsing, A. (2003). Soy and isoflavone consumption in relation to prostate cancer risk in China. *Cancer Epidemiology, Biomarekers and Prevention*, *12*(7), 665-68. http://cebp.aacrjournals.org/content/12/7/665.long

Lee, L., Pan, C., Cheng, C., Chi, C., & Liu, T. (2001). Expression of cyclooxygenase-2 in prostate adenocarcinoma and benign prostatic hyperplasia. *Anticancer Research, 21(2B)*, 1291-1294. http://www.ncbi.nlm.nih.gov/pubmed/11396201

Leewansangtong, S., & Soontrapa, S. (1999). Hormonal ablation therapy for metastatic prostatic carcinoma: A review. *Journal of the Medical Association of Thailand, 82(2),* 192-205. http://www.ncbi.nlm.nih.gov/pubmed/10087729

Lombardi, G., Zarrilli, S., Colao, A., Paesano, L., DiSomma, C., Rossi, F., & DeRosa, M. (2001). Estrogens and health in males. *Molecular and Cellular Endocrinology, 178*(1-2), 51–55. doi.org/10.1016/S0303-7207(01)00420-8

Lycette, J., Bland, L., Garzotto, M., & Beer, T. (2006). Parenteral estrogens for prostate cancer: Can a new route of administration overcome old toxicities? *Clinical Genitourinary Cancer, 5*(3), 198–205. http://dx.doi.org/10.3816/CGC.2006.n.037

Marks, L., Kojima, M., Demarzo, A., Heber, D., Bostwick, D., Qian, J., ..., Partin, A. (2004). Prostate cancer in native Japanese and Japanese-American men: Effects of dietary differences on prostatic tissue. *Urology*, *64*(4), 765-771. doi.org/10.1016/j.urology.2004.05.047

Maskarinec, G., Morimoto, Y., Hebshi, S., Sharma, S., Franke, A., & Stanczyk, F. (2006). Serum prostate-specific antigen but not testosterone levels decrease in a randomized soy intervention among men. *European Journal of Clinical Nutrition, 60*(12), 1423-1429. doi:10.1038/sj.ejcn.1602473

Medscape. (2013). Estradiol. Retrieved from http://emedicine.medscape.com/article/2089003-overview

Mentor-Marcel, R., Lamartiniere, C., Eltoum, I., Greenberg, N., & Elgavish, A. (2001). Genistein in the diet reduces the incidence of poorly differentiated prostatic

adenocarcinoma in transgenic mice (TRAMP). *Cancer Research, 61*(18), 6777-6782. http://cancerres.aacrjournals.org/content/61/18/6777

Merrick, G., Butler, W., Wallner, K., Galbreath, R., Allen, Z., & Adamovich, E. (2006) Androgen-deprivation therapy does not impact cause-specific or overall survival after permanent prostate brachytherapy. *International Journal of Radiation Oncology*Biology*Physics*, *65*(3), 669-677. doi:10.1016/j.ijrobp.2006.01.030

Michelin, R.S., & Gutierrez, M. (2013). The effect of phytoestrogen chemoprevention on prostate cancer. (Unpublished doctoral dissertation). Walden University, Minneapolis, Minnesota.

Mitchell, S., Zhu, W., & Young, C. (1999). Resveratrol inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Cancer Research, 59*(23), 5892-5895. http://cancerres.aacrjournals.org/content/59/23/5892

Morrissey, C., Bektic, J., Spengler, B., Galvin, D., Christoffel, V., Klocker, H., …, Watson, R. (2004). Phytoestrogens derived from *Belamcanda chinensis* have an antiproliferative effect on prostate cancer cells *in vitro*. *The Journal of Urology, 172*(6 Pt 1), 2426-2433. doi.org/10.1097/01.ju.0000143537.86596.66

Munoz-Espada, A., & Watkins, B. (2006). Cyanidin attenuates PGE2 production and cyclooxygenase-2 expression in LNCaP human prostate cancer cells. *Journal of Nutritional Biochemistry, 17*(9), 589-596. doi:10.1016/j.jnutbio.2005.10.007

Musey, P., Adlercreutz, H., Gould, K., Collins, D., Fotsis, T., Bannwart, C., … , Hase, T. (1995). Effect of diet on lignans and isoflavonoid phytoestrogens in chimpanzees. *Life Sciences*, *57*(7), 655-664. doi.org/10.1016/0024-3205(95)00317-Y

National Cancer Institute (NCI), (2012). What are estrogens? Retrieved from http://www.cancer.gov/cancertopics/understandingcancer/estrogenreceptors/page1

NCI. (2013). Prostate cancer. Retrieved from http://www.cancer.gov/cancertopics/types/prostate

Ndubuisi, S., Kofie, V., Andoh, J., & Schwartz, E. (1995). Black-white differences in the stage at presentation of prostate cancer in the District of Columbia. *Urology, 46*(1), 71-77. doi:10.1016/S0090-4295(99)80162-9

News Medical. (2012). Estrogen types. Retrieved from http://www.news-medical.net/health/Estrogen-Types.aspx.

Ockrim, J., Lalani, E., Kakkar, A., & Abel, P. (2005). Transdermal estradiol therapy for prostate cancer reduces thrombophilic activation and protects against thromboembolism. *The Journal of Urology*, *174(2), 527-533.* doi.org/10.1097/01.ju.0000165567.99142.1f

Ockrim, J., Lalani, E., Banks, L., Svensson, W., Blomley, M., Patel, S., Laniado, M., …… Abel, P. (2004). Transdermal estradiol improves bone density when used as single agent therapy for prostate cancer. *The Journal of Urology*, *172(6), 2203-2207.* doi.org/10.1097/01.ju.0000145511.56476.00

Oh, H., Leem, J., Yoon, S., Yoon, S., & Hong, S. (2010). Lipid raft cholesterol and genistein inhibit the cell viability of prostate cancer cells via the partial

contribution of EGFR-Akt/p70S6k pathway and down-regulation of androgen receptor. *Biochemical and Biophysical Communications, 393(2),* 319 – 324. doi: 10.1016/j.bbrc.2010.01.133

Parkin, D., Pisani, P., & Ferlay, J. (1999). Global cancer statistics. *CA Cancer J Clin., 49*(1), 33 – 64. http://www.ncbi.nlm.nih.gov/pubmed/10200776

Pendleton, J., Tan, W., Anai, S., Chang, M., Hou, W., Shiverick, K., ..., Rosser, C. (2008). Phase II trial of isoflavone in prostate-specific antigen recurrent prostate cancer after previous local therapy. *BMC Cancer, 8,* 132-141. doi: 10.1186/1471-2407-8-132

Qin, L., Wang, P., Kaneko, T., Hoshi, K., Sato, A. (2003). Estrogen: One of the risk factors in milk for prostate cancer. *Medical Hypotheses, 62*(1), 133 – 142. doi:10.1016/S0306-9877(03)00295-0

Ryan, C., & Small, E. (2003). Role of secondary hormonal therapy in the management of recurrent prostate cancer. *Urology*, *62*(1), 87-94. doi:10.1016/j.urology.2003.10.002

Sanofi Aventis (2010). Cancer information: Fight cancer with knowledge. Retrieved from www://prostate.cancerinfromation.com.

Schröder, F., Roobol, M., Boevé, E., de Mutsert, R., Zuijdgeest-van Leeuwen, S., Kersten, I., ..., van Helvoort, A. (2005). Randomized, double-blind, placebo-controlled crossover study in men with prostate cancer and rising PSA: Effectiveness of a dietary supplement. *European Urology*, *48*(6), 922-931. http://dx.doi.org/10.1016/j.eururo.2005.08.005

Science Daily. (2010). New research model of human prostate shows cancer development. Retrieved from http://www.sciencedaily.com/releases/2010/07/100701072700.htm

Sgambato, A., Ardito, R., Faraglia, B., Boninsegna, A., Wolf, F., & Cittadini, A. (2001). Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage. *Mutation Research, 496*(1-2), 171-180.

Sharpley, C., Bitsika, V., & Christie, D. (2011). How prostate cancer patients cope with the effects of diagnosis and treatment: Development of the effects of prostate cancer coping strategies scale. *Journal of Men's Health*, *8(1),* 56-65. doi:10.1016/j.jomh.2010.12.003

Shu, X., Jin, F., Dai, Q., Wen, W., Potter, J., Kushi, L., . . . Zheng, W. (2001). Soyfood intake during adolescence and subsequent risk of breast cancer among chinese women. *Cancer Epidemiology, Biomarkers & Prevention*, 10, 483-488. http://cebp.aacrjournals.org/content/10/5/483

Singh, P., Matanhelia, S., & Martin, F. (2008). A potential paradox in prostate adenocarcinoma: Oestrogen as the initiating driver. *European Journal of Cancer, 44*(7), 928 – 936. doi: 10.1016/j.ejca.2008.02.051

Singh-Gupta, V., Zhang, H., Yunker, C., Ahmad, Z., Zwier, D., Sarkar, F., . . . Hillman, G. (2010). Effect on hormone refractory prostate cancer in vitro and in vivo compared to genistein and soy extract: Potentiation of radiotherapy. *Pharmaceutical Research, 27*(6), 1115-1127. doi: 10.1007/s11095-010-0107-9

Smith, H. T. (2010). *Surviving doctoral studies*. Spokane, WA: Rosemary Books.

Staszewski, J., & Haenszel, W. (1965). Cancer mortality among Polish-born in the United states. *Journal of the National Cancer Institute, 35*(2), 291-297. http://www.ncbi.nlm.nih.gov/pubmed/5827237

Swami, S., Krishnan, A., Moreno, J., Bhattacharyya, R., Gardner, C., Brooks, J., . . . , Feldman, D. (2008). Inhibition of prostaglandin synthesis and actions by genistein in human prostate cancer cells and by soy isoflavones in prostate cancer patients. *International. Journal Of Cancer, 124*(9), 2050-2059. doi: 10.1002/ijc.24161

Thapa, D., & Ghosh, R. (2012). Antioxidants for prostate cancer chemoprevention: Challenges and opportunities. *Biochemical Pharmacology, 83*(10), 1319 - 1330. doi: 10.1016/j.bcp.2011.12.027

Turner, E., Lane, J., Metcalfe, C., Down, L., Donovan, J., Hamdy, F., ... , Vedhara, K. (2009). Psychological distress and prostate specific antigen levels in men with and without prostate cancer. *Brain, Behavior, and Immunity*, *23*(8), 1073-1078. doi: 10.1016/j.bbi.2009.01.009

Vaishampayan, U., Hussain, M., Banerjee, M., Seren, S., Sarkar, F., Fontana, J., . . . , Kucuk, O. (2007). Lycopene and soy isoflavones in the treatment of prostate cancer. *Nutrition and Cancer, 59*(1), 1-7. doi: 10.1080/01635580701413934

Vitamins and Health Supplements Guide (2012). Soy and isoflavones. Retrieved from http://www.vitamins-supplements.org/phytochemicals/soy-isoflavones.php.

Wang, X., Clubbs, E., & Bomser, J. (2006). Genistein modulates prostate epithelial cell proliferation via estrogen-and extracellular signal-regulated kinase dependent

pathways. *Journal of Nutritional Biochemistry, 17*(3), 204-210. doi:10.1016/j.jnutbio.2005.07.005

Weihua, Z., Warner, M., & Gustafason, J. (2002). Estrogen receptor beta in the prostate. *Molecular and Cellular Endocrinology, 193*(1-2), 1-5. doi.org.ezp.waldenulibrary.org/10.1016/S0303-7207(02)00089-8

Willis, M., & Wians, F. (2003). The role of nutrition in preventing prostate cancer: A review of the proposed mechanism of action of various dietary substances. *Clinica Chimica Acta, 330(1-2),* 57-83. doi.org.ezp.waldenulibrary.org/10.1016/S0303-7207(02)00089-8

Wu, A., Wan, P., Hankin, J., Tseng, C., Yu, M., Pike, M. (2002). Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. *Carcinogenesis, 23*(9), 1491-1496. doi: 10.1093/carcin/23.9.1491

Wuttke, W., Jarry, H., Becker, T., Schultens, A., Christoffel, V., Gorkow, C., & Seidlová-Wuttke, D. (2003). Phytoestrogens: endocrine disrupters or replacement for hormone replacement therapy? *Maturitas*, *44*(1), *S9-S20.* doi:10.1016/S0378-5122(02)00344-4

Watanabe, S., Uesugi, S., & Kikuchi, Y. (2002). Isoflavones for prevention of cancer, cardiovascular diseases, gynecological problems and possible immune potentiation. *Biomedicine & Pharmacotherapy*, *56*(6), 302-312. http://www.ncbi.nlm.nih.gov/pubmed/19434888

White, R., Tsodikov, A., Stapp, E., Soares, S., Fujii, H., & Hackman, R. (2010). Effects of a high dose aglycone-rich soy extract on prostate-specific antigen and serum

isoflavone concentrations in men with localized prostate cancer. *Nutrition Cancer, 62*(8), 1036-1043. doi: 10.1080/01635581.2010.492085

White, R., Hackman, R., Soares, S., Beckett, L., Li, Y., & Sun, B. (2004). Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. *Urology, 63*(2), 259-263. doi:10.1016/j.urology.2003.09.061

Willis, M., & Wians, F. (2003). The role of nutrition in preventing prostate cancer; A review of the proposed mechanism of action of various dietary substances. *Clinica Chimica Acta*, *330*(1–2), 57-83. doi.org/10.1016/S0009-8981(03)00048-2

World Health Organization. (2012). Cancer mortality and morbidity: Situation and trends. Retrieved from http://www.who.int/gho/ncd/mortality_morbidity/cancer_text/en/

Appendix A: Manual and Study Inclusion Form

Intervention Characteristics of Study: Selected studies should be RCT designed. Study should specifically show intervention from use of the phytoestrogens (isoflavones) genistein and daidzein among the components. Response of PSA and Estradiol in treatment or placebo should be the primary main dependent variables been studied. They are the important variables for the intervention strategy and are the primary measured variables. Their association relating to effect size or magnitude and direction of treatment effect and CaP chemoprevention is important.

Research Subjects: The RCTs should include subjects that are over 50 years old. However as indicated prior one study had an age range between 49.8 and 85.4 years old.

Valuable study variables: These should include the independent variable isoflavones (genistein and daidzein) and dependent variables PSA and estradiol.

Research methods: Studies needed to have both treatment and placebo groups. Baseline, pre-study, post-study information throughout the study was important. However, any lack of information on after study completion regarding follow-up would not deter selection. The treatment group should be matched by a placebo or control group that remain absent of any similarity to the employed treatment strategy.

Cultural and Language Concerns: Studies should be published in an English scientific peer-reviewed journal. Publications can be from any country where such research was conducted.

Publication Type: Primarily peer-reviewed RCTs

Publication time frame: Published within the past 15 years.

Coding Form

Study ID: Publication Type – Published scientific journal articles

Study Description: Randomized-controlled trials (RCTs)

Study Setting: Studies were conducted in several settings including medical and radiation oncology clinics; Urology clinics, Minnesota Veterans Administration medical Center; local urologists; Gastroenterology clinics; Los Angeles surgical patients; Local hospitals; University of Massachusetts Teaching Hospital; University of California, Davis; Moffitt Community Clinical Oncology program, Florida.

Age Range of Participants:

Mean Age of Participants:

Standard Deviation:

Target Population:

i) universal
ii) selective
iii) inclusive

Total confidence of target population: very weak; weak, moderate, strong, very strong

Total sample size (beginning of study (BS)):

Treatment group at BS:

Control group at BS:

Events: Reported study events

Adverse events (AEs):

Intervention Type:

i) effect identification;
ii) magnitude of effect;
iii) direction of effect

Intervention strategy Assurance/Confidence:

i) very low confidence;
ii) low confidence;
iii) moderate confidence;
iv) high confidence; and
v) very high confidence

Did information associating Phytoestrogens (Daidzein & Genistein) with PSA and estradiol responses identified in the studies?

1) Yes – in all studies
2) No – in most studies; in a few studies
3) Some exclusions

Nature of the control groups:

a) No treatment
b) Mixed
c) Placebo
d) Other (specify)
    a. time-delayed;
    b. volume-delayed;
    c. concentration-delayed

Duration of Intervention: (days; weeks; months; and years)

a) Single session (specify)
b) Multiple sessions (specify)
c) Continuous sessions (specify if applicable)

Length of treatment:

Length of control:

Length of follow-up:

Total length of study and follow-up (if applicable):

Periods of assessment:

Pre-test, post-test

Post-test only

Pre-test, post-test, follow-up

Appendix B: Effect Size Criteria Description

Intervention Criteria	Assigned # for Criteria	Description of Criteria
Intervention characteristics	1	Both daidzein and genistein used in intervention
	2	Only one isoflavone used in intervention
	3	None used
	4	Different isoflavones
Respondents to Research	5	Diagnosed with CaP or HRC
	6	Undergoing therapy or scheduled for surgery/therapies with increasing PSA
	7	Completed therapy/active surveillance with increasing PSA
Primary Variables	8	PSA levels
	9	Estradiol levels
	10	Available data for computing Effect Size data
Research Methodology	11	Meets the requirements for designing and conducting RCTs including randomization and blinding process
	12	Experimental groupings including Treatment and placebo/control subjects
	13	Presently available statistics employed in data analysis
Language/Cultural Requirements	14	Unpublished or published in English
Published or unpublished Time Frame	15	≤ 15 years ie. 1999 and later publications or unpublished work
Publication Type	16	Publication in peer-reviewed scientific journal

Appendix C: Effect Size and Study Coding Form

Study Level Coding

Level of effect size coding

1. Study ID
2. Effect size variables
   Duration of phytoestrogen therapy
   Concentration of components

3. Type of effect size
   a. 2 groups -- contrast in groups
   b. 3 or more groups -- contrast among groups
4. Effect size data page
5. Data providing Effect size:
   a. Means and SD
   b. *t*-value
   c. Correlation
   d. Hedges *g*
   e. Others
6. Contrast in group designs:
7. Sample size (N)
8. Pre-Intervention Means
9. Post-Intervention Means
10. SDs
11. Pooled Variance
12. ES uncorrected
13. Values of Effect Sizes
14. Corrected SMD
15. Standard Error of Means
16. Inverse variance (weight)

Characteristics and descriptive feature of study

1. Bibliography
2. Study
3. Format published a) journal; b) unpublished
4. Country published
5. Year published

Features of publication methodology

1. Study Design
    a. Experimental;
    b. Quasi-experiment;
    c. Cross-over
2. Control group
    a. Similar intervention;
    b. Placebo;
    c. Other treatment;
    d. No treatment

6. Study Design
    a. Experiment;
    b. Quasi-experiment
    c. Cross-over
    d. RCT
7. Diagnostic and assessment instruments for CaP diagnosis
8. Blinding of Researcher
    a. Yes;
    b. Nno;
    c. Partial, and
    d. Not reported

Subject Assignment in study

a) Random
b) Non-random (explain)
c) Other i.e. Cross-over (explain)

Defining CaP inclusion threshold:

a) Yes: indicate methods
b) No: indicate methods

Timelines for Data collection

a) Baseline/pre-treatment
b) During treatment
c) Post-treatment
d) Follow-up

Role of experimenter during treatment

a) Provides treatment
b) Non-contact with subjects
c) Directed treatment
d) Uncertain i.e. not reported

Appendix D: Permission to Use REM Figure

Subject : **Re: re. Use of Random Effects Model figure**

Date : Tue, May 21, 2013 09:02 AM CDT

From : Michael Borenstein <MichaelB@Meta-Analysis.com>

To : Ruel Michelin <ruel.michelin@waldenu.edu>

Thank you for your note.

You are welcome to use any results achieved from our software, however, please be sure to cite our program as follows:
Comprehensive Meta Analysis Version 2
Borenstein, M., Hedges, L., Higgins, J., & Rothstein, H.
Biostat, Englewood, NJ 2005

Thank you again.

Vivian Vargas
Assistant Administrator

At 07:42 PM 5/20/2013, you wrote:
>Dear Dr. Borenstein,
> Good day. I would be extremely
> appreciative if you could provide an email or attached word
> document response giving permission to use a copy of your random
> effects model figure in the dissertation research document been
> prepared for ProQuest submission. Your quick response will be
> highly appreciated.
>
>Respectfully,
>Ruel Michelin.
>

Curriculum Vitae

Ruel Slyfield Michelin

Summary of Qualifications and Experience

Biomedical Research

- Conducted more than seven (7) years of biomedical science research in academia devoting over five (5) years with primary focus on cancer research
- Integral in discovery of novel *Bacillus mojavensis* strain and developing and conducting biomedical research with the bacterium fermented metabolite. Strain is now employed in graduate research projects
- Experienced conducting established cellular confirmatory assays and employing established techniques for high quality research data
- Experienced designing and providing expertise conducting extensive research employing several mammalian tumor cell lines
- Experienced writing grant proposals and have collaborated on several published scientific articles
- Selected and recommended by university dean to participate as initial contributing member in proposed collaborative university cancer research team

Research Mentor

- Mentored undergraduate and graduate students in public health and biomedical science research
- Reviewer of scientific abstracts submitted for undergraduate/graduate research symposium and journal publication
- Assisted designing and lecturing undergraduate university research course

Public Health Research

- Invited guest lecturer at cancer and public health research conferences and symposia
- Presented research findings on public health issues including cancer and diabetes at major conferences including the **AACR, ASM, CACMID** and the **APHA**
- Developing research projects relating to cancer, diabetes and selected infectious diseases such as Lyme's and tuberculosis; conducting meta-analysis of selected published data for future presentation/publication and for requesting of research grants

Education

2013 PhD, Public Health, Walden University, Minneapolis MN

2011	MPH (Honors), Environmental Health, American Public University, Charles Town, WV
2009	Pre-Clinical Medical Studies, IUHS, St. Kitts, West Indies
2006	Diploma, Clinical Tropical Medicine and Parasitology, West Virginia University, School of Medicine, Morgantown, WV
2004	Graduate Certificate, Biotechnology and Microarray, FAES at NIH, Bethesda, MD
2000	BS, Liberal Studies, Excelsior College, Albany, NY
1992	Associate Diploma, Horticulture, University of Guelph, Ontario, Canada

Professional Memberships

2002 – Present	Member, Assoc. of Southeastern Biologists (ASB)
2003 – Present	Member, American Society of Microbiology (ASM)
2004 – Present	Canadian Association of Clinical Microbiology and Infectious Diseases (CACMID)
2007 – Present	Member, American Public Health Association (APHA)
2007 – Present	Member, European Association of Cancer Research (EACR)
2010 – Present	Member, Doctors for America, (DFA)

Selected Abstracts

1. **Michelin, R.,** Johnson, C., Leitner, W., Whittaker, J., & Gutierrez, M. Survivability and the opportunity for disease transmission: Observations of the *Ixodes pacificus*. Book of Abstracts. 2012 American Society of Tropical Medicine & Hygiene conference, November 11 – 15, 2012, Atlanta, Georgia.

2. **Michelin, R.,** Johnson, C., Leitner, W., Whittaker, J., & Gutierrez, M. Effect of phytoestrogen intake duration on PSA level and prostate cancer recurrence: A meta-analysis of randomized studies. Book of Abstracts. 2012 World Cancer Congress, August 27-30, 2012, Montréal, Canada.

3. **Michelin, R.,** Johnson, C., Leitner, W., Frederick, L., Shureiqi, I., Whittaker, J., & Gutierrez, M. Effects of climate change on *Aspergillus* species and consequences for agriculture and human health: A meta-analysis. Southeastern Biology, 59(2), 154. ASB 73rd Annual Meeting, April 4-7, 2012, The University of Athens, GA.

4. **Michelin R,** Frederick L, Benjamin E, Adelore T and Williams A. Purification and Partial-Characterization of an Antifungal Metabolite from an Axenic Culture of an Unidentified Species of *Bacillus.* Book of Abstracts. IFBLS 26th World Congress, Stockholm, Sweden. June 13-18, 2004.

5. **Michelin R,** Lobban K, Jenkins M, Adelore T, Frederick L and Williams A. Comparative *in vitro* Analysis of Novel Antifungal Compound using Etest and Disk Diffusion Assay. Book of Abstracts: Canadian Assoc of Clinical Microbiol and Infect Diseases 7th Conf. Regina, Nova Scotia, Canada. March 12-16, 2004.

6. **Michelin R,** Frederick L, Oladeinde F, Kinyua A and Williams A. Isolation and Purification Does Not Affect Cytotoxic Ability of Protein Fraction. ISPPP 2005, 25th

International Symposium & Exhibit on the Separation of Proteins, Peptides & Polynucleotides, November 6 – 9, 2005, St. Pete Beach, Florida, USA.

7. **Michelin R,** Frederick L, Kinyua A, Stewart J, Oladeinde F and Williams A. Nematicidal Activity of Compounds from New Strain of *Bacillus mojavensis*. The American Society of Tropical Medicine and Hygiene (ASTMH), 54th Annual Meeting, December 11 – 15, 2005, Hilton Washington Hotel & Towers, Washington, DC, USA.

8. **Michelin R,** Frederick L, Adelore T, Benjamin E, Jenkins M and Williams A. Identification, Purification and *in vitro* Analysis of an Antifungal Metabolite from an Unidentified *Bacillus* species. 72nd Conjoint Meeting on Infectious Diseases. Canadian Association for Clinical Microbiology and Infectious Diseases, November 7-10, 2004, Delta Regina Hotel, Regina, Saskatchewan.

9. **Michelin R,** Frederick L, Batta E, Benjamin E and Williams A. Comparative *in vitro* Assays Using Novel Antibacterial Compound against *Clostridium perfringens*. Book of Abstracts, ASM BioDefense Research Meeting. Baltimore Marriot Waterfront, March 7-10, 2004, Baltimore, Maryland.

10. Oladeinde F, Kinyua A, Laditan A, **Michelin R**, Makinde M, Williams A, Kennedy A, Taylor E and Bronner Y. Effect of *Cnidoscolus aconitifolius* (*Euphorbiaceae*) on blood glucose levels in Alloxan-induced Diabetic mice. Abstr. 4th Annual Levine Symposium: Advances in Diabetics Research, Nov. 4-8, 2003, Universal City, California.

11. Arzu-Thompson, K., Austin, L., **Michelin, R.**, & Frederick, L. (2007). Observations on the effect of culture filtrate of a strain of *Bacillus mojavensis* on germination of spores of *Alternaria* and *Ulocladium*. *Southeastern Biology,* 54(2), 136.

12. Knight, C., Austin, L., **Michelin, R.**, & Frederick, L. (2007). The induction of a dark strain of *Bacillus mojavensis* as an endophyte in corn and cotton plants. *Southeastern Biology*, 54(2), 136.

Selected Publications

Oladeinde, F., Kinyua, A., Laditan, **A., Michelin**, R., Makinde, M., Williams, A., et al. (2003). Phytochemical and anti-diabetic studies of *Cnidoscolus aconitifolius* (*Euphorbiaceae*), *West Indian Medical Journal*, 53 (Suppl 1): 30.

Oladeinde, F., Kinyua, A., Laditan, A., **Michelin,** R., Bryant, J., Denaro, F., et al. (2007). Effect of *Cnidoscoluis aconitifolius* leaf extract on blood glucose and insulin levels of inbred type 2 diabetic mice. *Cellular and Molecular Biology*, 53(3), 34-41.

Johnson, C., Oladeinde, F., Kinyua, A., **Michelin, R**., Makinde, J., Jaiyesimi, A., et al. (2008). Comparative assessment of total phenolic content in selected medicinal plants. *Nigerian Journal of Natural Products and Medicine*, 12, 40-42.

Johnson, C., Long-ze, L., Harnly, J., Oladeinde, F., Kinyua, A., **Michelin, R**., & Bronner, Y. (2011). Identification of the Phenolic Components of *Vernonia amygdalina* and *Russelia equisetiformis, Journal of Natural Products,* 4, 57-64.

Work Experience

2012–Present	Executive Director, Cariam Health Consulting, LLC, Rockville MD
2003–2008	Research Assistant, Dept. of Biology, Morgan State University, Baltimore, MD
2001–2003	Graduate Research, Dept. of Biology, Howard University, Washington, DC
1998--1999	Surgical Technician, BMT Unit, University of Michigan Hospitals, Ann Arbor, MI
1997–1998	Medical Assistant, University of Michigan Hospitals, Comprehensive Cancer Clinic, Ann Arbor, MI