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Single Nucleotide Polymorphisms in DNA Repair and Oxidative Stress Genes, and Their Interaction with Antioxidants on Prostate Cancer Aggressiveness

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SINGLE NUCLEOTIDE POLYMORPHISMS IN DNA REPAIR AND OXIDATIVE STRESS GENES, AND THEIR INTERACTION WITH ANTIOXIDANTS ON PROSTATE CANCER AGGRESSIVENESS

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ABSTRACT

Prostate cancer is the leading invasive malignancy and the second most common cause of cancer death among American men. Despite compelling evidence that oxidative stress, ineffective DNA damage repair, and habitually low antioxidants intake may act in tandem to influence prostate carcinogenesis, few studies have examined gene-diet interactions involving these risk factors. Even fewer studies have examined such interactions in relation to prostate cancer aggressiveness. This study investigated whether single nucleotide polymorphisms (SNPs) in DNA repair- and oxidative stress-related genes modulated associations between antioxidant intake and prostate cancer aggressiveness. We utilized data from the North Carolina-Louisiana Prostate Cancer Project (PCaP) among African-American (n = 948) and European-American (n = 1,016) men. Antioxidant intake was assessed using a food frequency questionnaire, and genotypes of 30 germline SNPs were examined.

Effect modification by certain polymorphic variants were observed with some variations by race, including variants in *XRCC1* (rs2854508, T > A), *XPA* (rs3176644, G > T), *NOS3* (rs1799983, G > T), *OGG1* (rs1805373, G > A) and *NQO1* (rs689453, C > T). For example, significant interaction was observed between *XRCC1* (rs2854508) genotype and α-tocopherol intake among African Americans and European Americans, such that among those with the TT genotype, higher α-tocopherol intake was inversely related to prostate cancer aggressiveness, while higher α-tocopherol intake was positively related to

high aggressive prostate cancer among those who harbor the AA or AT genotype. A similar pattern of effect modification by *XRCC1* (rs2854508) was observed for the association between γ-tocopherol and prostate cancer aggressiveness, but only among African Americans. Lower odds of high aggressive prostate cancer was observed among European Americans who possess the CT or TT genotype of *NQO1* (rs689453) and had higher lycopene intake, but not European Americans with the CC genotype, and there was no evidence of effect modification among African Americans.

Reduced odds and increased odds of high aggressive prostate cancer were observed with higher intakes of certain antioxidants (i.e., α-tocopherol, γ-tocopherol and lycopene) dependent on genotype, indicating potentially differential dietary recommendations based on genetic susceptibility. Because germline genotype is unalterable, these findings underscore the importance of considering genetic risk variability as part of dietary intervention strategies to identify the subgroup of men who are likely to benefit from such interventions.

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LIST OF ABBREVIATIONS

ACSM ..... American College of Sports Medicine

AICR ..... American Institute for Cancer Research

ATBC ..... Alpha-Tocopherol, Beta-Carotene Cancer Prevention Trial

BER ..... Base Excision Repair

BMI ..... Body Mass Index

BPH ..... Benign Prostatic Hyperplasia

CARET ..... Carotene and Retinol Efficacy Trial

CDC ..... Centers for Disease Control and Prevention

CEPH ..... Centre d'Etude du Polymorphisme Humain

CEU ..... Utah residents with ancestry from Northern and Western Europe

CGEMS ..... Cancer Genetic Markers of Susceptibility

CHAMPS ..... Community Health Activities Model Program for Seniors Physical Activity Questionnaire

CHB ..... Han Chinese in Beijing, China

CIDR ..... Center for Inherited Disease Research

DHT ..... Dihydrotestosterone

DNA ..... Deoxyribonucleic Acid

DoD ..... Department of Defense

EPIC ..... European Prospective Investigation into Cancer and Nutrition

ERCC8 ..... Excision Repair Cross-Complementing Rodent Repair Deficiency, Complementation Group 8

Fe/MnSOD ..... Iron, Manganese Superoxide Dismutase

GPX ..... Glutathione Peroxidase

GWAS ..... Genome-Wide Association Studies

HGPIN ..... Prostatic Intraepithelial Neoplasia

hOGG1 ..... Human 8-oxoguanine DNA Glycosylase

HPLC ..... High-performance Liquid Chromatography

HPV ..... Human Papilloma Virus

JPT ..... Japanese in Tokyo, Japan

LA ..... Louisiana

LD ..... Linkage Disequilibrium

LNCaP ..... Lymph Node Carcinoma of the Prostate

MAF ..... Minor Allele Frequency

MiRNA ..... Micro-Ribonucleic Acid

MMR ..... Mismatch Repair

NC ..... North Carolina

NCI-DHQ ..... National Cancer Institute Diet History Questionnaire

NER ..... Nucleotide Excision Repair

NHANES ..... National Health and Nutrition Examination Survey

NOX3 ..... Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase 3

NPC ..... National Prevention of Cancer Trial

NQO1 ..... Nicotinamide Adenine Dinucleotide Phosphate: Quinone Oxidoreductase 1

PC-3 ..... Prostate Cancer Cell line 3

PCa ..... Prostate Cancer

PCaP ..... North Carolina-Louisiana Prostate Cancer Project

PIA ..... Proliferative Inflammatory Atrophy

PIN ..... Prostate Intraepithelial Neoplasia

PLCO ..... Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

PPARG ..... Peroxisome Proliferator-Activated Receptor Gamma

PSA ..... Prostate-specific Antigen

RAD51C ..... RAD51 Paralog C

ROS.....Reactive Oxygen Species

SELECT.....Selenium and Vitamin E Cancer Prevention Trial

SNP ..... Single Nucleotide Polymorphism

SOD..... Superoxide Dismutase

VAF.....Variant Allele Frequency

WCRF ..... World Cancer Research Fund

XPA.....Xeroderma Pigmentosum, Complementation Group A

XPC.....Xeroderma Pigmentosum, Complementation Group C

XRCC.....X-ray Repair Complementing Defective Repair in Chinese Hamster cells

YRI.....Yoruba in Ibadan, Nigeria

CHAPTER 1

INTRODUCTION/BACKGROUND

1.1 INTRODUCTION

Prostate cancer (PCa) is the most prevalent invasive cancer and a leading cause of death in American men [1]. It is estimated that about 2.8 million American men are living with PCa with an estimated 241,740 new cases of PCa diagnosed in the U.S. in 2012 [1, 2]. Due to improved early detection methods, particularly with the advent and subsequent widespread use of prostate-specific antigen (PSA) test for PCa screening and improvements in cancer treatment, the majority of men diagnosed with PCa die with, rather than of, the disease. Thus, PCa is often regarded as an indolent disease of aging [3]. However, aggressive forms of PCa are very lethal. PCa aggressiveness generally refers to the extent of cancer invasiveness and migration, and often is defined based on tumor grade (Gleason score), cancer stage, and PSA level at diagnosis [4, 5]. Men with high aggressive PCa have been reported to have as much as a 14-fold increased risk of dying from the disease when compared to those with less aggressive PCa [5]. It is also estimated that about 30% of prostate tumors progress aggressively [6]. Given the prevalence and magnitude of fatalities associated with aggressive PCa, much research has been devoted to identifying biomarkers that distinguish indolent disease from aggressive disease [7, 8]. On the contrary, less research has been dedicated to understanding the interplay between genetic susceptibility and environmental factors that influence PCa aggressiveness. Specifically, less attention has been given to interactions between single

nucleotide polymorphisms (SNPs) within coding regions of genes that have been implicated in carcinogenesis and dietary micronutrients such as antioxidants, which may offer new insights for preventing or reversing PCa aggressiveness.

Similarly, researchers have, until recently, paid very little attention to the potential salutary effects of antioxidant-rich diet on PCa progression in men with biochemical recurrence of PCa. Biochemical recurrence of PCa is determined by the rising level of PSA after post-treatment nadir (lowest detectible level of PSA after treatment), and often an indicator of metastasis [9-12]. Men with biochemically recurrent PCa are traditionally treated with androgen ablation therapy (medical or surgical) to slow the disease progression and delay time to metastasis [9, 11-13]. However, androgen ablation is not a cure for PCa, does not always slow the disease progression, and it has been associated with life-altering side effects including erectile dysfunction, loss of bone density and cognitive decline; and long-term use of androgen ablation has also been associated with obesity, diabetes and cardiovascular disease [14-16]. Widespread recognition of these side effects has brought to the fore the need for new treatment options that could prevent, delay or reverse PCa progression in men with rising PSA levels after curative treatment for PCa without the side effects of androgen ablation. Epidemiologic studies suggest that diet is one of the important environmental factors that influence PCa initiation and progression [17]. Diets rich in antioxidants have also been shown to have chemopreventive effects [18]. Although promising, there is conflicting evidence on whether a plant-based, antioxidant-rich diet can halt PCa progression in men with biochemically defined PCa recurrence [9, 19-21]. However, these studies were limited by their inability to evaluate effects of bioactive antioxidants status (as markers of

antioxidant intake) on PCa progression. Thus, there is a need for well-designed, randomized clinical trials to examine effects of changes in bioactive levels of dietary antioxidant on PCa progression.

1.2 EPIDEMIOLOGY OF PROSTATE CANCER

PCa is the most commonly diagnosed invasive cancer and the second most virulent cancer in American men [1]. In 2012, PCa accounted for an estimated 29% (241,740) of all newly diagnosed cancers and 9% (28,170) of cancer deaths in American men [1]. At present, American men have an estimated 16% (one in six) risk of developing PCa during their lifetime, and a 3% (one in 36) risk of dying from the disease [22]. The introduction of PSA screening test in the late 1980's, and subsequent widespread use of PSA testing for PCa diagnosis led to about a 10% rise in the number of reported cases of PCa each year in the U.S. from 1986 until 1992, when the incidence rate peaked at 237 new cases per 100,000 men [23]. Since 1992, the number of newly diagnosed cases of PCa has been declining, which suggests that the screening effect is diminishing as the pool of men with previously undiagnosed latent disease who were captured with the advent of the PSA screening test may have been exhausted [24-26]. Even with the decline in PCa incidence rate, the disease remains the most frequent malignancy in American men, with the exception of superficial skin cancer [27]. This has been attributed to a multiplicity of factors including increasing life-expectancy, increased prevalence of environmental carcinogens, and improved diagnostic technology, particularly in the area of transurethral ultrasonography and biopsy technology [26, 28].

PCa is a multifactorial disease with etiology involving both genetic and environmental components. The well-established risk factors for PCa are aging, race/ethnicity and family history. Other less-established, but "highly probable" risk factors (due to reasonably consistent evidence of their involvement in PCa) include androgens, inflammation, diet, physical activity/obesity, and tobacco use. Purported risk factors such as vasectomy, sexually transmitted diseases, benign prostatic hyperplasia (BPH), and human papilloma virus (HPV) infections are a subject of both persistent controversy and active research (reviewed in [26, 28, 29]).

Perhaps the most distinguishable feature of PCa is its inseparable association with aging. PCa risk increases much faster with age than does other types of cancer [26]. In the U.S., the average age of prostate cancer diagnosis is 67 years. It is rarely diagnosed in men younger than age 50 years (3%); however, after this age the incidence increases exponentially with about 97% of the cases diagnosed in men ≥ 50 years old, and 60% diagnosed in men ≥ 65 years old [1]. The probability of developing PCa for men ≤ 39 years old is one in 8,499. This increases to one in 38 for men 40-50 years old, one in 15 for men 60-69 years old, and one in eight for men ≥ 70 years of age. The risk of death from PCa also increases substantially with aging, with men ≥ 70 years old having the highest proportion of death from the disease [1]. Indeed, autopsy reports have shown that about 70% of men who die by age 80 or older have histological evidence of latent PCa [30, 31].

African ancestry is an established risk factor for PCa incidence, aggressiveness and mortality from the disease [1]. Men of African descent are more likely to be

diagnosed with PCa at an early age [1, 27], present with advance-staged disease [32, 33], higher PSA levels [34, 35], poorly differentiated tumors [36, 37], and are more likely to die from PCa as compared to men of European ancestry [38, 39]. In two recent studies of PCa mortality-to-incidence ratio in South Carolina and Georgia, African-American men were found to have 58% and 55% higher PCa specific mortality given incidence, respectively, when compared to European Americans [20, 40]. Reasons behind these marked racial differences remain unclear; however, plausible explanations include inherited genetic susceptibility along racial lines, as well as differences in environmental and socioeconomic factors [41].

The hereditary component of PCa is also well-documented. Men with family history of PCa, especially among first-degree relatives (father or brother) have about a three-fold increased risk of developing the disease regardless of race/ethnicity [42, 43]. First-degree male relatives of a PCa patient also tend to be diagnosed with PCa an average of 6 to 7 years earlier than those without family history of PCa [44]. Family history of PCa is also a risk for developing the more aggressive forms of the disease [45, 46]. Additionally, familial clustering for PCa has also been found to be stronger than that of breast cancer and colon cancer, two malignancies that are well-recognized for their familial aggregation [47]. Also, several studies have reported significant associations between having a first-degree female relative (mother or a sister) with history of breast cancer and risk of PCa, as well as an increased risk for aggressive PCa [48-50].

PCa is often described as an androgen-dependent disease. This is largely attributable to the Nobel Prize winning study of urologist Charles Huggins, which led to

the use of androgen deprivation therapy for treatment of advanced PCa [51]. While the evidence remains inconclusive, androgens, particularly testosterone, have been implicated in the initiation and progression of PCa [52]. As is the case with normal prostate growth and function, the induction and progression of PCa largely depends on androgens and androgen receptor signaling [53]. Experimental studies suggest that the enzyme 5 alpha-reductase which is found in the prostate gland converts testosterone into dihydrotestosterone (DHT), a more active androgen which binds to genomic deoxyribonucleic acid (DNA) and regulates the expression of oncogenes that cause PCa [54]. The role of androgens in prostate carcinogenesis is also supported by epidemiological studies that have found that eunuchs (i.e., males who are castrated before puberty) do not develop PCa, primarily because the main source of androgens has been removed from the body [26, 28]. It is, however, important to note that recent studies have reported conflicting evidence on the role of androgens in PCa, and its aggressiveness [55, 56]. Similarly, the definitive role of specific androgens, timing of their effect, and their underlying mechanisms remains unclear [56].

Chronic inflammation has been implicated in the etiology of malignancy in several organs including the esophagus, lungs, pancreas, liver, stomach, colon, and urinary bladder [57]. Emerging evidence suggests that chronic inflammation may play a role in the neoplastic transformation of the prostate, and in PCa aggressiveness [10, 58]. The suggested mechanisms of inflammatory effect on prostate carcinogenesis include induction of oxidative stress-induced DNA damage, rapid cellular turnover and angiogenesis [58, 59]. Molecular and genetic studies also suggest that the sequelae of inflammatory effect on PCa involves formation of proliferative inflammatory atrophy

(PIA) in the prostate epithelium, which transitions into prostate intraepithelial neoplasia (PIN), a known precursor lesion of PCa [60]. Epidemiological studies have also reported associations between chronic prostatitis (inflammation of the prostate) and PCa [61, 62].

Several parallels have been drawn between dietary patterns and PCa; however, the evidence can, at best, be described as probable, rather than conclusive. A litany of epidemiological studies have found that excessive intake of energy (calories), calcium and dairy products (e.g., milk, cheese, cream, butter, etc.), animal fat and red meat increases risk for PCa and PCa aggressiveness (reviewed [17, 63, 64]). On the other hand, a diet rich in whole-grains, fruits, vegetables, isoflavones, nuts, seeds and berries appear to offer protection against PCa incidence [65, 66] and aggressiveness [67-69]. These protective effects are often attributed to the antioxidant micronutrients found in these diets. Antioxidants appear to modulate PCa incidence and aggressiveness by reducing oxidative DNA damage and gene mutations [70, 71]. However, the three largest randomized controlled trials conducted to examine associations between specific antioxidants and PCa (i.e., ATBC trial, the SELECT study, the Physicians' Health Study II) failed to establish definitive chemopreventive effects of antioxidants on prostate carcinogenesis [72-74]. Notwithstanding, a number of studies have reported significant associations between dietary antioxidants, particularly vitamin E, β-carotene, vitamin C, lycopene, and selenium, and PCa incidence [75-77] and aggressiveness [67, 78, 79]. Nonetheless, the association between dietary antioxidants and PCa remain an issue of intense scrutiny and ongoing research.

Despite biologically plausible relations, there are inconsistent findings on whether physical activity reduces PCa risk (reviewed in [80, 81]). It has been postulated that regular physical activity modulates prostate carcinogenesis by reducing serum testosterone levels, mitigating oxidative DNA damage, and enhancing immune defenses against genomic alterations [82]. There has also been increasing evidence that regular, moderate-to-vigorous physical activity may reduce the risk of developing aggressive PCa [83-85]. Several studies have also reported associations between obesity and prostate cancer risk [86, 87] and aggressiveness [88, 89]. However, the evidence remains inconclusive.

Tobacco smoke is a known carcinogen which is traditionally associated with lung cancer, but has also been associated with other malignancies, including cancers of the bladder, pancreas, esophagus, stomach, colon, and breast [90]. In recent years, there has been growing evidence that tobacco smoke is not only associated with PCa risk [91], but also PCa aggressiveness [92-94], recurrence [95, 96], and PCa specific mortality [38, 97]. The timing of the effect of tobacco smoke on PCa likely spans decades, and may be modulated by genetic susceptibility and/or gene-environment interaction. Nonetheless, at present, the epidemiological evidence on the association between tobacco smoke and PCa is not consistent (reviewed in [91, 92]).

1.3 STUDY RATIONAL AND SIGNIFICANCE

Dietary Antioxidant Intake and Prostate Cancer Progression

Primary management of PCa involves radical prostatectomy or radiation treatment, with curative intent [98, 99]. Unfortunately, about one in three men treated

with radiation or prostatectomy will experience PCa recurrence within 10 years of treatment. This increases to one in two men after 15 years of definitive therapy [100]. A rising level of serum PSA is the earliest sign of PCa recurrence, and often referred to as biochemical recurrence of PCa [100, 101]. Although the definition of biochemical recurrence of PCa has been a subject of debate, it is generally defined as having three or more successive rises in PSA above 0.2-0.4 ng/ml from the lowest detectable level after definitive therapy [101-103]. Sustained rise in PSA level after definitive therapy is also an early sign of metastatic disease and poorer prognosis [9, 104]. Biochemical recurrence of PCa is often treated with androgen ablation to delay time to metastasis [99, 105]. However, androgen ablation is not a cure for PCa, is not always effective, and often inflicts severe side effects including erectile dysfunction, osteoporosis, gynecomastia, cognitive decline, weight gain, diabetes, and cardiovascular disease [14-16]. Thus, there is considerable interest in a search for novel biomarker-driven treatment options that would halt PCa progression and prevent metastases without the side effects of androgen ablation.

There is ample evidence that a plant-based, antioxidant-rich diet, including a diet rich in fruits, vegetable, whole grains, soy and soy products may halt PCa progression in men with established disease [106]. Suggested mechanisms of dietary antioxidants' effect on PCa progression include mitigation of oxidative DNA damage and suppression of LNCaP cell growth by down-regulating male sex hormones [18, 70]. While serum PSA level remains a controversial diagnostic test for PCa since a rising level of PSA is not exclusive to prostate carcinoma, but also common with benign prostatic hyperplasia (BPH) and prostatitis [107], sustained rise in PSA level is an established biomarker of

PCa progression [108]. Findings from recent studies suggest that antioxidant-rich dietary interventions may benefit men with PCa recurrence, as evidenced by a decline in serum PSA level [9, 19, 109, 110]. However, the evidence remains inconclusive thus far, with a number of studies reporting null associations [13, 111-113]. Perhaps these inconsistent findings may be due to the inability of these studies to measure and evaluate the therapeutic effects of bioavailable antioxidants as markers of antioxidant intake. Thus, there is a need for well-designed randomized trials to evaluate the effects of biomarkers of antioxidants intake such as serum carotenoids and tocopherols on PSA dynamics, and by extension, PCa progression.

Oxidative Stress, Antioxidant Defense, and DNA Repair Pathways

Oxidative DNA damage is a major focus of ongoing etiologic PCa research. Oxidative stress refers to a state of imbalance in intracellular levels of reactive oxygen species (ROS), and biochemical antioxidants, in favor of ROS [71]. While oxygen is essential for normal cell growth and function, molecular oxygen-overload often results in excessive production of ROS, which causes damage to cellular components of genetic material particularly, protein, lipids, and nucleic acid [71, 114, 115]. Under normal physiological conditions, excess molecular oxygen is reduced to water (H_2O) through an elaborate system of electron transport involving series of oxygen-reduction reactions (redox) [70]. However, incomplete or partial reduction of oxygen results in the production of singlet oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}HO$), superoxide anion radical (O_2^{-}) and hydrogen peroxide (H_2O_2), collectively referred to as oxygen free radicals or reactive oxygen species (ROS) [70, 71, 116].

It is estimated that about 5% of molecular oxygen load are eventually converted to ROS during normal mitochondrial oxygen metabolism [70]. High levels of ROS are often produced as a result of endogenous processes such as chronic inflammation and oxidative phosphorylation, or through exposure to exogenous substances such as ionizing radiation, environmental toxins and pharmaceuticals [70, 117]. To counterbalance elevated levels of ROS, antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants (such as dietary sources of α-tocopherol, β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin, etc.), break down ROS to restore intracellular redox homeostasis [70]. Oxidative stress occurs when intracellular ROS levels exceed antioxidant defense capacity, resulting in oxidative DNA damage, altered gene expression, and cell death [70, 71]. Oxidative stress has been implicated in the etiology of several chronic diseases including cancer, neurodegenerative disorders, diabetes, arteriosclerosis, and pulmonary diseases [118].

Oxidative stress has been linked to PCa through its intimate association with known or potential risk factors for PCa, such as aging, androgens, and inflammation. Aging is the strongest risk factor for PCa, and is associated with inexorable decline in body functions including decline in antioxidant defense capacity [119]. Elevated levels of ROS and consequential oxidative DNA damage has been observed in aging tissues, including the prostate [117, 120]. Similarly, elevated levels of androgens can alter intracellular redox status in favor of ROS [70, 71]. This is also supported by findings from studies that show that androgen deprivation therapy reduces ROS levels in PCa cells (LNCaP), resulting in delayed disease progression [121, 122]. Likewise, chronic inflammation, particularly inflammation of the prostate (i.e., prostatitis), also appears to

increase ROS production through activation of phagocytes, such as neutrophils and macrophages, resulting in increased oxygen-uptake which, in turn, increases ROS production in prostate epithelial cells [70, 117]. It has also been clearly demonstrated that oxidative stress in PCa cells is essential for their aggressiveness (i.e., invasion and migration), with aggressive forms of PCa displaying higher degree of oxidative stress than do less aggressive forms [123].

Oxidative DNA damage is marked by the formation of oxidized base lesions or adducts which, if unrepaired, can result in mutation, altered gene expression, and transformation of normal cells into malignancy [8, 70]. Cellular response to oxidative DNA damage involves activation of several oxidative stress-mediating genes/pathways, including DNA repair pathways and antioxidant enzymes, in order to prevent further DNA damage, recover from mutagenesis, and restore genomic stability [124, 125]. Four categories of DNA repair pathways are known to operate on different types of DNA damage by reversing and/or removing damaged elements: (1) base excision repair (BER), which repairs small lesions such as oxidized DNA bases and nonbulky adducts; (2) nucleotide excision repair (NER), which removes bulky lesions and damaged single-stranded fragments from environmental and oxidative stress-induced damage; (3) mismatch repair (MMR) that corrects DNA replication errors, specifically mispaired DNA bases; and (4) double-stranded breaks, which are repaired through complex pathways involving homologous recombination and end-joining DNA repair mechanisms [124-126].

Single nucleotide polymorphisms (SNPs) in genes involved in oxidative stress and DNA repair pathways may influence susceptibility to PCa [8, 10]. Several studies have reported associations between SNPs in DNA repair and oxidative stress genes, and overall risk of PCa (summarized in a recent meta-analysis [127]). Existing evidence also suggests that associations between these SNPs and overall PCa risk may be modulated by dietary antioxidants [128-130]. Similarly, there is growing evidence that some of the SNPs in DNA repair and oxidative stress genes confer greater risk for developing aggressive PCa [131, 132]. However, just a handful of studies have investigated joint effects of these SNPs and antioxidant status in relations to PCa aggressiveness, with mixed findings [133-135]. These studies were limited by small sample size, lack of racial diversity, and inability to control for multiple potential confounders such as body mass index (BMI), physical activity, dietary factors including calcium and energy (calorie) intake. Thus, there is a need for a large, racially diverse, population-based study, with detailed data on demographics, personal and family health history, lifestyle, dietary patterns, PCa screening history, and clinical attributes of PCa.

1.4 OBJECTIVES AND HYPOTHESES

OBJECTIVE 1: To examine whether changes in dietary antioxidant intake (baseline to 3-months) as measured by plasma concentrations of carotenoids and tocopherols are associated with PCa progression (assessed by changes in serum PSA level as an intermediate prognostic marker of disease progression) in African-American and European-American men with biochemically defined PCa recurrence after definitive therapy.

Hypotheses:

i. Higher levels of plasma carotenoids and tocopherols at baseline will be associated with lower serum PSA levels at baseline.
ii. Higher post-intervention plasma carotenoids and tocopherol levels (at 3 months) will be associated with lower post-intervention PSA levels (at 3 months, and at 6 months), after adjusting for baseline PSA level.
iii. Change in plasma carotenoids and tocopherol levels (baseline to 3 months) will inversely correlate with serum PSA levels at 3 months and at 6 months.

OBJECTIVE 2: To examine associations between antioxidant levels in adipose tissue, plasma and diet, and PCa aggressiveness among African American and European-American men.

Hypothesis: Lower levels of antioxidants in adipose tissue, plasma and diet are associated with high aggressive PCa, and these associations do not vary by race.

OBJECTIVE 3: To examine whether SNPs in DNA repair and oxidative stress genes modulate associations between antioxidants in adipose tissue, plasma and diet, and PCa aggressiveness among African-American and European-American men.

Hypothesis: SNPs in DNA repair and oxidative stress genes modify associations between antioxidants in adipose tissue, plasma and diet, and PCa aggressiveness, and the degree of the effect modification vary by race.

CHAPTER 2

LITERATURE REVIEW

2.1 DIETARY INTERVENTION TRIALS FOR RECURRENT PROSTATE CANCER AFTER DEFINITIVE

Clinically localized PCa is traditionally managed with definitive therapy which consists of prostatectomy or radiation therapy, or prostatectomy followed by radiation therapy [9, 12, 100]. However, over a third of PCa patients treated with definitive therapy develop biochemically defined disease recurrence within 10 years of treatment [100]. Biochemical recurrence of PCa is marked by sustained rise in PSA after post-treatment nadir (lowest detectable level of PSA after treatment) [100-102]. A rising level of PSA is also a surrogate serum marker of PCa progression [9]. Men with biochemically recurrent PCa and rising level of PSA are often treated with androgen ablation with the hope of delaying the disease progression. Nonetheless, there is little evidence supporting the efficacy of androgen ablation in this population of men [136-138]. Androgen ablation has also been associated with severe, life-altering side effects [14, 15]. These reasons have motivated the search for new and innovative treatment strategies that could halt or delay the progression of recurrent PCa without the side effects of androgen ablation [9].

There is growing evidence that recurrent PCa may be a diet-sensitive disease stage because a number of dietary intervention trials have found inverse associations between increased consumption of a plant-based, antioxidant-rich diet and serum PSA

levels [18, 19, 109, 139]. This suggests that plant-based diets that are rich in antioxidants may offer an alternate treatment option for recurrent PCa. However, the current evidence is limited and inconclusive, as other dietary intervention studies including well-designed randomized clinical trials have also found null associations between antioxidant-rich dietary interventions and PCa progression in men with biochemically defined recurrence ([13, 112, 113], also reviewed in [111]). Thus, the exact role of dietary antioxidants in relations to PCa progression remains unclear.

In a recent dietary intervention trial of men with biochemically defined PCa recurrence and rising PSA levels, Saxe et al. [9] investigated the effect of dietary modification involving a plant-based, antioxidant-rich diet, together with stress management training, on PCa progression. The study consisted of 13 men (supported by their spouses) who opted not to use conventional therapy for treatment of recurrent disease. The study participants were recruited through the Veterans Affairs hospitals in San Diego, California and followed over a 6-month period. The investigators utilized a pre-post design with each participant serving as his own control. The intervention in this study consisted of increased intake of whole grains, fruits, vegetables, and legumes, along with lessons on how to shop for, and cook the study-compliant diet. Study participants in the intervention group were also offered individual dietary counseling, instructional materials, and a series of group meetings to help reinforce the intervention. Stress management training was also offered to the intervention group to help relieve any form of stress that may have been associated with change in diet palatability and culinary habits. Pre-intervention PSA values were obtained from participants' medical records, and rates of pre-intervention PSA rise were calculated based on these PSA values,

including the initial PSA values used to establish biochemical recurrence of the disease (i.e., three successive PSA values, at least 1 month apart) and all documented PSA values thereafter, until the start of intervention. The intervention rate of PSA rise was estimated based on PSA values measured at baseline, at 3-months, and at 6-months. The pre-intervention and intervention rates of PSA rise, and PSA doubling time were estimated for each patient as the natural log of PSA by time (months) and natural log of two divided by the rate of change in PSA, respectively, using linear regression analyses. Results of the study show a significant decline in rates of PSA rise during intervention when compared with the pre-intervention rates of PSA rise ($p< 0.01$). Additionally, significant improvements were made in PSA doubling time during the intervention, which increased from a median doubling time of 11.9 months (pre-intervention) to 112.3 months over the 6-month intervention period. This study suggests that plant-based, antioxidant-rich dietary modification combined with stress reduction may slow the progression of PCa as evidenced by decline in rates of PSA rise and prolonged PSA doubling time.

Carmody et al. [21] also investigated whether men with recurrent PCa and rising level of PSA can make sustainable dietary changes after attending series of cooking lessons integrated with mindfulness training, and whether these dietary changes can influence quality of life and PCa progression. The study population consisted of 36 male residents of Massachusetts with biochemically defined PCa recurrence after receiving definitive therapy who had not received any disease-directed therapy after PCa recurrence. The study participants were randomized into intervention (n=17) and control (n =19) groups, and followed over a 3-month period. Participants in the intervention group (supported by spouses or partner of choice to help with dietary change) were

offered 11 weekly diet and cooking lessons which emphasized increased intake of plant-based diets that are rich in antioxidants, particularly foods that are rich in carotenoids, soy, and isoflavones; including vegetables, whole grains, and soybean and soybean products. The intervention also encouraged consumption of fish, especially salmon due to its ω-3 fatty acids content, and discouraged intake of poultry and dairy products (including meat) because of saturated fat and calcium contents, which have been associated with PCa incidence, aggressiveness, and progression [17]. Since dietary changes can be onerous and often difficult to adhere to, mindfulness training was offered as part of the intervention to help promote participants' sense of control over change in dietary patterns. The intervention group was compared to a "wait-list" control group, who were offered an opportunity for the intervention at the end of the study. Diet assessment in both arms of the study was done using 24-hour diet recall interviews which provided estimates of nutrient intake including antioxidants, fat, protein, calcium and fiber at baseline, after 11 weeks of intervention, and at the end of the intervention (3 months). Change in quality of life was assessed using functional assessment questionnaires eliciting information on physical/functional status at the three study time points (baseline, 11 weeks, and 3-months). Pre-intervention PSA values were obtained from participants' medical records, and three additional PSA values were measured at the three study time points. The investigators calculated average pre-intervention PSA slopes for the intervention and control groups separately based on the two most current PSA values in participant's medical records. Similarly, they estimated the average intervention PSA slopes between the two groups based on PSA assays measured at the three study time points. PSA velocity was also calculated as the natural log of PSA by time. Results of the

study show that the intervention group was able make positive dietary changes including increased intake of antioxidants, particularly lycopene ($p = 0.05$), carotenoids ($p = 0.05$), and decreased intake of animal protein ($p = 0.03$), saturated fat ($p < 0.01$) and calcium ($p = 0.01$). Additionally, the intervention group had significant improvements in quality of life when compared with the control group ($p = 0.02$). However, there were no differences in change in PSA slope between the two study groups ($p = 0.28$). Nonetheless, the average PSA doubling time was significantly prolonged for the treatment group by about 172% (from 21.5 months at baseline to 58.5 months at 3-months), while that of the control group only increased by 1.6% (from 18.4 months at baseline to 18.7 months at 3-months). These findings suggest that men with recurrent PCa and rising PSA levels can make beneficial dietary changes involving antioxidants, and when integrated with mindfulness practice and partner support, these dietary modifications may improve quality of life and delay the progression of PCa.

Hébert et al. [13] also investigated the efficacy of plant-based, antioxidant-rich dietary intervention, integrated with physical activity and stress reduction, on PCa progression in men with biochemically defined recurrence. This study (EASE Study) was conducted in South Carolina and consisted of 47 men who had been previously treated with definitive therapy for histologically confirmed adenocarcinoma of the prostate. The study participants were randomized into intervention ($n = 26$) and control ($n = 21$), and followed over a 6 month period. Participants in the intervention group were enrolled in the study along with a spouse or partner-of-choice to offer support in the process of lifestyle change. Those in the control arm of the study had the usual care with an opportunity for the intervention at the end of the study. The intervention consisted of

individual diet and physical activity counseling sessions, as well as group sessions involving discussions on how to shop for, and cook study-compliant meals, physical activity goals, and practice of meditation that cultivates mindfulness. The diet aspect of the intervention emphasized increased intake of plant-based foods such as whole grains, fruits, vegetables, and legumes (particularly soybeans and soybean products), all of which are rich in antioxidant micronutrients. The intervention also emphasized reduced intake of meat and dairy products. The investigators integrated meditation and physical activity as part of the intervention to help reduce stress associated with comprehensive dietary change and to promote the overall well-being of the participants. Diet assessment was done using 24-hour diet recall interviews at baseline, and at 3 months and 6 months. Physical activity questionnaires were also used to ascertain activity levels and serum PSA assays were measured at each of the study time points (baseline, and at 3 months and 6 months). Effectiveness of the intervention on serum PSA levels was evaluated using an intent-to-treat, mixed-effects model with repeated measures analysis of variance. *Post hoc* analyses were also performed using signal detection (i.e., decision tree) methods to examine effects of individual dietary components on serum PSA level. Results of the study show that there were no significant differences in change in serum PSA levels between the intervention and control groups ($p = 0.45$). However, the intervention group made positive dietary modifications including increased intake of fruits, vegetables, and fiber (albeit, statistically non-significant); and decreased intake of calories ($p = 0.01$), total fat ($p = 0.02$), and saturated fat ($p < .01$). Results from the signal detection analyses also show that irrespective of intervention status, 56% of men who increased fruit intake had no rise in serum PSA levels compared to 29% of men who did not increase fruit

intake. Similarly, 56% of men who increased fruit and fiber intake and decreased saturated fat intake had no rise in serum PSA levels compared to 44% of men who did not. Thus, this study provides suggestive evidence that increased consumption of fruits and fiber, along with decreased intake of saturated fat, may offer protection against PCa progression.

Over the last two decades, a total of ten papers have been published defined by the authors as plant-based dietary interventional studies in relations to PCa progression [9, 11, 13, 18, 21, 112, 139-142], representing eight independent studies on plant-based diet intervention trials that have been conducted in the last 20 years. Five of these eight studies reported potential inhibitory effect of dietary modification on PCa progression as measured by change in serum PSA level [9, 11, 18, 21, 141]. Three of these studies found no effect of dietary modifications on PCa progression [13, 112, 140]. These mixed findings can be attributed to multiple factors, including the fact that conventional dietary interventions such as those described above are multifaceted, involving different combinations of diet, stress reduction, and physical activity which make it difficult to evaluate the independent effect of the prescribed diet. Secondly, diets used in dietary intervention trials usually have several components, such as increasing fruits, vegetables, and fiber intake, while decreasing consumption of meat and dairy products at the same time, which makes it difficult to evaluate independent effects of specific food constituents, including micronutrients such as antioxidants. There is also the possibility of treatment “contamination” between the intervention group and the control group, where health-conscious persons in the control group may have knowingly or inadvertently adopted the behavior change prescribed for the intervention group. These

limitations provide justification for further high-impact translational research involving biomarkers of dietary antioxidant intake and predictive markers of PCa progression to help establish potential etiologic relationship. Such research would provide more definitive evidence on the efficacy of dietary intervention on PCa progression. Additionally, this could provide valuable information to clinicians that can facilitate the development of structured and monitored dietary intervention strategies as a cost-effective treatment alternative for men with biochemically recurrent disease.

Table 2.1 Summary of findings from plant-based, dietary intervention trials in relation to prostate cancer progression among men with biochemically defined prostate cancer recurrence (PSA endpoint studies)

Author	Intervention	Subjects and design	Results
		Randomized trials	
Hébert et al., 2012	Diet, Physical activity & Stress reduction	A six month follow-up study: 2.5-hr weekly sessions over 3 months, monthly booster sessions continued for another 3 months. Intervention (n = 26) Control (n = 21) Intervention emphasized increased intake of antioxidant-rich diet such as whole grains, fruits, vegetables, and legumes (particularly soybeans and soybean products).	No difference in change in PSA level between the intervention and control group (p = 0.45) <u>Mean PSA at 3-months</u>: Intervention : 1.09 (0.54-2.18) Control : 0.77 (0.36-1.68) <u>Mean PSA at 6-months</u>: Intervention : 0.78 (0.36-1.70) Control : 0.84 (0.42-1.68)
Carmody et al., 2008	Diet, Stress reduction	A 23-week follow-up study of 36 male residents of Massachusetts randomized into intervention (n = 17) and control (19). Active intervention: 11weekly 2.5-hr sessions, and followed for additional 12 weeks. Intervention emphasized increased intake of carotenoids, soy, and isoflavones; including vegetables, whole grains, soybean, soybean products and fish (particularly salmon).	No difference in change in PSA slope (p = 0.28) <u>PSA doubling time</u>: Intervention: ↑ from 21.5 months (pre-study) to 58.5 months (during intervention) Controls: ↑ from 18.4 (pre-study) to 18.7 months (during study period)

Table 2.2 (continued): Summary of findings from plant-based, dietary intervention trials in relation to prostate cancer progression among men with biochemically defined prostate cancer recurrence (PSA endpoint studies)

Author	Intervention	Subjects and design	Results
		Non-randomized trials (no controls)	
Saxe et al., 2001	Diet, Stress reduction	Ten Massachusetts male residents followed over 4 months. Twelve weekly intervention of 3-4 hrs each Intervention emphasized increased intake of whole grains, legumes, fresh green and yellow vegetables, seeds, legumes, soy products and fruit.	Rate of PSA rise decreased in 8 of 10 men. Median PSA doubling time increased by 2.7 folds [from 6.5 months (pre-study) to 17.7 months]
Saxe et al., 2006	Diet, Stress reduction	Thirteen men from VA hospitals in San Diego, CA Ten 3 hour meetings over 6-months: Once per week for first month Once per month for month 2 to 5 Two in month 6 Intervention emphasized same diets as Saxe et al., 2001.	Rate of PSA rise decline during intervention compared with the pre-intervention rates of PSA rise ($p < 0.01$). Median PSA doubling time increased by 9.4 folds [from 11.9 months (pre-study) to 112.3 months]
Nguyen et al., 2006	Diet, Stress reduction	Same population as Saxe et al., 2006 Examined rates of PSA rise at different intervals	Rate of PSA rise decreased when comparing pre-study (0.06) to 0- to 3-months (–0.002, $P < .01$). However, the rate of PSA rise increased when comparing the interval 0- to 3-months (-0.002) with 3- to 6-months (0.03, $P = .43$).

2.2 ASSOCIATIONS OF ANTIOXIDANTS AND PROSTATE CANCER RISK AND AGGRESSIVENESS

Extensive research on carcinogenesis and chemoprevention (i.e., the use of diet, nutritional supplements and/or medications to prevent cancer) has led to a firm conclusion that one of the mechanisms by which diet modifies PCa risk and aggressiveness is through the consumption of antioxidant micronutrients, which are found in many foods including fruits, vegetables, and legumes [17, 18, 117, 123]. It has been clearly established that antioxidants protect cellular DNA from damage by oxidative stress through the elimination of intracellular reactive oxygen species (ROS) [8, 70]. Sustained oxidative stress (i.e., prolonged period of elevated levels of ROS beyond antioxidant repair capacity) can lead to changes to the prostatic microenvironment, including DNA base damage, DNA strand breaks, altered gene expression, and ultimately, PCa [70, 117]. Although numerous studies have examined associations between dietary antioxidants and PCa with some promising results, there is a lack of consistency in the outcome of these studies (reviewed in [64, 76, 143-149]). These inconsistencies emphasize the need for well-designed and well-executed studies to help delineate the role of individual antioxidants in PCa and PCa aggressiveness before widespread use of antioxidants for chemoprevention is encouraged. This summary includes reviews of benchmark studies and the current state of the science on relationships between antioxidants and PCa, with special emphasis on PCa aggressiveness.

Vitamin E

Vitamin E is thought to play an important role in PCa prevention because of its antioxidant effect. Vitamin E is a complex fat-soluble compound occurring in eight different chemical forms; four tocopherols [alpha (α), beta (β), gamma (γ), and delta (δ)] and four tocotrienols (α-, β-, γ-, and δ-tocotrienols) with varying levels of bioavailability and biologic significance [150]. The most bioactive form of vitamin E in human serum and tissues is α-tocopherol, which is also the most extensively researched form of vitamin E in relation to PCa [150-152]. The most common form of vitamin E in American diet is γ- tocopherol, while α-tocopherol is the most common type found in nutritional supplements [150, 153, 154]. Vitamin E is found in the human diet in various plant seeds, nuts, and oils [154]. Consumption of vitamin E from diet and nutritional supplements as well as serum and tissue levels of individual tocopherols and tocotrienols have been associated with PCa and PCa aggressiveness (reviewed in [64, 76, 154-158]). Unfortunately, the evidence is conflicting and often difficult to reconcile.

Several clinical trials have examined antioxidant supplementation in relation to prostate cancer incidence. The Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Trial is one of the benchmark studies in this area of research. The ATBC trial a randomized, double-blinded, placebo-controlled trial conducted among 29,133 Finish male smokers, aged 50-69 years, with a primary aim of exploring chemoprevention of lung cancer. The study participants were randomized into four treatment groups; (1) α-tocopherol 50mg/daily; (2) β-carotene 20 mg/daily; (3) α-tocopherol 50mg/daily and β-carotene 20 mg/daily; and (4) placebo. Although the primary end-point was lung cancer, the investigators observed that after 5 to 8 years of follow-up, smokers assigned to the

α–tocopherol group had a 32% (95% confidence interval [CI] = −47% to −12%) reduced risk of PCa incidence and 41% (95% CI = −65% to −1%) lower risk of death from PCa when compared to the placebo group [159]. Analysis of a 6-year post-intervention follow-up of this study showed that the protective effect in the α–tocopherol group reduced from 36% (RR = 66; 95% CI = 0.51-1.04) during the trial to 27% (RR = 0.73; 95% CI = 0.51 – 1.04) 3 years post-trial, and finally to 6% (RR = 0.96; 95% CI = 0.72 – 1.24) 6 years post-trial. This observation led to the conclusion that the beneficial effects of α–tocopherol against PCa require long-term use [160]. In a recent analysis of the ATBC trial with 19-year follow-up data, using biomarkers of antioxidant intake, Weinstein et al. [79] observed that higher levels of serum α–tocopherol reduced the risk of PCa by 20% (RR = 0.80; 95% CI = 0.66 - 0.96; highest vs. lowest quintile; P_{trend} = 0.03). Higher levels of serum α–tocopherol were also found to reduce the risk of advanced PCa by 44% (RR = 0.56; 95% CI, 0.36-0.85; P_{trend} = 0.002). Other studies have also provided evidence on the beneficial effects of vitamin E on PCa [74, 161, 162]. For example, in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), Kirsh et al. [68] observed that among current and former smokers, daily intake of 400 IU of vitamin E was associated with a 71% (RR = 0.29; 95% CI = 0.12 to 0.68; P_{trend} = .01) decreased risk of advanced PCa (i.e., Gleason score ≥ 7 or cancer stage II/IV) compared to a placebo group. Similarly, in a case-control study nested within the Physicians' Health Study, male physicians with the highest levels of serum α–tocopherol were found to have a 36% lower risk of aggressive PCa, albeit, statistically non-significant (OR = 0.64; 95% CI = 0.38 - 1.07). A subgroup analysis showed that the inverse association for

aggressive PCa was stronger and statistically significant for current/former smokers (OR = 0.51; 95% CI = 0.26 - 0.98; highest vs. lowest quintile) [163].

However, these findings are contradicted by results from the Selenium and Vitamin E Cancer Prevention Trial (SELECT), which was established based on the promising results of the ATBC trial [73]. SELECT recruited 35,533 male residents of the US, Canada, and Puerto Rico, aged ≥ 50 years, who were randomized into selenium (200 µg/day) only, vitamin E (α–tocopherol, 400 IU/day) only, a combination of selenium (200 µg/day) and vitamin E (α–tocopherol, 400 IU/day) or placebo. This trial was designed to span a minimum of seven years and maximum of 12 years, but did not continue after the initial phase of seven years due to a suspicion that the intervention with α-tocopherol may have been putting the study participants at risk for developing PCa [73]. After the 7-year follow-up period, those taking vitamin E alone had a 13% increased risk of PCa, albeit statistically non-significant (HR = 1.13; 99% CI = 0.95 -1.35) [73]. Analysis of data from additional 1.5 years of follow-up of SELECT showed a stronger and statistically significant increased risk of PCa in the study participants who received vitamin E alone (HR = 1.17; 95% CI = 1.004 - 1.36, $P_{trend} = 0.008$) [164]. However, in another large randomized, placebo-controlled trial (The Physicians' Health Study II), supplemental vitamin E (400 IU) taken every other day over a 10-year period did not have an effect on PCa incidence (HR = 0.97; 95% CI = 0.85-1.09) or PCa-specific mortality (HR = 1.01; 95% CI = 0.64-1.58) in US male physicians [74]. Additionally, analysis of data from the Nutritional Cohort of the Cancer Prevention Study II showed that daily intake of supplemental vitamin E (≥ 400 IU) does not have an effect on overall risk of PCa (RR = 0.98; 95% CI = 0.89-1.08) or risk of advanced-stage disease (RR =

0.97; 95% CI = 0.74 – 1.26) [165]. A number of observational studies, particularly case-control and cohort studies have also examined associations of vitamin E and PCa incidence [78, 152, 166-168] and advanced/aggressive PCa [169-171] with mixed findings. However, it is important to note that after reviewing evidence from cohort, case-control, and ecological studies involving serum/plasma-based, questionnaire-based, and aggregate data, the World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) expert panel concluded in 2007 that although the evidence remains inconsistent, it is "probable" that vitamin E protects against PCa [172].

Carotenoids

It has been suggested that carotenoids may decrease the risk and aggressiveness of PCa by neutralizing ROS, which play a role in PCa through oxidative DNA damage [117, 123]. The most common carotenoids in the Western diet are α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin, and retinol (vitamin A), all of which are found in a variety of foods [173, 174]. Carrots, pumpkin, and winter squash are the primary sources of α-carotene; while β-carotene, β-cryptoxanthin, lutein and zeaxanthin are most abundant in deep-yellow/orange fruits such as apricots, cantaloupes, and mangoes, as well as in dark-green leafy vegetables, such as spinach, kale, broccoli, Brussels sprouts, green beans, peas, and zucchini. Lycopene is primarily found in tomatoes and tomato-based products, particularly processed tomatoes [173, 174]. Several studies have examined associations between carotenoids and PCa. However, the evidence remains inconsistent, emphasizing the need for high-impact research to help delineate relationships between individual carotenoids and PCa; especially PCa aggressiveness, which has received very little attention in the literature.

The US Physicians' Health Study is one of the longest prospective studies conducted to examine associations between plasma concentrations of all the major carotenoids and PCa risk. Gann et al. [163] conducted a case-control study nested within the Physicians' Health Study, involving 578 men with PCa who were age-matched to 1294 controls. The investigators examined associations between plasma levels of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein and retinol, and PCa risk and observed that none of these carotenoids were associated with overall risk of PCa. However, lycopene was strongly and inversely associated with PCa aggressiveness (OR = 0.56; 95% CI = 0.34 - 0.92, p = 0.02; highest vs. lowest quintile). Similarly, in a recent population-based, case-control study, Zhang et al. [175] investigated associations between plasma concentrations of *α*-carotene, *β*-carotene, *β*-cryptoxanthin, lycopene, and lutein/zeaxanthin and PCa, and found that only plasma lycopene was inversely and significantly associated with PCa risk (OR = 0.45; 95% CI = 0.24 - 0.85; highest vs. lowest quartile; $P_{trend} = 0.042$).

Lycopene is considered a potent antioxidant, which in addition to its ability to neutralize ROS, is also thought to protect against PCa through the inhibition of IGF-mediated cell proliferation and suppression malignant prostate cell growth [176, 177]. Lycopene consumption has been inversely associated with PCa in both serum-based and questionnaire-based studies; however, the evidence remains mixed (reviewed in [64, 143, 145, 178]). In a meta-analysis of 10 cohort studies and 11 case-control studies, lycopene consumption appeared to offer protection against PCa. In the cohort studies, cooked tomato products were associated with a 19% (RR = 0.81; 95% CI = 0.71 - 0.92) reduced risk of PCa cancer, and consumption of raw tomatoes (200g/day) was associated with a

22% (RR = 0.78; 95% CI = 0.66 - 0.92) lower risk of PCa in the case-control studies. Other sources of lycopene were not associated with PCa [179]. In addition, a fairly recent prospective study, lycopene consumption was found to reduce the risk of PCa in men with a family history of the disease (P_{trend} = 0.04) [180]. In the European Prospective Investigation into Cancer and Nutrition (EPIC), plasma lycopene was inversely associated with advance-staged PCa (RR = 0.40; 95% CI = 0.19 - 0.88, highest vs. lowest quintile), but not localized disease (RR = 1.40; 95% CI = 0.89 - 2.21; lowest vs. highest quintile) [181]. Similarly, in the Third National Health and Nutrition Examination Survey (NHANES III), serum lycopene was associated with a 63% lower risk of aggressive PCa (RR = 0.37; 95% CI = 0.15–0.94; highest vs. lowest quartile; P_{trend} = 0.04), but not overall PCa risk (RR = 0.65; 95% CI = 0.36–1.15; highest vs. lowest quartile; P_{trend} = 0.09) [182]. However, in a case-control study nested within the PLCO trial, serum lycopene status had no association with PCa risk (P_{trend} = 0.28) or PCa aggressiveness (P_{trend} = 0.43) [183]. Other studies have also reported null associations between serum/plasma- and questionnaire-based studies on lycopene level and PCa risk and aggressiveness [184-187]. Together, the weight of the evidence suggests that lycopene likely offers protection against PCa and may have greater influence on PCa aggressiveness; however, more research is needed for definitive conclusion.

Perhaps the most puzzling association between individual carotenoids and PCa is that of β-carotene and PCa risk. In the ATBC trial, β-carotene was associated with a 23% (95% CI = −4% to −59%) increased risk of PCa and a 15% (95% CI = −30% to −89%) increased risk of death from PCa [159]. Also, in a nested case-control study using data from the PLCO trial, higher levels of serum β-carotene was associated with a 67% (OR =

1.67; 95% CI = 1.03 - 2.72, highest vs. lowest quintile) increased risk of aggressive PCa (Gleason sum ≥ 7 or stage III or IV). This increased risk was much higher for advanced-stage (stage III or IV) disease (OR = 3.16; 95% CI = 1.37 - 7.31, highest vs. lowest quintile) [183]. In another prospective study, high serum level of β-carotene was associated with a 2.3 fold increased risk of PCa (RR = 2.3; 95% CI = 1.12 - 4.66, highest vs. lowest tertile, P_{trend} = 0.023) [187]. While assessing PCa as a secondary aim in the Carotene and Retinol Efficiency Trial (CARET) it was observed that daily supplementations of 30mg of β-carotene plus 25,000 IU of retinyl palmitate was associated with a 52% increased risk of aggressive PCa (RR = 1.52; 95% CI = 1.03 - 2.24) [188]. Interestingly, other studies have reported inverse associations between β-carotene and PCa. Kirsh et al. [68] observed that supplemental β-carotene of at least 2000 μg/day was associated with a 48% lower risk of PCa in men with low dietary β-carotene intake (RR = 0.52; 95% CI = 0.33 - 0.81). In addition, a nested case-control study, high plasma β-carotene was also associated with a 69% PCa risk reduction (OR = 0.31; 95% CI = 0.15 – 62; higher than median vs. lower than median) [189]. However, in a recent meta-analysis, β-carotene was not associated with PCa incidence (RR = 0.99, 95% CI = 0.91 - 1.07) [190]. Similarly, there is conflicting evidence on associations between serum/plasma levels and dietary intake of α-carotene, *β*-carotene, *β*-cryptoxanthin, lutein/zeaxanthin, and retinol on PCa risk and aggressiveness [17, 76, 190]. For example, Lu et al. [191] reported a 70% to 80% PCa risk reduction in men with higher plasma levels of *β*-cryptoxanthin, lutein and zeaxanthin, but retinol, α- and β-carotene were not associated with PCa risk. However, in a prospective cohort study, dietary intake of β-cryptoxanthin was associated with an increased risk of PCa in a dose-response fashion

($P_{trend} < 0.01$). There were no discernible associations between retinol, α- and β-carotene, lutein/zeaxanthin and PCa [171]. These apparent discrepancies may be explained by baseline antioxidant status of the studied populations. That is, antioxidant supplementation in a population that is nutritionally replete may not have a beneficial effect, whereas supplementing a population that is nutritionally deficient might have substantial health benefits [192-195].

Selenium

Selenium is an essential micronutrient found in many food items, particularly plant-based foods such as bread and cereal (depending on soil concentrations), and in meat and fish products as well as nutritional supplements [17, 158]. It has been suggested that selenium may protect against PCa by inducing the antioxidant enzyme glutathione peroxidase, which neutralizes the free radical superoxide anion ($^{\cdot}O_2^-$) and also by inhibiting PCa cell proliferation through the suppression of angiogenesis [158, 196]. Dong et al. [197] have also demonstrated a dose dependent effect of selenium on PCa cell growth inhibition and apoptosis. Nonetheless, the role of selenium in prostate carcinogenesis remains a subject of ongoing debate.

Interest in the role of selenium in the prevention of PCa was stimulated by the National Prevention of Cancer (NPC) trial, which was primarily designed to investigate the effect of selenium on the recurrence of nonmelanoma skin cancer [198]. In this double-blinded, randomized, placebo-controlled trial, 1,312 patients recruited from dermatology clinics located in the eastern seaboard of the US were randomized into treatment (200μg of selenium daily) and placebo. After 13 years of follow-up, it was

observed that selenium did not have any effect on skin cancer recurrence; however, selenium was inversely associated with overall risk of PCa (RR = 0.51; 95% CI = 0.29 - 0.87). Nonetheless, results from a subgroup analysis showed that the PCa risk reduction was only in men with baseline PSA level ≤ 4 ng/ml (RR = 0.33; 95% CI = 0.14 - 0.79), but not those with baseline PSA ≥ 4 ng/ml (RR = 0.95; 95% CI = 0.42 – 2.14). In a matched case-control study, Yoshizawa et al. [199] observed inverse associations between toenail levels of selenium and advanced-stage PCa (OR = 0.35, 95% CI = 0.16 - 0.78, highest vs. lowest quintile, $P_{trend} = 0.03$). Van den Brandt et al. [200] also examined association between toenail level of selenium and PCa risk in a cohort of 1,211 men and found that elevated toenail levels of selenium was protective against PCa (RR = 0.69, 95% CI = 0.48 - 0.99, highest vs. lowest quintile, $P_{trend} = 0.008$). However, subgroup analysis in this study also showed that the protective effect was only evident in former smokers ($P_{trend} = 0.003$), and not current ($P_{trend} = 0.383$) or non-smokers ($P_{trend} = 0.412$). Three recent meta-analysis involving serum/plasma-, toenail- and questionnaire-based studies indicated that men with high intake of selenium have lower risk of developing PCa [144, 146, 201]. However, it is important to note that assessment of selenium intake in epidemiological studies is fraught with problems arising from the way food is prepared, digested and absorbed.

In the SELECT trial (described above), daily supplementation of 200 µg selenium did not have an effect on PCa risk (HR = 1.09; 99% CI = 0.93 - 1.27, $P_{trend} = 0.18$), neither did daily intake of 200 µg of selenium in combination with 400 IU of vitamin E in supplement form (HR = 1.05; 99% CI = 0.89 - 1.22, $P_{trend} = 0.46$) [164]. In another randomized, placebo-controlled trial, Marshall et al. [202] investigated the effect of daily

intake of 200 µg of selenium on high-grade prostatic intraepithelial neoplasia (HGPIN), a premalignant lesion of PCa. After a 3-year follow-up, it was observed that while selenium appears to be protective against PCa, the effect was not statistically significant (RR = 0.82; 95% CI = 0.40 - 1.69). In a nested case-control study conducted within the EPIC study, no associations were found between plasma selenium levels and PCa risk (RR = 0.96; 99% CI = 0.70 - 1.31, highest vs. lowest quintile, P_{trend} = 0.25) [203]. Chan et al. also examined associations between plasma selenium level, SOD2 gene variants, and PCa aggressiveness in men with localized and locally advanced PCa, and found that selenium appears to increase the risk of developing aggressive PCa (RR = 1.35, 95% CI = 0.99 – 1.84) [134]. After reviewing the evidence from randomized controlled trails, Klein et al. [204] concluded that selenium does not appear to have any effect on PCa risk. However, in another review of the evidence from clinical and observational studies, Richman and Chan [147] concluded that while the relationship between selenium and PCa remain inconsistent, selenium is likely more relevant to the etiology of aggressive or advanced-stage PCa. Nevertheless, research on selenium intake and PCa aggressiveness is very limited, which emphasizes the need for more well-designed studies in this area involving the use of validated biomarkers that reflect long-term selenium intake.

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble vitamin and a potent antioxidant that may confer protection against PCa by preventing oxidative DNA damage through the scavenging of ROS [205, 206]. In vitro and in vivo studies have also demonstrated dose and time dependent effects of vitamin C on PCa through the inhibition of PCa cell lines (i.e., LNCaP and PC-3) [207, 208]. Vitamin C is abundantly found in many plant and

animal foods such as fruits (e.g., citrus, cantaloupe, pineapple, kiwi, and berries), vegetables (e.g., broccoli, brussels sprouts, potatoes, tomatoes, winter squash, and cauliflower) and organ meat (e.g., liver and kidney) [206, 209]. Although vitamin C has been associated with reduced risk of other cancers [206], the role of vitamin C in PCa is controversial because of conflicting findings from various studies. In a recent case-control study, dietary intake of vitamin C was inversely associated with PCa risk (OR = 0.60, 95% CI = 0.41 – 0.88), although the protective effect appeared to be confined only to European Americans (OR = 0.56, 95% CI = 0.38 – 0.85) and not African Americans (OR = 1.19, 95% CI = 0.34 – 4.24) [210]. In another case-control study, increased intake of vitamin C was strongly and inversely associated with PCa (OR = 0.49, 95% CI = 0.33 – 0.74) [211]. Other studies have reported inverse associations between vitamin C and PCa risk [166, 212].

However, two double-blinded, randomized, placebo-controlled trials have investigated the role of vitamin C in PCa and have reported null effect [68, 74]. In the Physicians' Health Study II trial, Gaziano et al. found that daily supplementation of 500 mg of vitamin C did not have an effect on PCa incidence (HR; 1.02, 95% CI = 0.90 – 1.15), but rather appeared to increase the risk of death from PCa (HR; 1.46, 95% CI = 0.92 – 2.13) [74]. In the PLCO cancer screening trial, daily supplementation of 500 mg of vitamin C was also found not to be associated with PCa incidence (RR = 1.01; 95% CI = 0.87 – 1.17, highest vs. lowest quartile, P_{trend} = 0.98). A meta-analysis summarizing data from these two trials also indicated that vitamin C has no effect on PCa risk (RR = 0.98, 95% CI = 0.91 – 1.06) [167]. In a case-control study conducted in Italy, prospective assessment of dietary intake of vitamin C was not associated with the incidence of PCa

(OR = 0.88, 95% CI = 0.78 – 1.07, P_{trend} = 0.09). Stratified analysis by age, BMI, and family history of PCa in that study also did not show any discernible associations between vitamin C and PCa [162]. Hodge et al. [213] and Schuurman et al. [171] have also investigated association between vitamin C intake and PCa incidence in a population-based case-control study and a cohort study, respectively, and both studies reported null associations. It is important to note that relative to the other antioxidants, fewer studies have been conducted on associations between vitamin C and PCa. Additionally, a thorough literature search did not show any study on vitamin C and PCa aggressiveness within the last two decades. Thus, in the light of these conflicting findings, research into vitamin C and PCa aggressiveness may offer much clearer evidence on the nature of the relationship.

2.3 POLYMORPHISMS IN DNA REPAIR AND OXIDATIVE STRESS GENES AND THEIR INTERACTION WITH ANTIOXIDANTS IN RELATION TO PROSTATE CANCER

Extensive research into prostate carcinogenesis has led to the discovery of several deleterious molecular events such as oxidative stress, which allow the occurrence of genetic aberrations including the formation of oxidized DNA base lesions, DNA strand breaks, altered gene expression, deletions, and ultimately mutagenesis [6, 8, 70]. Several lines of evidence suggest that oxidative DNA damage is causally linked to PCa, and the extent of DNA damage correlates with the degree of PCa aggressiveness [117, 123]. Different DNA repair pathways are known to operate on different types of DNA damage. For example, base excision repair (BER) pathway removes small lesions, such as nonbulky adducts. Nucleotide excision repair (NER) removes bulky lesions and damaged single-stranded fragments. Mismatch repair (MMR) corrects DNA replication errors such

as mispaired bases, and double-stranded breaks are repaired through complex pathways involving homologous recombination and end-joining DNA repair mechanisms [124-126]. It has been clearly demonstrated that genes involved in DNA repair harbor polymorphisms that are functionally relevant to PCa because of their pro- or anticarcinogenic properties [131, 214, 215]. Thus, individual variations in the capacity to repair oxidative DNA damage, and by implication, the ability to inhibit the initiation and progression of PCa may be due to polymorphisms in the DNA repair gene pathways. However, these polymorphisms may act alone or in combination with environmental factors, such as dietary antioxidants, to influence the occurrence and clinical behavior of PCa.

Multiple polymorphisms in DNA repair genes have been investigated in relation to PCa susceptibility and aggressiveness with equivocal results [131, 132, 214, 215]. Additionally, studies have shown that the expressions of natural antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase, which mitigate the harmful effects of oxidative stress, are lower in PCa tissues than in healthy prostate tissues [216, 217]. Hence, it is reasonable to speculate that increased intake of antioxidants may help reduce oxidative DNA damage, and by extension, PCa risk and aggressiveness. However, epidemiological data regarding the independent effect of antioxidants on PCa also remains largely inconsistent [17]. Perhaps, investigations of the joint effect of polymorphisms in DNA repair gene pathways and antioxidant intake may provide better understanding of how these factors interact to influence PCa.

At least three recent case-control studies have examined joint effects of genetic variants of the superoxide dismutase 2 (SOD2) gene (a member of the mitochondrial

Fe/MnSOD family and an important component of the BER defense system) and plasma antioxidant status in relation to PCa risk and aggressiveness [130, 133, 134]. First, Li et al. [130] investigated associations between valine (Val)/alanine (Ala) polymorphism (rs4880) in the SOD2 gene and prediagnostic plasma levels of selenium, lycopene, and α-tocopherol on PCa risk and aggressiveness in a nested case-control study within the Physicians' Health Study. In this study, none of the SOD2 genotypes (*Val/Val, Val/Ala,* and *Ala/Ala*) had independent associations with overall risk of PCa or PCa aggressiveness. However, among men with *Ala/Ala* genotype, high prediagnostic plasma selenium level was associated with a 67% lower risk of PCa (RR = 0.33; 95% CI = 0.16-0.68; P_{trend} = 0.002) and an 82% reduced risk of aggressive PCa (RR = 0.18; 95% CI = 0.07-0.48; P_{trend} <0.001) when compared to those with low levels. These inverse associations were weaker in men with *Val/Val + Val/Ala* genotypes ($P_{interaction}$ = 0.01). No evidence of effect modification by SOD2 genotype was observed for associations between plasma lycopene and α-tocopherol status, and PCa risk or aggressiveness. However, a combined antioxidant score computed based on quartile levels of plasma concentrations of selenium, lycopene, and α-tocopherol showed significant interaction between SOD2 genotype and prediagnostic antioxidant status on over risk of PCa ($P_{interaction}$ = 0.02) and PCa aggressiveness ($P_{interaction}$ = 0.01).

Subsequently, in a different population of PCa patients, using plasma selenium levels measured at the time of the cancer diagnosis or shortly thereafter, Chan et al. [134] observed a non-significant 40% lower risk of aggressive PCa in men with *Ala/Ala* genotype of the SOD2 gene (rs 4880) and high plasma selenium levels (RR = 0.60, 95% CI = 0.32 - 1.12; highest vs. lowest quintile, P_{trend} = 0.06), which is consistent with the

previous findings by Li et al. [130]. However, there is a major difference between the two studies. Chan et al. observed that men with *Val/Val + Val/Ala* genotype and high plasma selenium concentration had an 82% increased risk of aggressive PCa (RR = 1.82; 95% CI = 1.27 - 2.61; highest vs. lowest quintile, $P_{trend} = 0.0003$), while this population of men had non-significant weak inverse association with aggressive PCa in the Li et al. study. Possible explanations for this inconsistency is that Li et al. focused on prediagnostic plasma selenium concentrations, and contrasted PCa cases with controls; while Chan et al. utilized plasma selenium levels measured at the time of PCa diagnosis or shortly thereafter, and contrasted low aggressive PCa with intermediate/high aggressive PCa. Nonetheless, together, the evidence suggests that SOD2 variants modulate associations between plasma antioxidant levels and PCa risk/aggressiveness. More specifically, having *Ala/Ala* genotype of the SOD2 gene may be beneficial to men with high intake of selenium and perhaps other antioxidants.

In a more recent study, Abe et al. [133] investigated the joint effect of SNPs in DNA repair genes (i.e., *GPX1, GPX4, PPARGC1A, PPARGC1B, SOD1, SOD2, SOD3* and *XRCC1*) and plasma selenium levels on PCa aggressiveness. Two SNPs in the *SOD1* gene were independently associated with PCa aggressiveness. One of these SNPs (rs17884057) was inversely associated with aggressive PCa (RR = 0.83; 95% CI = 0.70-0.99), while the other (rs4816407) was associated with an increased risk of aggressive PCa (RR = 1.27; 95% CI = 1.02–1.57). No associations were found between SNPs in the other genes and PCa aggressiveness. However, two additional SNPs; one in SOD1 (rs10432782) and another in SOD2 (rs2758330); were found to have modifying effects on associations between plasma selenium status and PCa aggressiveness ($P_{trend} = 0.04$

and <.0001, respectively), which suggest that these SNPs act in combination with antioxidants to influence PCa aggressiveness.

Goodman et al. [129] also examined whether an association between lycopene and PCa can be modified by x-ray repair cross-complementing protein 1(XRCC1) genotype, a gene involved with BER. These investigators observed a borderline statistically significant inverse association between lycopene intake and PCa (OR = 0.49; 95% CI = 0.24-0.99; highest vs. lowest tertile, $P_{trend} = 0.05$). However, a stronger and statistically significant protective effect of lycopene was observed in men with *Arg/Arg* genotype (OR = 0.21; 95% CI = 0.06-0.71; highest vs. lowest tertile, $P_{trend} < 0.01$), but not in those with *Arg/Gln + Gln/Gln* genotype (OR = 0.83; 95% CI = 0.33-2.01; highest vs. lowest tertile; $P_{trend} = 0.79$). A much stronger inverse association was observed for combined antioxidant exposure (lycopene + α-tocopherol + β-carotene) and PCa among men with *Arg/Arg* genotype (OR = 0.11; 95% CI = 0.02-0.65; above vs. below median; $P_{interaction} = 0.01$). However, the combined antioxidant exposure appeared to increase risk of PCa in men with *Arg/Gln + Gln/Gln* genotype (OR = 2.08; 95% CI = 0.46-9.43; above vs. below median); suggesting that the beneficial effect of antioxidant intake may be limited to men with *Arg/Arg* genotype.

Van Gils et al. [218] also examined whether three common polymorphisms of the XRCC1 gene at codon 194 (*Arg/Trp*), codon 280 (*Arg/His*), and codon 399 (*Arg/Gln*) modify the effect of antioxidant intake on PCa risk. None of the polymorphisms examined at each of the three codons had independent associations with PCa. However, there was evidence of effect modification by XRCC1 genotype. Among men with *Arg/Arg* genotype, low vitamin E intake was associated with a 2.4-fold increased risk of

PCa (OR = 2.4; 95% CI = 1.0-5.6, p = 0.04); however, a much lesser and statistically non-significant increased risk was observed in men with *Arg/Gln + Gln/Gln* genotype with low vitamin E intake (OR = 1.2; 95% CI = 0.5-2.8, p = 0.65).

Similarly, Zhang et al. [128] examined joint associations between variants of XRCC1 and hOGG1(two genes that are involved with BER) in relation to PCa. In this population based-study, *Arg/Gln + Gln/Gln* genotype of the XRCC1 gene were found to increase risk of PCa by an estimated 56% (OR = 1.56; 95% CI = 1.01-2.45, p = 0.049), while a non-significant inverse association was observed between *Ser/Cys + Cys/Cys* genotype of the hOGG1gene and PCa risk (OR = 0.72; 95% CI = 0.46 - 1.10, p = 0.13). When stratified by plasma antioxidant status, *Arg/Gln + Gln/Gln* genotype of the XRCC1 gene was associated with over 2-fold increased risk of PCa among men with lower than median levels of lutein/zeaxanthin (OR = 2.15; 95% CI = 1.17- 4.01, p = 0.015), β-cryptoxanthin (OR = 2.64; 95% CI = 1.40-5.07, p = 0.003), and lycopene (OR = 2.05; 95% CI = 1.07-3.98, *P* = 0.032) as compared to those with the *Arg/Arg* genotype. On the other hand, having *Ser/Cys + Cys/Cys* genotype of the hOGG1gene and lower than median levels of β-cryptoxanthin, lycopene, α-carotene, and α-tocopherol were associated with statistically significant 48–62% reductions in PCa risk when compared with the *Ser/Ser* genotype. Other studies have also reported similar effect modification and/or interaction between DNA repair gene variants and antioxidants on PCa [135, 219, 220].

These findings suggest that potential etiologic associations between antioxidant intake and PCa most likely depend on polymorphisms or genetic variants within DNA repair gene pathways. Larger studies using biomarkers reflecting long-term antioxidant

intake are required to provide supporting evidence and help better understand the underlying mechanisms for targeted interventions. Nonetheless, data on joint associations between antioxidant intake and polymorphisms in other genes that are involved in oxidative stress and DNA repair in relations to PCa are lacking. Thus, additional studies are also needed to examine the interplay between antioxidants and variants of these other genes such as those involved in double-stranded DNA break repair (e.g., XRCC2, XRCC4, and RAD51C); nucleotide excision repair (e.g., ERCC8, XPA, and XPC), and oxidative stress (e.g., NQO1, NOX3, and PPARG) in relation to PCa, particularly aggressive PCa, which is associated with high disease-specific mortality [5].

Table 2.2 Summary of findings on gene-diet studies; interactions between antioxidants and oxidative stress/DNA repair genes in relation to prostate cancer

Author	Gene & Dietary Agent	Subjects and Design	Results
Studies examining prostate cancer aggressiveness			
Li et al., 2005	Gene: polymorphism (rs4880) in SOD2 gene: valine (V) → alanine (A) Diet: prediagnostic plasma selenium, lycopene, and α-tocopherol	Nested case-control study within the Physicians' Health Study (PHS) PCa cases, n = 567 Controls, n = 764 No data on race groups; however, the PHS is 93% European American	<u>Stratified analysis by genotype</u> *AA* genotype + high selenium: lower overall risk of PCa (RR= 0.33; 95% CI, 0.16-0.68), and lower risk of aggressive PCa (RR= 0.18; 95% CI, 0.07-0.48). Weak, non-significant inverse association for men with (*VV + VA)*, and high selenium in relation to overall risk of PCa (RR = 0.6; 95% CI = 0.4 - 1.0), and PCa aggressiveness (RR= 0.7; 95% CI = 0.4 -1.2). A significant interaction observed between combined antioxidant score (selenium + lycopene + α-tocopherol) and SOD2 in relation to PCa aggressiveness ($P_{interaction} = 0.01$).
Chan et al., 2009	Gene: polymorphism (rs4880) in SOD2 gene: valine (V) → alanine (A) Diet: plasma selenium level (collect at, or immediately after diagnosis)	A nested case-control study Data from Dana-Farber Cancer Institute: 489 locally advanced PCa cases. <u>Aggressiveness</u>: Low n = 276; Intermediate n = 167; High = 146. Whites n = 468 Other n = 18 Unknown n = 3	*AA* genotype + high plasma selenium; 40% lower risk of aggressive PCa (RR = 0.60, 95% CI = 0.32-1.12). *VV + VA* genotype, and high selenium: increased risk of aggressive PCa (RR = 1.82; 95% CI = 1.27 - 2.61).

Table 2.2 (continued) Summary of findings on gene-diet studies; interactions between antioxidants and oxidative stress/DNA repair genes in relation to prostate cancer

Author	Gene & Dietary Agent	Subjects and Design	Results
Studies examining prostate cancer aggressiveness			
Abe et al., 2011	Genes: *GPX1, GPX4, PARGC1A, PPARGC1B, SOD1, SOD2, SOD3* and *XRCC* Diet: plasma selenium	Same population as Chan et al. 2009 Design: nested case control study (n = 753) Aggressiveness: Low n = 394 Intermediate n = 259 High = 100 Whites n = 719 Other n = 30 Unknown n = 4	SOD2 SNP (rs17884057) was inversely associated with high aggressive PCa (RR = 0.83; 95% CI = 0.70-0.99). SOD2 SNP (rs4816407) associated with increased risk of aggressive PCa (RR = 1.27; 95% CI = 1.02–1.57). Two additional SNPs; SOD1 (rs10432782) and SOD2 (rs2758330) had modifying effects on associations between selenium and PCa aggressiveness (P_{trend} = 0.04 and <.0001, respectively).
Mikhak et al., 2008	Gene: SOD2 Ala16Val polymorphism Diet: Plasma carotenoids: α-carotene; β-carotene; lycopene; β-cryptoxanthin; lutein/zeaxanthin	Nested case-control study within the Health Professionals Follow-up Study (HPFS) Controls (n = 612) matched to PCa cases (n = 612) on year of birth, year of blood draw, history of PSA screening. No data on race groups; however, HPFS is 97% European American. Aggressive PCa: advanced stage ($T3_C$, T4, N0M0, TN (1-3), T M1 or Gleason sum ≥ 7. None aggressive PCa: all others	No association between SOD2 genotype and risk of total or aggressive PCa No significant interaction between SOD2 genotype and any of the carotenoids

Table 2.2 (continued) Summary of findings on gene-diet studies; interactions between antioxidants and oxidative stress/DNA repair genes in relation to prostate cancer

Author	Gene & Dietary Agent	Subjects and Design	Results
Studies examining prostate cancer risk			
Goodman et al., 2006	Gene: XRCC1 genotypes; *Arg/Arg*, *Arg/Gln*, and *Gln/Gln* Diet: lycopene from FFQ, α-tocopherol and β-carotene from plasma	Case-control study conducted in North Carolina. Controls (n= 174) were age-matched to cases (n = 77) [± 5 years]. Race groups: Blacks, n = 20 Whites, n = 231	Protective effect of lycopene observed in men with *Arg/Arg* genotype and high lycopene (OR = 0.21; 95% CI = 0.06-0.71; $P_{trend} < 0.01$), but not those with *Arg/Gln* + *Gln/Gln*, and high lycopene (OR = 0.83; 95% CI = 0.33-2.01; $P_{trend} = 0.79$). Much stronger inverse association observed for combined antioxidant exposure (lycopene + α-tocopherol + β-carotene) and PCa risk among men with *Arg/Arg* genotype (OR = 0.11; 95% CI = 0.02-0.65; above vs. below median; $P_{interaction} = 0.01$). Combined antioxidant exposure appeared to increase risk of PCa in men with *Arg/Gln* + *Gln/Gln* (OR = 2.08; 95% CI = 0.46-9.43; above vs. below median).
Van Gils et al., 2002	Gene: polymorphisms in XRCC1 gene at codon 194 (*Arg/Trp*), codon 280 (*Arg/His*), and codon 399 (*Arg/Gln*) Diet: vitamin A, C & E, β-carotene and lycopene	Case-control study among North Carolina residents Controls (n = 183) were age-matched to cases (n = 77) [± 5 years].	None of the polymorphisms had independent associations with PCa risk. Among men with *Arg/Arg* genotype, low vitamin E intake was associated with an increased risk of PCa (OR = 2.4; 95% CI = 1.0-5.6, p = 0.04). A much lesser and non-significant increased risk observed in men with *Arg/Gln* + *Gln/Gln* and low vitamin E intake (OR = 1.2; 95% CI = 0.5-2.8, p = 0.65). No effect modification by the other antioxidants

Table 2.2 (continued): Summary of findings on gene-diet studies; interactions between antioxidants and oxidative stress/DNA repair genes in relation to prostate cancer

Author	Gene & Dietary Agent	Subjects and Design	Results
Studies examining prostate cancer risk			
Zhang et al., 2010	Gene: XRCC1 (Arg399Gln polymorphism), and hOGG1 (Ser326Cys polymorphism) Diet: α-carotene, β-carotene, β-cryptoxanthin, lycopene, Lutein/zeaxanthin and α-tocopherol	A case-control study conducted in Arkansas. Cases, n = 193 Controls, n = 197 <u>Race groups</u>: Whites, n = 198 Blacks, n = 192	*Arg/Gln* + *Gln/Gln* genotype of the XRCC1 gene associated with an increased risk of PCa among men with lower than median levels of lutein/zeaxanthin (OR = 2.15; 95% CI = 1.17- 4.01, p = 0.015), β-cryptoxanthin (OR = 2.64; 95% CI = 1.40-5.07, p = 0.003), and lycopene (OR = 2.05; 95% CI = 1.07-3.98, p = 0.032) compared to those with the *Arg/Arg* genotype. Men with *Ser/Cys* + *Cys/Cys* genotype of the hOGG1gene and had lower than median levels of β-cryptoxanthin, lycopene, α-carotene, and α-tocopherol had 48–62% reductions in PCa risk when compared with the *Ser/Ser* genotype.

2.4 DIETARY ASSESSMENT METHODS, ADVANTAGES AND DISADVANTAGES

Food Frequency Questionnaire

Epidemiological studies support hypotheses of associations between diet and chronic diseases such as cancer [17, 63-66, 221]. To better understand these associations, a measure of usual dietary intake is needed. Usual dietary intake is often measured by different dietary assessment instruments including food records, multiple 24-hour recalls, and food frequency questionnaires (FFQs) [222]. FFQs are the most suitable dietary assessment method for measurement of usual intake in large nutritional epidemiological studies due to issues of cost, time and feasibility [222-225]. FFQs are designed to measure usual intake of specific foods, food groups and nutrients over an extended period [222, 226, 227]. The rationale behind the FFQ approach is that long-term dietary intake, such as usual eating patterns over weeks, months or years are conceptually more relevant determinants of chronic diseases than intake on one or a few designated days [222]. The FFQ is commonly used to rank individuals according to their intake of specific foods or nutrients, and it has widespread applicability in case-control, cross-sectional and cohort studies of diet and diet-disease associations [228-230].

There are several FFQ instruments in circulation, some of which have been adapted and modified for different populations and purposes [231]. The most commonly used FFQs are the Willett FFQ [232], the Block FFQ [233], the Fred Hutchinson Cancer Research Center FFQ [234], and the National Cancer Institute Diet History Questionnaire (NCI-DHQ), a cognitive-based FFQ designed to enhance respondents comprehension in order to facilitate accurate reporting [235]. Despite continuous modification of existing standard FFQs and development of new FFQs, the overall design and analytic methods

remain the same. Typically, FFQ instruments contains a listed of predefined food groups listed as line items. Respondents are asked to report frequency of consumption and portion sizes over a designated period (e.g., usually the previous year) [222, 235]. Questions on food purchasing habits and preparation methods may also be asked [235]. FFQs require a robust and appropriate nutrient database for translation of reported dietary intakes into nutrients, and for ranking individuals on specific foods and nutrients [236, 237]. Nutrient intake from a FFQ are generally estimated by summing the product of a multiplication between the frequency of intake by nutrient density, and by portion size (if asked) over all foods consumed (i.e., nutrient intake = frequency x portion size x nutrient density) [222, 235, 238]. FFQs also require validation or calibration against other detailed and more accurate dietary assessment tools such as 7-day food records or multiple 24-hour dietary recalls [222, 238]. Several "validation" studies have found the FFQ approach to be a reasonably accurate method for estimating food and nutrient intake when compared to multiple 24-hour recalls or food records as reference instruments [223, 235, 238-244]. However, validation studies using recovery biomarkers as reference instruments suggest that FFQs may have significant measurement errors [245, 246].

Advantages and disadvantages of food frequency questionnaires

The merits of the FFQ approach to dietary assessment are a subject of ongoing debate [247-252]. It is important to note that FFQs differ widely, especially in terms of food list and level of detail, and some FFQs may be better than others with regards to food and nutrient assessment [235, 250, 253]. However, in general, FFQs are a convenient and relatively inexpensive approach to dietary assessment [222, 235, 254]. Additionally, FFQ instruments are often designed to be self-administered with low

respondent burden (require about 45 minutes to complete), and can be administered either electronically or completed on scanner-readable forms which reduces the time, effort and cost of data collection [254, 255]. Moreover, the use of standardized responses facilitates quick data analysis [222]. Long-term exposures such as habitual eating patterns are conceptually more relevant in studies of chronic disease, which makes FFQ more suitable for investigating associations between diet and chronic diseases [222, 228, 233, 254]. Also, because FFQs solicit information about past dietary intake, they are able to circumvent recent dietary changes which may have been motivated by a recent diagnosis unrelated to the outcome of interest [255]. Statistically, FFQs are considered to be the only dietary assessment tool that can minimize intra-person, day-to-day variations in nutrient intake without the need for assessment of actual intake over several days [256, 257]. Furthermore, inclusion of open-ended questions allows reporting of foods that are not listed on the FFQ [235]. In addition, standard FFQs are easy to modify to include foods that are commonly consumed by specific ethnic minority groups or populations of interest [258].

The limitations of FFQ have been discussed extensively, including substantial measurement errors resulting from incomplete listing of foods consumed by study participants, and inaccuracies in the estimation of portion size and frequency of intake [225, 227]. Incompleteness of food listing in FFQ is particularly problematic when the missing foods are major sources of nutrients in the study population, leading to considerable inaccuracies in nutrient estimates for the group [225]. Quantitative studies also suggest that portion size estimation by study participants are often influenced by the type of food been considered, the role of the food item a meal (e.g., appetizer, main dish,

or dissert) and personal food preferences, all of which can affect the accuracy of reporting [259]. Also, portion sizes in FFQ are generally categorized into small, medium and large, which may have different meaning for different respondents [260]. Additionally, when food is eaten as a mixed dish, it is often difficult to estimate portion sizes for individual components of the dish [261]. Studies have also shown that aggregation of food items in FFQs affects recall of food intake. For example, respondents are more likely to recall intake of beef when it is separated from lamb, pork and ham, than when they are all grouped together as a line item [253]. Reporting food intake can also be affected by social desirability bias, which can result in under-reporting of "unhealthy" foods and over-reporting of "healthy" foods [262]. Finally, given the retrospective nature of FFQ, reporting of intake generally rely on long-term memory with questionable accuracy, particularly among older adults. Despite these limitations, carefully developed FFQs have a conceptual advantage over other dietary assessment tools such 24-hour recalls and food records by providing estimates of usual intake over an extended period. Additionally, FFQs are practically and economically more feasible for large nutritional epidemiological studies, and impose lesser burden on participants relative to the other assessment tools [248, 250, 263, 264].

Biochemical measures of dietary intake

Nutritional epidemiologists are acutely aware of the numerous methodological issues and limitations of the traditional dietary assessment methods such as FFQs, food records, and 24-hour dietary recall. The reality is that, a gold standard method of dietary assessment is lacking [250]. However, the traditional methods are fraught with considerable systematic and random measurement errors, which have increasingly

motivated the use of nutritional biomarkers to complement the traditional methods [222, 265-267]. Nutritional biomarkers can be loosely defined as biochemical indicators of dietary intake which reflects not only food and nutrient consumption, but also metabolism and biological effects of dietary intake [268]. The underlying assumption for the use of nutritional biomarkers is that, they are responsive to dietary intake, sensitive to intake levels, and independent of some kinds of biases and measurement errors [245, 269]. An "ideal" nutritional biomarker will accurately reflect actual dietary intake levels, and be applicable to different populations [270]. However, existing nutritional biomarkers are not "ideal", but are functional and have far-flung relevance in nutritional epidemiology including their use as reference measurements for validation of the traditional dietary assessment methods [268-271].

Nutritional biomarkers are generally categorized into four groups; recovery, predictive, concentration and replacement biomarkers [269, 270, 272]. Recovery biomarkers are thought to provide absolute estimates of intake based on the concept that recovery of nutrient analytes from biologic samples are directly related to intake due to a fixed metabolic balance between intake and output over a specific period of time [273]. In other words, recovery biomarkers are not subject to individual differences in metabolism over a specified time window, hence considered as the gold standard of biomarkers [222]. However, very few of these biomarkers actually exist, including doubly labeled water (use to measure total energy expenditure and metabolic rate), and urine nitrogen/potassium (for measuring total protein and total potassium intake, respectively) [269, 270, 272]. Predictive biomarkers are similar to recovery biomarkers in terms of responsiveness to intake, ability to reflect intake levels (dose-response relations),

and time sensitivity. However, predictive biomarkers generally have lower recovery of analytes from biological samples [270]. Predictive biomarkers have recently been used as reference tool for correlation of sugars intake and recovery of sucrose and fructose from 24-hour urinary samples [271]. Concentration biomarkers generally do not reflect absolute intake, but often correlate with the corresponding food or nutrient intake levels [271, 274]. Examples of concentration biomarkers include serological markers (i.e., plasma and serum) and adipose tissue levels of nutrients, as well as urinary electrolytes [269, 275]. Replacement biomarkers are also similar to concentration biomarkers. The name “replacement” is generally used when food composition data are either unavailable or unsatisfactory for specific nutrients such as aflatoxins [276], and some phytochemicals [277]. Several studies have examined the efficacy of the various types of nutritional biomarkers; however, this review will focus on concentration biomarkers in relation to antioxidants recovery from blood, adipose tissue, and toenail clippings.

Advantages and disadvantages of biomarkers of antioxidant exposure

Epidemiological studies involving biomarkers of antioxidant intake often rely on blood samples (i.e., plasma or serum), adipose tissue, and toenail clippings to measure exposure level [278, 279]. Plasma/serum antioxidant levels are thought to reflect short-term intake, while adipose tissue and toenail antioxidant concentrations reflect long-term intake [280]. The use of these biomarkers in epidemiological research has several advantages. First, nutritional biomarkers are known to improve the validity of exposure estimates by eliminating information bias, particularly differential recall of intake in case-control studies [279]. Secondly, since the bioavailability of antioxidants is influenced by individual differences in metabolism and absorption, nutritional biomarkers provide an

assessment of the “biologically effective dose” of the nutrient of interest [279, 280]. Additionally, nutritional biomarkers are particularly advantageous for evaluating dose-response associations as they are known to be responsive to intake, correlate with the corresponding food or nutrient intake levels, and measure the bioactive dose of the nutrient of interest [245, 271, 281]. Also, nutritional biomarkers are frequently used in dietary intervention studies to assess compliance and individual response to the intervention being studied [282]. Nonetheless, nutritional biomarkers are imperfect measures of dietary intake.

Plasma/serum and adipose tissue antioxidant levels are often measured with high performance liquid chromatography (HPLC) [283, 284]. A major concern with the use of plasma/serum markers of antioxidant intake is that, they reflect recent rather than long-term dietary intake. Hence, plasma/serum biomarkers are not suitable for evaluating etiological or temporal relationships between antioxidant intake and slow-progressing diseases [222, 285]. Also, plasma/serum antioxidant levels may be altered by the disease being studied, in which case some biomarkers would be inappropriate for use in case-control studies [222]. Several lines of evidence also suggest that plasma/serum antioxidant levels are influenced by a variety of physiological and lifestyle factors independent of dietary intake such smoking status, obesity, and alcohol use [286-289]. Studies also suggest that adipose tissue sampling with the needle biopsy can lower participation rates in research studies [290]. Moreover, saponification of the adipose tissue specimen is generally required before HPLC can be performed, which often increases the cost of laboratory analysis [278]. Other limitations associated with biomarkers of specific nutrients and nutrient groups are discussed below.

Carotenoids such as β-carotene, α–carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthine have been studied extensively in relation to PCa [67, 106, 175, 183]. Carotenoids levels in blood are influenced cooking methods (reviewed in [291]). For example, mild heating (such as steaming) is known to promote the extraction of β-carotene from vegetables and increases its bioavailability in serological markers [292]. Similarly, processing of raw tomatoes into tomato paste or tomato sauce with mild heat treatment has been shown to increase the bioavailability of lycopene in plasma [293, 294]. However, stir frying of green leafy vegetables has been found to reduce lutein content by as much as 89%, while cooking these vegetables for 8 minutes has also been found to reduce lutein content by up to 428% [295]. Carotenoids are known to accumulate in adipose tissues because of their fat-soluble properties; however, their turnover rates remain unknown [278, 296]. Hence, the actual or average exposure time remains unclear. Studies have also shown that the distribution of carotenoids in adipose tissue differ by body fat sites (e.g., abdomen, buttock, and thigh) due to differences in carotenoid uptake and retention between the fat sites [280, 296]. Some researchers have suggested that the differential distribution of carotenoids in adipose tissue sites accounts for the poor reproducibility of carotenoid measurements from adipose tissue across studies [280, 297, 298].

Vitamin E is primarily transported in the human blood by plasma lipoproteins, and performs a biologic function of protecting lipids form oxidative degradation [299]. Due to its close association with lipids, plasma/serum lipids (especially total cholesterol) must be measured and controlled for in multivariate models in studies involving plasma vitamin E levels [278]. Relative to dietary questionnaires, plasma/serum levels of vitamin

E may represent a more relevant biologic measure of systemic exposure; however, it correlates poorly with estimates from dietary intake [278]. It has been suggested that observed correlations between intake estimates and plasma levels of vitamin E are largely due to vitamin E intake in supplement form, rather than from food intake [300, 301]. For example, Ford and Sowell examined associations between plasma α-tocopherol levels and dietary intake of α-tocopherol using 24-hour dietary recall in NHANES III and observed that these two measures do not correlate [300]. Another study of vitamin E supplementation showed that unlike dietary intake, the human blood responds well to vitamin E intake in supplement form [302]. However, it is reasonable to speculate that the poor correlation between dietary intake and plasma concentrations of vitamin E is likely due to food handling and preparation methods. Studies have also suggested that adipose tissue vitamin E concentrations have a low turnover rate [302]; however, findings from studies attempting to verify this hypothesis remain inconclusive [297, 303].

Unlike carotenoids and vitamin E which are fat-soluble, vitamin C is a water-soluble micronutrient, thus easily destroyed by food handling practices such as cutting, shredding, chopping or peeling of fruits and vegetables as well as cooking with excessive water or excessive heat [304, 305]. Plasma/serum ascorbate (i.e., ascorbic acid) is often used to measure vitamin C intake by HPLC methods. However, the use of plasma ascorbate is problematic for a number of reasons. First, to avoid degradation of ascorbate in serological markers, blood samples must be preserved in an acid stabilizer (e.g., metaphosphoric acid) immediately after collection of the samples. This makes many archived plasma/serum samples that were not treated this way unsuitable for analysis of ascorbate concentrations [278]. Secondly, the human body is unable to produce vitamin C

naturally, and since vitamin C is often eliminated from the body through urine, continuous dietary supply of vitamin C is required to maintain adequate levels in the body [306]. Thus, ascorbic acid levels in the blood fluctuate in response to dietary intake and renal clearance, which makes the use of fasting blood sample essential [278]. However, fasting blood samples usually estimate ascorbate at levels much lower than actual dietary intake levels [278]. The fluctuation of ascorbic acid in serological markers also implies that plasma/serum ascorbate is not a reliable measure of long-term intake [278, 279, 307]. Additionally, because of the water-soluble properties of vitamin C, they are not stored in adipose tissue [278, 307]. For these reasons, most researchers prefer the used dietary questionnaires such as the FFQ to assessment of vitamin C.

Assessment of selenium intake with dietary questionnaires is also fraught with problems. Selenium content in food vary considerably according to the levels of selenium in the soil, and hence of crops and animal forage grown in the soil [308]. Selenium content in the same foods from different geographic locations can vary by many folds, in some cases by over 10-fold [309]. Thus, many researchers prefer biomarkers of selenium intake such as selenium levels in plasma/serum, urine or toenail clippings [310]. However, these biologic markers are influenced by factors other than selenium intake, such as general state of health, metabolism, past and present disease history, and smoking status [286, 311]. The use of toenail clippings is relatively more attractive than the other markers because it is easy to sample and store, and the sampling of toenail clippings usually does not cause discomfort to subjects [147, 312]. Moreover, toenail clippings are thought to reflect long-term intake. However, toenail levels of selenium are also influenced by factors such as the size and thickness of the nail plate, rate of nail growth

and metabolism of selenium in nail beds [313, 314] In addition, the average time of selenium exposure reflected in toenail clippings remains unknown [314].

Despite these limitations, nutritional biomarkers are very appealing to most researchers because of their ability to estimate internal dose of the nutrient of interest. However, some researchers have suggested that it may be best to measure biomarkers of dietary intake in target tissues of the disease of interest, rather than the use of surrogate markers of systemic exposure [310]. All issues considered, use of multiple measures of dietary intake such as plasma, adipose tissue and FFQ would provide complementary information and perhaps offer a more robust dietary assessment than any single measure of intake.

CHAPTER 3

STUDY METHODS

3.1 OBJECTIVES AND HYPOTHESES

OBJECTIVE 1: To examine whether changes in dietary antioxidant intake (baseline to 3-months) as measured by plasma concentrations of carotenoids and tocopherols are associated with PCa progression (assessed by changes in serum PSA level as an intermediate prognostic marker of disease progression) in African-American and European-American men with biochemically defined PCa recurrence after definitive therapy.

Hypotheses:

i. Higher levels of plasma carotenoids and tocopherols at baseline will be associated with lower serum PSA levels at baseline.

ii. Higher post-intervention plasma carotenoids and tocopherol levels (at 3 months) will be associated with lower post-intervention PSA levels (at 3 months, and at 6 months), after adjusting for baseline PSA level.

iii. Change in plasma carotenoids and tocopherol levels (baseline to 3 months) will inversely correlate with serum PSA levels at 3 months and at 6 months.

OBJECTIVE 2: To examine associations between antioxidant levels in adipose tissue, plasma and diet, and PCa aggressiveness among African American and European-American men.

Hypothesis: Lower levels of antioxidants in adipose tissue, plasma and diet are associated with high aggressive PCa, and these associations do not vary by race.

OBJECTIVE 3: To examine whether SNPs in DNA repair and oxidative stress genes modulate associations between antioxidants in adipose tissue, plasma and diet, and PCa aggressiveness among African-American and European-American men.

Hypothesis: SNPs in DNA repair and oxidative stress genes modify associations between antioxidants in adipose tissue, plasma and diet, and PCa aggressiveness, and the degree of the effect modification vary by race.

3.2 STUDY DESIGN AND METHODS

OBJECTIVE 1

Design & Data Source

The first objective utilizes data from a previous study by Hébert et al. [13], which is a 6-month intervention trial involving diet, exercise and stress reduction conducted in South Carolina (the EASE Study). In brief, sixty men with histologically confirmed PCa and serum PSA levels after primary therapy with radical prostatectomy or radiation were recruited along with a partner of choice. Four participants were enrolled for the run-in period of the intervention to ascertain feasibility and potential issues with compliance. Of the remaining 54 participants, 29 (together with their partners) were randomized to intervention (dietary modification, physical activity and meditation practice) and 25 were randomized to usual care with an opportunity to receive the intervention at the end of the

study. Full details of the randomization process and progression of the participants have been provided in Figure-1.

Setting: The study participants were recruited from major urology practices located in the Midlands Region of South Carolina, covering seven counties [Richland county (67%),Lexington county (9%), Newberry (6%), Kershaw (6%), Orangeburg (6%), Sumter (4%) and Fairfield (2%) counties]. The intervention was administered under the supervision of the South Carolina Cancer Prevention and Control Program. Clinical data on the study participants were collected in the facilities of the Cancer Prevention and Control Program in Columbia.

Study Population: Eligible participants are men with histologically-confirmed, adenocarcinoma of the prostate who: (1) have been treated by radical prostatectomy or radiation therapy as primary treatment for PCa; (2) have had 3 successive rise in serum PSA level of at least 1.5 ng/ml from post-treatment nadir (usually at or close to zero) measured at 2- to 3-month intervals; (3) were free of other malignancy in the previous 5 years (except non-malignant skin cancer and primary PCa for which they were treated by radical prostatectomy or radiation therapy); (4) had not been taking thyroid medication, steroids, antibiotics or diuretics; (5) spoke English as first language; (6) were able to read at sixth grade level; (7) were of sound mind, memory and understanding; (8) were willing to be randomized to intervention or usual care. Prospective participants were excluded from the study if they: (1) had received post-operative hormonal therapy for PCa; (2) had a diagnosis or symptoms of cardiovascular disease, pulmonary disease, Crohn's disease, active ulcerative colitis or metabolic disease; (3) had experienced unexpected weight loss of 5 pounds or more within the previous 3 months; (4) plan to use

hormone supplements, fish oil, or other ω-3 fatty acids based supplements; or (5) had a diagnosis of post-traumatic stress disorder (PTSD).

Intervention: Participants were assigned to intervention or control group using block randomization by age (± 5 years) and race (African American/European American). The study spanned 6 months, which included a 3-month period of active intervention followed by monthly booster session for the following 3 months. The intervention consisted of dietary modifications, physical activity, and mindfulness-based stress reduction training. The 3-month active phase of the intervention involved individual diet and physical activity counseling and goal setting sessions, as well as twelve weekly group meetings that included cooking classes and shared model meals. In addition, participants were given weekly assignments on how to shop for and cook study-compliant meals, attain physical activity goals, and practice meditation for stress reduction.

The diet aspect of the intervention emphasized increased intake of plant-based foods such as whole grains, fruits, vegetables, and legumes (particularly soybeans and soybean products) along with decreased intake of meat and dairy products. The physical activity aspect involved working with participants to identify activities that they enjoyed and reinforce those activities to promote physical fitness and overall well-being. The goal of the exercise routine was to ensure that each participant attain the Centers for Disease Control and American College of Sports Medicine (CDC/ACSM) recommendations of at least 30 minutes of moderate intensity physical activity for 5 days or more per week [315]. Because comprehensive dietary change can be difficult to maintain, participants were taught to meditate in a way that inculcates mindfulness about decisions concerning

food choices in order to promote their sense of control over the change in diet and culinary habits [316]. Partner support was integrated to provide an encouraging environment for the process of change. Following the 3-month active phase, monthly booster sessions were held in a supportive group environment for another 3 months. These included frequent telephone calls to each participant and his partner for wellness checks and encouragement to sustain the intervention.

Control condition: Participants in the control group underwent the same general assessment as those in the intervention group. There were no attempts made to restrict their access to psychosocial support or educational resources available to PCa patients in the community. These participants and partners were given the opportunity to take the intervention at the end of the 6-month study period at no cost to them.

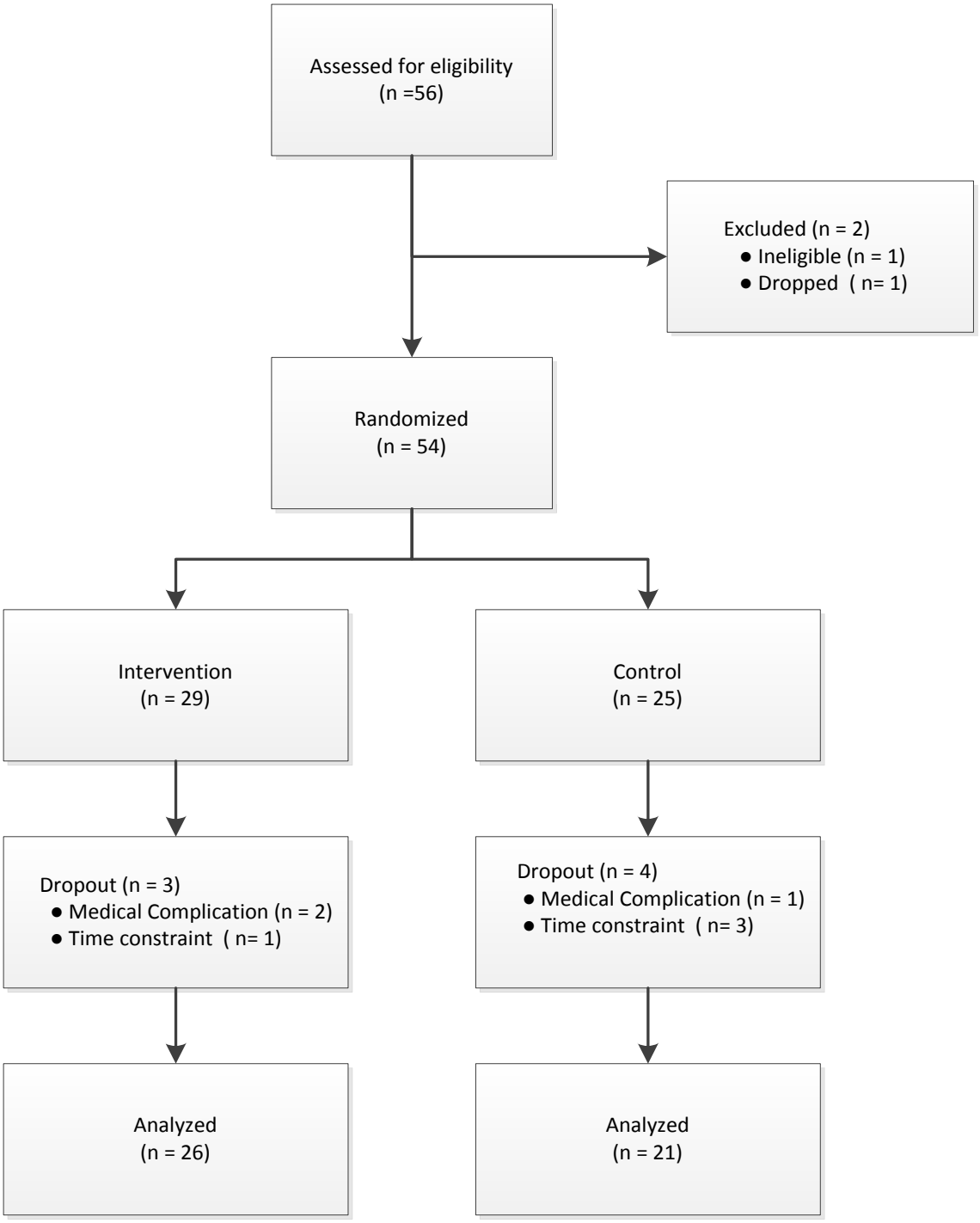

FIGURE 3.1 Consort diagram showing participants recruitment, screening, randomization, and retention [13].

Exposure and outcome variables: Plasma carotenoid and tocopherol levels at baseline and at 3 months were the main exposure of interest. As part of the study requirements, participants provided peripheral blood samples at baseline, 3 months and 6 months for analysis of biomarkers of food and nutrient intake as well as for analysis of serum PSA levels. The blood samples were collected by a trained phlebotomist after obtaining consent from the study participants. PSA was measured in serum at baseline, at 3 months and at 6 months. Carotenoids and tocopherols were measured in plasma using high performance liquid chromatography (HPLC) by Craft Technologies [317]. Because of limited availability of samples, data on carotenoids and tocopherols were only measured at baseline and at 3 months. The following carotenoids and tocopherols were measured: α- and γ-tocopherol, α-carotene, *cis*- and *trans*-β-carotene, lutein, zeaxanthin, *cis*-lutein/zeaxanthin, α- and β-cryptoxanthin, *cis*- and all-*trans*-lycopene.

Other study measures: Data on clinical and pathologic attributes of PCa were abstracted from participants' medical records obtained from referring urologists. At baseline, participants responded to questionnaires that solicited information on demographics and health-related behaviors, including age, race, education, marital status, employment, and smoking status. Data on diet, physical activity, and anthropometry were obtained at each of the three study checkpoints: baseline, 3 months, and 6 months. Diet assessments used 24-hour dietary recalls on three randomly selected days that included two weekdays and one weekend day, as this method better captures daily variation in intake [318, 319]. Physical activity was assessed using a questionnaire designed for older adults that had been tested for reliability [320]. Physical activity was expressed as metabolic equivalent (MET) value based on description of the activity using the

Compendium of Physical Activities [321], with one MET being equivalent to resting metabolic rate. Total METs of physical activity were estimated for each participant's sum of METs from light, moderate, and vigorous physical activity per week. The anthropometric measurements obtained were standing height (cm), weight (kg), waist and hip circumference (cm) used to calculate waist-to-hip ratio, and bioelectric impedance measures of percent body fat and lean body mass.

OBJECTIVE 2 & 3

Data Source & Design

Data from the North Carolina–Louisiana Prostate Cancer Project (PCaP) was used to evaluate objectives 2 and 3. PCaP is a large, racially diverse, population-based, multidisciplinary, cross-sectional, case-only, incident PCa study, designed to investigate racial/ethnic differences in PCa outcomes among African Americans and European Americans. The study population and methods have been published [4]. A total of 2,258 men (African-American, n = 1,130; European-American; n =1,128) with histologically confirmed incident PCa were recruited through rapid case ascertainment in North Carolina and Louisiana. In North Carolina, 1,031 men (African Americans, n = 505; European Americans, n = 526) were recruited between July 2004 and October 2007. In Louisiana, the study participants were recruited in two phases due to the devastation caused by Hurricane Katrina. Pre-Hurricane Katrina cases were recruited between May 2004 and June 2005 (n = 213; African Americans, n = 119; European Americans, n = 94), and Post-Hurricane Katrina cases were recruited between January 2006 and July 2009 (n = 1,014; African Americans, n = 506; European Americans n = 508). Although PCaP is a

case-only study, a case-control analysis was used to compare men with high aggressive PCa (higher Gleason score, higher clinical stage, and higher PSA level) to those with low/intermediate aggressive PCa.

Study population: Residents of the study catchment areas in North Carolina and Louisiana were eligible to participate in PCaP if they: (1) had a first diagnosis of histologically confirmed adenocarcinoma of the prostate; (2) were between the ages 40-79 years at the time of diagnosis; (3) self-reported race/ethnicity as white/Caucasian American or as black/African American; (4) Spoke English as a first language; and (5) did not reside in an institution (e.g., nursing home). Prospective participants were excluded if they: (1) were cognitively impaired or in a severely debilitated physical state; (2) were under the influence of alcohol, severely medicated, or had apparent psychosis as evaluated by recruiting staff.

Exposures: Three measures of antioxidant intake were assessed: (1) dietary assessment of average daily nutrient intake using a modified National Cancer Institute Diet History-Food Frequency Questionnaire (NCI-DHQ) [322]; (2) Supplemental antioxidants intake assessed with a validated questions [323]; and (3) abdominal adipose tissue antioxidant concentrations. The decision to use more than one measure of antioxidant intake was based on the inherent limitations of each of these measures. First, food frequency questionnaire (FFQ) is an imperfect dietary assessment tool because: (a) reporting of food intake relies on respondent's memory which may not accurately reflect actual intake; (b) there is the possibility of over-reporting of "healthy" foods and under-reporting of "unhealthy" foods which may contribute to random error; (c) FFQ's are generally less sensitive to absolute intake of specific nutrients due to the use of standard

portion sizes; (d) categorization of foods in the FFQ limits reporting of details about specific foods which may be relevant to nutrient estimation; (e) exclusion of foods that are major contributors of certain nutrients may affect the validity of the nutrient estimates [247, 261, 324, 325]. Reporting of supplement intake is also prone to recall bias [323]. Second, although adipose tissue concentrations of antioxidants are useful biomarker of internal dose, individual variations in the absorption and metabolism of antioxidants can affect bioavailability, bioconversion, and bioefficiency of these micronutrients [266, 326, 327]. Thus, concentration biomarkers do not reflect total antioxidant exposure. Therefore, examining three separate measures of antioxidant exposure would provide more comprehensive and perhaps more reliable data about the association between antioxidant intake and PCa severity than would any one of these measures alone.

The NCI-DHQ was modified to include Southern foods, and had questions pertaining to frequency of intake and portion sizes for 124 different food items as well as questions about methods of food preparation. The questionnaire asked study participants to recall food intake over the year prior to PCa diagnosis. Responses to the questions were linked to an updated NCI nutrient database through which nutrient intake were estimated using NCI Diet*Calc software [4]. Adipose tissue antioxidant concentrations were measured from samples collected by PCaP study nurses during in-home visits after obtaining written consent from the participants. Adipose tissue samples were collected from the abdominal area. PCaP research nurses who were specifically trained for adipose tissue sampling, followed a standardized procedure involving the insertion of a 15-gauge needle into the subcutaneous fat and applying negative pressure by a 15 ml vacutainer tube after prepping the overlying skin. The aspirated tissue was trapped in the needle and

luer lock adapter, which was placed in a separate cryovial and transported on ice immediately after collection to a designated storage facility where aliquots were prepared and stored at -80^{o}C until assayed. Individual carotenoids were measured by high performance liquid chromatography at the Nutrition Analyses Laboratory of Craft Technologies, Incorporated (Wilson, NC) using methods outlined by Craft et al. [328, 329]. The adipose tissue contents of α-, γ-, and δ-tocopherol, α-carotene, cis- and trans-β-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein, zeaxanthin, and cis- and trans-lycopene were quantified at a minimum detection limit of 0.07 μg/g for tocopherols and 0.003 μg/g for carotenoids.

Outcome classification: The outcome of interest for objectives 2 and 3 was PCa aggressiveness; defined by a combination of PSA level at diagnosis, clinical stage of PCa at diagnosis and Gleason sum [4]. Information on these clinical attributes of PCa were abstracted from participant's medical records by trained personnel. To assess consistency of abstractors and to ensure data quality, about 10% of the medical records were selected at random and abstracted by a second staff member [330]. PCa aggressiveness was categorized into three groups: high aggressive (Gleason sum ≥8 or PSA >20 ng/mL, or Gleason sum ≥ 7 and clinical stage T3–T4); low aggressive (i.e., Gleason sum < 7 and stage T1-T2 and PSA<10 ng/ml); and intermediate aggressive (all others).

SNP Selection: Candidate SNPs selected and genotyped by the PCaP consortiums were used for executing objective 3. The SNPs were selected using the SNPinfo web server (http://snpinfo.niehs.nih.gov) previously described in detail elsewhere [331]. In brief, SNPinfo provides a platform that allows investigators to specify genes or linkage

regions of interest and select SNPs based on results from genome-wide association studies (GWAS), population-specific linkage disequilibrium (LD) structure, and detailed functional predictions including coding, transcription factor binding, micro-ribonucleic acid (miRNA) binding, and splicing [331]. The PCaP consortium used six separate SNP selection procedures [131]. The first selection process was based on primary and secondary data from GWAS and validation studies, which identified 286 SNPs that were associated with PCa in published literature at the time of the SNP selection. The second process involved a thorough review of literature on candidate genes associated with PCa etiology or aggressiveness. Eight hundred and forty eight candidate genes were identified through this process. Three hundred and six of these genes were retained for SNP selection because: (a) they were listed in the CGEMS GWAS project as having p-values < 0.05 (associated with PCa susceptibility or aggressiveness); or (b) had inadequate SNP coverage in the 550K GWAS panel to sufficiently assess the gene. The SNPinfo's candidate gene SNP selection pipeline (GenePipe) was subsequently used to select 583 SNPs determined to be functionally significant to PCa based on p-values and multiple population LD tag SNPs for both European Americans and African Americans from the 306 candidate genes. The third process utilized prioritized selection of SNPs in the CGEMS GWAS 550K panel in conjunction with SNPinfo's GWAS functional SNP selection pipeline (GenomePipe). SNPs were selected from the CGEMS GWAS 550K panel if they had small p-value (associated with PCa susceptibility or aggressiveness) and were predicted to have functional effects, or had small p-value and were in high LD with SNPs predicted to have functional effects. Six thousand and thirty four SNPs were identified from the CGEMS GWAS 550K panel as having small p-values ($p \leq 0.01$).

SNPinfo's GenomePipe was used to identify 41,755 SNPs that were in high LD ($r^2 \geq 0.8$) with at least one of the SNPs from the CGEMS GWAS 550K panel. Of these, 379 common SNPs (minor allele frequency (MAF ≥ 0.05) predicted to have functional effects by at least one of the biological function prediction methods were selected.

The fourth selection procedure was based on linkage regions. At the time of the SNP selection, 43 non-overlapping linkage regions were reported to be associated with PCa. The CGEMS GWAS 550K panel p-values (based on PCa susceptibility or aggressiveness) were used together with SNPinfo's GWAS SNP selection in linkage loci pipeline (LinkPipe) to select a maximum of seven SNPs from each of the 43 non-overlapping linkage regions. The fifth involved overlap between small p-values for SNPs that were reported in multiple PCa GWAS. Only the Framingham GWAS and the CGEMS GWAS project had publicly available data on SNPs associated with PCa at the time of the SNP selection. Thus, fifty-eight SNPs with p-values <0.01 in the CGEMS project and the Framingham GWAS were included.

Finally, fifty ancestry informative markers (AIM) were selected using allele frequency data in HapMap phase I + II (http://hapmap.ncbi.nlm.nih.gov) to control for stratification in three populations: Utah residents with ancestry from Northern and Western Europe (CEU) from the Centre d'Etude du Polymorphisme Humain (CEPH) collection representing European ancestry; Yuroba individuals in Ibadan Nigeria (YRI) representing African ancestry; and individuals from Han, China (CHB) and Tokyo, Japan (JTP) collectively representing Asian ancestry (HapMap CHB plus JTP). Twenty-five of these SNPs were monoallelic [variant allele frequency (VAF = 0)] in CEU, rare in Asians (VAF < 0.01) but common YRI (VAF > 0.25). The other 25 SNPs were monoallelic in

(YRI) (VAF = 0), rare in Asians (VAF <0.5) and but very common in CEU (VAF > 0.5). The SNP selection process has been described in sufficient detail elsewhere [131]. Additional information on the ancestry informative markers for the PCaP study participants is presented in Table 3.1.

Genotyping: Germline DNA was extracted from blood samples (n = 1,630) or buccal cells (n = 118) by the University of North Carolina (UNC, Chapel Hill) Biospecimen Processing Facility or from peripheral blood mononuclear cells immortalized by the UNC Tissue Culture Facility (n = 216). Genotyping was done at the National Institutes of Health (NIH) Center for Inherited Disease Research (CIDR) using a custom designed Illumina GoldenGate array. There was an excellent genotyping call rate (99.93%) and inter-assay agreement with blinded duplicates (99.99%). Further details of the genotyping process and quality control measures have been published [332].

SNPs in DNA repair and oxidative stress genes

The PCaP consortium maintains a repository containing over 1,536 GWAS and candidate SNPs among European Americans and African Americans. Data on SNPs in DNA repair pathways (i.e., base excision repair, nucleotide excision repair, and double-stranded DNA break repair) and oxidative stress-related genes, which were considered for analysis. A complete list of the SNPs that were evaluated is presented in Table 3.2.

Table 3.1 Ancestry informative markers proportions by race and geographic region in the North Carolina-Louisiana Prostate Cancer Project (PCaP) [333].

Self-Reported Race	Study site	Mean YRI (%)	Mean CEU (%)	Mean CHB plus JTP (%)	p-value [2]
African Americans n = 1043	Louisiana n = 594	86.9	11.9	1.2	0.03
	North Carolina n = 449	89.5	9.3	1.2	
European Americans n = 1063	Louisiana n = 582	1.8	96.9	1.3	0.001
	North Carolina n = 481	0.8	98.4	0.8	
Non-ethnic African Americans [1] n = 930	Louisiana n = 485	89.2	9.5	1.3	0.78
	North Carolina n = 445	89.4	9.4	1.2	
Non-ethnic European Americans [1] n = 824	Louisiana n = 354	1.5	97.3	1.2	0.002
	North Carolina n = 470	0.8	98.6	0.6	

Abbreviations: YRI- Yoruba individuals in Nigerians (represents African ancestry), CEU- Utah residents with ancestry from Northern and Western Europe (represents European Americans), CHB plus JTP - individuals from Han, China and Tokyo, Japan (representing Asian ancestry).

[1] includes ONLY individuals reporting “no” ethnicity membership.

[2] One-way multivariate analysis of variance (MANOVA) models comparing mean CEU and YRI ancestry estimates between research subjects in North Carolina and Louisiana.

3.3 SAMPLE SIZE CALCULATION

OBJECTIVE 1

The data for Objective 1 (EASE study) has complete information on plasma carotenoid and tocopherol levels for 39 participants at baseline and 35 participants at 3 months. No data on carotenoids or tocopherols are available for the 6-month time point; thus, the 3–month data on tocopherols and carotenoids was analyzed in relation the 6-month PSA levels. Calculation of statistical power for this Objective assumed unmatched data. The outcomes for computing statistical power were mean PSA levels at baseline, and at 3 months and 6 months comparing participants with high *versus* low plasma carotenoid and tocopherol level. The null hypothesis was that mean PSA values are equal in both groups (H_0: $\mu_1 = \mu_2$). Based on findings from a previous study [11], a two-sided test at level $\alpha = 0.05$ and sample size of 39 and 35, a power of 86% and 81%, respectively, were achieved to observe a difference as large as that observed in the previous study (mean change PSA level of 0.09). The power calculation was performed using the PASS software version 12.

OBJECTIVE 2

Complete data on dietary antioxidants intake and PCa aggressiveness were available for 2102 PCaP study participants (African American, n = 1,023; European Americans, n = 1,079). Analysis were performed separately for African Americans (high aggressive PCa, n = 206; low/intermediate aggressive PCa, n = 817) and European Americans (high aggressive PCa, n = 164; low/intermediate aggressive PCa, n = 915). Previous studies have reported as much as 63–80% lower overall risk of PCa or lower

risk high aggressive PCa among men with higher antioxidant intake [191, 334]. Thus, assuming a 63% difference in odds of high aggressive PCa among men with low *versus* high intake of antioxidants, based on a two-sided test at significance level $\alpha = 0.05$ and sample sizes of 1,023 and 1,079, a statistical power of 87% and 81% for African Americans and European Americans respectively.

OBJECTIVE 3

Data on SNP genotypes and PCa aggressiveness also were available for 1,964 PCaP participants (African American, n = 948; European Americans, n = 1,016). Similar to the analysis of main effect of the antioxidants, the gene-dietary analysis were performed separately for African Americans (high aggressive PCa, n = 188; low/intermediate aggressive PCa, n = 760) and European Americans (high aggressive PCa, n = 153; low/intermediate aggressive PCa, n = 863). Calculation of statistical power for gene-diet interaction was informed by findings from two recent studies. Li et al. [130] observed a statistically significant 82% lower risk of aggressive PCa in men with *Ala/Ala* genotype of the SOD2 gene polymorphism (rs4880) with high selenium intake. Goodman et al. [129] also observed a statistically significant 89% lower risk of PCa in men with *Arg/Arg* genotype of the XRCC1 gene and high antioxidant intake (lycopene + α-tocopherol + β-carotene). Therefore assuming 89% difference in odds of high aggressive PCa among men with low *versus* high intake of antioxidants by gene (dichotomous: homozygous wild-type allele *vs*. homozygous + heterozygous variants), based on a two-sided test at significance level $\alpha = 0.05$ and sample sizes of 948 and 1,016, 42% and 43% power was achieved for African Americans and European Americans respectively. The analyses based on these data were underpowered and may have concealed some modest

association. Thus, replication in larger studies is encouraged. The power calculations for specific objectives 2 and 3 were performed using the NCI power and sample size calculation software version 3.0.

3.4 STATISTICAL ANALYSES

OBJECTIVE 1

Differences in patient characteristics were compared between the intervention and control groups using *t*-test for continuous variables and Fisher's exact test for categorical variables. Linear regression models were used to estimate least squares means and *P* values for test of difference between group means, modeling PSA values as continuous variable. Natural log transformation was performed on the positively skewed PSA data in order to achieve normality; results were back transformed for presentation. Plasma carotenoids and tocopherols were categorized into binary groups (< vs. ≥ median) because of nonlinear distribution pattern assessed by the generalized additive model procedure in SAS (PROC GAM). A total antioxidant score also was computed as a measure of overall antioxidant status following the method described by Li et al. [335]. In estimating the antioxidant score, the carotenoid and tocopherol variables (i.e., α- and γ-tocopherol, α-carotene, *cis*- and *trans*-β-carotene, α- and β-cryptoxanthin, lutein, zeaxanthin, and *cis*- and *trans*-lycopene) were categorized into quartiles and scores were assigned to each quartile in multiples of 3 (i.e., 3 to 12, from low to high). The scores were summed for each participant across all carotenoids and tocopherols, and then categorized into median groups (< vs. ≥ median).

Analyses were performed in minimally adjusted (adjusting for age, race and randomized group) and multivariable adjusted linear regression models. Covariates selected for inclusion in the multivariable adjusted models were age (continuous); race (African American, European American); education (high school graduate or less, high school and some college, college graduate); marital status (Married or with partner, Widowed, divorced, or single); employment (yes, full time; yes, part time; no); smoking status (never, former, current); Gleason score (<5,5–6, ≥7); BMI (continuous); physical activity (metabolic equivalent/week); energy intake (continuous); and randomized condition (treatment, control). These variables were selected based on evaluation of confounding effect (>10% change in effect estimates) in conjunction with the backward elimination method. Additional variables considered but not included in the final analyses were: type of PCa treatment received; body fat mass; fruit, vegetables, fiber and dairy intake; and total dietary fat and omega-3 fatty acids intake., fiber and dairy intake; and total dietary fat and ω-3 fatty acids intake.

Analysis for hypothesis-1 was based on baseline data, which compared mean PSA values between participants with high *versus* low carotenoid or tocopherol using *P* values as a for differences between group means. For hypothesis 2, mean PSA values levels at 3 months and at 6 months (modeled separately) were compared between participants with high *versus* low carotenoid or tocopherol at 3 months, adjusting for baseline PSA values. Hypothesis 3, examined percent change in carotenoid and tocopherol levels (from baseline to 3 months) in relation to PSA levels at 3 months and at 6 months, adjusting for baseline PSA values. The sign for the percent change values was reserved to ensure that a positive value represented an increase in plasma carotenoid and tocopherol levels. The

percent change variables were also categorized into binary (increase *vs*. decrease) as well as tertile [decrease, minimal increase (1–20%), or substantial increase (>20 %)] groups for evaluation of whether mean PSA level vary with substantial increase in plasma levels.

Statistical models for Objective 1:

Models-1: $Y_1 = \beta_0 + \beta_1 (\text{antioxidant}_i) + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + \beta_k (X_k) + \varepsilon$

Models-2: $Y_2 = \beta_0 + \beta_1 (\text{antioxidant}_{ii}) + \beta_2 (\text{baseline PSA level}) \ldots\ldots + \beta_k (X_k) + \varepsilon$

Model-3: $Y_2 = \beta_0 + \beta_1(\text{antioxidant}_\Delta) + \beta_2 (\text{baseline PSA level}) \ldots\ldots + \beta_k (X_k) + \varepsilon$

$$\varepsilon \overset{iid}{\sim} N(0, \sigma^2)$$

Where Y_1 = serum PSA level at baseline

Y_2 = serum PSA level at 3 months and 6 months modeled separately

β_0 = intercept, β_j, j = 1……....k, = slope

antioxidant_i = plasma antioxidants at baseline (i.e., α- and γ-tocopherol, α-carotene, *cis*- and *trans*-β-carotene, lutein, zeaxanthin, *cis*-lutein/zeaxanthin, α- and β-cryptoxanthin, *cis*- and all-*trans*-lycopene and antioxidant score).

antioxidant_{ii} = individual plasma antioxidants at 3 months, and antioxidant score at 3 months

$\text{antioxidant}_\Delta$ = percent change in antioxidants from baseline to month 3

X_j, j = 1.....k, = age, race, education, marital status, employment, smoking status, Gleason score, BMI, physical activity, energy intake and randomized group).

OBJECTIVE 2 & 3

All analyses were conducted separately for African Americans and African European Americans because of significant interaction between race and lycopene intake.

Distributions of research subjects' characteristics by the levels of PCa aggressiveness were examined using Students' *t*-test and chi-square tests for continuous and categorical variables, respectively. Objective 2: Associations between antioxidants intake and PCa aggressiveness were examined using a case-control design by treating low/intermediate aggressive PCa cases as "control" or comparison group, and high aggressive PCa as "cases". Unconditional logistic regression models were used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs). Dietary and adipose tissue tocopherol levels (i.e., α, β, γ and δ) as well as supplemental vitamin E intake (α-tocopherol equivalent) were categorized into quartiles, while carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene) from diet, supplements and adipose tissue were categorized into tertiles. These data were categorized based on distribution among "controls" (i.e., low/intermediate aggressive PCa cases).

All analysis were performed in age-adjusted and multivariable models. The following variables were evaluated for inclusion in multivariable models: age (continuous), study site (NC, LA); BMI (in kg/m^2); pre-diagnostic PSA screening history (0, 1-7, >7 screenings); comorbidities (0, 1, 2, ≥3); family history of PCa in a first degree relative (none *vs*. at least one); whether PCa treatment had started at the time of the interview (yes, no); smoking status (never, former, current); education (less than high school education, high school graduate/some college, college graduate); annual household income (< $20,000, $20,001 - $40,000, $40,001 - $70,000, >$70,000); NSAIDs use in the five years prior to diagnosis (yes, no); physical activity in the year prior to diagnosis [total metabolic equivalents (METs) of light, moderate, and vigorous exercise categorized as: ≤ 10.2, 10.3-29.0, > 29.0 METs/week]; total fat intake

(grams/day); and alcohol intake (grams/day). As with objective 1 analysis, the multivariable models were constructed first by evaluating the confounding effect of each variable based on a 10% change in effect estimate of the main exposure variables with the removal of the covariate from the model. Variables determined to be confounders and those that are biologically relevant to PCa were then placed in an elaborate model for final model selection. A combination of the backward elimination model selection method and likelihood ratio tests were then used to select covariates for the final models consisting of age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total fat intake, and study site. Further adjustment for family history of PCa, comorbidities, and PCa treatment status were done in models examining associations between adipose carotenoid/tocopherol levels and PCa aggressiveness.

Statistical model for specific objective 2:

Model-1: $\ln(\frac{\pi}{1-\pi}) = \beta_0 + \beta_1 (\text{antioxidant}_i) + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + \beta_k X_k$

Where β_0 = intercept,

β_j, j = 1……....k, = slope (coefficient)

antioxidant_i = each antioxidant from diet, supplement and adipose tissue were modeled separately

X_j, j = 1 ……k, covariates

Objective 3: Gene-nutrients analyses were performed for α-tocopherol, γ-tocopherol and lycopene only because of significant findings for these nutrients observed in the analysis of the dietary data. Similar to specific objective 2, a case-control design

was used to examine whether associations between dietary intakes of α-tocopherol, γ-tocopherol and lycopene, and PCa aggressiveness are modulated by SNPs in DNA repair and oxidative stress genes, and whether the effect modification varies by race. *A priori* SNPs selected and genotyped by the PCaP consortium were utilized (see Table 4). Hardy-Weinberg equilibrium (HWE) test was not performed in this analysis because of lack of a disease-free control group. The "control group" as defined by this study is not an ideal population for HWE test because of the possibility that some of the SNP allelic variants may contribute to the expression of different PCa phenotypes [336, 337]. An *a priori* decision was made to exclude SNPs with low minor allele frequency (MAF, < 0.05).

In order to maximize sample size, a dominant model was assumed by collapsing the genotype variables into two groups [i.e., minor allele heterozygous + homozygous *versus* homozygous common allele (referent group)]. Effect modification of associations between α--tocopherol, γ-tocopherol, and lycopene with PCa aggressiveness, was examined in series of stratified analyses by genotype (binary groups). This was done by comparing high *versus* low levels of the nutrients intake in each stratum of genotype groups. Likelihood ratio tests (LRTs) were used to examine interaction on the multiplicative scale by comparing the difference in -2 log likelihood values of logistic regression models with and without the interaction terms. Interaction p-values were considered statistically significant at $p < 0.10$ to compensate for small sample size [336]. All results were adjusted for adjusted for multiple testing using the false discovery rate (FDR) method [338]. All statistical analysis described in the document were performed using SAS version 9.3 (SAS Inc., Cary, NC, USA) with statistical significance set at $\alpha = 0.05$ (two-tailed).

Statistical model for specific objective 3:

Model-1(stratified analysis by genotype groupings: wild-type *versus* variant genotype):

$$\ln\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 (\text{antioxidant}_i) + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots + \beta_k X_k$$

Where β_0 = intercept, β_j, j = 1……....k, = slope (coefficient)

antioxidant$_i$ = each dietary antioxidants (α- and γ-tocopherol, and lycopene)

Genotype groupings = homozygous wild-type allele (reference group) vs. homozygous + heterozygous variant alleles

X_j = j……..k, covariates

CHAPTER 4

PLASMA CAROTENOIDS AND TOCOPHEROL LEVELS IN RELATION TO PROSTATE-SPECIFIC ANTIGEN (PSA) LEVELS IN MEN WITH BIOCHEMICAL PROSTATE CANCER RECURRENCE

4.1 INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed visceral tumor and the second most lethal malignancy among American men [339]. Most patients diagnosed with PCa in the United States present with clinically localized disease (about 94%), and often are treated with radical prostatectomy or radiation with curative intent [340, 341]. Unfortunately, about 25–40% of these patients develop biochemical recurrence of the disease within five years of definitive treatment [342-345]. Biochemical recurrence of PCa is identified by rising serum prostate-specific antigen (PSA) level on three or more successive tests after achieving post-treatment PSA nadir (lowest detectible level) [346]. PSA-defined PCa relapse following definitive therapy is often an early sign of metastasis, and precedes pathological and radiographic evidence of metastasis by several years [347, 348]. Thus, the identification of PSA-defined PCa recurrence provides ample time for intervention.

Although there is no known cure for biochemically recurrent PCa, it is often managed with surgical or medical androgen ablation to delay the time to metastasis and prolong survival [349, 350]. Androgen ablation is often ineffective in controlling the disease progression as most patients become hormone-refractory within two years,

resulting in continuous rise in PSA [351, 352]. Severe side effects are also associated with the use of androgen ablation [349, 351]. Thus, there is continued interest in the search for adjuvant and neoadjuvant therapies for biochemical PCa relapse [13]. Epidemiologic data from migrant studies indicate that in addition to age, race/ethnicity and a positive family history, diet plays an important role in PCa [353, 354]. Greater intake of cruciferous vegetables, fruits, and specific dietary nutrients such as lycopene, soy isoflavones and polyhenols have been associated with modest reduction in PCa risk, while energy imbalance and increased consumption of fat, meat, calcium and dairy products have been associated with increased risk of PCa [17, 355, 356].

Few epidemiologic studies have investigated whether the progression of biochemically recurrent PCa can be altered using plant-based, dietary intervention [11, 13, 357-361]. Most of these intervention trials incorporated supporting interventions such as stress reduction [11, 358-360] and physical activity [13] to reinforce the dietary modification. Five reported potential inhibitory effect of the intervention on PCa progression [11, 357-360], while two reported null results [13, 361]. However, because these trials involved different combinations of diet, stress reduction, and physical activity, it is difficult to determine whether study diet or other factors were responsible for the potential beneficial effects reported by some studies. Although others studies have investigated effects of dietary modifications alone among men with biochemical recurrence (reviewed in [355, 362, 363]), the diets used in these studies also had different components, such as increasing fruits, vegetables, legumes, and whole grains intake while decreasing meat and dairy intake, which also makes it difficult to examine the independent effects of specific food components. Thus, additional work is needed to

evaluate the role of specific foods and nutrients. Of particular interest are biomarkers of antioxidant intake, which have been inversely associated with PCa risk in some studies [364, 365] and therefore may be associated with reduction in progression of recurrent PCa [362].

Our team previously reported results of a pilot intervention trial conducted in South Carolina to investigate whether a plant-based dietary intervention integrated with physical activity and stress reduction could alter the progression of PCa in men with biochemical recurrence of PCa after definitive therapy [13]. The current report is an expansion of that work. We investigated whether plasma carotenoids (including all major carotenoids) and tocopherol (α– and γ–tocopherols) levels were associated with PSA levels in these patients.

4.2 MATERIALS AND METHODS

Study Population

Participants of the intervention trial were men with histologically confirmed localized adenocarcinoma of the prostate who had completed primary therapy (i.e., radical prostatectomy or radiation) and had experienced increasing serum PSA levels, a minimum of three successive increases of 1.5 ng/mL above the post-treatment PSA nadir, with each increase at 2- to 3-month intervals [13]. Participants were deemed eligible if they were free of other malignancy in the previous 5 years (with the exception of non-malignant skin cancer); spoke English as a first language; were able to read at a sixth grade level; were of sound mind, memory, and understanding; had not been taking thyroid medication, steroids, antibiotics, or diuretics; and were willing to be randomized to intervention or control (with an option to obtain the intervention at the end of the

study). The participants were required to enter the study with their spouse or partner of choice to provide support for compliance with the study protocol. Prospective participants were excluded if they had received post-operative hormonal therapy for treatment of PCa; had a current diagnosis or symptoms of active ulcerative colitis or cardiovascular, pulmonary, Crohn's, or metabolic disease; had experienced weight loss of 5 pounds or more within the previous 3 months; planned to use hormone supplements, fish oil, or other ω-3 fatty acids-based supplements; or had a diagnosis of post-traumatic stress disorder (PTSD). All participants provided informed consent prior to enrollment. The research protocol of the parent study was reviewed and approved by the Institutional Review Boards (IRBs) of the University of South Carolina (USC) and Palmetto Health; the current analysis also was approved by the USC IRB.

All participants were recruited from major urological practices of seven counties in the Midlands region of SC (Richland, Lexington, Orangeburg, Kershaw, Sumer, Fairfield, and Newberry). The majority of participants were from Richland (67%) and Lexington (9%) counties, which are the two most densely populated counties in the greater Columbia area. The intervention was conducted at locations close to the recruitment sites under the auspices of the primary investigator (JRH). All clinical and anthropometric data were collected at the facilities of the Cancer Prevention and Control Program at USC.

Study Design

Details of the study design and methods have been published [13]. In brief, participants were assigned to intervention or control group using block randomization by

age (± 5 years) and race (African American/European American). The study spanned 6 months, which included a 3-month period of active intervention followed by monthly booster session for the following 3 months. The intervention consisted of dietary modifications, physical activity, and mindfulness-based stress reduction training. The 3-month active phase of the intervention involved individual diet and physical activity counseling and goal setting sessions, as well as twelve weekly group meetings that included cooking classes and shared model meals. In addition, participants were given weekly assignments on how to shop for and cook study-compliant meals, attain physical activity goals, and practice meditation for stress reduction. The diet aspect of the intervention emphasized increased intake of plant-based foods such as whole grains, fruits, vegetables, and legumes (particularly soybeans and soybean products) along with decreased intake of meat and dairy products. The physical activity aspect involved working with participants to identify activities that they enjoyed and reinforce those activities to promote physical fitness and overall well-being. The goal of the exercise routine was to ensure that each participant attain the Centers for Disease Control and American College of Sports Medicine (CDC/ACSM) recommendations of at least 30 minutes of moderate intensity physical activity for 5 days or more per week [315]. Because comprehensive dietary change can be difficult to maintain, participants were taught to meditate in a way that inculcates mindfulness about decisions concerning food choices in order to promote their sense of control over the change in diet and culinary habits [316]. Partner support was integrated to provide an encouraging environment for the process of change. Following the 3-month active phase, monthly booster sessions were held in a supportive group environment for another 3 months. These included

frequent telephone calls to each participant and his partner for wellness checks and encouragement to sustain the intervention.

Participants in the control group underwent the same general assessment as those in the intervention group. There were no attempts made to restrict their access to psychosocial support or educational resources available to PCa patients in the community. These participants and partners were given the opportunity to take the intervention at the end of the 6-month study period at no cost to them.

Data Collection and Phlebotomy

Data on clinical and pathologic attributes of PCa were abstracted from participants' medical records obtained from referring urologists. At baseline, participants responded to questionnaires that solicited information on demographics and health-related behaviors, including age, race, education, marital status, employment, and smoking status. Data on diet, physical activity, and anthropometry were obtained at each of the three study checkpoints: baseline, 3 months, and 6 months. Diet assessments used 24-hour dietary recalls on three randomly selected days that included two weekdays and one weekend day, as this method has been found to be least prone to dietary measurement error [318, 319]. Physical activity was assessed using a questionnaire designed for older adults that had been tested for reliability [320]. Physical activity was expressed as metabolic equivalent (MET) value based on description of the activity using the *Compendium of Physical Activities* [321], with one MET being equivalent to resting metabolic rate. Total METs of physical activity were estimated for each participant's sum of METs from light, moderate, and vigorous physical activity per week. The anthropometric measurements obtained were standing height (cm), weight (kg), waist-to-

hip ratio, and bioelectric impedance measures of percent body fat and lean body mass [13]. Body mass index (BMI) was subsequently calculated as weight (kg)/height (m^2).

Each participant provided a 5 ml vial of blood from venipuncture obtained by a trained phlebotomist at each of the three study timepoints. The samples were fractionated by centrifuge, frozen at -80^{oc} within 1 hour of collection, and transported on ice within 1week via overnight courier to Quest ® Laboratories for analysis. PSA was measured in serum at baseline, at 3 months and at 6 months. Carotenoids and tocopherols were measured in plasma using high performance liquid chromatography (HPLC). Because of limited availability of samples, data on carotenoids and tocopherols were only measured at baseline and at 3 months. The following carotenoids and tocopherols were measured: α- and γ-tocopherol, α-carotene, *cis*- and *trans*-β-carotene, lutein, zeaxanthin, *cis*-lutein/zeaxanthin, α- and β-cryptoxanthin, *cis*- and *trans*-lycopene.

Statistical Methods

Differences in baseline characteristics were assessed using Student's *t*-test to compare means of continuous variables and Fisher's exact test for categorical variables. Means and standard deviations (SDs) of plasma carotenoids and tocopherols at baseline and at 3 months also were calculated and compared by intervention group. Because carotenoids and tocopherols are transported in the blood by lipoproteins [278], we corrected for circulating lipid levels by dividing each carotenoid and tocopherol (μg/ml) by total plasma cholesterol level (mg/dL). These variables were subsequently categorized into binary groups (< vs. ≥ median) because of nonlinear distribution pattern; assessed by the generalized additive model procedure in SAS (PROC GAM). A total antioxidant

score was computed as a measure of overall antioxidant status following the method described by Li et al. [335]. In estimating the antioxidant score, the carotenoid and tocopherol variables (i.e., α- and γ-tocopherol, α-carotene, *cis*- and *trans*-β-carotene, α- and β-cryptoxanthin, lutein, zeaxanthin, and *cis*- and *trans*-lycopene) were categorized into quartiles and scores were assigned to each quartile in multiples of 3 (i.e., 3 to 12, from low to high). The scores were summed for each participant across all carotenoids and tocopherols, and then categorized into median groups (< vs. ≥ median).

The relations between serum PSA levels and plasma carotenoids and tocopherols were examined in three sets of analyses. First, we considered how baseline carotenoid and tocopherol levels are related to baseline PSA level. Second, we explored whether carotenoid and tocopherol levels at 3 months are related to PSA levels at 3 months and at 6 months, adjusting for baseline PSA level, as baseline PSA is related to subsequent PSA values [366]. Finally, we examined percent change in carotenoid and tocopherol levels (from baseline to 3 months) in relation to PSA levels at 3 months and at 6 months, adjusting for baseline PSA values. The sign for the percent change values was reserved to ensure that a positive value represented an increase in plasma carotenoid and tocopherol levels. The percent change variables also were categorized into binary (increase *vs*. decrease) as well as tertile [decrease, minimal increase (1–20%), or substantial increase (>20 %)] groups. Linear regression was used for all analyses to estimate least squares means and *P* values for test of difference between group means, modeling PSA values as continuous variable. Natural log transformation was performed on the positively skewed PSA data in order to achieve normality; however, results were back transformed for presentation.

The analyses were performed in minimally adjusted (i.e., "crude model", adjusting for age, race and randomized group) and in multivariable adjusted models. Covariates chosen for inclusion in the multivariable adjusted models were age, race, education, marital status, employment, smoking status, Gleason score, BMI, physical activity, energy intake and randomized group, and modeled as continuous or categorical variables as presented in Table 4.1. These variables were selected based on evaluation of confounding effect (>10% change in effect estimates) in conjunction with the backward elimination method. Additional variables considered but not included in the final analyses type of PCa treatment received; body fat mass; fruit, vegetables, fiber and dairy intake; and total dietary fat and omega-3 fatty acids intake. All statistical tests were two sided; statistical significance was set at $\alpha = 0.05$, and all analyses performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

4.3 RESULTS

Full details of the randomization procedure have been reported [13]. Overall, 54 men with a history of localized PCa and rising PSA levels after definitive treatment with radical prostatectomy, radiation or both were successfully randomized to intervention (n = 29) and control (n = 25). Of these participants, seven were lost to follow-up (intervention, n = 3; control, n = 4). Of the remaining 47 participants, data on plasma carotenoid and tocopherol levels were available for 39 participants at baseline and 35 participants at 3 months.

Differences in the distribution of baseline characteristics and PSA levels at all three timepoints are presented in Table 4.1. The mean age of the study sample was 70 years (SD = 8), with mean BMI of 29.75 kg/m^2 (SD = 5.21), and included 28 (72%)

European Americans and 11 (28%) African Americans. Fifteen percent of the participants underwent radical prostatectomy, 39% had radiation only, and 46% had both radiation and prostatectomy prior to enrollment in the study. Mean serum PSA levels were 3.91, 5.01, and 4.72 ng/mL at baseline, at 3 months, and at 6 months, respectively. None of the baseline characteristics including education, marital status, employment, smoking status, and tumor grade, differed significantly by intervention status.

The plasma carotenoid and tocopherol concentrations did not vary significantly between the intervention and control groups at baseline or at 3 months (Table 4.2). Analysis of baseline data also did not show any significant difference in mean PSA levels between participants with high *versus* low carotenoid/tocopherol levels or total antioxidant score (Table 4.3).

Tables 4.4 and 4.5 presents results for associations of plasma carotenoids and tocopherols at 3 months in relation to serum PSA levels at 3 months and 6 months, respectively, after adjusting for baseline PSA level in addition to age, race, education, marital status, employment, smoking status, Gleason score, BMI, physical activity and randomization status. Participants with higher carotenoid and tocopherol levels at 3 months, tended to have lower PSA levels at 3 months as compared to those with lower carotenoid and tocopherol levels at 3 months, though the association with PSA levels at 3 months after adjustment was statistically significant only for cis-lutein/zeaxanthin (P = 0.008). The 3-month carotenoid and tocopherol levels appeared to be more strongly associated with serum PSA levels at 6 months, as participants with high plasma levels of α-tocopherol (P = 0.01), β-cryptoxanthin (P = 0.01), all-*trans*-lycopene (P = 0.004), and

total antioxidant score ($P = 0.003$) showed significantly lower mean PSA levels than those with low levels of these micronutrient antioxidants.

We further examined whether percent change in carotenoid and tocopherol levels from baseline to month 3 was associated with PSA levels at 3 months and at 6 months, adjusting for baseline PSA level (Table 4.6 and 4.7, respectively). These results show that participants who experienced an increase in carotenoid and tocopherol levels generally had lower mean PSA levels at 3 months compared to those who had a decrease in carotenoid and tocopherol levels. The evidence of inverse relation with serum PSA at 3 months was particularly strong for α-tocopherol ($P = 0.0007$). Although significantly lower mean PSA levels were observed for higher levels of all-*trans*-β-carotene and α-cryptoxanthin in relation to PSA level at 3-months, significant findings in the tertile categories was confined to participants who had a minimal increase in their plasma levels (i.e., 1–20% increase). In the analysis of 6-month PSA values, percent increase in carotenoid/tocopherol level was inversely related to mean PSA level for α-tocopherol, *trans*-β-carotene, β-cryptoxanthin, *cis*-lutein/zeaxanthin, *trans*-lycopene, and total antioxidant score.

4.4 Discussion

In this study, we examined the relations between plasma carotenoid and tocopherol levels, and serum PSA levels among men with biochemical recurrence of PCa who were enrolled in a 6-month diet and lifestyle intervention trial in South Carolina. In an analysis of baseline data, no significant differences in mean PSA levels were observed between participants with high *versus* low carotenoid or tocopherol levels. We further

explored whether carotenoid and tocopherol levels at 3 months (during the study period) were associated with PSA levels at 3 months and at 6 months, adjusting for baseline PSA values. Results from this analysis showed that participants with higher *cis*-lutein/zeaxanthin level at 3 months had statistically lower mean PSA level at 3 months. Additionally, participants with higher plasma levels of α-tocopherol, β-cryptoxanthin, all-*trans*-lycopene, and higher antioxidant score at 3 months, had significantly lower mean PSA level at 6 months. Finally, we examined whether percent change in plasma carotenoid and tocopherol levels from baseline to month 3 were inversely related to PSA levels at 3 months and at 6 months, independent of baseline PSA values. These results showed significantly lower mean PSA values at 3 months and at 6 months for participants with an increase in α-tocopherol and *trans*-β-carotene levels compared to who had a decrease in the levels of these nutrients. In addition, those with an increase in β-cryptoxanthin, *cis*-lutein/zeaxanthin, *trans*-lycopene and antioxidant score had significantly lower mean PSA values at 6 months. Overall, higher plasma levels of certain carotenoids and tocopherols paralleled with lower PSA level at various time points, with stronger findings for associations with the 6-month PSA values. This suggests that it may take a few months before a clinical benefit on PSA is observed from a dietary intervention.

The idea of using dietary agents as an alternate therapy or as a neoadjuvant to delay the use of more traditional therapy such as androgen ablation is a prospect that would be appealing to most patients because of the severe side effects associated with traditional therapy [349, 350]. While it is plausible that intake of certain carotenoids and tocopherols may influence serum PSA levels, it is possible that these nutrients could alter

PSA levels without affecting cancer progression. Interestingly, declines in PSA have been found to correlate with inhibition of the androgen-sensitive LNCaP prostate tumor cell growth in animal and human studies [357, 367, 368]. Secretion of PSA and hormone-dependent LNCaP activity are both modulated by androgens [369, 370]. Higher blood levels of antioxidants such as lycopene and α-tocopherol have been found to down-regulate serum androgen levels [371-373]. Thus, the suppression of androgens may be an underlying mechanism for the potential effect of carotenoids and tocopherols on PSA, and possibly, PCa progression. Other mechanisms involving antioxidative and anti-inflammatory activities have also been proposed [374, 375].

Prior studies on men with biochemically recurrent PCa have focused primarily on multiple interventions involving diet, exercise, and stress reduction [11, 13, 357-361]. There is very little published literature on associations of carotenoids and tocopherols intake in relation to PSA levels among men with PCa relapse (reviewed in [355, 362, 363]). Data on carotenoids and tocopherols in relation to PSA progression among men with PCa relapse are lacking. The vast majority of the available data are from studies examining the potential benefits of supplemental or dietary lycopene. In a study involving 71 men with biochemical recurrence who were randomized to intervention with supplemental lycopene alone (15 mg) or together with soy isoflavones capsule (40 mg) taken twice daily for 6 months, no decline in serum PSA level was observed in either group [376]. In that same study, however, the rate of PSA rise decreased in 95% of patients in the lycopene group and 67% of those in the lycopene and soy isoflavones group [376]. In another study where 36 men with biochemical recurrence of PCa were

given varying doses of lycopene (15, 30, 45, 60, 90 and 120 mg/day) for one year, no change in serum PSA was observed across all the six dose groups [377].

In a related study, Chen et al. [374] investigated the effect of lycopene on cancer progression among 32 patients with incident PCa treated tomato sauce-based diet containing 30 mg of lycopene per day for 3 weeks before their scheduled prostatectomy. The results showed significant reduction in serum PSA levels as well as declines in markers of oxidative DNA damage measured in leukocytes and prostate tissue, when comparing pre- and post-intervention measurements [374]. Ansari and Gupta [378] evaluated the effect of lycopene and orchiectomy *versus* lycopene alone in 54 patients with metastatic PCa, and found significantly lower PSA levels in the lycopene group after 6 months of follow-up. Others have reported that supplemental lycopene intake decreases PSA velocity and may prolong PSA doubling time [379]. Among studies conducted in disease-free men, one found an inverse association between serum α-carotene levels and percent free PSA level (OR = 0.49, 95% CI = 0.32–0.76), but not total PSA, and no inverse association was found for other carotenoids [380]. Another found no association between tocopherol intake and serum PSA level or PSA velocity [381]. The variability is these findings may be related to the source of the nutrients (e.g., supplement *versus* diet for lycopene) or the possibility that these nutrients may have varying effect on different disease states.

To our knowledge, this is the first study to examine biomarkers of carotenoids and tocopherols in relation to PSA levels among men with biochemical recurrence of PCa. The results show that after controlling for baseline PSA values, certain plasma carotenoids and tocopherol were associated with low mean PSA values at various

timepoints. Despite these findings, it is conceivable that these nutrients may have served as surrogates for higher consumption of fruits and vegetables which contain other beneficial dietary factors. Of note, the original EASE intervention study did not find a beneficial effect of the diet and lifestyle intervention on PSA [13]. Challenges associated with conducting clinical trials of lifestyle interventions, such as lack of large enough contrast between the intervention and control group due to contamination or suboptimal compliance [382], may partially explain this finding. The current study results suggest that higher exposure to certain dietary antioxidants may have a beneficial effect on PSA rise following prostatectomy and should be confirmed in other larger studies.

Both strengths and limitation of the study deserve mention. Given the small sample size and the multiple comparisons made, there is a possibility that some of the findings could be due to chance. Because humans consume foods containing multiple nutrients, there is also the possibility that the study results may be reflecting interactions between plasma nutrients, rather than the effect of a specific nutrient *per se* [383]. The short duration of the study and lack of carotenoid and tocopherol data at 6 months prohibited evaluation of temporal trends over long periods. Restricting the study to a subgroup of PCa patients with strictly defined disease attributes precludes generalizability of the findings to the larger population of men with PCa. However, since the study participants had already undergone radical prostatectomy and/or radical radiation for the treatment of organ-confined disease, continuous rise in serum PSA level as defined in this study most likely reflect progressive disease (which was the intent of the study), rather than residual normal tissue left from radiation or spared during prostatectomy. Other strengths of the study include the use of biomarkers of nutrient

intake, which are more reliable measures of nutritional status relative to self-reported intake [384]. Several potential confounders including BMI, smoking, physical activity, tumor grade and race were controlled for in the analysis. The study findings add to the limited data on potentially beneficial dietary factors for men with biochemically recurrent PCa.

4.5 CONCLUSIONS

Higher plasma levels of α-tocopherol, β-cryptoxanthin, *trans*-β-carotene, cis-lutein/zeaxanthin, and *trans*-lycopene were associated with lower PSA levels among men with biochemically defined PCa recurrence. A higher antioxidant score, used as a measure of total antioxidant status, also was associated with lower PSA levels at various timepoints. These findings suggest that increasing intake of these micronutrients, which are found in many fruits and vegetables, may slow the progression of PSA in men with a biochemical recurrence of PCa. Considering the small sample size and short duration, additional work in larger cohorts with longer follow-up time is warranted.

Table 4.1 Baseline characteristics of study subjects and changes in PSA levels				
	All subjects (n = 39)	Intervention (n = 22)	Control (n = 17)	*P* §
	Mean ± SD	Mean ± SD	Mean ± SD	
Age, years	70 ± 8	69 ± 9	71 ± 7	0.51
BMI, kg/m^2	29.75 ± 5.21	29.49 ± 4.86	30.09 ± 5.77	0.73
Energy, kcal/day	1683.90 ± 414.24	1741.24 ± 367.52	1609.68 ± 468.92	0.33
Physical activity, total METs/week	44.60 ± 35.51	52.02 ± 41.29	35.43 ± 24.96	0.13
	n (%)	n (%)	n (%)	
Race				
White/European American	28 (72)	17 (77)	11 (65)	0.48
Black/African American	11 (28)	5 (23)	6 (32)	
Education				
High school graduate or less	8 (20)	4 (18)	4 (23)	0.70
High school and some college	12 (31)	8 (36)	4 (23)	
College graduate	19 (49)	10 (45)	9 (53)	
Marital status				
Married or with partner	31 (79)	16 (73)	15 (88)	0.43
Widowed, divorced, or single	8 (21)	6 (27)	2 (12)	
Employment				
Yes, full time	7 (18)	3 (14)	4 (23)	0.68
Yes, part time	4 (10)	2 (9)	2 (12)	
No	28 (72)	17 (77)	11 (65)	
Smoking status				
Never	14 (37)	8 (36)	7 (41)	0.80

Former	21 (53)	11 (50)	9 (53)	
Current	4 (10)	3 (14)	1 (6)	
Tumor grade (Gleason score)				
Well differentiated (<5)	1 (3)	1 (5)	0 (0)	0.95
Moderately differentiated (5–6)	9 (23)	5 (23)	4 (24)	
Poorly differentiated (≥7)	20 (51)	12 (54)	8 (47)	
Missing	9 (23)	4 (18)	5 (29)	
Type of treatment				
Prostatectomy	6 (15)	3 (14)	3 (18)	0.99
Prostatectomy and radiation	18 (46)	10 (45)	8 (47)	
Radiation only	15 (39)	9 (41)	6 (35)	
PSA levels, mean (range) ng/mL [a]				
Baseline	3.91 (0.10-52.00)	3.24 (0.10-37.90)	4.78 (0.10-52.00)	0.61
At 3-months	5.01 (0.10-68.30)	4.37 (0.10-44.70)	5.85 (0.10-68.30)	0.70
At 6-months	4.72 (0.10-67.20)	4.26 (0.10-54.40)	5.27 (0.10-67.20)	0.80

Abbreviations: PSA – prostate-specific antigen; SD – standard deviation; METs – metabolic equivalent task per week from physical activity

[a] Data represents actual PSA values, not logarithm transformed values.

§ *P* value comparing intervention and control groups using Student's *t*-test for continuous variables and Fisher's exact test for categorical variables

Table 4.2 Means and standard deviations of plasma carotenoid and tocopherol levels at baseline and at 3 months post-intervention

	Baseline				Post-intervention (at 3 months)			
Plasma carotenoids and tocopherols (μg/ml)	All subjects (n = 39)	Intervention (n = 22)	Control (n = 17)	*P* [§]	All subjects (n = 35)	Intervention (n = 20)	Control (n = 15)	*P* [§]
α-tocopherol	14.91 ± 5.15	15.23 ± 5.56	14.51 ± 4.71	0.67	14.35 ± 5.17	14.64 ± 5.48	13.96 ± 4.87	0.71
γ-tocopherol	1.70 ± 1.01	1.67 ± 1.01	1.73 ± 1.04	0.86	1.65 ± 0.99	1.60 ± 0.89	1.70 ± 1.13	0.78
α-carotene	0.04 ± 0.03	0.04 ± 0.03	0.05 ± 0.03	0.60	0.05 ± 0.04	0.04 ± 0.03	0.05 ± 0.05	0.64
cis-β-carotene	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.07	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.32
Trans-β-carotene	0.20 ± 0.13	0.18 ± 0.12	0.24 ± 0.15	0.17	0.20 ± 0.14	0.20 ± 0.16	0.21 ± 0.12	0.83
α-cryptoxanthin	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.77	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.86
β-cryptoxanthin	0.11 ± 0.08	0.11 ± 0.09	0.10 ± 0.07	0.71	0.10 ± 0.07	0.10 ± 0.08	0.09 ± 0.06	0.87
Lutein	0.11 ± 0.06	0.10 ± 0.06	0.12 ± 0.07	0.40	0.12 ± 0.07	0.12 ± 0.07	0.12 ± 0.08	0.92
Zeaxanthin	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.30	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.47
Cis-lutein/zeaxanthin	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.59	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.07
Cis-lycopene	0.18 ± 0.13	0.18 ± 0.11	0.18 ± 0.15	0.94	0.17 ± 0.11	0.19 ± 0.10	0.15 ± 0.13	0.26
Trans-lycopene	0.19 ± 0.12	0.20 ± 0.11	0.19 ± 0.14	0.81	0.19 ± 0.11	0.22 ± 0.10	0.16 ± 0.12	0.12

Abbreviation: SD, standard deviation
[§] *P* value comparing intervention and control groups based on Student's *t*-test

Table 4.3 Baseline PSA levels by baseline carotenoid and tocopherol levels

			Crude model [a]		Adjusted model [b]	
Plasma tocopherols and carotenoids at baseline [c]		n	Mean (95% CI) [d]	*P* §	Mean (95% CI) [d]	*P* §
α-tocopherol	low	19	0.80 (0.39-1.62)	0.35	0.53 (0.25-1.16)	0.40
	high	20	1.34 (0.57-3.14)		0.79 (0.30-2.07)	
γ-tocopherol	low	20	1.40 (0.59-3.30)	0.30	0.71 (0.29-1.71)	0.50
	high	19	0.78 (0.38-1.58)		0.52 (0.23-1.20)	
α-carotene	low	19	1.10 (0.52-2.34)	0.67	0.49 (0.20-1.19)	0.45
	high	20	0.88 (0.42-1.88)		0.70 (0.30-1.63)	
Cis-β-carotene	low	20	0.77 (0.35-1.67)	0.37	0.67 (0.26-1.70)	0.71
	high	19	1.27 (0.58-2.78)		0.55 (0.23-1.31)	
Trans-β-carotene	low	20	0.91 (0.42-1.95)	0.75	0.50 (0.21-1.18)	0.44
	high	19	1.07 (0.49-2.34)		0.72 (0.30-1.74)	
α-cryptoxanthin	low	21	0.94 (0.44-2.00)	0.86	0.87 (0.35-2.19)	0.20
	high	18	1.03 (0.48-2.22)		0.46 (0.20-1.05)	
β-cryptoxanthin	low	20	0.92 (0.45-1.89)	0.76	0.56 (0.23-1.35)	0.80
	high	19	1.08 (0.49-2.37)		0.66 (0.23-1.89)	
Lutein	low	19	0.91 (0.40-2.06)	0.79	0.70 (0.29-1.68)	0.52
	high	20	1.05 (0.51-2.14)		0.51 (0.22-1.22)	
Zeaxanthin	low	18	0.96 (0.43-2.16)	0.93	0.53 (0.20-1.43)	0.74
	high	21	1.01 (0.49-2.05)		0.63 (0.29-1.37)	
Cis-lutein/zeaxanthin	low	21	0.94 (0.46-1.91)	0.82	0.61 (0.28-1.34)	0.90
	high	18	1.05 (0.47-2.38)		0.58 (0.23-1.42)	
Cis-lycopene	low	21	1.39 (0.60-3.22)	0.30	0.46 (0.14-1.54)	0.48
	high	18	0.72 (0.28-1.87)		0.30 (0.11-0.85)	
Trans-lycopene	low	20	1.27 (0.35-2.07)	0.50	0.42 (0.16-1.07)	0.14
	high	19	0.85 (0.54-3.03)		0.22 (0.09-0.56)	
Antioxidant score [e]	low	19	1.16 (0.55-2.42)	0.51	0.77 (0.32-1.85)	0.31
	high	20	0.82 (0.38-1.79)		0.45 (0.19-1.12)	

Abbreviations: PSA – prostate-specific antigen, CI – confidence interval

[a] Adjusted for age, race and randomized group.

[b] Adjusted for age, race, education, marital status, employment status, smoking status, Gleason score, body mass index, total metabolic equivalent (MET) per week of physical activity, energy intake, and randomized group.

[c] Categorized by median splits as less than median (low) versus greater than or equal to median (high).

[d] Data are reported as least square means.

[e] Antioxidant score; low : 57 – 83, high: 84 –123.

§ *P* values from regression model comparing mean difference between low and high tocopherol/carotenoid categories

Table 4.4 Associations of carotenoid and tocopherol levels at 3 months in relation to PSA levels at 3 months adjusting for baseline PSA level

			PSA levels at 3 months [a]			
Plasma tocopherols and carotenoids at 3 months [b]		n	Crude model [c]		Adjusted model [d]	
			Means (95% CI)	*P* [§]	Means (95% CI)	*P* [§]
α-tocopherol	low	18	0.98 (0.74-1.29)	0.09	0.62 (0.45-0.85)	0.10
	high	17	0.68 (0.49-0.94)		0.42 (0.27-0.65)	
γ-tocopherol	low	17	0.70 (0.50-0.98)	0.16	0.56 (0.39-0.83)	0.82
	high	18	0.97 (0.73-1.28)		0.53 (0.33-0.83)	
α-carotene	low	18	1.00 (0.76-1.33)	0.07	0.65 (0.45-0.93)	0.13
	high	17	0.69 (0.50-0.93)		0.44 (0.30-0.66)	
Cis-β-carotene	low	17	1.04 (0.77-1.41)	0.05	0.66 (0.45-0.96)	0.16
	high	18	0.69 (0.52-0.92)		0.49 (0.34-0.68)	
Trans-β-carotene	low	18	1.03 (0.78-1.35)	0.03	0.63 (0.43-0.92)	0.25
	high	17	0.66 (0.49-0.90)		0.50 (0.35-0.70)	
α-cryptoxanthin	low	18	0.97 (0.73-1.30)	0.15	0.57 (0.41-0.80)	0.65
	high	17	0.72 (0.54-0.97)		0.50 (0.30-0.83)	
β-cryptoxanthin	low	18	0.99 (0.74-1.32)	0.13	0.56 (0.39-0.83)	0.82
	high	17	0.72 (0.54-0.97)		0.53 (0.36-0.78)	
Lutein	low	17	1.01 (0.75-1.36)	0.09	0.61 (0.41-0.93)	0.45
	high	18	0.71 (0.54-0.95)		0.51 (0.35-0.73)	
Zeaxanthin	low	17	0.92 (0.66-1.29)	0.48	0.60 (0.43-0.82)	0.17
	high	18	0.79 (0.59-1.05)		0.44 (0.29-0.68)	
Cis-lutein/zeaxanthin	low	18	1.02 (0.77-1.35)	0.05	0.75 (0.52-1.07)	**0.008**

	high	17	0.67 (0.49-0.92)		0.45 (0.33-0.62)	
Cis-lycopene	low	17	0.97 (0.72-1.30)	0.20	0.61 (0.43-0.88)	0.29
	high	18	0.72 (0.52-0.99)		0.49 (0.34-0.71)	
Trans-lycopene	low	17	0.90 (0.66-1.22)	0.57	0.58 (0.40-0.82)	0.60
	high	18	0.78 (0.56-1.10)		0.51 (0.33-0.78)	
Antioxidant score [e]	low	17	1.03 (0.77-1.37)	0.05	0.62 (0.44-0.87)	0.18
	high	18	0.69 (0.52-0.92)		0.47 (0.32-0.68)	

Abbreviation: PSA – prostate-specific antigen, CI – confidence interval

[a] Data are reported as least square means
[b] Categorized by median splits as less than median (low) versus greater than or equal to median (high).
[c] Adjusted for age, race randomized group and baseline PSA level.
[d] Adjusted for age, race, education, marital status, employment status, smoking status, Gleason score, body mass index, total metabolic equivalent (MET) per week of physical activity, energy intake, randomized group and baseline PSA level.
[e] Antioxidant score; low : 45 – 80, high: 81 –111.

§ *P* values from regression model comparing mean difference between low and high tocopherol/carotenoid categories

Table 4.5 Associations of carotenoid and tocopherol levels at 3 months in relation to PSA levels at 6 months, adjusting for baseline PSA level

Plasma tocopherols and carotenoids at 3 months [b]		n	PSA levels at 6 months [a]			
			Crude model [c]		Adjusted model [d]	
			Means (95% CI)	*P* [§]	Means (95% CI)	*P* [§]
α-tocopherol	low	18	1.00 (0.45-2.21)	0.11	0.76 (0.28-2.01)	**0.01**
	high	17	0.38 (0.16-0.93)		0.13 (0.03-0.48)	
γ-tocopherol	low	17	0.75 (0.32-1.72)	0.62	0.64 (0.16-2.59)	0.45
	high	18	0.55 (0.21-1.39)		0.33 (0.10-1.08)	
α-carotene	low	18	1.04 (0.45-2.40)	0.12	0.88 (0.26-2.95)	0.08
	high	17	0.42 (0.19-0.95)		0.23 (0.07-0.73)	
Cis-β-carotene	low	17	0.74 (0.32-1.68)	0.65	0.51 (0.18-1.50)	0.52
	high	18	0.56 (0.23-1.39)		0.33 (0.10-1.15)	
Trans-β-carotene	low	18	0.85 (0.37-1.95)	0.36	0.46 (0.12-1.70)	0.87
	high	17	0.49 (0.21-1.17)		0.41 (0.14-1.23)	
α-cryptoxanthin	low	18	0.67 (0.29-1.57)	0.90	0.69 (0.15-3.22)	0.46
	high	17	0.63 (0.26-1.50)		0.36 (0.12-1.04)	
β-cryptoxanthin	low	18	0.69 (0.27-1.44)	0.86	0.97 (0.33-2.86)	**0.01**
	high	17	0.62 (0.29-1.63)		0.17 (0.05-0.53)	
Lutein	low	17	0.80 (0.33-1.91)	0.53	0.77 (0.22-2.65)	0.17
	high	18	0.55 (0.24-1.26)		0.28 (0.09-0.86)	
Zeaxanthin	low	17	0.56 (0.23-1.37)	0.63	0.59 (0.15-2.30)	0.55
	high	18	0.76 (0.32-1.78)		0.38 (0.13-1.08)	
Cis-lutein/zeaxanthin	low	18	1.05 (0.46-2.35)	0.09	0.76 (0.23-2.50)	0.16

	high	17	0.37 (0.15-0.91)		0.31 (0.11-0.86)	
Cis-lycopene	low	17	0.77 (0.34-1.75)	0.54	0.73 (0.25-2.15)	0.08
	high	18	0.52 (0.20-1.36)		0.43 (0.07-0.73)	
Trans-lycopene	low	17	0.77 (0.34-1.75)	0.54	0.89 (0.33-2.37)	**0.004**
	high	18	0.51 (0.18-1.42)		0.10 (0.03-0.37)	
Antioxidant score [e]	low	17	0.84 (0.36-1.96)	0.38	0.86 (0.32-2.25)	**0.003**
	high	18	0.51 (0.22-1.17)		0.14 (0.04-0.44)	

Abbreviation: PSA – prostate-specific antigen, CI – confidence interval

[a] Data are reported as least square means
[b] Categorized by median splits as less than median (low) versus greater than or equal to median (high).
[c] Adjusted for age, race randomized group and baseline PSA level.
[d] Adjusted for age, race, education, marital status, employment status, smoking status, Gleason score, body mass index, total metabolic equivalent (MET) per week of physical activity, energy intake, randomized group and baseline PSA level.
[e] Antioxidant score; low : 45 – 80, high: 81 –111.

§ *P* values from regression model comparing mean difference between low and high tocopherol/carotenoid categories

Table 4.6 Percent change in carotenoid and tocopherol levels from baseline to 3 months in relation to PSA levels at 3 months, adjusting for baseline PSA level

		PSA level at 3 months [a]				
			Means (95% CI)			
Change in plasma tocopherols and carotenoids from baseline to 3 months		n	Crude model [b]		Adjusted model [c]	
				P [§]		*P* [§]
α-tocopherol	Decrease	13	1.13 (0.80-1.59)	ref	0.84 (0.58-1.21)	ref
	Increase	21	0.73 (0.56-0.95)	**0.04**	0.47 (0.36-0.62)	**0.0007**
	Decrease	13	1.13 (0.53-1.03)	ref	0.88 (0.61-1.26)	ref
	Minimal increase (1-20%)	14	0.74 (0.53-1.03)	0.08	0.54 (0.37-0.77)	**0.008**
	Substantial increase (> 20%)	7	0.71 (0.45-1.12)	0.10	0.40 (0.26-0.61)	**0.004**
γ-tocopherol	Decrease	17	0.96 (0.69-1.32)	ref	0.56 (0.39-0.80)	ref
	Increase	17	0.77 (0.56-1.05)	0.34	0.52 (0.36-0.76)	0.73
	Decrease	17	0.95 (0.69-1.31)	ref	0.56 (0.39-0.80)	ref
	Minimal increase (1-20%)	8	0.70 (0.45-1.08)	0.25	0.50 (0.32-0.77)	0.64
	Substantial increase (> 20%)	9	0.84 (0.56-1.28)	0.66	0.55 (0.33-0.89)	0.93
α-carotene	Decrease	29	0.85 (0.67-1.09)	ref	0.52 (0.38-0.71)	ref
	Increase	5	0.88 (0.47-1.64)	0.92	0.67 (0.33-1.36)	0.50
	Decrease	29	0.85 (0.67-1.09)	ref	0.53 (0.38-0.73)	ref
	Minimal increase (1-20%)	3	0.91 (0.30-2.26)	0.39	0.79 (0.32-1.90)	0.38
	Substantial increase (> 20%)	2	0.83 (0.44-1.90)	0.53	0.54 (0.19-1.51)	0.98
Cis-β-carotene	Decrease	19	0.97 (0.75-1.03)	ref	0.92 (0.48-0.99)	ref

	Increase	15	0.76 (0.50-0.96)	0.29	0.79(0.33-0.87)	0.52
	Decrease	19	0.97 (0.75-1.03)	ref	0.90 (0.51-0.93)	ref
	Minimal increase (1-20%)	7	0.67 (0.41-1.09)	0.16	0.68 (0.15-0.86)	0.65
	Substantial increase (> 20%)	8	0.81 (0.46-1.07)	0.28	0.87(0.53-0.98)	0.94
Trans-β-carotene	Decrease	19	1.08 (0.82-1.42)	ref	0.72 (0.51-1.04)	ref
	Increase	15	0.63 (0.47-0.86)	**0.009**	0.44 (0.32-0.60)	**0.01**
	Decrease	19	1.08 (0.82-1.42)	ref	0.71 (0.51-0.99)	ref
	Minimal increase (1-20%)	9	0.63 (0.43-0.92)	**0.02**	0.34 (0.23-0.49)	**0.0005**
	Substantial increase (> 20%)	6	0.65 (0.40-1.08)	0.08	0.67 (0.42-1.06)	0.85
α-cryptoxanthin	Decrease	10	1.00 (0.68-1.48)	ref	0.73 (0.44-1.20)	ref
	Increase	24	0.79 (0.60-1.04)	0.33	0.51 (0.38-0.69)	0.16
	Decrease	10	1.01 (0.68-1.48)	ref	0.73 (0.45-1.18)	ref
	Minimal increase (1-20%)	5	0.64 (0.37-1.10)	0.18	0.37 (0.23-0.61)	**0.03**
	Substantial increase (> 20%)	19	0.84 (0.62-1.15)	0.49	0.60 (0.42-0.85)	0.45
β-cryptoxanthin	Decrease	18	0.85 (0.63-1.16)	ref	0.62 (0.43-0.89)	ref
	Increase	16	0.86 (0.61-1.20)	0.97	0.47 (0.32-0.67)	0.18
	Decrease	18	0.84 (0.62-1.14)	ref	0.62 (0.43-0.89)	ref
	Minimal increase (1-20%)	7	1.00 (0.61-1.64)	0.57	0.45 (0.26-0.79)	0.29
	Substantial increase (> 20%)	9	0.77 (0.50-1.18)	0.71	0.47 (0.31-0.72)	0.25
Lutein	Decrease	15	0.81 (0.59-1.12)	ref	0.47 (0.32-0.69)	ref
	Increase	19	0.90 (0.66-1.24)	0.65	0.59 (0.42-0.83)	0.29

	Decrease	15	0.82 (0.60-1.12)	ref	0.47 (0.32-0.69)	ref
	Minimal increase (1-20%)	6	0.65 (0.47-2.31)	0.22	0.64 (0.35-1.18)	0.37
	Substantial increase (> 20%)	13	0.77 (0.54-1.10)	0.80	0.58 (0.40-0.83)	0.33
Zeaxanthin	Decrease	22	0.82 (0.62-1.08)	ref	0.48 (0.35-0.67)	ref
	Increase	12	0.94 (0.64-1.37)	0.55	0.53 (0.46-0.99)	0.10
	Decrease	22	0.80 (0.61-1.05)	ref	0.47 (0.35-0.63)	ref
	Minimal increase (1-20%)	7	1.16 (0.71-1.87)	0.20	0.54 (0.40-1.00)	0.21
	Substantial increase (> 20%)	5	0.71 (0.41-1.23)	0.68	0.48 (0.29-0.77)	0.97
Cis-lutein/zeaxanthin	Decrease	11	0.81 (0.54-1.22)	ref	0.63 (0.39-1.00)	
	Increase	23	0.88 (0.66-1.16)	0.76	0.51 (0.38-0.71)	0.42
	Decrease	11	0.81 (0.54-1.22)	ref	0.63 (0.39-1.01)	ref
	Minimal increase (1-20%)	8	0.83 (0.53-1.29)	0.94	0.51 (0.33-0.79)	0.48
	Substantial increase (> 20%)	15	0.90 (0.64-1.28)	0.68	0.52 (0.36-0.74)	0.45
Cis-lycopene	Decrease	14	0.77 (0.53-1.11)	ref	0.55 (0.37-0.81)	ref
	Increase	19	0.93 (0.70-1.25)	0.40	0.54 (0.37-0.79)	0.97
	Decrease	14	0.76 (0.54-1.07)	ref	0.77 (0.44-1.36)	ref
	Minimal increase (1-20%)	8	0.73 (0.61-1.11)	0.87	0.59 (0.40-0.87)	0.40
	Substantial increase (> 20%)	11	0.70 (0.49-1.00)	0.75	0.48 (0.33-0.71)	0.34
Trans-lycopene	Decrease	14	0.79 (0.55-1.11)	ref	0.55 (0.37-0.83)	ref
	Increase	20	0.91 (0.68-1.21)	0.52	0.53 (0.38-0.75)	0.89
	Decrease	14	0.80 (0.57-1.12)	ref	0.56 (0.37-0.83)	ref
	Minimal increase (1-20%)	7	1.23 (0.76-1.99)	0.14	0.64 (0.39-1.06)	0.61

	Substantial increase (> 20%)	13	0.79 (0.56-1.10)	0.95	0.50 (0.34-0.72)	0.63
Antioxidant score [d]	Decrease	14	0.87 (0.55-0.35)	ref	0.64 (0.40-1.02)	ref
	Increase	19	0.85 (0.66-1.11)	0.96	0.51 (0.37-0.70)	0.37
	Decrease	14	0.85 (0.55-1.31)	ref	0.65 (0.42-1.01)	ref
	Minimal increase (1-20%)	8	1.07 (0.73-1.59)	0.43	0.73 (0.47-1.14)	0.69
	Substantial increase (> 20%)	11	0.71 (0.50-1.00)	0.53	0.43 (0.30-0.60)	0.10

Abbreviation: PSA – prostate-specific antigen, CI – confidence interval
[a] Data are reported as least square means and confidence intervals
[b] Adjusted for age, race, randomized group and baseline PSA level
[c] Adjusted for age, race, education, marital status, employment status, smoking status, Gleason score, body mass index, total metabolic equivalent (MET) per week of physical activity, energy intake, randomized group and baseline PSA level
[e] Antioxidant score; low : 45 – 80, high: 81 –111.

[§] *P* values from regression models comparing mean difference between decrease in tocopherol/carotenoid categories with an increase, minimal increase or substantial increase respectively

Table 4.7 Percent change in carotenoid and tocopherol levels from baseline to 3 months in relation to PSA levels at 6 months, adjusting for baseline PSA level

			PSA level at 6 months [a]			
			Means (95% CI)			
Change in plasma tocopherols and carotenoids from baseline to 3 months		n	Crude model [b]		Adjusted model [c]	
				P [§]		*P* [§]
α-tocopherol	Decrease	13	0.82 (0.63-1.06)	ref	0.89 (0.72-1.10)	ref
	Increase	21	0.63 (0.52-0.77)	0.11	0.51 (0.44-0.60)	**<0.0001**
	Decrease	13	0.81 (0.63-1.05)	ref	0.92 (0.74-1.13)	ref
	Minimal increase (1-20%)	14	0.66 (0.52-0.84)	0.25	0.55 (0.45-0.67)	**<0.0001**
	Substantial increase (> 20%)	7	0.57 (0.40-0.80)	0.09	0.45 (0.35-0.58)	**<0.0001**
γ-tocopherol	Decrease	17	0.70 (0.55-0.90)	ref	0.62 (0.48-0.79)	ref
	Increase	17	0.68 (0.55-0.85)	0.87	0.54 (0.42-0.71)	0.38
	Decrease	17	0.70 (0.55-0.89)	ref	0.62 (0.48-0.79)	ref
	Minimal increase (1-20%)	8	0.61 (0.45-0.84)	0.20	0.50 (0.37-0.68)	0.20
	Substantial increase (> 20%)	9	0.75 (0.56-1.00)	0.19	0.60 (0.44-0.84)	0.91
α-carotene	Decrease	29	0.71 (0.60-0.84)	ref	0.59 (0.47-0.73)	ref
	Increase	5	0.52 (0.32-0.85)	0.23	0.55 (0.33-0.94)	0.82
	Decrease	29	0.72 (0.61-0.85)	ref	0.62 (0.50-0.76)	ref
	Minimal increase (1-20%)	3	0.67 (0.37-1.24)	0.32	0.82 (0.44-1.54)	0.35
	Substantial increase (> 20%)	2	0.37 (0.19-0.74)	0.36	0.34 (0.17-0.67)	0.11
Cis-β-carotene	Decrease	19	0.84 (0.69-1.02)	ref	0.74 (0.61-0.88)	ref
	Increase	15	0.73 (0.44-0.87)	0.39	0.68 (0.34-0.82)	0.84
	Decrease	19	0.84 (0.70-1.02)	ref	0.75 (0.63-0.90)	ref

	Minimal increase (1-20%)	7	0.79 (0.42-0.89)	0.56	0.71 (0.36-0.89)	0.28
	Substantial increase (> 20%)	8	0.66 (0.39-0.78)	0.15	0.63 (0.29-0.86)	0.63
Trans-β-carotene	Decrease	19	0.88 (0.74-1.04)	ref	0.84 (0.69-1.03)	ref
	Increase	15	0.49 (0.41-0.60)	**<0.0001**	0.45 (0.38-0.54)	**<0.0001**
	Decrease	19	0.89 (0.75-1.04)	ref	0.84 (0.69-1.03)	ref
	Minimal increase (1-20%)	9	0.55 (0.43-0.72)	**0.002**	0.46 (0.37-0.58)	**<0.0001**
	Substantial increase (> 20%)	6	0.43 (0.32-0.58)	**<0.0001**	0.44 (0.34-0.57)	**<0.0001**
α-cryptoxanthin	Decrease	10	0.76 (0.56-1.02)	ref	0.90 (0.64-1.27)	ref
	Increase	24	0.67 (0.55-0.81)	0.48	0.77 (0.46-0.67)	0.39
	Decrease	10	0.76 (0.56-1.02)	ref	0.90 (0.65-1.26)	ref
	Minimal increase (1-20%)	5	0.66 (0.45-0.96)	0.56	0.69 (0.48-0.92)	0.43
	Substantial increase (> 20%)	19	0.67 (0.53-0.84)	0.51	0.64 (0.41-0.64)	0.18
β-cryptoxanthin	Decrease	18	0.62 (0.50-0.79)	ref	0.66 (0.52-0.84)	ref
	Increase	16	0.77 (0.61-0.97)	0.21	0.49 (0.37-0.65)	0.07
	Decrease	18	0.61 (0.49-0.76)	ref	0.67 (0.53-0.83)	ref
	Minimal increase (1-20%)	7	1.01 (0.74-1.38)	0.21	0.70 (0.47-1.04)	0.82
	Substantial increase (> 20%)	9	0.63 (0.48-0.82)	0.89	0.44 (0.33-0.58)	**0.009**
Lutein	Decrease	15	0.76 (0.60-0.96)	ref	0.60 (0.46-0.80)	ref
	Increase	19	0.63 (0.51-0.79)	0.27	0.68 (0.45-0.73)	0.74
	Decrease	15	0.76 (0.61-0.96)	ref	0.61 (0.45-0.81)	ref
	Minimal increase (1-20%)	6	0.78 (0.48-1.25)	0.95	0.75 (0.35-0.98)	0.75
	Substantial increase (> 20%)	13	0.60 (0.47-0.77)	0.16	0.63 (0.44-0.86)	0.77
Zeaxanthin	Decrease	22	0.61 (0.50-0.74)	ref	0.57 (0.45-0.70)	ref
	Increase	12	0.65 (0.49-1.09)	0.33	0.63 (0.48-0.89)	0.29
	Decrease	22	0.61 (0.50-0.74)	ref	0.55 (0.44-0.68)	ref

	Minimal increase (1-20%)	7	0.66 (0.58-1.29)	0.25	0.77 (0.54-1.16)	0.65
	Substantial increase (> 20%)	5	0.64 (0.53-1.09)	0.29	0.54 (0.37-0.80)	0.86
Cis-lutein/zeaxanthin	Decrease	11	0.84 (0.63-1.11)	ref	0.80 (0.60-1.07)	
	Increase	23	0.63 (0.52-0.77)	0.11	0.52 (0.42-0.64)	**0.003**
	Decrease	11	0.84 (0.64-1.11)	ref	0.78 (0.60-1.02)	ref
	Minimal increase (1-20%)	8	0.72 (0.52-0.98)	0.46	0.64 (0.49-0.83)	0.26
	Substantial increase (> 20%)	15	0.59 (0.47-0.75)	0.06	0.47 (0.38-0.57)	**0.0004**
Cis-lycopene	Decrease	14	0.77 (0.59-1.00)	ref	0.73 (0.57-0.94)	ref
	Increase	19	0.68 (0.55-0.85)	0.49	0.67 (0.39-0.86)	0.19
	Decrease	14	0.76 (0.59-0.99)	ref	0.78 (0.65-0.98)	ref
	Minimal increase (1-20%)	8	0.83 (0.60-1.14)	0.68	0.72 (0.56-1.01)	0.69
	Substantial increase (> 20%)	11	0.59 (0.45-0.78)	0.17	0.64 (0.34-0.82)	0.28
Trans-lycopene	Decrease	14	0.73 (0.57-0.93)	ref	0.69 (0.53-0.91)	ref
	Increase	20	0.67 (0.54-0.83)	0.59	0.53 (0.42-0.66)	0.07
	Decrease	14	0.73 (0.57-0.93)	ref	0.69 (0.55-0.87)	ref
	Minimal increase (1-20%)	7	0.76 (0.53-1.10)	0.85	0.73 (0.55-0.97)	0.72
	Substantial increase (> 20%)	13	0.63 (0.49-0.81)	0.39	0.45 (0.36-0.56)	**0.002**
Antioxidant score [d]	Decrease	14	0.81 (0.59-1.10)	ref	0.93 (0.72-1.21)	ref
	Increase	19	0.65 (0.54-0.79)	0.26	0.50 (0.42-0.60)	**<0.0001**
	Decrease	14	0.81 (0.59-1.10)	ref	0.92 (0.72-1.16)	ref
	Minimal increase (1-20%)	8	0.64 (0.47-0.87)	0.31	0.62 (0.50-0.78)	0.01
	Substantial increase (> 20%)	11	0.66 (0.52-0.84)	0.30	0.44 (0.37-0.53)	**<0.0001**

Abbreviation: PSA – prostate-specific antigen, CI – confidence interval
[a] Data are reported as least square means and confidence intervals
[b] Adjusted for age, race, randomized group and baseline PSA level
[c] Adjusted for age, race, education, marital status, employment status, smoking status, Gleason score, body mass index, total

metabolic equivalent (MET) per week of physical activity, energy intake, randomized group and baseline PSA level

[d] Antioxidant score; low : 45 – 80, high: 81 –111.

[§] *P* values from regression models comparing mean difference between decrease in tocopherol/carotenoid categories with an increase, minimal increase or substantial increase respectively

Table 4.8 Percent change in carotenoid and tocopherol levels from baseline to 3 months in relation to post-intervention PSA levels (at 3 months and at 6 months), adjusting for baseline PSA level — Mixed models

		Means (95% CI) [a]				
Change in plasma tocopherols and carotenoids from baseline to 3 months		n	Crude model [b]		Adjusted model [c]	
				P [§]		*P* [§]
α-tocopherol	Decrease	13	0.91 (0.73-1.13)	ref	0.84 (0.59-1.19)	ref
	Increase	21	0.72 (0.65-0.80)	0.08	0.47 (0.36-0.63)	**0.003**
	Decrease	13	0.91 (0.73-1.13)	ref	0.85 (0.60-1.21)	ref
	Minimal increase (1-20%)	14	0.74 (0.65-0.84)	0.13	0.52 (0.40-0.69)	**0.004**
	Substantial increase (> 20%)	7	0.67 (0.61-0.73)	**0.02**	0.39 (0.23-0.64)	**0.01**
γ-tocopherol	Decrease	17	0.83 (0.69-1.01)	ref	0.56 (0.38-0.82)	ref
	Increase	17	0.74 (0.66-0.84)	0.35	0.52 (0.35-0.76)	0.69
	Decrease	17	0.83 (0.68-1.01)	ref	0.56 (0.38-0.81)	ref
	Minimal increase (1-20%)	8	0.72 (0.59-0.87)	0.29	0.50 (0.35-0.72)	0.60
	Substantial increase (> 20%)	9	0.77 (0.69-0.86)	0.57	0.54 (0.32-0.90)	0.88
α-carotene	Decrease	29	0.85 (0.70-1.03)	ref	0.62 (0.44-0.89)	ref
	Increase	5	0.72 (0.65-0.80)	0.16	0.45 (0.30-0.69)	0.10
	Decrease	29	0.84 (0.70-1.01)	ref	0.56 (0.37-0.84)	ref
	Minimal increase (1-20%)	3	0.77 (0.69-0.86)	0.46	0.58 (0.39-0.87)	0.88
	Substantial increase (> 20%)	2	0.64 (0.57-0.73)	**0.04**	0.32 (0.18-0.60)	**0.01**
Cis-β-carotene	Decrease	19	0.87 (0.75-1.00)	ref	0.67 (0.47-0.96)	ref
	Increase	15	0.68 (0.61-0.76)	0.28	0.65 (0.28-0.68)	0.71
	Decrease	19	0.87 (0.75-1.00)	ref	0.67 (0.46-0.97)	ref
	Minimal increase (1-20%)	7	0.74 (0.66-0.84)	0.22	0.66 (0.24-0.88)	0.27

	Substantial increase (> 20%)	8	0.66 (0.58-0.75)	0.18	0.65 (0.34-0.76)	0.35
Trans-β-carotene	Decrease	19	0.90 (0.78-1.04)	ref	0.72 (0.50-1.05)	ref
	Increase	15	0.65 (0.59-0.72)	**0.002**	0.44 (0.32-0.61)	**0.01**
	Decrease	19	0.90 (0.77-1.03)	ref	0.69 (0.50-0.95)	ref
	Minimal increase (1-20%)	9	0.69 (0.60-0.79)	**0.03**	0.61 (0.41-0.91)	0.56
	Substantial increase (> 20%)	6	0.57 (0.47-0.69)	**<0.0001**	0.22 (0.09-0.53)	**0.02**
α-cryptoxanthin	Decrease	10	0.75 (0.68-0.83)	ref	0.50 (0.34-0.73)	ref
	Increase	24	0.84 (0.70-1.03)	0.26	0.60 (0.42-0.86)	0.36
	Decrease	10	0.75 (0.68-0.83)	ref	0.54 (0.35-0.82)	ref
	Minimal increase (1-20%)	5	0.91 (0.68-1.22)	0.22	0.72 (0.40-1.31)	0.25
	Substantial increase (> 20%)	19	0.74 (0.64-0.86)	0.19	0.48 (0.32-0.72)	0.71
β-cryptoxanthin	Decrease	18	0.78 (0.66-0.92)	ref	0.62 (0.40-0.96)	ref
	Increase	16	0.79 (0.72-0.87)	0.91	0.47 (0.31-0.70)	0.24
	Decrease	18	0.78 (0.66-0.92)	ref	0.63 (0.41-0.97)	ref
	Minimal increase (1-20%)	7	0.85 (0.73-0.99)	0.44	0.51 (0.29-0.89)	0.44
	Substantial increase (> 20%)	9	0.74 (0.65-0.83)	0.65	0.44 (0.29-0.66)	0.21
Lutein	Decrease	15	0.78 (0.70-0.86)	ref	0.47 (0.32-0.68)	ref
	Increase	19	0.79 (0.67-0.93)	0.83	0.59 (0.43-0.81)	0.16
	Decrease	15	0.77 (0.70-0.85)	ref	0.46 (0.29-0.73)	ref
	Minimal increase (1-20%)	6	0.91 (0.64-1.28)	0.38	0.62 (0.41-0.93)	0.35
	Substantial increase (> 20%)	13	0.71 (0.60-0.84)	0.32	0.55 (0.32-0.94)	0.27
Zeaxanthin	Decrease	22	0.84 (0.75-0.98)	ref	0.67 (0.46-0.97)	ref
	Increase	12	0.76 (0.64-0.94)	0.28	0.58 (0.42-0.88)	0.25
	Decrease	22	0.75 (0.66-0.86)	ref	0.64 (0.42-0.93)	ref

	Minimal increase (1-20%)	7	89 (0.77-1.04)	0.09	0.60 (0.33-0.78)	0.25
	Substantial increase (> 20%)	5	0.74 (0.65-0.84)	0.84	0.58 (0.43-0.99)	0.30
Cis-lutein/zeaxanthin	Decrease	11	0.79 (0.68-0.92)	ref	0.63 (0.42-0.94)	ref
	Increase	23	0.78 (0.68-0.90)	0.89	0.52 (0.36-0.75)	0.32
	Decrease	11	0.79 (0.68-0.92)	ref	0.63 (0.42-0.97)	ref
	Minimal increase (1-20%)	8	0.84 (0.68-1.03)	0.66	0.52 (0.36-0.74)	0.52
	Substantial increase (> 20%)	15	0.76 (0.65-0.90)	0.77	0.49 (0.25-0.94)	0.32
Cis-lycopene	Decrease	14	0.80 (0.68-0.94)	ref	0.54 (0.35-0.83)	ref
	Increase	19	0.76 (0.67-0.85)	0.56	0.54 (0.38-0.76)	0.94
	Decrease	14	0.80 (0.67-0.94)	ref	0.58 (0.39-0.87)	ref
	Minimal increase (1-20%)	8	0.75 (0.67-0.84)	0.30	0.74 (0.46-1.22)	0.39
	Substantial increase (> 20%)	11	0.70 (0.62-0.79)	0.15	0.47 (0.36-0.63)	0.24
Trans-lycopene	Decrease	14	0.78 (0.69-0.93)	ref	0.55 (0.35-0.86)	ref
	Increase	20	0.76 (0.68-0.85)	0.58	0.53 (0.38-0.75)	0.87
	Decrease	14	0.78 (0.69-0.93)	ref	0.59 (0.39-0.87)	ref
	Minimal increase (1-20%)	7	0.76 (0.68-1.85)	0.26	0.66 (0.54-1.81)	0.39
	Substantial increase (> 20%)	13	0.72 (0.55-1.09)	0.91	0.46 (0.33-0.66)	0.30
Antioxidant score [d]	Decrease	14	0.85 (0.74-0.97)	ref	0.64 (0.46-0.91)	ref
	Increase	19	0.65 (0.55-0.75)	0.01	0.41 (0.30-0.58)	**0.004**
	Decrease	14	0.85 (0.74-0.97)	ref	0.67 (0.50-0.89)	ref
	Minimal increase (1-20%)	8	0.62 (0.57-0.76)	0.06	0.62 (0.45-0.85)	0.29
	Substantial increase (> 20%)	11	0.58 (0.66-0.80)	**0.02**	0.31 (0.21-0.47)	**<0.0001**

Abbreviation: PSA – prostate-specific antigen, CI – confidence interval
[a] Data are reported as least square means and confidence intervals
[b] Adjusted for age, race, randomized group and baseline PSA level
[c] Adjusted for age, race, education, marital status, employment status, smoking status, Gleason score, body mass index, total

metabolic equivalent task (MET) per week of physical activity, energy intake, randomized group and baseline PSA level

[d] Antioxidant score; low : 45 – 80, high: 81 –111.

§ *P* value comparing mean difference between decrease in tocopherol/carotenoid categories with an increase, minimal increase or Substantial increase respectively

CHAPTER 5

DIETARY, SUPPLEMENT, AND ADIPOSE TISSUE TOCOPHEROL LEVELS IN RELATION TO PROSTATE CANCER AGGRESSIVENESS

5.1 INTRODUCTION

Prostate cancer (PCa) is the leading invasive malignancy and the second most fatal cancer in American men [385]. International variations in PCa incidence as well as changes in the disease risk patterns among migrant populations in Western countries indicate the importance of environmental factors in PCa, particularly the role of dietary factors [354, 386, 387]. Vitamin E, a fat-soluble antioxidant found in vegetable oils, seeds, nuts, leafy green vegetables and whole grains, contributes to the body's defenses against reactive oxygen species (ROS), which may play a role in PCa by causing oxidative DNA damage [117, 158, 388-390].

It has long been recognized that vitamin E, the collective name for eight naturally occurring compounds consisting of four tocopherols (i.e., α-, β-, γ- and δ-tocopherol) and corresponding four tocotrienols, has potent antioxidant properties that may inhibit carcinogenesis [158, 388, 391]. Studies examining associations between vitamin E and PCa have focused primarily on PCa incidence, but these yielded conflicting findings, including results from randomized controlled trials (reviewed in [64, 392-394]). Recently, there has been increasing awareness of the remarkable heterogeneity of PCa.

Owing to the widespread use of prostate-specific antigen (PSA) blood test for early detection, most newly diagnosed PCa cases are latent disease and often remain indolent over a lifetime, similar to those observed at autopsy [395, 396]. Few of these tumors progress aggressively (approximately 30%) and are associated with poorer prognosis [5, 6]. There is the possibility that vitamin E may have differential effect on aggressive PCa versus indolent disease, and thus, prior conflicting findings on PCa incidence may be due to mixing of different disease states [397].

Distinguishing the modifiable factors of virulent PCa from that of indolent disease is particularly important for addressing racial disparities in PCa as African Americans (AAs) have greater burden of virulent PCa compared to European Americans (EAs) [398]. Therefore, this study investigated whether higher intakes of tocopherols from diet and supplements (α-tocopherol equivalent), and higher adipose tissue tocopherol levels are inversely associated with PCa aggressiveness among AA and European American (EA) men.

5.2 MATERIALS AND METHODS

Study Population

The North Carolina-Louisiana Prostate Cancer Project (PCaP) is a population-based, cross-sectional, case-only, incident PCa study, designed to investigate racial and geographical differences in PCa aggressiveness. The methods and design of PCaP have been described [399]. Briefly, using a rapid case ascertainment system, men with a first diagnosis of histologically confirmed adenocarcinoma of the prostate were recruited in North Carolina (NC) and Louisiana (LA) between July 1, 2004 and August 31, 2009. Residents of North Carolina and Louisiana were eligible if they resided within the study

catchment areas, and were (1) between 40-79 years old at diagnosis; (2) self-identified race as AA/Black or Caucasian/White (EA); (3) able to complete study interview in English; (4) did not live in an institution (e.g., nursing home); and (5) were mentally and physically able to complete the interview. Written informed consents were obtained from all research subjects prior to participation. Approximately equal numbers of AAs and EAs were enrolled from NC (AAs n = 505; EAs n = 527) and LA (AAs n = 632; EA n = 603), with participation rates of 62% for NC, 72% for pre- and 63% for post-Hurricane Katrina Louisiana. The PCaP study protocols were approved by Institutional Review Boards (IRB) of the University of North Carolina at Chapel Hill, Louisiana State University Health Sciences Center, and the Department of Defense Prostate Cancer Research Program. The current analyses also were approved by the University of South Carolina IRB as exempt.

Data Collection

Consenting research subjects completed structured in-home interviews with trained research nurses who administered study questionnaires covering various information including demographics, pre-diagnostic PCa screening history, comorbidities, family health history, healthcare access, and behavioral factors such as physical activity and smoking status. The research nurses obtained anthropometric measurements (height and weight) using standard protocols. Medical records were obtained from diagnosing physicians and abstracted by trained personnel for information including cancer stage at diagnosis, Gleason sum and prostate-specific antigen (PSA) level at diagnosis. To ensure abstractor consistency, a random sample of the abstracted medical records (approximately 10%) were abstracted a second time by another staff

member. In PCaP, PCa aggressiveness is defined by a combination of Gleason sum, cancer stage and PSA level at diagnosis as (1) high aggressive (Gleason sum ≥8 or PSA >20 ng/mL, or Gleason sum ≥ 7 and cancer stage T3–T4); (2) low aggressive (Gleason sum < 7 and stage T1-T2 and PSA<10 ng/ml), and (3) intermediate aggressive PCa (all others). For the present analyses, a case-control study design was used to contrast research subjects with high aggressive PCa ("cases") to those with low/intermediate aggressive PCa (comparison group or "controls").

Dietary Assessment

The food frequency questionnaire was based on the National Cancer Institute Diet History Questionnaire (NCI-DHQ) modified to include Southern foods was used to assess food intake in the year prior to PCa diagnosis and included questions pertaining to frequency of intake, portion sizes and methods of food preparation for over 124 food items [400]. Responses to the questions were linked to an updated NCI nutrient database through which food compositions of α-, β-, γ-, and δ-tocopherol were estimated using the NCI Diet*Calc software [401].

Assessment of Dietary Supplement Use

Information on dietary supplement use was solicited via a validated questionnaire [323] administered by the research nurses during in-home visits. Data on supplemental vitamin E intake were derived from response to questions about the use of multivitamins containing vitamin E and use of single-nutrient vitamin E supplements. For multivitamins, research subjects were asked whether they had taken multivitamin supplements in the 12 months prior to PCa diagnosis (no, less than once a week, yes); and if yes, the frequency of use (1-2, 3-4, 5-6, 7 days/week). Forty-five percent of the

research subjects reported multivitamin supplement use in the previous 12 months, and were asked to identify the most often used brand from a list of common multivitamin brands in the U.S., which included an open-ended option for unlisted brands. Subsequently, these research subjects were asked to provide the multivitamin supplement bottle for recording of nutrient contents and dose. Research subjects who were unable to provide the multivitamin bottle (about 5% of users) were assigned the vitamin E dose listed on manufacturer label of the stated brand. When the manufacturer label could not be found (less than 1%), research subjects were assigned the vitamin E dose of the most commonly used brand among multivitamin supplement users; this value was 50 IU (i.e., from Centrum Silver). In subsequent questions, research subjects were asked about the use of single nutrient supplements; and if yes (13% of subjects), the frequency of use (same categories as above). Research subjects who were unable to provide the supplement bottle were asked to indicate the usual dose taken; dose choices for single-nutrient vitamin E supplements were 30, 100, 200, 400, 600 or 800 IU/day, and an open-ended option for unlisted dose. Research subjects who reported using single-nutrient vitamin E supplement but could not provide the supplement bottle or unable to report usual dose (4% of users) were assigned the mode dose (i.e., 400 IU) among single-nutrient vitamin E supplement users. Total vitamin E supplement intake was estimated as the sum of vitamin E from single-nutrient supplement and multivitamins, and converted as 1 IU = 0.45 mg of α-tocopherol [402]. Total α-tocopherol exposure was subsequently calculated as the sum of dietary α-tocopherol intake and total vitamin E supplement intake (i.e., diet + supplement).

Adipose Tissue Sampling and Analysis

Adipose tissue samples were obtained from the abdominal region of consenting research subjects who were not allergic to the local anesthesia solution (2% lidocaine). After the overlying skin was anesthetized, a 15-gauge needle was inserted into the subcutaneous fat and suction was applied using 15 ml vacutainer tube. The aspirated tissue was trapped in the needle and luer lock adapter, which was placed in separate cryovials for transportation. The collected samples were transported on ice to the assigned storage facility within 24 hours of collection and stored at -80^{o}C. The samples were later transported on ice to Craft Technologies, Incorporated in Wilson, NC for high-performance liquid chromatography (HPLC) analysis. The average time between sample collection and storage was 24 hours, and average time from storage to analysis was 6 months. Adipose tissue concentrations of α-, γ- and δ-tocopherol were expressed as mcg per gram of tissue at tocopherols detection limit of 0.07 mcg/g.

Statistical Methods

The analytic population was drawn from 2,173 PCaP research subjects with data on PCa aggressiveness. Prior to data analysis, research subjects with implausibly low or high daily caloric intake (< 500 or > 6,000 kcals, n = 71) were excluded, leaving a final study sample of 2,102 (AAs n = 1,023, EAs n = 1,079). Of these research subjects, data on adipose tissue tocopherol levels were available for 945 subjects (AA n = 361, EAs n = 584).

Descriptive statistics were compared by level of PCa aggressiveness as means (continuous variables) and proportions (categorical variables) using *t* and χ^2 tests, respectively. All tocopherol exposure variables were categorized into quartiles, separately

for AAs and EAs, based on distribution among low/intermediate aggressive cases in the respective race group. Hence, analyses were conducted separately for AAs and EAs. The decision to categorize the exposures separately by race was informed by preliminary analysis indicating different dietary and supplement use patterns between AAs and EAs. Unconditional logistic regression was used to estimate crude (age-adjusted) and multivariable-adjusted odds ratios (ORs) and 95% confidence interval (CIs).

In selecting the multivariable-adjusted models, the following variables were considered as potential confounders based on review of the literature: pre-diagnostic PSA screening history (0, 1-7, >7 screenings); family history of PCa (number of affected first degree relative: none *vs*. at least one); prevalence of comorbidities (Charlson Comorbidity Index: 0, 1, 2, ≥3); whether PCa treatment had started at time of interview (yes, no); smoking status (never, former, current); education (less than high school education, high school graduate/vocational school, some college/college graduate, graduate degree); annual household income (< $20,000, $20,001 - $40,000, $40,001 - $60,000, $60,001 - $80,000, >$80,000, unknown); multivitamin use in the year prior to diagnosis (yes, no); non-steroidal anti-inflammatory drug (NSAID) use in the five years prior to diagnosis (yes, no); physical activity in the year prior to diagnosis [total metabolic equivalents (METs) of light, moderate and vigorous exercise categorized as: ≤ 10.2, 10.3-29.0, > 29.0 METs/week]; body mass index (BMI: kg/m^2, continuous); study site (NC, LA); energy intake (kcal/day); dairy intake (servings/day); and alcohol intake (grams/day). These variables were first examined for confounding effect (i.e., ≥10% change in effect estimates of each exposure variable with age in the model). Next, variables determined to be confounders and those that are biologically relevant to PCa

were placed in an elaborate model simultaneously for final model selection using a combination of the backward elimination method and likelihood ratio tests to remove one variable at a time. Through this process, the following variables were included in the final adjusted model for analysis of dietary tocopherols and vitamin E supplement use associations: age (continuous), pre-diagnostic PSA screening history, BMI, smoking status, education, income, NSAIDs use, dietary fat intake, and study site. Additional adjustment of family history of PCa, comorbidities and PCa treatment status were done for associations of adipose tocopherol levels and PCa aggressiveness. Tests for linear trend (P_{trend}) were performed by modeling the median values of each tocopherol category as continuous variable. Family history of PCa, pre-diagnostic PSA screening history, BMI and NSAIDs use were examined for potential effect modification by assessing stratum-specific ORs in stratified multivariable analyses, and including evaluation of interaction terms between these factors and the main exposures using likelihood ratio tests. All analyses were performed with SAS version 9.3 (Cary, NC, USA) with statistical significance set at $\alpha = 0.05$ (two-tailed).

5.3 RESULTS

Differences in distribution of research subject characteristics are presented by level of PCa aggressiveness separately for AAs and EAs in Table 5.1. AA subjects with high aggressive PCa were slightly older, had higher intakes of energy and dietary fat, included a greater proportion of current smokers and lower incomes, more often reported no PSA screening prior to diagnosis, but less often reported vitamin E supplement use compared to those with low/intermediate aggressive disease. EA subjects with high aggressive PCa were older, had slightly higher BMI, higher proportion had started PCa

treatment by start of study and more often reported vitamin E supplement use compared to those with low/intermediate aggressive PCa. In both AAs and EAs, research subjects with high aggressive PCa had lower educational level than those with low/intermediate aggressive PCa.

Table 5.2 presents mean difference in tocopherol levels and supplemental vitamin E intake by race and by level of PCa aggressiveness. Overall, AA subjects tended to have higher dietary intakes of γ- and δ-tocopherol but lower intakes of supplemental vitamin E and total α-tocopherol compared to EAs. Mean adipose α-tocopherol level was 75% higher in EAs than to AAs. While no differences in dietary, supplement or adipose tocopherol levels were observed by the level of PCa aggressiveness among EAs, AA subjects with low/intermediate aggressive PCa had higher intakes of supplemental vitamin E and total α-tocopherol compared to their counterparts with high aggressive PCa.

Multivariable-adjusted ORs for high aggressive PCa were estimated by quartiles of dietary tocopherols and supplemental vitamin E intake with lower quartiles as the referent group (Table 5.3). No significant associations were observed among AAs, although there were some suggestive inverse associations, particularly in the highest quartiles of dietary α-tocopherol and vitamin E supplement intake. Among EA subjects, a dose-response inverse association was observed between dietary α-tocopherol intake and PCa aggressiveness, showing 66% lower odds of high aggressive PCa in the highest quartile. However, neither vitamin E supplement intake nor total α-tocopherol intake was associated with PCa aggressiveness among EAs. Dietary δ-tocopherol intake also was

inversely and linearly associated with PCa aggressiveness among EAs. A nearly statistically significant inverse associations was observed in the highest quartile of β-tocopherol intake (OR = 0.56, 95% CI = 0.30-1.02). There also was a nearly significant trend for lower odds of high aggressive PCa with increasing consumption of γ-tocopherol among EAs (P_{trend} = 0.05).

Research subjects with and without data on adipose tocopherol levels did not differ substantially. In sensitivity analyses, similar associations were observed between dietary tocopherol intake and PCa aggressiveness among research subjects with and without data on adipose tocopherols (Table 5.5 and 5.6). Adipose tissue tocopherol levels also were categorized and analyzed separately for AAs and EAs (Table 5.4). While none of the associations was statistically significant, higher adipose α-tocopherol level appeared to be inversely associated with high aggressive PCa among AAs (OR = 0.66, 95% CI = 0.27-1.62, highest *vs*. lowest quartile), but positively associated among EAs (OR = 1.43, 95% CI = 0.66-3.11, highest *vs*. lowest quartiles). Evaluations of potential modifying effects of family history of PCa, pre-diagnostic PSA screening history, smoking status, BMI and NSAIDs use did not show effect modification by these factors (data not shown).

5.4 DISCUSSION

In this population-based, case-only, study of PCa aggressiveness, higher dietary intake of α- and δ-tocopherol was inversely associated with high aggressive PCa among EAs. Nearly statistically significant inverse associations also were observed between higher dietary intake of γ- and β-tocopherol, and high aggressive PCa among EAs. None

of the dietary tocopherols was associated with PCa aggressiveness among AAs. Similarly, no significant association was observed for supplemental and total (diet and supplement) α-tocopherol intake among AAs or EAs. Interestingly, there was a suggestion that a higher adipose tissue α-tocopherol level was inversely associated with high aggressive PCa among AAs, but positively associated among EAs.

Tocopherols are thought to have strong chemopreventive properties that may protect against PCa by preventing or mitigating the deleterious effects of oxidative stress, specifically oxidative damage to DNA, proteins and lipids [158, 388, 391, 394]. Many reports indicate that tocopherols interact with a variety of ROS, notably peroxyl radicals, to form relatively innocuous compounds, thereby mitigating oxidative stress [388, 403]. Other proposed anticarcinogenic properties of tocopherols include enhancing the immune system's surveillance and destruction of tumor cells, regulation of genes involved in tumor cell growth, inhibition of protein kinase C, modulation of apoptosis and cell cycle signaling pathways, and down-regulating inflammatory responses [158, 164, 403, 404]. However, clinical trials investigating the efficacy of supplemental α-tocopherol intake for the prevention of PCa also have yielded contradictory results with some showing beneficial effect [159], no benefits [74, 405], and even possible harm [164, 406]. In particular, the Alpha Tocopherol Beta-Carotene (ATBC) Cancer Prevention Trial, originally designed to investigate lung cancer incidence, reported a 32% reduced risk of PCa and 41% decreased mortality from PCa among Finnish male smokers taking 50 mg/day of supplemental vitamin E (α-tocopherol) over 5-8 years compared to placebo [159]. In contrast, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) reported a 17% increased risk of PCa among healthy males taking 400 IU/day of α-

tocopherol over a 7-year median follow-up time compared to placebo [164]. Two other clinical trials have reported null associations between vitamin E supplementation and PCa incidence [74, 405]. The epidemiologic data relating to associations of tocopherols intake and PCa incidence also are equivocal (reviewed in [64, 393, 394]).

Data on tocopherols from diet, supplements, and adipose tissue provide complementary information about the role of tocopherols in PCa; however, as shown in this analysis, they can yield mixed results because these data are different measures of tocopherol status. While dietary and supplement use questionnaires can provide estimates of usual intake patterns, typically in the recent past; they do not reflect day-to-day variations or longer periods of intake [384]. On the other hand, fat-soluble antioxidants are known to selectively accumulate in human adipose tissue and turn to turn over at a low rate [407]. Thus, adipose tissue serves as an objective marker of tocopherol status and can quantify systemic exposure over longer periods, although influenced by individual differences in absorption and metabolism [290, 407].

The mean α-tocopherol intake level (10.5 mg/day) in this study population is comparable to that of a study conducted among AAs and EAs in NC [408]. The inverse association between increased dietary intake of α-tocopherol and PCa aggressiveness also concurs with previous literature on PCa incidence [64, 393, 409]. It was somewhat surprising that although AAs and EAs had similar dietary intakes of α-tocopherol (Tables 4.2.2 and 4.2.3); α-tocopherol was not associated with PCa aggressiveness among AAs. This discrepancy may be explained, at least in part, by differences in food sources of α-tocopherol between the two groups. Exploratory analysis showed that a greater

proportion of EAs in the highest quartile of the dietary α-tocopherol had higher intake of plant-based foods containing high amounts of α-tocopherol such as nuts, seeds, olive oils and other healthy food sources of α-tocopherol. By contrast, AAs in this category tended to consume higher amounts of foods from less healthy sources of α-tocopherol, particularly processed foods containing high amounts of saturated fat including potato and corn chips, and dark green vegetables prepared with fatback and lard. Besides racial difference in dietary patterns, there is also the possibility of gene-nutrient interactions involving polymorphisms in genes that regulate α-tocopherol activity [410], those implicated in PCa aggressiveness [131, 411] or both, which may vary by race.

Despite the strong inverse association for dietary α-tocopherol among EAs, supplemental vitamin E and total α-tocopherol intake from both diet and supplements were not associated with PCa aggressiveness among EAs or AAs. Epidemiologic studies regarding vitamin E supplement use and PCa incidence have often reported null results [412-414]; few have reported protective associations but this has been limited to smokers [68, 170] who may have greater need for vitamin E because of increased exposure to ROS from tobacco smoke [415]. In the present study, however, subgroup analysis did not show effect modification by smoking status, which may have been limited by small sample size especially since analyses were stratified by race. The lack of significant associations for total α-tocopherol may be because research subjects who consumed high amounts of α-tocopherol from diet may have consumed low amounts from supplements or *vice versa*, which would lead to classification differences into low and higher quartiles when dietary and supplemental intakes were combined into one category.

The nearly significant inverse association in the highest quartile of dietary β-tocopherol and nearly significant inverse linear trend for γ-tocopherol among EAs suggest a potential beneficial role for these tocopherols or food sources of these tocopherols in PCa aggressiveness. Dietary intakes of γ-tocopherol were actually higher than α-tocopherol; consistent with the general observation that the amounts of γ-tocopherol in American diet are higher than that of α-tocopherol [416]. Nonetheless, blood concentrations of α-tocopherol are about ten times higher that γ-tocopherol, which has been attributed to the preferential transfer of α-tocopherol to the blood by the hepatic α-tocopherol transfer protein (α-TTP) [388, 417]. Thus, perhaps higher intakes of the other tocopherols may be needed to increase their bioavailability and subsequent antioxidant activity. Alternatively, α-tocopherol may have more potent anticarcinogenic properties than the other tocopherols [391].

There was a suggestion that higher adipose α-tocopherol levels were inversely associated with high aggressive PCa among AAs, but positively associated among EAs. A possible explanation for these seemingly conflicting results is the significant difference in adipose α-tocopherol levels among AAs and EAs. EAs had a 75% higher mean adipose α-tocopherol level than AAs (Table 5.2). It is unclear what constitutes “normal” adipose tocopherol levels. Mean α-tocopherol levels in EAs in PCaP were slightly higher than those reported in breast tissue from Malaysian women in a previous study [418] and lower than those reported in adipose tissue from European males in the EURAMIC study [419]. It is reasonable to speculate that long-term use of dietary supplements (the most common source of α-tocopherol) may have been the major

contributor of the adipose α-tocopherol levels among EAs, especially since a much greater proportion of EAs reported vitamin E supplement use compared to AAs. This speculation could not be verified due to lack of data on long-term duration of supplement use in PCaP.

Although the mechanisms by which higher physiological levels of α-tocopherol may be influencing PCa aggressiveness have yet to be clarified, laboratory studies suggest that α-tocopherol may have dual function as an antioxidant and as a pro-oxidant such that at very high levels, α-tocopherol tends to exhibit pro-oxidant properties that promote oxidative stress [420]. A recent study in mouse model suggest that α-tocopherol supplementation in nutritionally replete organisms can promote cancer cell proliferation by suppressing the expression of p53, a major tumor suppressor gene, which can lead to cancer cell escape from apoptosis [421]. These reports suggest that a fine balance between ROS and antioxidants is needed to maintain intracellular homeostasis, and provide mechanistic support to the finding in SELECT where α-tocopherol supplementation in healthy men was associated with increased risk of PCa [164, 406].

Notable strengths the present study includes its design to measure PCa aggressiveness, which minimizes potential confounding by disease heterogeneity (i.e., the mixing of indolent and aggressive disease). The evaluation of three complementary measures of tocopherol intake allowed for a comprehensive assessment of tocopherol status in PCa aggressiveness. Additionally, the assessment of individual tocopherols rather than the mixing of tocopherols and tocotrienols helps delineate the role of each tocopherol in PCa aggressiveness. The use of an ethnically diverse population with

approximately equal numbers of AAs and EAs also made it possible to explore whether associations between tocopherols and PCa aggressiveness differed by race. Moreover, the potential for selection bias and selective survival were minimized because participation rates were reasonably high at both study sites and research subjects were recruited shortly after diagnosis via rapid case-ascertainment; an average of five months from the time of diagnosis to time of interview.

The following limitations are also worth consideration. Imprecise measurements of dietary tocopherols could have influenced the study results to some extent. Because exposure assessment for tocopherols were done independent of the extent of PCa aggressiveness, differential misclassification bias is unlikely; however, non-differential exposure misclassification may have occurred, resulting in underestimation of ORs and failure to show modest associations [422]. Diet was assessed using a food frequency questionnaire. It is known that these structured instruments may be biased according to response sets [423], which in turn, may be related to psychological traits that either may exert a direct effect on cancer outcomes or indirectly affect other factors that may influence carcinogenesis [424]. There is also the concern that adipose tocopherol levels may be altered by the presence of a tumor; however, a study examining the effect of breast tumor proximity on breast adipose tocopherol levels did not find significant differences in adipose tocopherol levels at different quadrants of breast tissue, including sites proximal and distal to the tumor [425]. Moreover, although adipose tocopherol levels are good markers for internal dose, they may not reflect prostatic tocopherol levels. Thus, results should be interpreted with this in mind. Other limitations include the failure to control for cholesterol levels, in particularly, low density lipoprotein which function as

transport vehicles for tocopherols [388] and abdominal adiposity which may influence the adipose tocopherol levels. Nonetheless, this might have been indirectly considered by adjusting for total dietary fat intake and BMI. The influence of individual differences in metabolism and absorption, interactions between individual tocopherols compounds and other micronutrients, as well as potential modifying effects of genetic variants acting via similar mechanisms [383, 407, 426] were beyond the scope of this study. The possibility exists that some of the findings may be spurious owing to the sample size and multiple testing.

5.5 CONCLUSIONS

In summary, dietary intakes of α- and δ-tocopherol were inversely associated with PCa aggressiveness among EAs. There was no evidence that vitamin E supplement use protects against high aggressive PCa. However, higher adipose α-tocopherol levels appear to be inversely associated with high aggressive PCa among AAs, but positively associated among EA which may be due to a significantly higher adipose α-tocopherol level among EAs. Future work with larger samples and involving evaluation of interaction between measures of tocopherol intake and functional gene polymorphisms in oxidative stress and DNA repair pathways may help to elucidate the etiologic relevance of tocopherols on PCa aggressiveness.

Table 5.1 Distribution of demographic and patient characteristics by race and prostate cancer aggressiveness among men in the North Carolina – Louisiana Prostate Cancer Project (PCaP)

Characteristics	**African Americans** n = 1,023					**European Americans** n = 1,079				
	High aggressive (n=206)		Low/intermediate aggressive (n=817)		P ‡	High aggressive (n=164)		Low/intermediate aggressive (n=915)		P ‡
	Mean (SD)		Mean (SD)			Mean (SD)		Mean (SD)		
Age, years	63 (8)		62 (8)		0.004	67 (8)		64 (8)		<0.0001
Energy Intake, kcals/day	2799.6 (1232.4)		2593.0 (1146.0)		0.02	2339.3 (952.0)		2320.5 (865.7)		0.80
Dietary fat intake, grams/day	103.9 (52.1)		94.8 (48.4)		0.02	94.5 (42.4)		91.1 (39.2)		0.31
Body mass index (BMI), kg/m^2	29.9 (6.7)		29.2 (5.4)		0.16	30.5 (5.1)		29.0 (4.8)		0.0006
	N	%	N	%		N	%	N	%	
Study Site										
NC	92	45	386	47	0.51	73	45	448	49	0.29
LA (pre & post Katrina)	114	55	431	53		91	55	467	51	
Family History of Prostate Cancer										
No affected 1st degree relative	157	76	606	74	0.55	136	83	696	76	0.05
At least 1 affected 1st degree relative	49	24	211	26		28	17	219	24	
Prostate Cancer Screening History										
0 screenings	120	58	307	38	<0.0001	40	24	153	17	0.06
1-7 screenings	53	26	338	41		68	42	405	44	
> 7 screenings	33	16	172	21		56	34	357	39	
Comorbidities										
0	88	43	382	47	0.39	84	51	503	55	0.07

1	53	26	216	26		31	19	214	23	
2	36	17	106	13		29	18	98	11	
≥ 3	29	14	113	14		20	12	100	11	
Started PCa treatment at start of study										
No	20	10	112	14	0.20	11	7	99	11	0.007
Yes	163	79	599	73		146	89	720	79	
Unknown	23	11	106	13		7	4	96	10	
Education										
Graduate/professional degree	6	3	59	7	0.004	29	18	197	22	0.01
Some college or college graduate	50	24	239	29		71	43	377	41	
High school grad or voc/tech school	65	32	275	34		37	23	260	28	
Less than high school education	85	42	243	30		27	16	81	9	
Income Level										
≤ $20, 000	82	40	234	29	0.001	24	15	78	9	0.22
$20,001 - $40,000	52	25	212	26		33	20	184	20	
$40,001 - $60,000	20	10	132	16		24	15	154	17	
$60,001 - $80,000	12	6	70	8		20	12	124	13	
>$80,000	14	7	99	12		47	29	298	33	
Unknown	26	13	70	8		16	9	77	8	
Smoking Status										
Never	40	19	276	34	<0.0001	59	36	330	36	0.76
Former smokers	107	52	390	48		87	53	501	55	
Current smokers	59	29	151	18		18	11	84	9	
NSAID Use										
No	84	41	364	45	0.33	56	34	305	33	0.85
Yes	120	59	446	55		108	66	608	67	
Vitamin E Supplement Use [a]										
No	141	68	488	60	0.02	65	40	396	43	0.38

Yes	65	32	329	40		99	60	519	57	

Prostate cancer aggressiveness defined by a combination of Gleason sum, clinical stage, and PSA level at diagnosis and classified as follows: High aggressive (Gleason sum ≥8 or PSA >20ng/ml or Gleason sum ≥7 AND clinical stage T3 -T4); Low /Intermediate aggressive: all other cases.

[a] Both single nutrient vitamin E supplements and multivitamins containing vitamin E.
Abbreviations: PCa – Prostate Cancer; SD – Standard deviation; NC –North Carolina LA – Louisiana; NSAIDs – Nonsteroidal anti-inflammatory drugs.

[‡]Test for differences between low/intermediate and high aggressive cancers were done using Student's t-test for continuous variables and chi-square tests for categorical variables.

Table 5.2 Mean difference in tocopherol intake from diet and supplements, and adipose tissue tocopherol levels by race and level of prostate cancer aggressiveness

	African Americans n = 1,023	European Americans n = 1,079	% diff	African Americans			European Americans		
				High aggressive n = 206	Low/intermediate Aggressive n = 817	P †	High aggressive n = 164	Low/intermediate Aggressive n = 915	P †
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Dietary intake									
α-tocopherol (mg/day)	10.6 (5.9)	10.5 (5.3)	1	10.9 (6.5)	10.5 (5.8)	0.43	10.1 (5.3)	10.6 (5.3)	0.29
β-tocopherol (mg/day)	0.4 (0.2)	0.4 (0.2)	0	0.4 (0.3)	0.4 (0.2)	0.13	0.4 (0.2)	0.4 (0.2)	0.35
γ-tocopherol (mg/day)	21.0 (11.3)	18.6 (8.6)	11 ‡	22.2 (11.7)	20.7 (11.2)	0.09	18.6 (9.1)	18.6 (8.5)	0.95
δ-tocopherol (mg/day)	3.0 (1.7)	2.7 (1.5)	10 ‡	3.2 (1.8)	2.9 (1.7)	0.05	2.7 (1.5)	2.7 (1.5)	0.58
Supplemental vitamin E [a] (α-tocopherol, mg/day)	53.5 (82.4)	76.0 (100.4)	- 42 ‡	37.8 (54.2)	56.6 (86.6)	0.02	64.7 (94.2)	78.2 (101.4)	0.22
Total α-tocopherol (diet + supplement) mg/day	31.2 (57.8)	54.1 (85.2)	- 73 ‡	22.8 (36.1)	33.3 (61.9)	0.002	49.2 (79.6)	55.0 (86.2)	0.42
Adipose tissue biomarkers [b]									
α-tocopherol (mcg/g)	86.6 (144.9)	151.4 (198.0)	- 75 ‡	78.9 (138.0)	88.3 (146.6)	0.63	163.6 (221.3)	149.2 (193.7)	0.53
γ-tocopherol (mcg/g)	48.4 (74.1)	53.3 (54.5)	- 10	38.2 (38.2)	50.8 (79.9)	0.06	55.4 (56.5)	52.9 (54.1)	0.69
δ-tocopherol (mcg/g)	9.3 (10.2)	8.6 (7.8)	7	8.6 (9.9)	9.4 (10.3)	0.55	8.2 (6.6)	8.7 (8.0)	0.55

[a] Among vitamin E supplement users only (African Americans, n = 394; European Americans, n = 618). Converted as 1 IU of Vitamin E = 0.45 mg α-tocopherol [402].

[b] Among research subjects with data on adipose tocopherol levels (African Americans, n = 361; European Americans, n = 584).

‡ Significant p-values (< 0.05) for test of difference between African Americans and European Americans

† Chi-square test for difference by level of prostate cancer aggressiveness

Table 5.3 Associations between dietary and supplemental vitamin E intake and prostate cancer aggressiveness among African Americans (n = 1,023) and European Americans (n = 1,079)

			Crude [a]			Adjusted [b]		
		Cases/ controls [c]	OR	95% C.I.	P §	OR	95% C.I.	P §
Dietary α-tocopherol mg/day (median)								
African Americans								
	Q1: 0.51 - 6.37 (4.82)	51/205	1.00	(ref)	0.35	1.00	(ref)	0.20
	Q2: 6.38 - 9.25 (7.72)	42/204	0.86	0.55-1.35		0.73	0.43-1.22	
	Q3: 9.26 - 13.32 (10.85)	59/204	1.21	0.79-1.85		1.08	0.62-1.86	
	Q4: 13.33 - 44.62 (17.13)	54/204	1.13	0.73-1.74		0.58	0.28-1.19	
European Americans								
	Q1: 1.67 - 7.02 (5.55)	54/229	1.00	(ref)	0.18	1.00	(ref)	0.006
	Q2: 7.03 - 9.54 (8.35)	35/229	0.66	0.41-1.06		0.54	0.32-0.91	
	Q3: 9.55 - 12.79 (11.01)	39/229	0.77	0.49-1.22		0.51	0.29-0.88	
	Q4: 12.80 - 53.18 (16.28)	36/228	0.68	0.43-1.09		0.34	0.17-0.69	
Vitamin E supplements [d] α-tocopherol, mg/day (median)								
African Americans								
	Q1: non-users	141/488	1.00	(ref)	0.01	1.00	(ref)	0.15
	Q2: 1.13 – 11.25 (10.13)	24/91	0.95	0.58-1.55		1.09	0.65-1.84	
	Q3: 11.26 – 22.50 (22.50)	27/136	0.69	0.44-1.09		0.83	0.51-1.34	
	Q4: 22.51 – 472.50 (180.00)	14/102	0.48	0.26-0.87		0.64	0.34-1.21	
European Americans								
	Q1: non-users	65/396	1.00	(ref)	0.26	1.00	(ref)	0.38
	Q2: 0.96 – 20.25 (13.50)	37/178	1.24	0.79-1.94		1.40	0.87-2.24	
	Q3: 20.26 – 45.00 (22.50)	37/172	1.25	0.80-1.95		1.41	0.88-2.27	
	Q4: 45.01 – 540.00 (193.50)	25/169	0.83	0.50-1.37		0.93	0.55-1.58	
Total α-tocopherol Diet + supplement, mg/day (median)								
African Americans								
	Q1: 0.52 – 9.14 (6.64)	51/205	1.00	(ref)	0.27	1.00	(ref)	0.36

	Q2: 9.15 – 15.83 (11.93)	60/204	1.24	0.81-1.89		1.27	0.78-2.06	
	Q3: 15.84 – 28.54 (20.71)	52/204	1.07	0.69-1.65		0.88	0.50-1.52	
	Q4: 28.55 – 482.37 (42.87)	43/204	0.87	0.55-1.37		0.89	0.51-1.58	
European Americans								
	Q1: 2.40 – 11.40 (8.04)	43/229	1.00	(ref)	0.47	1.00	(ref)	0.55
	Q2: 11.41 – 23.34 (15.56)	39/228	0.94	0.58-1.51		0.85	0.50-1.42	
	Q3:23.35 – 39.69 (31.55)	44/230	0.98	0.62-1.56		0.99	0.59-1.65	
	Q4:39.70 – 558.17 (190.37)	38/228	0.84	0.52-1.36		0.83	0.49-1.40	
Dietary β-tocopherol mg/day (median)								
African Americans								
	Q1: 0.03 - 0.25 (0.19)	53/208	1.00	(ref)	0.05	1.00	(ref)	0.22
	Q2: 0.26 - 0.37 (0.31)	42/204	0.85	0.54-1.33		0.92	0.56-1.50	
	Q3: 0.38 - 0.52 (0.44)	46/206	0.91	0.58-1.41		1.04	0.62-1.77	
	Q4: 0.53 - 1.73 (0.69)	65/199	1.03	0.92-2.12		1.08	0.73-2.59	
European Americans								
	Q1: 0.07 - 0.28 (0.22)	53/242	1.00	(ref)	0.44	1.00	(ref)	0.09
	Q2: 0.29 - 0.38 (0.33)	37/239	0.71	0.44-1.12		0.64	0.39-1.05	
	Q3: 0.39 - 0.52 (0.45)	37/217	0.79	0.50-1.26		0.65	0.39-1.11	
	Q4: 0.53 - 1.62 (0.63)	37/217	0.79	0.50-1.26		0.56	0.30-1.02	
Dietary γ-tocopherol mg/day (median)								
African Americans								
	Q1: 0.03 - 12.37 (9.07)	46/204	1.00	(ref)	0.08	1.00	(ref)	0.59
	Q2: 12.38 - 18.55 (15.48)	47/205	1.08	0.68-1.70		1.02	0.61-1.71	
	Q3: 18.56 - 27.04 (22.78)	51/204	1.14	0.73-1.78		0.94	0.53-1.69	
	Q4: 27.05 - 67.95 (33.75)	62/204	1.44	0.94-2.22		0.83	0.39-1.80	
European Americans								
	Q1: 2.68 - 12.66 (9.83)	39/230	1.00	(ref)	0.82	1.00	(ref)	0.05
	Q2: 12.67 - 17.35 (15.14)	47/228	1.26	0.79-2.01		1.14	0.68-1.91	
	Q3: 17.36 - 22.85 (19.57)	41/229	1.10	0.68-1.78		0.77	0.43-1.37	
	Q4: 22.86 - 55.13 (28.34)	37/228	1.00	0.61-1.64		0.52	0.24-1.13	

Dietary δ-tocopherol mg/day (median)								
African Americans								
	Q1: 0.08 - 1.63 (1.22)	41/206	1.00	(ref)	0.04	1.00	(ref)	0.69
	Q2: 1.64 - 2.56 (2.11)	52/204	1.33	0.84-2.10		1.25	0.75-2.08	
	Q3: 2.57 - 3.71 (3.09)	48/204	1.21	0.76-1.93		0.92	0.52-1.65	
	Q4: 3.72 - 13.09 (4.92)	65/203	1.64	1.06-2.54		0.97	0.47-1.98	
European Americans								
	Q1: 0.34 - 1.69 (1.31)	36/231	1.00	(ref)	0.29	1.00	(ref)	0.007
	Q2: 1.70 - 2.37 (2.05)	56/229	1.64	1.03-2.60		1.40	0.85-2.30	
	Q3: 2.38 - 3.46 (2.84)	43/228	1.24	0.76-2.01		0.91	0.52-1.60	
	Q4: 3.47 - 10.92 (4.29)	29/227	0.89	0.52-1.50		0.45	0.21-0.95	

Cases: high aggressive prostate cancers; Controls: low and intermediate aggressive cancers

[a] Adjusted for age

[b] additional adjustment for PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[c] Some of the categories may not sum to the total sample size due to missing data

[d] Converted as 1 IU of Vitamin E = 0.45 mg α-tocopherol [402].

§ Trend *P* value

Table 5.4 Associations between adipose tissue tocopherol levels and prostate cancer aggressiveness among African (n = 361) and European (n = 584) Americans.

			Crude [a]			Adjusted [b]		
		Cases/ Controls [c]	OR	95% C.I.	p (trend)	OR	95% C.I.	p (trend)
α-tocopherol mcg/g (median)								
African Americans								
	Q1: 0.30 - 14.50 (6.45)	18/74	1.00	(ref)	0.41	1.00	(ref)	0.60
	Q2: 14.51 - 36.30 (24.10)	13/73	0.74	0.33-1.63		0.58	0.24-1.39	
	Q3: 36.56 - 108.90 (61.12)	22/74	1.18	0.58-2.41		0.98	0.44-2.16	
	Q4: 108.91 - 1313.10 (183.27)	13/73	0.68	0.31-1.50		0.66	0.27-1.62	
European Americans								
	Q1: 0.20 - 26.50 (12.50)	16/123	1.00	(ref)	0.71	1.00	(ref)	0.68
	Q2: 26.51 - 78.80 (45.60)	26/123	1.54	0.78-3.04		1.54	0.74-3.23	
	Q3: 79.81 - 204.70 (125.70)	26/123	1.39	0.70-2.76		1.58	0.75-3.33	
	Q4: 204.71 - 1585.60 (328.90)	21/123	1.12	0.55-2.27		1.43	0.66-3.11	
γ-tocopherol mcg/g (median)								
African Americans								
	Q1: 0.10 - 10.4 0 (5.29)	17/73	1.00	(ref)	0.60	1.00	(ref)	0.77
	Q2: 10.41- 28.90 (18.10)	19/72	1.21	0.58-2.54		1.03	0.45-2.35	
	Q3: 28.91- 64.10 (41.70)	15/72	0.86	0.40-1.87		0.72	0.31-1.69	
	Q4: 64.11 - 972.90 (92.42)	15/72	0.90	0.42-1.96		0.93	0.40-2.16	
European Americans								
	Q1: 0.20 - 13.40 (6.20)	16/122	1.00	(ref)	0.79	1.00	(ref)	0.91
	Q2: 13.41 - 37.90 (23.90)	28/122	1.70	0.87-3.32		1.39	0.68-2.83	
	Q3: 37.91 - 72.08 (52.10)	27/122	1.59	0.81-3.12		1.18	0.57-2.46	
	Q4: 72.34 - 318.70 (110.74)	19/122	1.14	0.55-2.33		1.20	0.56-2.55	
δ-tocopherol mcg/g (median)								
African Americans								
	Q1: 0.40 - 3.20 (2.00)	14/68	1.00	(ref)	0.45	1.00	(ref)	0.73

	Q2: 3.21 - 6.43 (4.66)	20/67	1.39	0.64-2.99		1.46	0.63-3.36	
	Q3: 6.44 - 11.54 (8.71)	14/67	0.97	0.43-2.20		1.17	0.48-2.85	
	Q4: 11.55 - 101.40 (17.80)	12/67	0.86	0.37-2.01		1.01	0.40-2.55	
European Americans								
	Q1: 0.30 - 2.99 (1.60)	17/117	1.00	(ref)	0.98	1.00	(ref)	0.81
	Q2: 3.00 - 6.70 (4.60)	26/119	1.37	0.70-2.68		1.20	0.58-2.49	
	Q3: 6.71 - 11.60 (8.69)	23/115	1.28	0.64-2.55		1.10	0.52-2.32	
	Q4: 11.61- 54.11 (16.10)	19/116	1.12	0.55-2.29		0.99	0.46-2.13	

[a] Adjusted for age
[b] Additional adjustment for education level, study site, BMI, smoking history, family history of PCa, PSA screening history, total fat intake, whether treatment started at time of interview, and comorbidities.
[c] Some of the categories may not sum to the total sample size due to missing data.

Table 5.5 Comparison of demographic and clinical attributes of prostate cancer between research subjects included and those excluded from the adipose tissue tocopherol and prostate aggressiveness analysis

	Included n = 945		**Excluded** n = 1157	
	Mean (SD)		Mean (SD)	
Age, years	63.4 (7.9)		63.1 (7.8)	
Energy Intake, kcals/day	2461.2 (1018.8)		2486.0 (1057.8)	
Dietary fat intake, grams/day	94.9 (44.4)		93.4 (44.9)	
Body mass index (BMI), kg/m^2	29.9 (5.2)		28.8 (5.3)	
	N	%	N	%
Race				
African American	361	38	662	57
European American	584	62	495	43
Prostate cancer aggressiveness				
Low aggressive	485	51	589	51
High aggressive	304	32	354	31
Intermediate aggressive	156	16	214	18
Study Site				
NC	407	43	592	51
LA (pre & post Katrina)	538	57	565	49
Family History of Prostate Cancer				
No affected 1st degree relative	696	74	899	78
At least 1 affected 1st degree relative	249	26	258	22
Prostate Cancer Screening History				
0 screenings	248	26	372	32
1-7 screenings	375	40	489	42
> 7 screenings	322	34	296	26
Comorbidities				
0	497	53	560	49
1	224	24	290	25
2	105	11	164	14
≥ 3	119	12	143	12
Started PCa treatment at start of study				
No	95	10	147	13
Yes	749	79	879	76
Unknown	101	11	131	11
Education				
Graduate/professional degree	155	17	136	12
Some college or college graduate	351	37	386	33
High school grad or voc/tech school	268	28	369	32
Less than high school education	171	18	265	23
Income Level				
≤ $20, 000	156	16	262	23

$20,001 - $40,000	188	20	293	25
$40,001 - $60,000	148	16	182	16
$60,001 - $80,000	124	13	102	9
>$80,000	233	25	225	19
Unknown	96	10	93	8
Smoking Status				
Never	355	37	350	30
Former smokers	471	50	614	53
Current smokers	119	13	193	17
NSAID Use				
No	361	38	448	39
Yes	584	62	698	61
Vitamin E Supplement Use [a]				
No	474	50	616	53
Yes	471	50	541	47

Prostate cancer aggressiveness defined by a combination of Gleason sum, clinical stage, and PSA level at diagnosis and classified as follows: High aggressive (Gleason sum ≥8 or PSA >20ng/ml or Gleason sum ≥7 AND clinical stage T3 -T4); Low /Intermediate aggressive: all other cases.

[a] Includes single nutrient vitamin E supplement and multivitamins containing vitamin E.

Abbreviations: PCa – Prostate Cancer; SD – Standard deviation; NC –North Carolina; LA – Louisiana; NSAIDs – Nonsteroidal anti-inflammatory drugs.

[‡] Student's *t* test for continuous variables and chi-square tests for categorical variables

Table 5.6 (Sensitivity Analysis**)** Associations between dietary vitamin E intake and prostate cancer aggressiveness among African Americans (n = 361) and European Americans (n = 584) with data on adipose tissue tocopherol levels.

			Crude [a]			Adjusted [b]		
		Cases/ controls [c]	OR	95% C.I.	P §	OR	95% C.I.	P §
Dietary α-tocopherol mg/day (median)								
African Americans								
	Q1: 0.51 - 6.37 (4.82)	15/69	1.00	ref	0.15	1.00	ref	0.37
	Q2: 6.38 - 9.25 (7.72)	11/78	0.69	0.29-1.61		0.50	0.19-1.32	
	Q3: 9.26 - 13.32 (10.85)	21/79	1.29	0.61-2.73		0.76	0.28-2.09	
	Q4: 13.33 - 44.62 (17.13)	19/69	1.44	0.67-3.11		0.43	0.11-1.64	
European Americans								
	Q1: 1.67 - 7.02 (5.55)	23/126	1.00	ref	0.65	1.00	ref	0.11
	Q2: 7.03 - 9.54 (8.35)	23/124	1.04	0.55-1.96		0.86	0.42-1.76	
	Q3: 9.55 - 12.79 (11.01)	23/113	1.17	0.62-2.22		0.84	0.38-1.85	
	Q4: 12.80 - 53.18 (16.28)	21/131	0.86	0.45-1.64		0.46	0.17-1.25	
Vitamin E supplements [d] α-tocopherol, mg/day (median)								
African Americans								
	Q1: non-users	45/185	1.00	ref	0.31	1.00	ref	0.65
	Q2: 1.13 – 11.25 (10.13)	8/28	1.32	0.55-3.12		1.04	0.40-2.70	
	Q3: 11.26 – 22.50 (22.50)	8/48	0.70	0.31-1.60		0.76	0.31-1.83	
	Q4: 22.51 – 472.50 (180.00)	5/34	0.61	0.22-1.66		0.79	0.27-2.34	
European Americans								
	Q1: non-users	36/208	1.00	ref	0.45	1.00	ref	0.46
	Q2: 0.96 – 20.25 (13.50)	17/94	1.09	0.58-2.06		1.22	0.62-2.42	
	Q3: 20.26 – 45.00 (22.50)	23/102	1.24	0.69-2.22		1.46	0.77-2.78	
	Q4: 45.01 – 540.00 (193.50)	14/90	0.83	0.42-1.63		0.89	0.43-1.85	
Total α-tocopherol Diet + supplement, mg/day (median)								
African Americans								
	Q1: 0.52 – 9.14 (6.64)	16/74	1.00	ref	0.93	1.00	ref	0.59
	Q2: 9.15 – 15.83 (11.93)	17/81	1.05	0.49-2.25		0.81	0.33-1.97	
	Q3: 15.84 – 28.54 (20.71)	18/67	1.40	0.65-3.01		0.85	0.31-2.34	
	Q4: 28.55 – 482.37 (42.87)	15/73	1.03	0.47-2.25		0.71	0.25-2.03	
European Americans								
	Q1: 2.40 – 11.40 (8.04)	24/122	1.00	ref	0.65	1.00	ref	0.60
	Q2: 11.41 – 23.34 (15.56)	16/123	0.71	0.36-1.41		0.57	0.26-1.23	
	Q3:23.35 – 39.69 (31.55)	29/124	1.11	0.61-2.04		1.11	0.56-2.20	
	Q4:39.70 – 558.17 (190.37)	21/125	0.82	0.43-1.57		0.74	0.35-1.57	
Dietary β-tocopherol mg/day (median)								
African Americans								
	Q1: 0.03 - 0.25 (0.19)	16/71	1.00	ref	0.16	1.00	ref	0.77
	Q2: 0.26 - 0.37 (0.31)	10/75	0.63	0.27-1.50		0.61	0.24-1.57	
	Q3: 0.38 - 0.52 (0.44)	19/69	1.36	0.64-2.90		1.25	0.51-3.10	
	Q4: 0.53 - 1.73 (0.69)	21/80	1.39	0.66-2.92		1.02	0.35-2.99	
European Americans								
	Q1: 0.07 - 0.28 (0.22)	26/131	1.00	ref	0.71	1.00	ref	0.16
	Q2: 0.29 - 0.38 (0.33)	22/128	0.85	0.45-1.59		0.67	0.34-1.33	
	Q3: 0.39 - 0.52 (0.45)	21/114	0.93	0.49-1.76		0.72	0.34-1.52	
	Q4: 0.53 - 1.62 (0.63)	21/121	0.86	0.45-1.62		0.50	0.21-1.20	
Dietary γ-tocopherol mg/day (median)								
African Americans								
	Q1: 0.03 - 12.37 (9.07)	15/70	1.00	ref	0.14	1.00	ref	0.38

	Q2: 12.38 - 18.55 (15.48)	12/73	0.84	0.37-1.95		0.68	0.26-1.74	
	Q3: 18.56 - 27.04 (22.78)	17/75	1.18	0.54-2.56		0.69	0.25-1.90	
	Q4: 27.05 - 67.95 (33.75)	22/77	1.56	0.74-3.30		0.52	0.14-1.90	
European Americans								
	Q1: 2.68 - 12.66 (9.83)	20/127	1.00	ref	0.62	1.00	ref	0.29
	Q2: 12.67 - 17.35 (15.14)	21/119	1.23	0.63-2.40		1.12	0.54-2.35	
	Q3: 17.36 - 22.85 (19.57)	29/134	1.49	0.79-2.79		0.98	0.44-2.18	
	Q4: 22.86 - 55.13 (28.34)	20/114	1.17	0.59-2.31		0.58	0.19-1.75	
Dietary δ-tocopherol mg/day (median)								
African Americans								
	Q1: 0.08 - 1.63 (1.22)	11/72	1.00	ref	0.02	1.00	ref	0.67
	Q2: 1.64 - 2.56 (2.11)	15/74	1.52	0.64-3.57		1.25	0.49-3.19	
	Q3: 2.57 - 3.71 (3.09)	15/77	1.38	0.59-3.23		0.95	0.33-2.72	
	Q4: 3.72 - 13.09 (4.92)	25/72	2.60	1.17-5.77		1.39	0.39-4.92	
European Americans								
	Q1: 0.34 - 1.69 (1.31)	18/128	1.00	ref	0.95	1.00	ref	0.21
	Q2: 1.70 - 2.37 (2.05)	25/121	1.62	0.83-3.15		1.37	0.65-2.86	
	Q3: 2.38 - 3.46 (2.84)	31/128	1.85	0.97-3.50		1.57	0.73-3.37	
	Q4: 3.47 - 10.92 (4.29)	16/117	1.08	0.52-2.24		0.57	0.20-1.61	

Cases: high aggressive prostate cancers; Controls: low and intermediate aggressive cancers

[a] Adjusted for age

[b] additional adjustment for PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[c] Some of the categories may not sum to the total sample size due to missing data

[d] Converted as 1 IU of Vitamin E = 0.45 mg α-tocopherol [402].

CHAPTER 6

CAROTENOIDS INTAKE AND ADIPOSE TISSUE CAROTENOID LEVELS IN RELATION TO PROSTATE CANCER AGGRESSIVENESS

6.1 INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed non-dermatological malignancy among men in Western countries [427]. Accumulated data on the relation between diet and cancer indicates that about 30-40% of cancer cases are preventable through healthy diet and weight control [428, 429]. Greater intake of fruits and vegetables has been associated with reduced risk of various types of cancer, including PCa [430-432]. Carotenoids are biologically active phytochemicals commonly found in fruits and vegetables, and they are thought to contribute to the inverse associations between fruits and vegetables intake and cancer incidence [433, 434]. However, findings from case-control and cohort studies summarized in recent reviews [17, 434-436], suggest that the pattern of association between carotenoids intake and PCa is largely unclear.

β-carotene and lycopene are the most commonly studied carotenoids, with lycopene, a carotenoid devoid of vitamin A activity, having the most favorable association with PCa [437-440], although study results are not entirely consistent [413, 441, 442]. Early studies focused primarily on β-carotene, a pro-vitamin A carotenoid; however, two large intervention trials failed to show a beneficial effect of β-carotene on

PCa incidence in secondary data analyses [159, 188].however, two large intervention trials failed to show a beneficial effect of β-carotene on PCa incidence in secondary data analyses [159, 188]. One reported a 23% increased risk of PCa among β-carotene intervention group versus placebo [159], and the other, which examined effects of β-carotene and retinol in tandem because of their close metabolic relationship, found a 52% increased risk of aggressive PCa (Gleason ≥ 7 or stage III/IV) in the intervention group compared with placebo [188]. The elevated risks associated with β-carotene supplementation were not evident in follow-up studies [188, 443], and the majority of observational studies have conflicted on β-carotene associations with PCa [17, 434-436, 444-447]. Carotenoids such as α-carotene, β-cryptoxanthin, lutein, and zeaxanthin have been associated with modest reductions in PCa risk, but as with lycopene and β-carotene, the study results are mixed [189, 448-451].

There is limited data regarding associations between carotenoids intake and PCa aggressiveness. As suggested by Giovannucci et al. [437], the dietary risk factors for aggressive PCa may differ from that of a non-aggressive disease, and thus, some carotenoids may differentially influence aggressive versus non-aggressive PCa. Given the growing interest in identifying modifiable risk factors for PCa, particularly among African Americans (AAs), a population with a high incidence of aggressive PCa [452], this study investigated associations of dietary, supplemental and adipose tissue carotenoid levels in relation to PCa aggressiveness among AA and European-American (EA) men in North Carolina and Louisiana.

6.2 MATERIALS AND METHODS

Study Population

A population-based, case-control study was conducted using data from the North Carolina-Louisiana Prostate Cancer Project (PCaP). One of the primary aims of PCaP, a multidisciplinary, cross-sectional, case-only, incident PCa study, was to investigate and compare factors associated with PCa aggressiveness among AAs and EAs. Residents of the study catchment areas in North Carolina and Louisiana were eligible to participate in PCaP if they had a first, histologically confirmed, diagnosis of adenocarcinoma of the prostate between July 1, 2004 and August 31, 2009, were 40-79 years of age at the time of diagnosis, and self-identified their race as AA/Black or Caucasian American/White (EA). The other eligibility criteria were having sufficient cognitive and physical functions to consent and complete the study interview in English, and not residing in an institution (e.g., nursing home). PCaP enrolled 2267 research subjects of whom approximately half were EAs (n= 1130) and half were AAs (n = 1137). All research subjects provided written informed consent before participating in the study. Participation rates were 62% in North Carolina, 72% for pre-Hurricane Katrina Louisiana and 63% for post- Hurricane Katrina Louisiana. Further details of the PCaP methods and design can be found elsewhere [399]. The PCaP study protocols were approved by Institutional Review Boards of all collaborating institutions, and the current study also received institutional approval from the University of South Carolina.

Data Collection

Structured in-person interviews were conducted by trained research nurses, usually in the home of the research subject or at a place of his choosing, soliciting various

information that included demographic and socioeconomic factors, personal health history, family history of PCa, pre-diagnostic PCa screening habits, smoking history, physical activity, usual dietary intake, and use of dietary supplements and non-steroidal anti-inflammatory drugs (NSAIDs). The research nurses measured each research subject's height, and weight at the end of each interview using a standardized protocol. Information on the cancer stage, Gleason grade and prostate-specific antigen (PSA) level at the time of diagnosis and other health data including comorbid conditions and disease-directed treatments were extracted from the research subjects' medical records which were obtained from diagnosing physicians after receiving consent. The medical record abstractions were performed by trained personnel and included a double abstraction of a randomly selected sample (approximately 10%) to ensure consistency between abstractors. PCa aggressiveness is defined in PCaP as high aggressive (Gleason sum ≥8 or PSA >20 ng/mL or Gleason sum ≥7 and cancer stage T3–T4), low aggressive (Gleason sum < 7 and cancer stage T1-T2 and PSA<10 ng/ml), and intermediate aggressive (all others). These categories were used in case-control analyses contrasting high aggressive PCa "cases" with low/intermediate aggressive PCa as the comparison group or "controls".

Dietary Assessment

Dietary carotenoid intakes were assessed using the National Cancer Institute Diet History-Food Frequency Questionnaire (NCI-DHQ) [453], which was modified to include Southern foods. The modified, 124-item DHQ solicited information about usual diet in the year before the diagnosis of PCa, including frequency of food intake, portion size, and food preparation methods. Responses to the questions were linked to an updated

NCI nutrient database through which the research subjects' usual daily intakes of various nutrients including α- and β-carotene, β-cryptoxanthin, lutein-zeaxanthin and lycopene were estimated using the NCI Diet*Calc software [401].

Data on supplemental carotenoid intake were derived using a questionnaire that has been tested for reliability [454]. The research subjects were asked about multivitamin and single-nutrient supplement use in the year preceding their diagnosis of PCa (no, less than once, yes) and those who answered "yes" were queried about the frequency of use (1-2, 3-4, 5-6, 7 days/week). Responses to the questionnaire were recorded by the nurse interviewers who also undertook an inventory of nutrient contents and listed dose information from the manufacturer label of each supplement type. When the supplement bottle was not available, subjects were asked to state the usual dose taken. Average daily intakes of supplemental β-carotene, lutein and lycopene were subsequently estimated based on contributions from multivitamin and single-nutrient supplements as frequency (days per week) x dose (in μg) x number of pills taken at each time / 7 [454]. Total daily intake of β-carotene, lutein and lycopene were estimated as the sum of intakes from diet and supplement (diet + supplement).

Adipose Tissue Sampling and Analysis

Approximately two grams of subcutaneous abdominal adipose tissue samples were obtained from consenting research subjects after anesthetizing the overlying skin with 2% lidocaine solution. The PCaP research nurses, who were specifically trained for adipose tissue sampling, followed a standardized procedure involving the insertion of a 15-gauge needle into the subcutaneous fat and applying negative pressure by a 15 ml

vacutainer tube after prepping the overlying skin. The aspirated tissue was trapped in the needle and luer lock adapter, which was placed in a separate cryovial and transported on ice immediately after collection to a designated storage facility where aliquots were prepared and stored at -80^{o}C until assayed. Individual carotenoids were measured by high performance liquid chromatography at the Nutrition Analyses Laboratory of Craft Technologies, Incorporated (Wilson, NC) using methods outlined by Craft et al. [328, 329]. The adipose tissue contents of α-carotene, cis- and trans-β-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein, zeaxanthin, and cis- and trans-lycopene were quantified at a minimum detection limit of 0.003 μg/g of tissue.

Statistical Methods

Before any analysis was performed, research subjects with incomplete data on PCa aggressiveness (n = 94) and those with implausible values for energy intake (< 500 or ≥ 6000 kcal/day, n = 71) were excluded from the total PCaP sample of 2267. The remaining 2,102 research subjects were included in the analyses; however, data on adipose carotenoid levels were available for only 939 cases (EAs n = 581, AAs n = 358).

Descriptive statistics were expressed as means for continuous variables and proportions for categorical variables using *t*-tests and chi-square tests, respectively. The carotenoid variables were categorized into tertiles according to their distributions among controls, and unconditional logistic regression was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) for increasing tertiles with the lowest tertiles as the referent group. Trend tests were performed by assigning each tertile its median value expressed as a continuous variable in the logistic regression models. All

associations were examined in crude (age-adjusted) and multivariable-adjusted models. The following known or suspected risk factors for PCa were considered for inclusion in the multivariable-adjusted models: age (continuous), study site (NC, LA); BMI (in kg/m^2); pre-diagnostic PSA screening history (0, 1-7, >7 screenings); comorbidities (0, 1, 2, ≥3); family history of PCa in a first degree relative (none *vs*. at least one); whether PCa treatment had started at the time of the interview (yes, no); smoking status (never, former, current); education (less than high school education, high school graduate/some college, college graduate); annual household income (< $20,000, $20,001 - $40,000, $40,001 - $70,000, >$70,000); NSAIDs use in the five years prior to diagnosis (yes, no); physical activity in the year prior to diagnosis [total metabolic equivalents (METs) of light, moderate, and vigorous exercise categorized as: ≤ 10.2, 10.3-29.0, > 29.0 METs/week]; total fat intake (grams/day); and alcohol intake (grams/day). The multivariable models were constructed first by evaluating the confounding effect of each variable based on a 10% change in effect estimate of the main exposure variables with the removal of the covariate from the model. Variables determined to be confounders and those that are biologically relevant to PCa were then placed in an elaborate model for final model selection. A combination of the backward elimination model selection method and likelihood ratio tests were then used to select covariates for the final models consisting of age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total fat intake, and study site. Further adjustment for family history of PCa, comorbidities, and PCa treatment status were done in models examining associations between adipose carotenoid levels and PCa aggressiveness.

Stratified analyses by BMI (< 24.9, 25-29.9, ≥ 30 kg/m2) and smoking status were performed to evaluate whether the associations differed by these factors. In order to retain enough sample size for the stratified analyses, the carotenoid variables were categorized into two levels (< or ≥ median) with the lowest levels as the referent group. The evaluation of effect modification included interaction terms between the BMI and smoking status variables, and each of the carotenoids (median splits), which were examined by likelihood ratio tests based on models with and without an interaction term. All statistical tests were two-sided and a *P* value less than 0.05 was considered statistically significant except for interaction p-values in the stratified analyses, which were considered significant at a *P* value less than 0.10. All analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC).

6.3 RESULTS

Characteristics of the research subjects are presented for AAs and EAs in Table 6.1. In both AAs and EAs, the research subjects with high aggressive PCa were older and less educated compared to those with low/intermediate aggressive PCa. EA research subjects with high aggressive PCa had a slightly higher BMI and were more likely to have started treatment for PCa compared to EAs with low/intermediate aggressive PCa. The AA research subjects with high aggressive PCa tended to have a higher intake of energy and total fat, were less likely to have had at least one pre-diagnostic PSA screening, and included a greater proportion of current and former smokers and low incomes compared to AAs with low/intermediate aggressive PCa.

Table 6.2 presents differences in carotenoid levels among AAs and EAs. In general, the daily intake of carotenoids varied significantly between AAs and EAs, such

that while EAs tended to have higher intakes of α-carotene, supplemental β-carotene and lutein, and higher intake of lycopene from diet and supplements, AAs had a higher dietary intake of β-carotene, β-cryptoxanthin and lutein + zeaxanthin. Adipose tissue carotenoid levels were generally higher in EAs than AAs with significant differences in the levels of zeaxanthin and lycopene (*cis* and *trans*). Few differences in carotenoid intake or adipose levels were observed by the levels of PCa aggressiveness among EAs and AAs.

Multivariable-adjusted ORs and corresponding 95% CIs for high aggressive PCa in relation to dietary and supplemental carotenoids intake are reported in Table 6.3. Because of the substantial differences in carotenoids intake between AAs and EAs, different cut-points were used to categorize each carotenoid by race, based on distributions among low/intermediate aggressive PCa research subjects in the respective race group. Hence, results are presented separately for AAs and EAs. Among EAs, dietary lycopene intake was associated with a 45% lower odds of high aggressive PCa in the highest compared with the lowest tertile. Although supplemental lycopene use was not independently associated with PCa aggressiveness among EAs, total lycopene intake from both diet and supplements was inversely related to high aggressive PCa (OR = 0.56, 95% CI = 0.34-0.90, highest versus lowest tertile, P_{trend} = 0.03). These significant associations were not observed among AAs. However, dietary β-cryptoxanthin intake was inversely associated with high aggressive PCa among AAs only (OR = 0.56, 95% CI = 0.36-0.87, highest versus lowest tertile, P_{trend} = 0.01). None of the other carotenoids was significantly associated with PCa aggressiveness among AAs or EAs.

Although data on adipose tissue carotenoid levels were available for a subgroup of the study population, this group did not differ substantially from the total study sample with regard to demographic and other variables (Table 6.5). Evaluation of associations between adipose tissue carotenoid levels and PCa aggressiveness showed a marginally significant linear trend toward lower odds of high aggressive PCa for the associations of adipose α-carotene (P_{trend} = 0.07) and lycopene (*cis* + *trans*, P_{trend} = 0.11) (Table 6.4). No apparent associations were observed between adipose carotenoid levels and PCa aggressiveness among AAs. To examine the impact of missing data on the observed associations, an alternative analysis of associations between dietary carotenoids intake and PCa aggressiveness was conducted among subjects with data on adipose carotenoids only. These results were very similar to those reported in Table 6.3 (see Table 6.6).

In the stratified analyses, the associations between all measured carotenoids and PCa aggressiveness did not vary by smoking status (data not shown). However, there were significant effect modifications by BMI for the associations of carotenoids intake and PCa aggressiveness (Table 6.7). Notably, there was suggestion of reduced odds of high aggressive PCa among EAs who were supplemental β-carotene users in the highest BMI category ($\geq$ 30 kg/m^2) but increased odds for supplemental β-carotene use was observed among EAs in the lowest BMI category (< 24.9 kg/m^2). Among both AAs and EAs, a similar interaction was observed for dietary and total lutein + zeaxanthin intake and BMI(all *P* values for interaction, < 0.10). Among EAs, it appeared that the reduced odds of high aggressive PCa with higher dietary lycopene was lowest among obese research subjects (*P* value for interaction =0.01); whereas among AAs, a significant interaction between BMI and dietary and total lycopene was observed such that increased

intake of lycopene was associated with increased odds of high aggressive PCa among research subjects in the normal weight BMI category but not in the other BMI categories.

6.4 DISCUSSION

This population-based, case-control study examined associations between carotenoid intake and adipose tissue carotenoid levels in relation to PCa aggressiveness among AAs and EAs in North Carolina and Louisiana. Inverse associations were observed between intake of lycopene and PCa aggressiveness among EAs, and between β-cryptoxanthin intake and PCa aggressiveness among AAs. Marginally significant linear trends in the direction of reduced odds of high aggressive PCa were observed for higher adipose levels of α-carotene and lycopene (*cis* + *trans*) among EAs only. Evaluation of effect modification by BMI indicated that men with higher BMI ($\geq$ 30 kg/m^2) may have a greater benefit from a higher intake of certain carotenoids, while higher lycopene intake among AAs and use of β-carotene supplements among EAs was associated with increased odds of high aggressive PCa among normal weight research subjects but not overweight or obese subjects.

Carotenoids are broadly categorized as pro-vitamin A (i.e., α-carotene, β-carotene, and β-cryptoxanthin) or non-pro-vitamin A (i.e., lutein, zeaxanthin and lycopene) depending on whether they are converted into retinol in the body [455]. These carotenoids have been shown in *in vitro* and *in vivo* studies to have biological functions that could prevent or suppress the progression of cancer [456]. Proposed mechanisms by which carotenoids may influence PCa aggressiveness include induction of the apoptosis of malignant cells, modulation of gene expression, up-regulation of gap-junctional

communication, mitigation of oxidative stress, and enhancement of antitumor immune responses [455-457]. Despite these mechanisms, the epidemiologic data relating to carotenoid intake and PCa incidence are largely inconsistent [17, 434-436]. The populations included in previous studies were predominantly of European decent, thus, the results may not apply to AAs. It is worth noting that although the current study shows some differences in carotenoid associations between AAs and EAs, comparisons were made within each race. This analytic approach minimizes confounding by unmeasured sociocultural factors, and possibly, biological factors that are inherently different between AAs and EAs [452, 458].

The current finding on lycopene among EAs is consistent with previous studies suggesting that lycopene may be beneficial in reducing the risk and aggressiveness of PCa. In a prospective cohort study of male health professionals, higher lycopene intake was associated a 21% lower risk of PCa and a high intake of tomato and tomato products, which are primary sources of lycopene, also was association with a 53% reduced risk of advance-staged PCa [449]. Gann et al. [438] found a lower risk of aggressive PCa in men with high plasma lycopene levels. In another prospective study, Kirsh et al. [441] reported an inverse association between lycopene intake and PCa incidence among men with a family history of PCa. Reports from some case-control studies suggest that lycopene may reduce the risk of PCa [450, 459], although others have failed to show an association [413, 460-462]. Lycopene, which in addition to tomatoes and tomato products can be obtained in modest amounts from watermelon, guava, and papaya, is considered the most potent antioxidant carotenoid due to its exceptional singlet oxygen quenching ability [463]. The bioavailability of lycopene increases with cooking, partly because

thermal treatment of vegetables enhances the extractability of lycopene from the vegetable fibers [464]. The act of processing tomatoes with oil and simultaneous ingestion of lycopene-based foods with fat have also been shown to promote the dissolution, absorption and subsequent bioavailability of lycopene [465]. Thus, the potential benefits of lycopene are dependent on food processing methods and dietary habits, which may explain the discrepancy in lycopene associations between AAs and EAs. As indicated in Table 2, the consumption of lycopene from food and supplements, as well as adipose lycopene concentrations were significantly higher in EAs than AAs. Hence, it appears that the potential benefits of lycopene in relation to PCa aggressiveness may be acquired only at higher levels of intake. Differences in lycopene associations between AAs and EAs also may have been influenced by gene-diet interactions that may vary by race. As demonstrated by Goodman et al. [466], polymorphic variants in *XRCC1*, a gene involved in base excision repair of DNA damage, can alter the ability of lycopene to decrease PCa risk. Evaluations of such gene-diet interactions between AAs and EAs would help elucidate how lycopene may differentially influence PCa among different population subgroups.

β-cryptoxanthin, which is commonly found in tangerines, oranges, grapefruit, mangoes, fruit juices and red peppers [467], was inversely related to PCa aggressiveness, but only among AAs. This discrepancy may be due to the greater intake of β-cryptoxanthin among AAs relative to EAs (Table 2). Studies have reported inverse [189, 459] as well as positive [448, 468] associations between β-cryptoxanthin and PCa risk. Reviews of the literature do not provide compelling evidence for or against a protective association between β-cryptoxanthin and PCa incidence [17, 434-436]. Plasma β-

cryptoxanthin has been found to correlate inversely with markers of oxidative damage to DNA and lipid peroxidation in humans [469]; factors that have been causally linked to PCa [470]. Perhaps examining β-cryptoxanthin associations with different PCa phenotypes, as done in this study, may help delineate the role of β-cryptoxanthin in prostate carcinogenesis. The associations of α-carotene, β-carotene and lutein + zeaxanthin, and PCa incidence have also varied across studies [17, 189, 434-436, 448-451]. However, evaluations of these carotenoids in the context of PCa aggressiveness are rare [413, 438, 440], but evolving, and may help clarify their role with PCa.

The use of adipose tissue biomarkers of nutrient intake in assessing disease risk has been done in a few studies [284, 471] and continues to receive increased attention because of the ability of adipose tissue to reflect long-term nutritional status [472]. Nonetheless, the uptake and turnover rates of carotenoids in adipose tissues remain unclear [472]. This study suggests that higher adipose α-carotene and lycopene concentrations are inversely related to PCa aggressiveness, which warrants further investigation in larger studies. However, the possibility that adipose α-carotene and lycopene may have acted as markers for increased consumption of fruits and vegetables or as surrogates for a healthy lifestyle in general cannot be ruled out.

Cigarette smoking has been associated with depletion of circulating carotenoid levels [473] and a high BMI also appear to increase the body's carotenoids requirement [474]; however, only BMI was found to have a modifying effect on associations between carotenoids intake and PCa aggressiveness. The results suggest that men with a high BMI may benefit from the intake of lycopene and lutein + zeaxanthin, while β-carotene

supplement intake may increase odds of aggressive disease among EAs with normal BMI and higher dietary lycopene may increase odds among normal BMI AAs. This is the first study to examine effect modification of carotenoids by BMI in relation to PCa aggressiveness, but of note, some studies suggest that β-carotene supplements may increase the risk of PCa among smokers [159, 188]. It is unclear why β-carotene supplements or dietary lycopene may be associated with increased odds of high aggressive disease among normal weight men but not overweight or obese men. Given the number of comparisons, the role of chance cannot be ruled out in these findings.

Diet was assessed using a food frequency questionnaire. It is known that these structured instruments may be biased according to response sets [475], which in turn, may be related to psychological traits that either may exert a direct effect on cancer outcomes or indirectly affect other factors that may influence carcinogenesis [476]. Other limitations of the current study include the fact that carotenoids likely do not act alone, and thus, the results shown here may reflect interactions between individual carotenoids or interactions with other food components or genetic variants [383, 466]. The recall of dietary intakes over the year prior to diagnosis of PCa also may have been influenced by changes in dietary patterns after the diagnosis with PCa. Such recall inaccuracies would have resulted in non-differential misclassification because the research subjects were not likely to consider the extent of their disease aggressiveness in answering questions relating to food and supplements intake. Moreover, laboratory personnel involved in the analyses of adipose carotenoid levels were blinded to the PCa attributes of the samples, eliminating the possibility for differential misclassification of adipose measurements. Therefore, non-differential, rather than differential, misclassification may have attenuated

the ORs to some extent. Because it is possible that some carotenoids may exert their beneficial effects in the early stages of carcinogenesis [456], the one-year reference period for the dietary assessment may not be etiologically relevant to PCa, but can provide an estimate of usual dietary patterns, [453], while adipose tissue concentrations reflect longer-term exposure. It is conceivable that the adipose tissue carotenoid levels can be altered through the metabolic processes of cancer; however, studies show that adipose carotenoid levels are less susceptible to changes due to the presence of a tumor [477]. Additionally, studies show that adipose carotenoid levels correlates inversely with body fat percentage [296]; thus, it would have been desirable to control for body fat mass. However, the potential confounding effect of body fat burden was partially considered by adjusting for BMI (in the BMI unstratified analyses). The small sample size may have reduced the statistical power of the study. Furthermore, since multiple comparisons were made there is the possibility of chance findings. Despite these limitations, the design of the study uniquely captures the complex pathological and clinical attributes of PCa, and the findings of this analysis add to the limited knowledge of the potential role of carotenoids in PCa aggressiveness within specific race groups.

6.5 CONCLUSIONS

In summary, this analysis shows a statistically significant inverse association between lycopene intake and PCa aggressiveness among EAs, and between β-cryptoxanthin and PCa aggressiveness among AAs. Higher adipose tissue α-carotene and lycopene (*cis* + *trans*) levels also appear to be inversely related to PCa aggressiveness among EAs. In addition, the results suggest that certain carotenoids may have greater beneficial impact among obese individuals with the possibility of detrimental effects among normal weight

men, findings that warrant further investigation in larger studies. Although some of the findings vary by race, this was likely due to the variations in the levels of carotenoid intake between AAs and EAs. Overall, the findings support suggestions that a higher consumption of fruits and vegetables, which are the main sources of carotenoids, may be inversely associated with CaP aggressiveness.

Table 6.1 Characteristics of research subjects by race and prostate cancer aggressiveness

Characteristics	European Americans n = 1,079					African Americans n = 1,023				
	High aggressive PCa (n=164)		Low/intermediate aggressive PCa (n=915)		P ‡	High aggressive PCa (n=206)		Low/intermediate aggressive PCa (n=817)		P ‡
	Mean (SD)		Mean (SD)			Mean (SD)		Mean (SD)		
Age, years	67 (8)		64 (8)		<0.0001	64 (8)		62 (8)		0.004
Energy intake, kcals/day	2339.3 (952.0)		2320.5 (865.7)		0.80	2799.6 (1232.4)		2593.0 (1146.0)		0.02
Dietary fat intake, grams/day	94.5 (42.4)		91.1 (39.2)		0.31	103.9 (52.1)		94.8 (48.4)		0.02
Body mass index (BMI), kg/m^2	30.5 (5.1)		29.0 (4.8)		0.0006	29.9 (6.7)		29.2 (5.4)		0.16
	N	**%**	**N**	**%**		**N**	**%**	**N**	**%**	
Study Site										
NC	73	45	448	49	0.29	92	45	386	47	0.51
LA (pre & post Katrina)	91	55	467	51		114	55	431	53	
Family History of Prostate Cancer										
No affected 1st degree relative	136	83	696	76	0.05	157	76	606	74	0.55
At least 1 affected 1st degree relative	28	17	219	24		49	24	211	26	
PSA Screening History										
0 screenings	40	24	153	17	0.06	120	58	307	38	<0.0001
1-7 screenings	68	42	405	44		53	26	338	41	
> 7 screenings	56	34	357	39		33	16	172	21	
Comorbidities										
0	84	51	503	55	0.05	88	43	382	47	0.39
1	31	19	214	23		53	26	216	26	
2	29	18	98	11		36	17	106	13	
≥ 3	20	12	100	11		27	14	109	14	

Started PCa treatment at start of study										
No	11	7	99	11	0.007	20	10	112	14	0.20
Yes	146	89	720	79		163	79	599	73	
Unknown	7	4	96	10		23	11	106	13	
Education										
Less than high school education	27	17	81	9	0.005	85	41	243	30	0.001
High school graduate/ some college	79	48	432	47		102	50	438	54	
College graduate	58	35	402	44		19	9	135	16	
Income Level										
≤ $20, 000	24	15	78	9	0.11	82	40	234	29	0.0005
$20,001 - $40,000	33	20	184	20		52	25	212	26	
$40,001 - $70,000	38	23	217	24		24	12	171	21	
>$70,000	53	32	359	39		22	11	130	16	
Unknown	16	10	77	8		26	12	70	8	
Smoking Status										
Never	59	36	330	36	0.76	40	19	276	34	<0.001
Former smoker	87	53	501	55		107	52	390	48	
Current smoker	18	11	84	9		59	29	151	18	
NSAID Use										
No	56	34	305	33	0.85	84	41	364	45	0.33
Yes	108	66	608	67		120	59	446	55	

Prostate cancer aggressiveness defined by a combination of Gleason sum, clinical stage, and PSA level at diagnosis and classified as follows: high aggressive (Gleason sum ≥8 or PSA >20ng/ml or Gleason sum ≥7 AND clinical stage T3 -T4); low/intermediate aggressive: all other cases.

Abbreviations: PCa – Prostate Cancer; LA – Louisiana; NC –North Carolina; NSAIDs – Nonsteroidal anti-inflammatory drugs; PSA – prostate specific-antigen;
SD – Standard deviation

‡Test for differences between high and low/intermediate PCa performed using t-test for continuous variables and chi-square tests for categorical variables.

Table 6.2 Mean difference in carotenoids from diet, supplements, and adipose tissue by race and prostate cancer aggressiveness

					European Americans			African Americans		
	European Americans n = 1,079	African Americans n = 1,023	% diff	High aggressive PCa n = 164	Low/intermediate aggressive PCa n = 915	P †	High aggressive PCa n = 206	Low/intermediate Aggressive PCa n = 817	P †
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
α-carotene μg/day									
dietary	661.9 (741.7)	596.3 (730.2)	10 ‡	610.0 (874.3)	671.1 (715.5)	0.39	597.6 (829.4)	595.9 (703.6)	0.98
β-carotene μg/day									
dietary	3914.5 (3028.0)	4788.6 (3898.9)	-22 ‡	3830.8 (3095.6)	3929.5 (3017.2)	0.70	4834.6 (3951.4)	4777.1 (3887.8)	0.85
supplement [a]	442.1 (1607.4)	235.0 (859.2)	47 ‡	300.9 (447.3)	467.4 (1734.2)	0.01	192.8 (1064.9)	245.6 (799.5)	0.50
diet + supplement	4356.5 (3477.9)	5023.6 (4028.0)	-15	4131.6 (3196.4)	4396.9 (3526.1)	0.37	5027.3 (4170.0)	5022.7 (3994.1)	0.99
β-cryptoxanthin μg/day									
dietary	162.9 (134.3)	223.5 (204.3)	-37 ‡	160.8 (117.8)	163.3 (137.1)	0.81	199.3 (171.8)	229.6 (211.4)	0.03
Lutein + zeaxanthin μg/day									
dietary	3230.5 (2708.2)	4231.4 (3533.4)	-31 ‡	3204.3 (2706.8)	3235.2 (2709.9)	0.89	4133.5 (3149.9)	4256.1 (3625.1)	0.63
supplement (lutein) [a]	81.7 (126.5)	45.5 (123.4)	44 ‡	84.0 (116.1)	81.3 (128.3)	080	32.8 (83.1)	48.8 (131.4)	0.03
diet + supplement	3312.2 (2736.6)	4277.0 (3550.0)	-29 ‡	3288.2 (2723.3)	3316.5 (2740.5)	0.90	4166.3 (3151.3)	4304.9 (3644.9)	0.62
Lycopene μg/day									
dietary	6715.8 (7841.9)	5538.9 (7790.7)	17 ‡	5993.8 (5770.4)	6845.3 (8153.6)	0.10	5439.7 (8294.3)	5563.9 (7663.6)	0.84
supplement [a]	85.3 (201.3)	66.7 (141.7)	22 ‡	85.0 (133.4)	85.3 (211.2)	0.97	60.5 (117.6)	68.3 (147.2)	0.42
diet + supplement	6801.1 (7855.4)	5605.6 (7794.4)	18 ‡	6078.8 (5773.4)	6930.6 (8168.5)	0.10	5500.3 (8302.8)	5632.2 (7666.0)	0.83
Adipose tissue carotenoid levels μg/day	N = 581 Mean (SD)	N = 358 Mean (SD)		N = 89 Mean (SD)	N = 492 Mean (SD)		N = 66 Mean (SD)	N = 292 Mean (SD)	
α-carotene	0.04 (0.05)	0.03 (0.05)	25	0.03 (0.06)	0.04 (0.05)	0.26	0.03 (0.03)	0.03 (0.05)	0.33
cis-β-carotene	0.10 (0.14)	0.10 (0.15)	0	0.09 (0.16)	0.10 (0.14)	0.42	0.10 (0.17)	0.09 (0.14)	0.81
trans-β-carotene	0.19 (0.27)	0.16 (0.27)	16	0.16 (0.27)	0.19 (0.28)	0.32	0.17 (0.32)	0.16 (0.26)	0.79
α-cryptoxanthin	0.03 (0.03)	0.02 (0.02)	33	0.02 (0.02)	0.03 (0.03)	0.16	0.02 (0.02)	0.02 (0.03)	0.49
β-cryptoxanthin	0.09 (0.11)	0.08 (0.10)	11	0.08 (0.09)	0.09 (0.11)	0.28	0.07 (0.08)	0.08 (0.10)	0.84

	Lutein	0.21 (0.22)	0.20 (0.24)	5	0.18 (0.17)	0.21 (0.23)	0.20	0.19 (0.23)	0.20 (0.24)	0.82
	Zeaxanthin	0.10 (0.16)	0.07 (0.08)	30 ‡	0.08 (0.07)	0.10 (0.17)	0.03	0.07 (0.08)	0.08 (0.09)	0.46
	Lycopene (*cis* + *trans*)	0.35 (0.40)	0.28 (0.34)	20 ‡	0.28 (0.32)	0.36 (0.42)	0.04	0.28 (0.32)	0.28 (0.35)	0.93
	cis-lycopene	0.22 (0.26)	0.17 (0.21)	23 ‡	0.17 (0.20)	0.22 (0.27)	0.05	0.17 (0.20)	0.17 (0.22)	0.96
	all-trans-lycopene	0.13 (0.15)	0.10 (0.13)	23 ‡	0.10 (0.12)	0.13 (0.15)	0.05	0.11 (0.12)	0.10 (0.13)	0.84

Abbreviations: PCa – prostate cancer; SD – standard deviation

[a] Among supplement users only

‡ Significant p-values (< 0.05) for test of difference between European Americans and African Americans

[†] Chi-square test for difference by level of prostate cancer aggressiveness

Table 6.3 Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between dietary and supplemental carotenoids intake and prostate cancer aggressiveness among European-American (n = 1,079) and African-American men (1,023)

Carotenoids	High aggressive/ low-intermediate aggressive	OR (95% CI) [a]	OR (95% CI) [b]	Trend p-value [c]
α-carotene				
Dietary μg/day				
European American				
27.20 - 324.13	64/305	1.00 (ref)	1.00 (ref)	0.33
324.14 - 626.76	48/305	0.74 (0.49-1.12)	0.72 (0.47-1.11)	
626.77 - 9812.26	52/305	0.80 (0.53-1.20)	0.76 (0.49-1.18)	
African American				
11.83 - 262.11	71/273	1.00 (ref)	1.00 (ref)	0.77
262.12 - 585.99	63/272	0.89 (0.61-1.30)	0.79 (0.52-1.21)	
586.00 - 9558.28	72/272	1.05 (0.72-1.52)	0.99 (0.64-1.52)	
β-carotene				
European American				
Dietary μg/day				
194.86 - 2327.81	61/305	1.00 (ref)	1.00 (ref)	0.96
2327.82 - 4046.15	44/305	0.68 (0.45-1.04)	0.67 (0.43-1.05)	
4046.16 - 25124.05	59/305	0.91 (0.61-1.35)	0.92 (0.60-1.43)	
Supplement μg/day				
non-users	91/536	1.00 (ref)	1.00 (ref)	0.44
63.00 - 600.00	61/301	1.20 (0.84-1.71)	1.39 (0.96-2.02)	
600.01 - 16470.00	12/78	0.88 (0.46-1.69)	1.00 (0.50-1.97)	
Diet + supplement μg/day				
346 .80 - 2566.41	60/304	1.00 (ref)	1.00 (ref)	0.75
2566.42 - 4458.51	48/306	0.76 (0.50-1.15)	0.76 (0.49-1.18)	
4458.52 - 25512.15	56/305	0.87 (0.58-1.30)	0.88 (0.56-1.37)	
African American				
Dietary μg/day				
286.48 - 2544.86	61/273	1.00 (ref)	1.00 (ref)	0.64
2544.87 - 5156.05	69/272	1.16 (0.79-1.70)	1.07 (0.70-1.62)	
5156.06 - 32901.26	76/272	1.22 (0.84-1.78)	1.12 (0.72-1.73)	
Supplement μg/day				
non-users	153/564	1.00 (ref)	1.00 (ref)	0.37
63.00 - 590.00	26/92	1.08 (0.67-1.74)	1.22 (0.74-2.03)	
590.01 - 15600	27/161	0.62 (0.40-0.98)	0.76 (0.48-1.21)	
Diet + supplement μg/day				
286.48 - 2773.39	67/273	1.00 (ref)	1.00 (ref)	0.88
2773.40 - 5405.28	69/272	1.05 (0.72-1.53)	0.98 (0.65-1.47)	

	5405.29 - 32901.26	70/272	1.02 (0.70-1.49)	0.96 (0.62-1.49)	
β-cryptoxanthin					
Dietary μg/day					
European American					
	6.04 - 86.14	51/305	1.00 (ref)	1.00 (ref)	0.62
	86.15 - 180.62	52/305	1.00 (0.65-1.52)	1.00 (0.64-1.55)	
	180.63 - 1082.55	61/305	1.18 (0.78-1.77)	1.11 (0.71-1.72)	
African American					
	3.53 - 116.43	87/273	1.00 (ref)	1.00 (ref)	**0.01**
	116.44 - 243.34	65/272	0.75 (0.52-1.08)	0.74 (0.50-1.11)	
	243.34 - 1594.39	54/272	0.63 (0.43-0.92)	**0.56 (0.36-0.87)**	
Lutein + Zeaxanthin					
European American					
Dietary μg/day					
	289.67 - 1830.51	60/305	1.00 (ref)	1.00 (ref)	0.49
	1830.52 - 3253.94	44/305	0.71 (0.47-1.09)	0.71 (0.45-1.12)	
	3253.95 - 30165.13	60/305	1.01 (0.68-1.50)	1.05 (0.67-1.63)	
Supplement μg/day					
	non-users	106/617	1.00 (ref)	1.00 (ref)	
	users	58/298	0.90 (0.64-1.28)	0.79 (0.55-1.14)	
Diet + supplement μg/day					
	289.67 - 1907.94	59/305	1.00 (ref)	1.00 (ref)	0.56
	1907.95 - 3318.23	46/305	0.75 (0.49-1.15)	0.75 (0.48-1.18)	
	3318.23 - 31665.13	59/305	1.01 (0.69-1.50)	1.04 (0.69-1.63)	
African American					
Dietary μg/day					
	112.25 - 2299.78	64/273	1.00 (ref)	1.00 (ref)	0.82
	2299.79 - 4408.18	72/272	1.15 (0.79-1.68)	1.11 (0.74-1.68)	
	408.19 - 36608.75	70/272	1.10 (0.75-1.61)	1.08 (0.70-1.65)	
Supplement μg/day					
	non-users	177/671	1.00 (ref)	1.00 (ref)	
	users	29/137	1.34 (0.87-2.06)	1.03(0.65-1.63)	
Diet + supplement μg/day					
	112.25 - 2309.42	63/273	1.00 (ref)	1.00 (ref)	0.85
	2309.43 - 4461.54	74/272	1.20 (0.82-1.75)	1.19 (0.78-1.79)	
	4461.55 - 37558.75	69/272	1.10 (0.75-1.61)	1.09 (0.71-1.67)	
Lycopene					
European American					
Dietary μg/day					
	344.77 - 3605.57	70/305	1.00 (ref)	1.00 (ref)	**0.02**
	3605.58 - 6299.34	48/305	0.73 (0.48-1.09)	0.67 (0.44-1.02)	
	6299.35 - 100250.76	46/305	0.74 (0.49-1.12)	**0.55 (0.34-0.89)**	
Supplement μg/day					

	non-users	114/664	1.00 (ref)	1.00 (ref)	0.27
	64.28 - 250.00	17/126	1.91 (0.80-4.57)	2.14 (0.87-5.24)	
	250.01 - 5000.00	33/125	1.09 (0.74-1.60)	1.22 (0.82-1.83)	
Diet + supplement μg/day					
	344.77 - 3649.40	70/305	1.00 (ref)	1.00 (ref)	**0.03**
	3649.41 - 6352.12	48/305	0.72 (0.48-1.08)	0.67 (0.44-1.02)	
	6352.13 - 100550.76	46/305	0.74 (0.49-1.12)	**0.56 (0.34-0.90)**	
African American					
Dietary μg/day					
	21.52 - 2390.06	61/273	1.00 (ref)	1.00 (ref)	0.58
	2390.07 - 5003.81	70/272	1.23 (0.84-1.81)	1.27 (0.83-1.94)	
	5003.82 - 85677.94	73/272	1.29 (0.88-1.89)	1.22 (0.77-1.93)	
Supplement μg/day					
	non-users	161/630	1.00 (ref)	1.00 (ref)	0.57
	64.28 - 250.00	12/88	0.86 (0.39-1.89)	0.97 (0.43-2.22)	
	250.01 - 1000.00	33/99	0.97 (0.65-1.45)	1.14 (0.74-1.73)	
Diet + supplement μg/day					
	21.52 - 2448.39	62/273	1.00 (ref)	1.00 (ref)	0.70
	2448.39 - 5064.89	70/272	1.22 (0.83-1.79)	1.21 (0.80-1.85)	
	5064.90 - 85677.94	72/272	1.25 (0.85-1.84)	1.16 (0.73-1.84)	

Abbreviation: PCa – prostate cancer
[a] Adjusted for age
[b] Additional adjustment for PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site
[c] Multivariable-adjusted trend p-value
[d] Categorized into two levels because limited variability in dose did not allow for creation of meaningful tertile categories

Table 6.4 Associations between adipose tissue carotenoid levels and prostate cancer aggressiveness among European Americans (n = 581) and African Americans (n = 358)

Carotenoids	High aggressive/ low-intermediate aggressive	OR (95% CI) [a]	OR (95% CI) [b]	Trend p-value [c]
α-carotene μg/g				
European American				
0.003 - 0.015	26/128	1.00 (ref)	1.00 (ref)	
0.016 - 0.039	30/116	1.15 (0.63-2.08)	1.52 (0.78-2.94)	
0.040 - 0.454	12/112	0.49 (0.24-1.03)	0.58 (0.25-1.32)	0.07
African American				
0.003 - 0.012	16/54	1.00 (ref)	1.00 (ref)	
0.013 - 0.025	7/53	0.34 (0.11-1.09)	0.45 (0.15-1.34)	
0.026 - 0.450	13/49	1.07 (0.39-2.92)	1.13 (0.43-3.00)	0.70
***cis*-β-carotene** μg/g				
European American				
0.003 - 0.032	28/138	1.00 (ref)	1.00 (ref)	
0.033 - 0.088	27/134	0.89 (0.49-1.61)	1.06 (0.57-1.99)	
0.089 - 1.233	27/135	0.88 (0.49-1.59)	1.16 (0.60-2.25)	0.65
African American				
0.003 - 0.028	23/71	1.00 (ref)	1.00 (ref)	
0.029 - 0.073	7/71	0.60 (0.12-1.74)	0.74 (0.28-1.22)	
0.074 - 1.163	24/71	0.93 (0.47-1.83)	1.13 (0.52-2.44)	0.71
***trans*-β-carotene** μg/g				
European American				
0.003 - 0.061	33/154	1.00 (ref)	1.00 (ref)	
0.062 - 0.171	34/152	0.95 (0.56-1.64)	1.11 (0.62-1.99)	
0.172 - 2.408	21/151	0.58 (0.32-1.06)	0.75 (0.39-1.45)	0.31
African American				
0.003 - 0.045	21/88	1.00 (ref)	1.00 (ref)	
0.046 - 0.126	17/88	0.85 (0.42-1.73)	0.70 (0.32-1.53)	
0.127 - 2.322	22/88	0.97 (0.49-1.91)	1.12 (0.52-2.39)	0.57
α-cryptoxanthin μg/g				
European American				
0.003 - 0.013	23/113	1.00 (ref)	1.00 (ref)	
0.014 - 0.025	18/112	0.73 (0.37-1.45)	0.84 (0.41-1.75)	
0.026 - 0.219	20/115	0.82 (0.42-1.60)	0.97 (0.46-2.02)	0.99
African American				
0.003 - 0.010	15/62	1.00 (ref)	1.00 (ref)	
0.011 - 0.024	14/62	0.92 (0.41-2.07)	0.94 (0.39-2.33)	
0.025 - 0.165	12/61	0.82 (0.35-1.90)	1.03 (0.40-2.68)	0.93
β-cryptoxanthin μg/g				
European American				
0.003 - 0.037	30/151	1.00 (ref)	1.00 (ref)	

	0.038 - 0.089	32/148	1.03 (0.59-1.79)	1.19 (0.64-2.19)	
	0.090 - 0.909	25/146	0.83 (0.46-1.49)	0.92 (0.48-1.76)	0.67
African American					
	0.003 - 0.030	22/86	1.00 (ref)	1.00 (ref)	
	0.031 - 0.070	17/82	0.82 (0.41-1.67)	0.71 (0.32-1.61)	
	0.071 - 0.638	19/83	0.90 (0.45-1.78)	1.00 (0.46-2.19)	0.77
Lutein μg/g					
European American					
	0.004 - 0.078	28/161	1.00 (ref)	1.00 (ref)	
	0.079 - 0.219	31/161	1.22 (0.69-2.14)	1.26 (0.69-2.31)	
	0.220 - 1.457	29/159	1.01 (0.57-1.79)	1.27 (0.68-2.35)	0.51
African American					
	0.003 - 0.069	23/95	1.00 (ref)	1.00 (ref)	
	0.070 - 0.204	22/94	0.96 (0.50-1.85)	0.95 (0.47-1.95)	
	0.205 - 2.033	20/94	0.89 (0.46-1.73)	1.02 (0.49-2.12)	0.92
Zeaxanthin μg/g					
European American					
	0.003 - 0.038	28/156	1.00 (ref)	1.00 (ref)	
	0.039 - 0.098	40/157	1.42 (0.83-2.44)	1.73 (0.96-3.09)	
	0.099 - 2.985	20/154	0.76 (0.41-1.43)	0.96 (0.49-1.88)	0.78
African American					
	0.003 - 0.028	24/93	1.00 (ref)	1.00 (ref)	
	0.029 - 0.073	20/93	0.83 (0.43-1.61)	0.87 (0.42-1.83)	
	0.074 - 0.713	19/91	0.83 (0.42-1.63)	1.01 (0.47-2.17)	0.89
Lycopene (*cis* + *trans*) μg/g					
European American					
	0.004 - 0.137	35/153	1.00 (ref)	1.00 (ref)	
	0.138 - 0.371	34/152	1.02 (0.60-1.73)	1.23 (0.69-2.18)	
	0.372 - 3.164	17/152	0.51 (0.27-0.96)	0.64 (0.34-1.31)	0.11
African American					
	0.004 - 0.100	23/91	1.00 (ref)	1.00 (ref)	
	0.101 - 0.272	15/87	0.74 (0.36-1.51)	0.69 (0.31-1.56)	
	0.273 - 3.013	23/88	1.08 (0.56-2.07)	1.11 (0.53-2.35)	0.62
***cis*-lycopene** μg/g					
European American					
	0.003 - 0.080	33/155	1.00 (ref)	1.00 (ref)	
	0.081 - 0.234	37/157	1.14 (0.67-1.93)	1.52 (0.85-2.71)	
	0.235 - 2.049	18/152	0.57 (0.31-1.07)	0.79 (0.40-1.54)	0.30
African American					
	0.003 - 0.059	25/92	1.00 (ref)	1.00 (ref)	
	0.060 - 0.168	16/88	0.74 (0.37-1.49)	0.74 (0.34-1.63)	
	0.169 - 1.879	22/90	0.94 (0.49-1.80)	0.97 (0.46-2.02)	0.96
***all-trans*-lycopene** μg/g					
European American					
	0.003 - 0.050	35/158	1.00 (ref)	1.00 (ref)	
	0.051 - 0.137	33/154	0.99 (0.58-1.69)	1.11 (0.63-1.99)	
	0.138 – 1.115	18/154	0.54 (0.29-1.01)	0.69 (0.36-1.36)	0.25

African American					
	0.003 - 0.037	22/95	1.00 (ref)	1.00 (ref)	
	0.038 - 0.102	17/90	0.84 (0.42-1.70)	0.85 (0.39-1.85)	
	0.103 - 1.134	22/92	1.07 (0.55-2.07)	1.10 (0.51-2.34)	0.72

[a] Some categories may not sum to total number of subjects because of missing data

[b] Adjusted for age

[c] additional adjustment for PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, study site, family history of prostate cancer, comorbidities, and prostate cancer treatment status.

[d] Multivariable-adjusted trend p-values

Table 6.5 Characteristics of subsample with data on adipose carotenoids compared to the total study sample

Characteristics	Total study sample n = 2,102		Subsample with data on adipose carotenoid levels n = 939		
	Mean (SD)		Mean (SD)		p-value
Age, years	63.2 (7.9)		63.3 (7.9)		0.66
Energy intake, kcals/day	2474.8 (1040.3)		2454.9 (1019.1)		0.62
Dietary fat intake, grams/day	94.0 (44.7)		94.7 (44.5)		0.68
Body mass index (BMI), kg/m^2	29.7 (5.3)		29.9 (5.2)		0.29
	n	%	n	%	
Race					
African American	361	38	662	57	<0.001
European American	584	62	495	43	
Prostate cancer aggressiveness					
Low aggressive	485	51	589	51	0.48
High aggressive	304	32	354	31	
Intermediate aggressive	156	16	214	18	
Study Site					
NC	999	48	408	43	0.04
LA (pre & post Katrina)	1103	52	531	57	
Family History of Prostate Cancer					
No affected 1st degree relative	1595	76	694	74	0.24
At least 1 affected 1st degree relative	507	24	245	26	
PSA Screening History					
0 screenings	620	30	244	26	0.02
1-7 screenings	864	41	375	40	
> 7 screenings	618	29	320	34	
comorbidities					
0	1057	50	497	53	0.53
1	514	25	220	24	
2	269	13	107	11	
≥ 3	255	12	115	12	
Started PCa treatment at start of study					
No	242	12	94	10	0.42
Yes	1628	77	745	79	
Unknown	232	11	100	11	
Education					
Less than high school education	436	21	169	18	0.03

High school graduate/ some college	1051	50	455	48	
College graduate	614	29	315	34	
Income Level					
≤ $20, 000	418	20	154	17	0.02
$20,001 - $40,000	481	23	188	20	
$40,001 - $70,000	450	21	209	22	
>$70,000	564	27	294	31	
Unknown	189	9	94	10	
Smoking Status					
Never	705	33	353	38	0.04
Former smoker	1085	52	470	50	
Current smoker	312	15	116	12	
NSAID Use					
No	809	39	361	38	0.90
Yes	1282	61	578	62	

Prostate cancer aggressiveness defined by a combination of Gleason sum, clinical stage, and PSA level at diagnosis and classified as follows: high aggressive (Gleason sum ≥8 or PSA >20ng/ml or Gleason sum ≥7 AND clinical stage T3 -T4); low /intermediate aggressive: all other cases.

Abbreviations: PCa – Prostate Cancer; LA – Louisiana; NC –North Carolina; NSAIDs – Nonsteroidal anti-inflammatory drugs; PSA – prostate specific-antigen; SD – Standard deviation

[‡]Test for differences between two study populations were done using t-test for continuous variables and chi-square tests for categorical variables.

Table 6.6 (Sensitivity Analyses) Associations between dietary carotenoids and prostate cancer aggressiveness among European Americans and African Americans with data on adipose tissue carotenoid level only (n = 939)

Carotenoids		High aggressive/ low-intermediate aggressive	OR (95% CI) [a]	OR (95% CI) [b]	Trend p-value [c]
α-carotene					
Dietary μg/day					
European American					
	27.20 - 324.13	35/173	1.00 (ref)	1.00 (ref)	0.33
	324.14 – 626.76	23/157	0.71 (0.40-1.27)	0.75 (0.41-1.41)	
	626.77– 9812.26	31/162	0.89 (0.52-1.52)	0.88 (0.48-1.64)	
African American					
	11.83 – 262.11	24/94	1.00 (ref)	1.00 (ref)	0.92
	262.12 – 585.99	17/107	0.60 (0.30-1.20)	0.40 (0.18-1.16)	
	586.00 – 9558.28	25/91	1.07 (0.57-2.01)	0.76 (0.36-1.62)	
β-carotene					
European American					
Dietary μg/day					
	194.86 - 2327.81	34/163	1.00 (ref)	1.00 (ref)	0.44
	2327.82 - 4046.15	19/179	0.48 (0.26-1.06)	0.50 (0.26-1.04)	
	4046.16 - 25124.05	36/150	1.05 (0.62-1.78)	1.07 (0.58-1.96)	
Supplement μg/day					
	non-users	48/277	1.00 (ref)	1.00 (ref)	0.15
	63.00 - 600.00	33/181	1.09 (0.67-1.77)	1.35 (0.80-2.28)	
	600.01 - 16470.00	8/34	1.36 (0.59-3.15)	1.73 (0.70-4.24)	
Diet + supplement μg/day					
	346 .80 - 2566.41	31/162	1.00 (ref)	1.00 (ref)	0.50
	2566.42 - 4458.51	24/179	0.86 (0.38-1.21)	0.71 (0.38-1.33)	
	4458.52 - 25512.15	34/151	1.08 (0.62-1.85)	1.10 (0.60-2.04)	
African American					
Dietary μg/day					
	286.48 - 2544.86	12/95	1.00 (ref)	1.00 (ref)	0.25
	2544.87 - 5156.05	27/95	2.27 (0.93-4.77)	1.64 (0.74-3.64)	
	5156.06 - 32901.26	27/102	2.03 (0.97-4.24)	1.81 (0.77-4.23)	
Supplement μg/day					
	non-users	50/211	1.00 (ref)	1.00 (ref)	0.83
	63.00 - 590.00	7/25	1.35 (0.55-3.35)	1.24 (0.46-3.38)	
	590.01 - 15600	9/56	0.69 (0.32-1.50)	0.87 (0.38-1.97)	
Diet + supplement μg/day					

	286.48 - 2773.39	16/94	1.00 (ref)	1.00 (ref)	0.69
	2773.40 - 5405.28	25/95	1.54 (0.77-3.09)	1.15 (0.54-2.43)	
	5405.29 - 32901.26	25/103	1.39 (0.70-2.77)	1.20 (0.54-2.68)	
β-cryptoxanthin					
Dietary μg/day					
European American					
	6.04 - 86.14	19/170	1.00 (ref)	1.00 (ref)	0.38
	86.15 - 180.62	38/159	2.10 (0.89-3.82)	1.94 (0.64-3.64)	
	180.63 - 1082.55	32/163	1.70 (0.92-3.15)	1.57 (0.80-3.07)	
African American					
	3.53 - 116.43	25/101	1.00 (ref)	1.00 (ref)	0.19
	116.44 - 243.34	22/102	0.88 (0.46-1.66)	0.75 (0.37-1.53)	
	243.34 - 1594.39	19/89	0.87 (0.45-1.70)	0.59 (0.27-1.28)	
Lutein + Zeaxanthin					
European American					
Dietary μg/day					
	289.67 - 1830.51	30/166	1.00 (ref)	1.00 (ref)	0.21
	1830.52 - 3253.94	24/166	0.72 (0.40-1.30)	0.82 (0.43-1.56)	
	3253.95 - 30165.13	35/160	1.20 (0.70-2.07)	1.34 (0.71-2.50)	
Supplement μg/day					
	non-users	59/325	1.00 (ref)	1.00 (ref)	
	users	30/167	1.04 (0.64-1.69)	0.90 (0.54-1.52)	
Diet + supplement μg/day					
	289.67 - 1907.94	28/168	1.00 (ref)	1.00 (ref)	0.26
	1907.95 - 3318.23	28/164	0.91 (0.51-1.63)	1.06 (0.57-1.99)	
	3318.23 -31665.13	33/160	1.23 (0.70-2.15)	1.40 (0.73-2.66)	
African American					
Dietary μg/day					
	112.25 - 2299.78	17/103	1.00 (ref)	1.00 (ref)	0.42
	2299.79 - 4408.18	24/90	1.57 (0.79-3.12)	1.11 (0.52-2.37)	
	4408.19 - 36608.75	25/99	1.53 (0.78-3.02)	1.35 (0.63-2.90)	
Supplement μg/day					
	non-users	55/248	1.00 (ref)	1.00 (ref)	
	users	11/44	0.89 (0.43-1.83)	0.69 (0.32-1.51)	
Diet + supplement μg/day					
	112.25 - 2309.42	17/102	1.00 (ref)	1.00 (ref)	0.42
	2309.43 - 4461.54	24/91	1.53 (0.77-3.04)	1.10 (0.51-2.35)	
	4461.55 - 37558.75	25/99	1.52 (0.77-2.99)	1.35 (0.63-2.88)	
Lycopene					
European American					
Dietary μg/day					

	344.77 - 3605.57	34/162	1.00 (ref)	1.00 (ref)	0.18
	3605.58 - 6299.34	28/154	0.87 (0.50-1.52)	0.83 (0.46-1.51)	
	6299.35 -100250.76	27/176	0.81 (0.46-1.41)	0.63 (0.32-1.23)	
Supplement μg/day					
	non-users	54/243	1.00 (ref)	1.00 (ref)	0.95
	64.28 - 250.00	13/114	1.40 (0.38-5.15)	1.46 (0.38-5.63)	
	250.01 - 5000.00	22/135	0.86 (0.51-1.47)	1.00 (0.57-1.76)	
Diet + supplement μg/day					
	344.77 - 3649.40	33/165	1.00 (ref)	1.00 (ref)	0.26
	3649.41 - 6352.12	29/150	0.98 (0.56-1.70)	0.96 (0.53-1.74)	
	6352.13 - 100550.76	27/177	0.85 (0.49-1.49)	0.69 (0.35-1.37)	
African American					
Dietary μg/day					
	21.52 - 2390.06	15/97	1.00 (ref)	1.00 (ref)	0.79
	2390.07 - 5003.81	23/87	1.82 (0.89-3.74)	1.69 (0.76-3.75)	
	5003.82 - 85677.94	26/108	1.72 (0.85-3.48)	1.36 (0.59-3.01)	
Supplement μg/day					
	non-users	42/205	1.00 (ref)	1.00 (ref)	0.70
	64.28 - 250.00	14/40	1.78 (0.60-4.76)	1.94 (0.52-6.32)	
	250.01 - 1000.00	10/47	0.99 (0.46-2.10)	1.10 (0.49-2.45)	
Diet + supplement μg/day					
	21.52 - 2448.39	16/98	1.00 (ref)	1.00 (ref)	0.79
	2448.39 - 5064.89	22/87	1.72 (0.84-3.51)	1.56 (0.71-3.44)	
	5064.90 - 85677.94	26/107	1.67 (0.84-3.34)	1.31 (0.58-2.96)	

[a] Adjusted for age
[b] Additional adjustment for PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site
[c] First value represents age-adjusted trend p-values and the second is the multivariable adjusted trend p-value
[d] Multivariable-adjusted trend p-value
[e] Categorized into two levels because limited variability in dose did not allow for creation of meaningful tertile categories

Table 6.7 Stratified analyses of associations between dietary carotenoids and prostate cancer aggressiveness by BMI among European Americans (n = 1,079) and African Americans (n = 1,023).

			European Americans		African Americans	
			High aggressive/ low-intermediate aggressive PCa	OR (95% CI) ‡	High aggressive/ low-intermediate aggressive PCa	OR (95% CI) ‡
Dietary α-carotene (μg/day) [a]						
BMI	≤ 24.9 kg/m^2	< median	9/79	1.00 (ref)	19/92	1.00 (ref)
		≥ median	11/80	0.73 (0.24-2.21)	21/79	0.92 (0.41-2.08)
	25 – 29.9 kg/m^2	< median	32/ 201	1.00 (ref)	36/176	1.00 (ref)
		≥ median	32/ 224	0.97 (0.55-1.72)	39/149	1.22 (0.67-2.17)
	≥ 30 kg/m^2	< median	45.174	1.00 (ref)	41/135	1.00 (ref)
		≥ median	32/153	0.76 (0.44-1.34)	43/179	0.88 (0.51-1.52)
			interaction (BMI by α-carotene) = 0.32		interaction (BMI by α-carotene) = 0.25	
Dietary β-carotene (μg/day) [b]						
BMI	≤ 24.9 kg/m^2	< median	5/83	1.00 (ref)	19/99	1.00 (ref)
		≥ median	15/76	1.86 (0.55-6.32)	21/72	1.10 (0.49-2.48)
	25 – 29.9 kg/m^2	< median	30/193	1.00 (ref)	37/167	1.00 (ref)
		≥ median	34/232	0.92 (0.51-1.64)	38/158	0.91 (0.51-1.63)
	≥ 30 kg/m^2	< median	45/178	1.00 (ref)	38/138	1.00 (ref)
		≥ median	32/149	0.93 (0.54-1.63)	46/176	0.90 (0.53-1.55)
			interaction (BMI by β-carotene) = **0.06**		interaction (BMI by β-carotene) = 0.35	
Supplemental β-carotene (μg/day) [c]						
BMI	≤ 24.9 kg/m^2	non-users	6/88	1.00 (ref)	32/116	1.00 (ref)
		users	14/71	3.50 (1.04-11.84)	8/55	0.45 (0.17-1.20)
	25 – 29.9 kg/m^2	non-users	32/249	1.00 (ref)	52/231	1.00 (ref)
		users	32/176	1.54 (0.87-2.73)	23/94	1.08 (0.59-1.97)
	≥ 30 kg/m^2	non-users	50/196	1.00 (ref)	62/213	1.00 (ref)
		users	27/131	0.81 (0.47-1.41)	22/101	0.85 (0.47-1.53)
			interaction (BMI by supplemental β-carotene) = **0.007**		interaction (BMI by supplemental β-carotene) = 0.42	
Total β-carotene (diet + supplement, μg/day) [d]						
BMI	≤ 24.9 kg/m^2	< median	6/78	1.00 (ref)	19/96	1.00 (ref)
		≥ median	14/81	1.33 (0.41-4.31)	21/75	0.99 (0.43-2.28)
	25 – 29.9 kg/m^2	< median	28/198	1.00 (ref)	37/166	1.00 (ref)
		≥ median	36/227	1.11 (0.61-2.00)	38/159	0.89 (0.50-1.59)
	≥ 30 kg/m^2	< median	44/178	1.00 (ref)	38/143	1.00 (ref)
		≥ median	33/149	0.93 (0.54-1.62)	46/171	0.94 (0.55-1.62)
			interaction (BMI by total β-carotene) = 0.10		interaction (BMI by total β-carotene) = 0.50	
Dietary β-cryptoxanthin (μg/day) [e]						
BMI	≤ 24.9 kg/m^2	< median 6/77		1.00 (ref)	23/91	1.00 (ref)

		≥ median	14/82	1.21 (0.37-4.00)	17/80	0.79 (0.34-1.84)
	25 – 29.9 kg/m^2	< median	32/208	1.00 (ref)	51/153	1.00 (ref)
		≥ median	32/217	0.82 (0.46-1.46)	24/172	0.32 (0.18-1.61**)**
	≥ 30 kg/m^2	< median	39/171	1.00 (ref)	39/161	1.00 (ref)
		≥ median	38/156	1.22 (0.72-2.06)	45/153	1.34 (0.77-2.23)
			interaction (BMI by dietary β-cryptoxanthin) = 0.51		interaction (BMI by dietary β-cryptoxanthin) = 0.19	
Dietary lutein + zeaxanthin (μg/day) [f]						
BMI	≤ 24.9 kg/m^2	< median	6/82	1.00 (ref)	15/99	1.00 (ref)
		≥ median	14/77	1.79 (0.51-6.35)	25/72	1.50 (0.66-3.39)
	25 – 29.9 kg/m^2	< median	31/205	1.00 (ref)	35/163	1.00 (ref)
		≥ median	33/220	1.06 (0.60-1.87)	40/162	0.99 (0.56-1.74)
	≥ 30 kg/m^2	< median	45/168	1.00 (ref)	40/142	1.00 (ref)
		≥ median	32/159	0.80 (0.46-1.39)	44/172	0.93 (0.54-1.60)
			interaction (BMI by dietary lutein + zeaxanthin) = **0.03**		interaction (BMI by dietary lutein + zeaxanthin) = 0.13	
Supplemental lutein (μg/day) [c]						
BMI	≤ 24.9 kg/m^2	non-users	9/100	1.00 (ref)	34/138	1.00 (ref)
		users	11/59	1.67 (0.53-5.26)	6/33	0.84 (0.28-2.54)
	25 – 29.9 kg/m^2	non-users	36/287	1.00 (ref)	63/274	1.00 (ref)
		users	28/138	1.74 (0.98-3.11)	12/51	1.29 (0.60-2.78)
	≥ 30 kg/m^2	non-users	59/227	1.00 (ref)	73/253	1.00 (ref)
		users	18/100	0.76 (0.42-1.39)	11/61	0.68 (0.33-1.41)
			interaction (BMI by supplemental lutein) = **0.02**		interaction (BMI by supplemental lutein) = 0.64	
Total Lutein + Zeaxanthin (diet + supplement, μg/day) [g]						
BMI	≤ 24.9 kg/m^2	< median	6/77	1.00 (ref)	15/101	1.00 (ref)
		≥ median	14/82	1.40 (0.40-4.93)	25/70	1.66 (0.74-3.74)
	25 – 29.9 kg/m^2	< median	34/207	1.00 (ref)	35/162	1.00 (ref)
		≥ median	30/218	0.92 (0.52-1.62)	40/163	1.00 (0.57-1.75)
	≥ 30 kg/m^2	< median	45/171	1.00 (ref)	41/141	1.00 (ref)
		≥ median	32/156	0.81 (0.47-1.42)	43/173	0.88 (0.51-1.50)
			interaction (BMI by total lutein + zeaxanthin) = **0.08**		interaction (BMI by total lutein + zeaxanthin) = **0.07**	
Dietary lycopene (μg/day) [h]						
BMI	≤ 24.9 kg/m^2	< median	9/86	1.00 (ref)	11/90	1.00 (ref)
		≥ median	11/73	0.86 (0.26-2.80)	29/81	2.90 (1.15-7.31)
	25 – 29.9 kg/m^2	< median	37/211	1.00 (ref)	32/168	1.00 (ref)
		≥ median	27/214	0.83 (0.46-1.50)	43/157	1.31 (0.72-2.40)
	≥ 30 kg/m^2	< median	45/158	1.00 (ref)	41/146	1.00 (ref)
		≥ median	32/169	0.68 (0.38-1.21)	43/168	1.03 (0.57-1.85)
			interaction (BMI by dietary lycopene) = **0.01**		interaction (BMI by dietary lycopene) = **0.02**	
Supplemental lycopene (μg/day) [c]						
BMI	≤ 24.9 kg/m^2	non-users	12/109	1.00 (ref)	32/126	1.00 (ref)
		users	8/50	1.51 (0.45-5.02)	8/45	0.70 (0.25-1.93)
	25 – 29.9 kg/m^2	non-users	39/309	1.00 (ref)	58/261	1.00 (ref)
		users	25/116	1.64 (0.90-2.99)	17/64	1.24 (0.64-2.42)
	≥ 30 kg/m^2	non-users	60/242	1.00 (ref)	64/237	1.00 (ref)

		users	17/85	0.91 (0.49-1.70)	20/77	1.01 (0.55-1.86)
			interaction (BMI by supplemental lycopene) = 0.16		interaction (BMI by supplemental lycopene) = 0.62	
Total Lycopene (diet + supplement, μg/day) [i]						
BMI	≤ 24.9 kg/m^2	< median	9/84	1.00 (ref)	12/91	1.00 (ref)
		≥ median	11/75	0.85 (0.26-2.75)	28/80	2.46 (0.99-6.07)
	25 – 29.9 kg/m^2	< median	37/212	1.00 (ref)	32/168	1.00 (ref)
		≥ median	27/213	0.85 (0.47-1.53)	43/157	1.34 (0.74-2.44)
	≥ 30 kg/m^2	< median	44/160	1.00 (ref)	40/146	1.00 (ref)
		≥ median	33/167	0.75 (0.43-1.33)	44/168	1.10 (0.62-1.97)
			interaction (BMI by total lycopene) = 0.25		interaction (BMI by total lycopene) = **0.05**	

[‡] Adjusted for age, PSA screening history, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[a] Dietary α-carotene: European Americans (range: 27.20-9812.26, median = 453.42 μg/day); African Americans (range: 11.83-9558.28, median = 396.03 μg/day)

[b] Dietary β-carotene: European Americans (range: 194.86-25124.05, median =3016.49 μg/day); African Americans (range: 286.48-32901.26, median = 3727.51 μg/day)

[c] Supplements: compared users to non-users because limited variability in dose did not allow for creation of meaningful categories

[d] Total β-carotene: European Americans (range: 346.80-25512.15, median = 3356.11 μg/day); African Americans (range: 286.48-32901.26, median = 3872.02 μg/day)

[e] Dietary β-cryptoxanthin: European Americans (range: 6.04-1082.55, median = 125.73 μg/day); African Americans (range: 3.53-1594.39, median = 179.15 μg/day)

[f] Dietary Lutein + Zeaxanthin: European Americans (range: 289.67-30165.13 , median = 2423.60 μg/day); African Americans (range: 112.25-36608.75, median = 3140.16 μg/day)

[g] Total Lutein + Zeaxanthin: European Americans (range: 289.6-31665.13 , median = 2535.48 μg/day); African Americans (range: 112.25-37558.75, median = 3185.08 μg/day)

[h] Dietary lycopene: European Americans (range: 344.77-100250.76, median = 4646.27 μg/day); African Americans (range: 4.90-106071.98, median = 3305.31 μg/day)

[i] Total Lycopene: European Americans (range: 344.77-100550.76 , median = 4708.89 μg/day); African Americans (range: 4.90-106071.98, median = 3336.95 μg/day)

CHAPTER 7

POLYMORPHISMS IN DNA REPAIR AND OXIDATIVE STRESS-RELATED GENES, DIETARY ALPHA- AND GAMMA-TOCOPHEROL INTAKE, AND PROSTATE CANCER AGGRESSIVENESS

7.1 INTRODUCTION

Among American men, prostate cancer (PCa) continues to have the highest incidence and second highest mortality of any other cancer [339]. Although the etiology of PCa remains largely unclear, considerable evidence indicates that oxidative stress may play a role in the disease initiation and progression [470, 478, 479]. Oxidative stress is a state of elevated intracellular levels of reactive oxygen and nitrogen species beyond antioxidant defense capacity, which can lead to the malignant transformation of normal epithelial cells [480, 481].

Several studies have shown parallels between oxidative stress and the known and potential risk factors of PCa. For example, exposure to reactive oxygen species (ROS), whether endogenously or exogenously generated, and accumulation of oxidative DNA damage are known to increase with age, which is the strongest risk factor for PCa [482]. Chronic inflammation, particularly chronic prostatitis, is thought to promote oxidative stress by activating inflammatory cells that increase the up-take of oxygen resulting in excessive production of ROS [483, 484]. Epidemiologic data regarding diet and PCa suggest that energy imbalance as well as high-fat and high-calorie diets promote PCa incidence and aggressiveness [17]. These factors also have been shown to increase oxidative stress through various metabolic pathways [470]. Studies show that the

expression of oxidative stress defense enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), are lower in malignant prostate tissues than in health prostate tissues [485-487]. Furthermore, high aggressive CaPs are found to have a greater degree of oxidative stress than less aggressive CaPs [479]. Together, these observations suggest that oxidative stress may be an underlying mechanism through which several risk factors influence PCa incidence and aggressiveness.

Sustained oxidative stress resulting from impaired antioxidant defense or excessive exposure to ROS causes damage to DNA and other important cellular components [481]. Oxidative DNA damage is repaired through complex DNA repair pathways involving base excision repair (BER) which repairs non-bulky adducts and single-strand breaks, nucleotide excision repair (NER) removes bulky and helix-distorting adducts, mismatch repair (MMR) corrects mispaired DNA bases, and homologous recombination and end-joining DNA repair mechanisms are involved in the repair of double-stranded breaks [488, 489]. There is ample evidence that genes involved in oxidative stress, antioxidant defense, and DNA repair mechanisms harbor polymorphisms that may have functional significance to PCa due to their pro- or anti-carcinogenic properties [490-492]. These polymorphisms may act in tandem with environmental factors to influence prostate carcinogenesis.

Habitually low antioxidant intake may promote oxidative stress and susceptibility to PCa, whereas greater intakes of antioxidants can boost antioxidant defense against oxidative stress [470]. There has been particular interest in the cancer prevention potential of alpha (α) and gamma (γ) tocopherol, which have strong antioxidant properties and are the major forms of vitamin E [391]; however, study results are varied

(reviewed in [17, 493-496]). It is possible that these nutrients offer protection only for men with certain genetic profiles, and are perhaps detrimental to others. Therefore, this study examined whether associations between α- and γ-tocopherol intake and PCa aggressiveness are modulated by single nucleotide polymorphisms (SNPs) in DNA repair and oxidative stress-related genes among African-American (AA) and European-American (EA) men in North Carolina and Louisiana.

7.2 METHODS

Study Population

This work was performed with data from the North Carolina-Louisiana Prostate Cancer Project (PCaP), which is a population-based, case-only, cross-sectional study of PCa aggressiveness. North Carolina (NC) and Louisiana (LA) residents with pathologist-confirmed, first diagnosis of adenocarcinoma of the prostate between July 2004 and August 2009, who were 40-79 years old at diagnosis and self-identified their race to be Black/AA or Caucasian American/White/EA were recruited via rapid case-ascertainment. Other eligibility criteria included the ability to complete study interview in English and being mentally and physically competent to give consent and participate in the study. A detailed description of the PCaP research protocol has been published [399]. All research subjects provided written informed consent, including consent for genetic studies, prior to enrollment. PCaP enrolled a total of 2258 research subjects with approximately equal numbers of AAs (n = 1,130) and EAs (n = 1,128). Analyses were restricted to the research subjects with genotyped data on the polymorphisms of interest (AA, n = 948; EA, n = 1,016). The study protocols were approved by Institutional Review Boards (IRBs) of the University of North Carolina at Chapel Hill (UNC-CH), the Louisiana State

University Health Sciences Center, and the Department of Defense Prostate Cancer Research Program. For the current analyses, additional IRB approval was obtained from the University of South Carolina.

Data Collection

Trained research nurses conducted structured, in-person interviews soliciting information on prostate-related health factors, including demographics, family history of PCa, pre-diagnostic PCa screening habits, personal and family health history, physical activity, smoking status, and use of vitamins and supplements, non-steroidal anti-inflammatory drugs (NSAIDs), and alcohol. The research nurses obtained anthropometric measurements (i.e., weight and height) using standardized protocol during in-person interviews and collected biological specimens that included peripheral blood and buccal cell samples for DNA analyses. The research subjects provided consent for review of their medical records, which were obtained from diagnosing physicians and abstracted by trained personnel to yield information relating to prostate-specific antigen (PSA) tests, Gleason scores and cancer stage at diagnosis as well as other health status information. Approximately 10% of the abstracted medical records were selected at random and abstracted a second time by a different staff member to ensure consistency between abstractors. In PCaP, a research subject is considered to have high aggressive PCa if he had a Gleason sum ≥8, PSA >20 ng/mL or a Gleason sum ≥7 and cancer stage T3–T4 at diagnosis. Low aggressive PCa was defined as research subjects with Gleason sum < 7 and cancer stage T1-T2 or PSA<10 ng/ml. All other cases are classified as intermediate aggressive PCa. The current analyses utilized a case-control design to compare high

aggressive PCa (cases) to low/intermediate aggressive disease (comparison group or controls).

Dietary Assessment

Dietary nutrient intake in the 12 months prior to PCa diagnosis was assessed using a modified version of the National Cancer Institute Diet History, Food-frequency Questionnaire (NCI-DHQ) [400]. The NCI-DHQ was modified to include Southern foods and assesses frequency of intake and usual portion sizes for 144 food items/groups. Nutrient values were assigned to various foods by linking the questionnaire responses to an updated NCI nutrient database. Usual daily intakes of various nutrients were estimate by the NCI Diet*Calc software [401], including intakes of α- and γ-tocopherol. None of the research subjects included in this analysis had implausible energy intake values (i.e., < 500 or > 6000 kcal/day).

SNP Selection

A set of 34 SNPs across 18 candidate genes involved in oxidative stress, antioxidant defense, and DNA repair with known or suspected functional significance in cancer based on previous association studies [2, 134, 466, 490-492, 497-499] were selected from the PCaP GWAS data bank [500]. These included four SNPs implicated in oxidative stress: *NOS3* (rs1799983, rs3918201, rs3918226), *NOX3||ARID1B* (rs9372014); and six SNPs involved in antioxidant defense: *GPX2* (rs4902346), *NQO1* (rs689453), *PPARG* (rs1801282), *SOD2* (rs10370, rs4880), and *USP4||GPX1* (rs8179172). SNPs in different DNA repair pathways were included, with nine in the BER pathway: *APEX1* (rs1048945, rs1130409), *APEX2* (rs28382675), *MUTYH* (rs3219489), *OGG1* (rs1805373), and *XRCC1* (rs1799778, rs1799782, rs2854508,

rs3213247); and seven in the NER pathway: *ERCC8* (rs4647100, rs4647102, rs976631), *XPA* (rs1800975, rs3176644), and *XPC* (rs2227998, rs2733537). Three are involved in homologous recombination repair: *RAD51C* (rs304269, rs6503874), and *XRCC2* (rs3218522). Five are in the non-homologous end-joining repair pathway: *XRCC4* (rs10474079, rs28360135, rs28360248, rs35268, rs3777018) [488, 489]. The selected SNPs were eliminated from further analysis if they had less than 5% minor allele frequency (MAF). Based on this criterion, the following SNPs were removed from analyses among AAs and EAs: *APEX1* (rs1048945), *NOS3* (rs3918201), and *XRCC4* (rs28360135, rs28360248). Additionally, *NOS3* (rs3918226), *PPARG* (rs1801282), and *XRCC1* (rs3213247, rs10474079, rs3777018) were eliminated among AAs only and *APEX2* (rs28382675), *OGG1* (rs1805373), *USP4||GPX1* (rs8179172), and *XPA* (rs3176644) were eliminated among EAs only.

Genotyping

DNA was extracted from each research subject's peripheral blood samples (n = 1,630) or buccal cells (n = 118) by the UNC-CH Biospecimen Processing Facility, or from immortalized lymphocytes by the UNC-CH Tissue Culture Facility (n = 216). Genotyping was done by the Johns Hopkins University's Center for Inherited Disease Research using a custom 1,536-SNP Illumina GoldenGate array. The genotyping data included 22 blinded duplicates and HapMap control samples consisting of a set of 11 YRI and 8 CEU trios for quality checks. Full details of the genotyping and quality control procedures has been described by Bensen et al. [500].

Statistical Methods

Differences in the distributions of research subjects' characteristics by their levels of PCa aggressiveness were assessed using *t* and χ^2 tests for continuous and categorical variables, respectively. Unconditional logistic regression was use to estimate odds ratios (ORs) and 95% confidence intervals (CIs) in models examining main effects of SNP genotypes and dietary α- and γ-tocopherol. Because the frequency of minor alleles of the SNPs were too low to allow for meaningful analysis, the genotypes were categorized into two groups assuming a dominant model, by combining the heterozygous and homozygous variants into one group and compared to the homozygous common genotype (referent group).

Associations between dietary α- and γ-tocopherol intake and PCa aggressiveness were examined by categorizing research subjects into "high" or "low" levels of intake based on median cutpoints among the low/intermediate aggressive research subjects in each race group. These variables were categorized separately for AAs and EAs because of an indication of different dietary patterns between these race groups. Accordingly, analyses were performed separately by race. *A priori* variables determined to be confounders in a previous PCaP study of associations between tocopherols intake and PCa aggressiveness were included in the multivariable-adjusted regression models. These include age (continuous), PSA screening history (0, 1-7, >7 screenings), body mass index (BMI, kg/m^2), smoking status (never, former, current), education (less than high school education, high school graduate, some college/college graduate, graduate degree), household income (<$20,000, $20,001-$40,000, $40,001-$60,000, $60,001-$80,000, >$80,000, unknown), use of NSADs in the five years before diagnosis (no, yes), total

dietary fat intake (grams/day), and study site (NC, LA). Other variables examined for confounding effects but not included in the final analyses were: number of first degree relatives with PCa (none, at least one), comorbidities (0, 1, 2, ≥3), PCa treatment status (started treatment, not started) and total metabolic equivalents (METs) of light, moderate and vigorous exercise in the year prior to diagnosis (≤ 10.2, 10.3-29.0, >29.0 MET-hours/week).

To examine effect modification of associations between α- and γ-tocopherol and PCa aggressiveness, a series of stratified analyses by genotype (categorized into two groups) were performed comparing high to low levels of tocopherol intake. Likelihood ratio tests (LRTs) were used to examine interaction on the multiplicative scale between the binary genotype variable for each SNP and binary tocopherol variables. In evaluating interaction, the difference in -2 log likelihood values of logistic regression models with and without the interaction terms was evaluated by χ^2 test with one degree of freedom. Interaction p-values were considered statistically significant at $p < 0.10$ to compensate for small sample size, which limits statistical power to detect significant interaction [336]. Results were adjusted for multiple testing by controlling for false discovery rate at 0.05 [338]. All statistical analyses were performed with SAS® (version 9.3, SAS Institute, Cary, NC).

7.3 RESULTS

Table 7.1 presents research subject characteristics by level of PCa aggressiveness among AAs and EAs. AA research subjects with high aggressive PCa were somewhat older, had higher dietary fat intake, were less educated, less likely to have had at least one

PSA screening test prior to diagnosis, and included a greater percentage of lower incomes and current smokers as compared to AAs with low/intermediate aggressive PCa. EAs with high aggressive PCa also were older and less educated, and had slightly higher BMI than EAs with low/intermediate aggressive disease.

Analysis of the main effect of the SNPs showed a nearly significant increased odds of high aggressive PCa among EAs with the heterozygous or homozygous variants of *XRCC4* (rs377018) (OR = 1.63, 95% CI = 1.00-2.64, AG+GG *versus* AA), but no other association was observed (Table 7.2). Similarly, dietary α-tocopherol intake was not associated with PCa aggressiveness among AAs (OR = 1.23, 95% CI = 0.79-1.93) or EAs (OR = 0.70, 95% CI = 0.44-1.11) when comparing high with low intake (Table 7.3). In analysis stratified by genotype, statistically significant interactions were observed between α-tocopherol intake and certain SNP genotypes, with some variations in gene-nutrient interaction by race (Table 7.3).

Effect modification by *NOS3* (rs1799983, G > T) was observed among EA research subjects, such that among the minor allele carriers, high α-tocopherol intake was associated with a 54% lower odds of high aggressive PCa compared to low intake, while no association was observed among those homozygous for the common allele ($P_{\text{interaction}}$ = 0.08), and no evidence of effect modification among AAs. *XPA* (rs3176644) was examined among AAs only because of less variability in genotype distribution among EAs (i.e., MAF < 0.05). The AA research subjects who harbor the homozygous common allele of *XPA* (rs3176644, G > T) and had high α-tocopherol intake as compared to lower intake, had an over two-fold increased odds of high aggressive PCa, whereas no

association was observed among those with one or two copies of the minor allele ($P_{interaction}$ = 0.08).

Statistically significant effect modification by *XRCC1* (rs2854508, T > A) was observed among both AAs and EAs ($P_{interaction}$ = 0.01 and 0.04, respectively). In the stratified analysis by *XRCC1* (rs2854508) genotype, α-tocopherol intake was inversely related to high aggressive PCa among EA research subjects who were homozygous for the common allele (OR = 0.46, 95% CI = 0.22-0.85; high *versus* low intake), while no association was observed among AAs with this genotype (OR = 0.95, 95% CI = 0.54-1.64; high *versus* low intake). In contrast, higher α-tocopherol intake appeared to be positively associated with high aggressive PCa among AA research subjects heterozygous or homozygous for the *XRCC1* (rs2854508) minor allele, with no association among EA research subjects with these genotypes. The other notable associations were observed among EAs only, wherein higher α-tocopherol intake was inversely associated with PCa aggressiveness among EA research subjects homozygous for the common allele of *APEX* (rs1130409, T > G), *PPARG* (rs1801282, C > G), *XPC* (rs2733537, A > G), and *XRCC1* (rs1799782, G > A). There were no associations among EA carriers of the minor alleles of these SNPs, and no evidence of interaction (all interaction *P* values > 0.10).

ORs for high *versus* low γ-tocopherol intake did not show significant associations with PCa aggressiveness among AAs or EAs (Table 7.4). However, among EA research subjects, higher γ-tocopherol intake was inversely related to PCa aggressiveness among carriers of the minor allele of *XRCC1* (rs1799782, G > A) and *XRCC2* (rs3218522, C >

T), but not those homozygous for the common allele of these SNPs ($P_{interaction}$ = 0.04 and 0.02, respectively). Among AAs, higher γ-tocopherol intake appears to be inversely related to high aggressive PCa among those who possess the minor allele of *OGG1* (rs1805373, G > A), but not the homozygous common allele carriers ($P_{interaction}$ = 0.08). Similar to the findings for α-tocopherol, a suggestion of positive association between higher γ-tocopherol intake and PCa aggressiveness was observed among AA research subjects with one or two copies of the minor allele for *XRCC1* (rs2854508, T > A) ($P_{interaction}$ = 0.03). In two instances among EAs, no interaction was observed, but higher γ-tocopherol intake was significantly and inversely associated with PCa aggressiveness among carriers of the minor allele of *ERCC8* (rs4647102, T > C) and *RAD51C* (rs6503874, C > G), while no associations were observed among EAs homozygous for the common allele. However, neither these associations nor those reported for α-tocopherol retained statistical significance after adjusting for multiple testing using false discovery rate of 0.05.

7.4 DISCUSSION

This population-based, case-only study of incident PCa showed that associations between dietary intakes of α- and γ-tocopherol and PCa aggressiveness may be modified by certain genotypes of SNPs in oxidative stress and DNA repair genes. The strongest evidence of effect modification for an association between α-tocopherol and PCa aggressiveness was observed among genotypes of *NOS3* (rs1799983), *XPA* (rs3176644), and *XRCC1* (rs2854508). The results indicate that higher α-tocopherol intake is associated with significantly lower odds of high aggressive among EAs who are

heterozygous or homozygous for the minor allele of *NOS3* (rs1799983, G > T), but not EA homozygous common allele, and there was no evidence of effect modification among AAs. Higher α-tocopherol intake also was associated with an increased odds of high aggressive PCa among AAs who harbor the homozygous common allele of *XPA* (rs3176644, G > T), with no associations observed among AAs who possess the at least one copy of the minor allele. Interaction by *XRCC1* (rs2854508, T > A) pointed towards an inverse association between higher α-tocopherol intake and PCa aggressiveness among EAs who were homozygous for the common allele, while a suggestion of an increased odds of high aggressive PCa was associated with higher intake of α- and γ-tocopherol among AAs who were heterozygous or homozygous for the minor allele of this SNP. Additional evidence of effect modification by variants in *OGG1* (rs1805373), *XRCC1* (rs1799782) and *XRCC2* (rs3218522) was observed for associations between γ-tocopherol intake and PCa aggressiveness.

Although there is compelling evidence that oxidative stress, ineffective DNA repair, and habitually low antioxidant intake may act synergistically to promote PCa [470, 491, 494, 495], few studies have investigated interactions between genetic variants in oxidative stress or DNA repair genes, and antioxidants intake in relation to PCa [2, 128, 219, 220, 466, 497]. Even fewer studies have evaluated such interactions within the context of PCa aggressiveness [133, 134, 335]. This is the first study to examine SNPs such as *XPA* (rs3176644), *XRCC1* (rs1799782, rs2854508), and *XRCC2* (rs3218522) for their potential modifying effect of associations between dietary tocopherols and PCa. Other polymorphisms in these genes have been examined in gene-nutrient interaction studies in relation to PCa [2, 128, 466].

In one study, van Gils et al. [2] observed that *XRCC1* (rs25487, *Arg399Gln*) genotype modulates the association between dietary vitamin E intake (α-tocopherol equivalent) and PCa incidence, such that lower vitamin E intake was associated with an increased risk of PCa among carriers of *Arg/Arg* (OR= 2.4, 95%CI = 1.0-5.6, < *versus* ≥ median), and not those with *Arg/Gln or Gln/Gln*. In contrast, Goodman et al. [466] did not observe a modifying effect by the *XRCC1 Arg399Gln* genotype on association between α-tocopherol intake and PCa risk. However, in an evaluation of combined antioxidant exposure (α-tocopherol + lycopene + β-carotene), higher antioxidant exposure was associated with lower risk of PCa among men with *Arg/Arg* genotype (OR= 0.11, 95%CI = 0.02-0.65, < *versus* ≥ median), while no association was observed among those with Arg*/Gln* or *Gln/Gln* genotype ($P_{interaction}$ = 0.01) [466]. However, it remains unclear if α-tocopherol acts differently depending on the biological activity of *XRCC1* variants. *In vitro* studies suggest that the allele substitution *Gln → Arg* at codon 399 appear to decrease the BER capacity of *XRCC1* (rs25487) [501, 502]. Evidence from animal studies indicate that adequate intake of α-tocopherol as well as other antioxidants can boost antioxidant defense and promote DNA repair functions [503]. Thus, α-tocopherol may compensate for defective DNA repair associated with the *Arg* allele [466]. Information on the functional effects of other *XRCC1* polymorphism such as (rs1799782, rs2854508) are lacking. Possibly, the DNA repair functionality of allelic variants of other *XRCC1* polymorphism also vary, and thus may differentially influence tocopherol associations with PCa aggressiveness.

Genetic variations in the NOS3, an oxidative stress-related gene, have been associated with several malignancies including breast and prostate cancer [499, 504-506].

The results from the main effect of *NOS3* (rs1799983, G > T) in the current study was suggestive of an increased odds of high aggressive PCa among EA who carry the minor allele; however, higher α-tocopherol intake in this population was associated with significantly lower odds of high aggressive PCa. Similar observation was made by Li et al. [499], where a positive relation between the *NOS3* (rs1799983) T allele and breast cancer risk in analysis of the main effect of genotype reversed towards a lower risk of breast cancer in the presence of higher fruit and vegetable intake ($P_{\text{interaction}} = 0.005$). Although the biological mechanisms are not clearly elucidated, a functional polymorphism in *NOS3* (894 G → T) at exon 7 has been shown to reduce pro-oxidant enzyme activity [507, 508]. Thus, it appears that the T allele may be sensitive to antioxidant intervention, and as suggested by the current findings, EAs who possess the *NOS3* (rs1799983) T allele may benefit from dietary α-tocopherol intake with respect to PCa aggressiveness.

The main effect of *XRCC4* (rs3777018, G > A) was suggestive of an increased odds of high aggressive PCa among EA carriers of the minor allele; however, there was no evidence of interaction with α- or γ-tocopherol intake. Perhaps *XRCC4* (rs3777018) may modulate associations of other dietary factors in relation PCa. It is also worth noting that while no interaction was observed between α-tocopherol intake and the genotypes of *APEX1* (rs1130409, T > G), *PPARG* (rs1801282, C > G), *XPC* (rs2733537, A > G) or *XRCC1* (rs1799782, G > A), higher α-tocopherol intake was associated with a significantly lower odds of high aggressive PCa among EA who possess the homozygous common alleles of these SNPs. This supports suggestions that the potential benefits of α-tocopherol and other antioxidants may be limited to men with certain genetic variants,

and may explain some of the inconsistencies in the epidemiologic literature when diet is examined in isolation of genetic factors [509].

Among the major findings for γ-tocopherol were significant interactions with *XRCC1* (rs1799782, G > A) and *XRCC2* (rs3218522, C > T) wherein higher γ-tocopherol intake was inversely associated with PCa aggressiveness among EA carriers of the heterozygous and homozygous minor alleles. The minor alleles of *ERCC8 (rs4647102*, T > C), *RAD51C* (rs6503874, C > G), *XRCC4* (rs35268, T > C) among EAs, and *OGG1* (rs1805373, G > A) among AAs also were inversely related to PCa aggressiveness with higher intake of γ-tocopherol. Nonetheless, results should be interpreted cautiously as some of the effect estimates are unstable due to the small sample size. Additionally, the observed associations did not retain statistical significance after correction for multiple comparisons.

Other limitations that are worth consideration include the use of a food frequency questionnaire to measure tocopherol intake, which in addition to identified response set biases [510-512] does not account for the bioavailability or bioefficiency of these nutrients because of inter-individual variability in absorption and metabolism [278]. The use of a single dietary assessment interview also could have introduced some misclassification of tocopherol exposure. However, because this is a case-only study, such misclassification would likely be non-differential, resulting in conservative OR estimates [513]. Because this analysis was limited to research subjects with data on the SNPs of interest, there is the possibility of selection bias, though included *versus* excluded research subjects did not vary substantially in their demographic characteristics suggesting selection bias due to lack of genotyping data is likely minimal (Supplemental

Table 1). Strengths of the study include its evaluation of PCa aggressiveness as opposed to overall risk of PCa. Aggressive PCa tends to have a strong genetic influence, and thus, aggressive and non-aggressive forms of PCa may differ in their etiology [458]. Hence, examining PCa aggressiveness reduces confounding by different disease states. The use of a multiethnic population spanning two states also increases the generalizability of the study findings. Although genotyping errors cannot be completely ruled out in any genetic study, the genotyped data had over 99% concordance with blinded duplicates [411], which adds to the strengths of the study.

7.5 CONCLUSIONS

This study provides evidence of effect modification of the association between α-tocopherol and PCa aggressiveness by *XRCC1* (rs2854508), *NOS3* (rs1799983) and *XPA* (rs3176644) genotypes. The results further suggest that an association between γ-tocopherol and PCa aggressiveness may be modified *OGG1* (rs1805373), *XRCC1* (rs2854508) and *XRCC2* (rs3218522) genotypes. Although the general applicability of these findings awaits verification in larger studies, they illustrate the complex interaction between α- and γ-tocopherol intake, and polymorphisms in oxidative stress and DNA repair genes in relation to PCa aggressiveness. The findings also underscore the importance of considering one's genetic makeup in dietary intervention to identify those mostly likely to benefit from such interventions.

Table 7.1 Characteristics of prostate cancer patients by level of the disease aggressiveness among African and European American men

Characteristics	African Americans N = 948					European Americans N = 1,016				
	High aggressive (n=188)		Low/intermediate aggressive (n= 760)		P ‡	High aggressive (n=153)		Low/intermediate aggressive (n= 863)		P ‡
	Mean (SD)		Mean (SD)			Mean (SD)		Mean (SD)		
Age, years	63 (7.6)		62 (7.6)		0.007	67 (7.5)		64 (7.7)		<0.0001
Energy Intake, kcals/day	2792.8 (1200.8)		2614.7 (1153.1)		0.06	2360.1 (963.4)		2322.1 (878.2)		0.63
Dietary fat intake, grams/day	103.8 (51.6)		95.5 (48.2)		0.04	95.7 (43.2)		91.2 (39.7)		0.20
Body mass index (BMI), kg/m^2	30.0 (6.7)		29.2 (5.4)		0.10	30.3 (5.1)		29.0 (4.8)		0.002
	N	**%**	**N**	**%**		**N**	**%**	**N**	**%**	
Study Site										
NC	80	43	347	46	0.44	64	42	413	48	0.17
LA	108	57	413	54		89	58	450	52	
PSA Screening History										
0 screenings	111	59	278	37	<0.0001	35	23	140	16	0.13
1-7 screenings	46	24	321	42		63	41	385	45	
> 7 screenings	31	17	161	21		55	36	338	39	
Education										
Graduate/professional degree	4	2	54	7	0.002	28	18	184	21	0.004
Some college or college graduate	49	26	227	30		65	42	361	42	
High school grad or voc/tech school	57	30	258	34		33	22	244	28	
Less than high school education	78	42	220	29		27	18	74	9	
Income Level										

≤ $20, 000	75	40	217	29	0.004	22	14	71	8	0.28
$20,001 - $40,000	47	25	199	26		30	20	171	20	
$40,001 - $60,000	18	10	120	16		24	16	149	17	
$60,001 - $80,000	11	6	65	8		19	12	120	14	
>$80,000	14	7	94	12		44	29	278	32	
Unknown	23	12	65	8		14	9	74	9	
Smoking Status										
Never	37	20	258	34	0.0003	54	35	314	36	0.69
Former smokers	99	52	358	47		82	54	472	55	
Current smokers	52	28	144	19		17	11	77	9	
NSAID Use										
No	76	41	336	44	0.38	52	34	289	34	0.92
Yes	110	59	420	56		101	66	572	66	

Prostate cancer aggressiveness defined by a combination of Gleason sum, clinical stage, and PSA level at diagnosis and classified as follows: High aggressive (Gleason sum ≥8 or PSA >20ng/ml or Gleason sum ≥7 AND clinical stage T3 -T4); Low /Intermediate aggressive: all other cases.

Abbreviations: LA – Louisiana; NC –North Carolina; NSAIDs – nonsteroidal anti-inflammatory drugs; PSA – prostate-specific antigen; SD – standard deviation

[‡]Test for differences between low/intermediate and high aggressive cancers were done using t-test for continuous variables and chi-square tests for categorical variables.

Table 7.2 Associations of single nucleotide polymorphisms (SNPs) in oxidative stress and DNA repair genes in relation to prostate cancer aggressiveness by race

		African Americans			European Americans		
Gene (SNP ID)	Genotype	Case/ Controls [b] 188/760	OR (95% CI) [c]	*P*	Case/ Controls [b] 153/863	OR (95% CI) [c]	*P*
APEX1 (rs1130409)	TT	81/305	1.00 (ref)		38/230	1.00 (ref)	
	GT+GG	107/455	0.88 (0.64-1.22)	0.44	115/633	1.10 (0.74-1.64)	0.63
ERCC8 (rs4647100)	AA	156/625	1.00 (ref)		87/481	1.00 (ref)	
	AG+GG	32/135	0.97 (0.64-1.49)	0.91	66/382	0.96 (0.67-1.36)	0.81
ERCC8 (rs4647102)	TT	75/315	1.00 (ref)		61/324	1.00 (ref)	
	CT+CC	113/443	1.10 (0.79-1.53)	0.57	92/539	0.90 (0.63-1.29)	0.57
ERCC8 (rs976631)	TT	101/434	1.00 (ref)		46/275	1.00 (ref)	
	CT+CC	85/325	1.09 (0.79-1.51)	0.58	107/585	1.12 (0.77-1.63)	0.56
GPX2 (rs4902346)	AA	70/266	1.00 (ref)		86/553	1.00 (ref)	
	AG+GG	118/494	0.90 (0.64-1.25)	0.53	67/310	1.39 (0.98-1.97)	0.07
MUTYH (rs3219489)	CC	107/434	1.00 (ref)		92/459	1.00 (ref)	
	CC+GG	81/326	1.02 (0.74-1.41)	0.89	61/404	0.76 (0.53-1.08)	0.12
NOS3 (rs1799983)	GG	152/598	1.00 (ref)		59/382	1.00 (ref)	
	GT+TT	36/160	0.89 (0.59-1.33)	0.56	94/475	1.31 (0.92-1.87)	0.13

NOS3 (rs3918226) [d]	CC	-	-	-	126/725	1.00 (ref)	
	CT+TT	-	-	-	27/138	1.09 (0.69-1.73)	0.70
NOX3||ARID1B (rs9372014) [d]	GG	-	-	-	77/403	1.00 (ref)	
	GT+TT	-	-	-	76/459	0.86 (0.60-1.21)	0.38
NQO1 (rs689453)	CC	171/673	1.00 (ref)		138/731	1.00 (ref)	
	CT+TT	17/87	0.75 (0.44-1.31)	0.31	15/132	0.62 (0.35-1.10)	0.10
OGG1 (rs1805373) [d]	GG	151/648	1.00 (ref)		-	-	-
	AG+AA	37/112	1.45 (0.96-2.20)	0.08	-	-	-
PPARG (rs1801282) [d]	CC	-	-	-	122/670	1.00 (ref)	
	CG+GG	-	-	-	30/193	0.84 (0.54-1.29)	0.42
RAD51C (rs304269)	GG	104/400	1.00 (ref)		57/360	1.00 (ref)	
	AG+AA	84/360	0.92 (0.67-1.27)	0.61	96/503	1.21 (0.85-1.73)	0.30
RAD51C (rs6503874)	CC	89/388	1.00 (ref)		136/767	1.00 (ref)	
	CG+GG	99/371	1.20 (0.87-1.65)	0.27	17/96	1.00 (0.57-1.73)	0.99
SOD2 (rs10370)	TT	145/549	1.00 (ref)		94/515	1.00 (ref)	
	GT+GG	43/209	0.79 (0.54-1.15)	0.21	59/348	0.96 (0.67-1.37)	0.83
SOD2 (rs4880)	AA	69/254	1.00 (ref)		42/197	1.00 (ref)	
	AG+GG	118/505	0.88 (0.63-1.23)	0.45	111/665	0.77 (0.52-1.14)	0.19
USP4||GPX1 (rs8179172) [d]	AA	153/640	1.00 (ref)		-	-	-
	AT+TT	35/120	1.22 (0.81-1.86)	0.34	-	-	-

XPA (rs1800975)	CC	121/470	1.00 (ref)		69/366	1.00 (ref)	
	CT+TT	67/289	0.90 (0.64-1.25)	0.52	84/497	0.90 (0.64-1.28)	0.56
XPA (rs3176644) [d]	GG	69/322	1.00 (ref)		-	-	-
	GT+TT	118/437	1.28 (0.92-1.79)	0.14	-	-	-
XPC (rs2227998)	CC	94/366	1.00 (ref)		78/480	1.00 (ref)	
	CT+TT	94/394	0.92 (0.67-1.27)	0.62	75/378	1.17 (0.83-1.66)	0.36
XPC (rs2733537)	AA	131/527	1.00 (ref)		86/401	1.00 (ref)	
	AG+GG	57/233	0.96 (0.68-1.37)	0.84	67/462	0.71 (0.50-1.01)	0.05
XRCC1 (rs1799778)	GG	102/462	1.00 (ref)		66/353	1.00 (ref)	
	GT+TT	85/294	1.31 (0.95-1.81)	0.10	87/509	0.91 (0.64-1.29)	0.58
XRCC1 (rs1799782)	GG	165/660	1.00 (ref)		131/735	1.00 (ref)	
	AG+AA	23/100	0.93 (0.57-1.52)	0.79	22/128	0.98 (0.60-1.60)	0.93
XRCC1 (rs2854508)	TT	123/478	1.00 (ref)		98/508	1.00 (ref)	
	AT+AA	65/281	0.90 (0.64-1.26)	0.54	55/355	0.80 (0.56-1.14)	0.22
XRCC1 (rs3213247) [d]	CC	-	-	-	136/772	1.00 (ref)	
	AC+AA	-	-	-	17/91	1.06 (0.61-1.84)	0.84
XRCC2 (rs3218522)	CC	122/529	1.00 (ref)		38/248	1.00 (ref)	
	CT+TT	66231	1.25 (0.89-1.75)	0.20	115/615	1.20 (0.80-1.78)	0.38
XRCC4 (rs10474079 [d]	GG	-	-	-	115/665	1.00 (ref)	
	AG+AA	-	-	-	38/197	1.07 (0.71-1.60)	0.74

XRCC4 (rs28360135 [d]	TT	-	-	-	144/792	1.00 (ref)	
	CT+CC	-	-	-	9/71	0.77 (0.37-1.59)	0.48
XRCC4 (rs35268)	TT	100/417	1.00 (ref)		116/670	1.00 (ref)	
	CT+CC	87/343	1.08 (0.78-1.49)	0.63	37/193	1.05 (0.70-1.59)	0.80
XRCC4 (rs3777018) [d]	AA	-	-	-	128/769	1.00 (ref)	
	AG+GG	-	-	-	25/94	**1.63 (1.00-2.64)**	0.05

[a] The most common genotype used as the reference category
[b] Some categories may not sum to total sample because of missing data
[c] Adjusted for age
[d] Results are not presented for SNPs with less than 5% minor allele frequency in the respective race group

Table 7.3 Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between dietary α-tocopherol intake and prostate cancer aggressiveness, and stratified by genotype of SNP in oxidative stress and DNA repair gene pathways

		African Americans (n = 948)				
		Cases/ Controls	OR (95% CI) [b]			
Dietary α-tocopherol mg/day [a]	low	84/379	1.00 (ref)			
	high	104/381	1.23 (0.79-1.93)			
Stratified analysis by SNP		Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele		Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409) T / G	low	34/158	1.00 (ref)	50/221	1.00 (ref)	
	high	47/147	1.58 (0.79-3.14)	57/234	1.06 (0.58-1.94)	0.44
ERCC8 (rs4647100) A / G	low	74/316	1.00 (ref)	10/63	1.00 (ref)	
	high	82/309	1.22 (0.74-1.99)	22/72	1.29 (0.41-4.09)	0.57
ERCC8 (rs4647102) T / C	low	39/159	1.00 (ref)	45/220	1.00 (ref)	
	high	36/156	0.80 (0.37-1.71)	68/223	1.52 (0.86-2.69)	0.60
ERCC8 (rs976631) T / C	low	46/212	1.00 (ref)	37/167	1.00 (ref)	
	high	55/222	1.08 (0.58-1.99)	48/158	1.39 (0.70-2.75)	0.63
GPX2 (rs4902346) A / G	low	32/125	1.00 (ref)	52/254	1.00 (ref)	

		high	38/141	1.17 (0.54-2.52)	66/240	1.32 (0.75-2.33)	0.80
MUTYH (rs3219489)	C / G	low	44/218	1.00 (ref)	40/161	1.00 (ref)	
		high	63/216	1.50 (0.82-2.72)	41/165	1.03 (0.51-2.05)	0.35
NOS3 (rs1799983)	G / T	low	69/308	1.00 (ref)	15/70	1.00 (ref)	
		high	83/290	1.38 (0.84-2.28)	21/90	0.61 (0.20-1.85)	0.66
NOS3 (rs3918226) [c]	C / T	low	-	-		-	
		high	-	-		-	
NOX3||ARID1B (rs9372014) [c]	G / T	low	-	-		-	
		high	-	-		-	
NQO1 (rs689453)	C / T	low	75/340	1.00 (ref)	9/39	1.00 (ref)	
		high	96/333	1.26 (0.79-2.00)	8/48	2.20 (0.26-18.27)	0.22
OGG1 (rs1805373) [c]	G / A	low	65/329	1.00 (ref)	19/50	1.00 (ref)	
		high	86/319	1.33(0.81-2.18)	18/62	1.06 (0.30-3.77)	0.15
PPARG (rs1801282) [c]	C / G	low	-	-		-	
		high	-	-		-	
RAD51C (rs304269)	G / A	low	48/201	1.00 (ref)	36/178	1.00 (ref)	
		high	56/199	1.65 (0.89-3.05)	48/182	0.82 (0.41-1.64)	0.68
RAD51C (rs6503874)	C / G	low	40/210	1.00 (ref)	44/169	1.00 (ref)	
		high	49/178	1.56 (0.78-3.12)	55/202	0.95 (0.52-1.74)	0.44
SOD2 (rs10370)	T / G	low	61/270	1.00 (ref)	21/107	1.00 (ref)	

		high	84/279	1.24 (0.74-2.07)	22/102	1.14 (0.41-3.18)	0.75
SOD2 (rs4880)	A / G	low	27/116	1.00 (ref)	56/262	1.00 (ref)	
		high	42/138	1.18 (0.54-2.56)	62/243	1.33 (0.76-2.34)	0.81
USP4||GPX1 (rs8179172) [c]	A / T	low	68/318	1.00 (ref)	16/61	1.00 (ref)	
		high	85/322	1.17 (0.72-1.92)	19/59	1.66 (0.46-6.06)	0.74
XPA (rs1800975)	.C / T	low	54/236	1.00 (ref)	30/142	1.00 (ref)	
		high	67/234	1.10 (0.63-1.95)	37/147	1.41 (0.67-2.99)	0.74
XPA (rs3176644) [c]	G / T	low	27/163	1.00 (ref)	56/213	1.00 (ref)	
		high	42/159	**2.19 (1.05-4.57)**	62/224	**0.80** (0.45-1.44)	**0.08**
XPC (rs2227998)	C / T	low	43/182	1.00 (ref)	41/197	1.00 (ref)	
		high	51/184	0.95 (0.49-1.83)	53/197	1.47 (0.78-2.76)	0.84
XPC (rs2733537)	A / G	low	58/267	1.00 (ref)	26/112	1.00 (ref)	
		high	73/260	1.19 (0.69-2.05)	31/121	1.55 (0.64-3.78)	0.75
XRCC1 (rs1799778)	G / T	low	47/227	1.00 (ref)	37/151	1.00 (ref)	
		high	55/235	1.23 (0.68-2.20)	48/143	1.15 (0.55-2.39)	0.74
XRCC1 (rs1799782)	G / A	low	72/336	1.00 (ref)	12/43	1.00 (ref)	
		high	93/324	1.33 (0.83-2.15)	112/57	0.43 (0.07-2.73)	0.19
XRCC1 (rs2854508)	T / A	low	61/229	1.00 (ref)	23/150	1.00 (ref)	
		high	62/249	**0.95** (0.54-1.67)	42/131	**2.04** (0.94-4.42)	**0.01**
XRCC1 (rs3213247) [c]	C / A	low	-	-		-	
		high	-	-		-	

XRCC2 (rs3218522) C / T	low	52/274	1.00 (ref)	32/105	1.00 (ref)	
	high	70/255	1.54 (0.88-2.71)	34/126	0.77 (0.35-1.70)	0.11
XRCC4 (rs10474079) [c] G / A	low	-	-		-	
	high	-	-		-	
XRCC4 (rs28360135) [c] T / C	low	-	-		-	
	high	-	-		-	
XRCC4 (rs35268) [c] T / C	low	47/204	1.00 (ref)	36/175	1.00 (ref)	
	high	53/213	1.08 (0.59-1.99)	51/168	1.63 (0.83-3.21)	0.16
XRCC4 (rs3777018) [c] A / G	low	-	-		-	
	high	-	-		-	

[a] Categorized based on median split (< vs. ≥ median) among controls for African Americans as low: 1.54 – 9.30 mg/day and high: 9.31 – 44.62 mg/day; and for European Americans: 1.67 – 9.50 and 9.51 – 53.18 mg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[c] Results not presented for SNPs with deviation from Hardy-Weinberg equilibrium (p-value <0.05) and for SNPs with less than 5% minor allele frequency among controls in the respective race group.

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and dietary α-tocopherol level (< vs. ≥ median) in multivariable adjusted models.

Table 7.3 (continued) Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between dietary α-tocopherol intake and prostate cancer aggressiveness, and stratified by genotype of SNP in oxidative stress and DNA repair gene pathways

		European Americans (n = 1,016)				
		Cases/ Controls	OR (95% CI) [b]			
Dietary α-tocopherol mg/day [a]	low	81/430	1.00 (ref)			
	high	72/433	0.70 (0.44-1.11)			
Stratified analysis by SNP		Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele		Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409) T / G	low	20/102	1.00 (ref)	61/328	1.00 (ref)	
	high	18/128	**0.31 (0.12-0.81)**	54/305	0.90 (0.53-1.54)	0.50
ERCC8 (rs4647100) A / G	low	46/244	1.00 (ref)	35/486	1.00 (ref)	
	high	41/237	0.62 (0.33-1.15)	31/196	0.71 (0.35-1.44)	0.99
ERCC8 (rs4647102) T / C	low	34/158	1.00 (ref)	47/272	1.00 (ref)	
	high	27/166	0.55 (0.26-1.16)	45/267	0.82 (0.46-1.49)	0.31
ERCC8 (rs976631) T / C	low	25/135	1.00 (ref)	56/294	1.00 (ref)	
	high	21/140	0.61 (0.26-1.40)	51/291	0.75 (0.43-1.33)	0.94
GPX2 (rs4902346) A / G	low	46/269	1.00 (ref)	35/161	1.00 (ref)	
	high	40/284	0.60 (0.32-1.09)	32/149	0.82 (0.39-1.72)	0.41

MUTYH (rs3219489) C / G	low	50/228	1.00 (ref)	31/202	1.00 (ref)	
	high	42/231	0.57 (0.31-1.06)	30/202	0.90 (0.44-1.82)	0.77
NOS3 (rs1799983) G / T	low	29/205	1.00 (ref)	52/222	1.00 (ref)	
	high	30/177	**1.09** (0.51-2.30)	42/253	**0.46 (0.25-0.84)**	**0.08**
NOS3 (rs3918226) [c] C / T	low	63/363	1.00 (ref)	18/67	1.00 (ref)	
	high	63/362	0.80 (0.48-1.32)	9/71	0.39(0.11-1.37)	0.12
NOX3||ARID1B (rs9372014) [c] G / T	low	39/200	1.00 (ref)	42/230	1.00 (ref)	
	high	38/203	0.93 (0.48-1.81)	34/229	0.52 (0.27-1.02)	0.74
NQO1 (rs689453) C / T	low	73/369	1.00 (ref)	8/61	1.00 (ref)	
	high	65/362	0.72 (0.44-1.17)	7/71	0.87 (0.17-4.40)	65
OGG1 (rs1805373) [c] G / A	low	-	-		-	
	high	-	-		-	
PPARG (rs1801282) [c] C / G	low	67/329	1.00 (ref)	14/101	1.00 (ref)	
	high	55/341	**0.59 (0.35-0.99)**	16/92	0.86 (0.26-2.80)	0.24
RAD51C (rs304269) G / A	low	28/181	1.00 (ref)	53/249	1.00 (ref)	
	high	29/179	0.86 (0.41-1.81)	43/254	0.64 (0.35-1.17)	0.42
RAD51C (rs6503874) C / G	low	70/381	1.00 (ref)	11/49	1.00 (ref)	
	high	66/386	0.74 (0.46-1.21)	6/47	0.39 (0.05-3.15)	0.53
SOD2 (rs10370) T / G	low	52/253	1.00 (ref)	29/177	1.00 (ref)	
	high	42/262	0.56 (0.31-1.03)	30/171	0.88 (0.42-1.84)	0.54
SOD2 (rs4880) A / G	low	23/114	1.00 (ref)	58/316	1.00 (ref)	

		high	19/83	1.29 (0.52-3.21)	53/349	0.59 (0.34-1.05)	0.49
USP4||GPX1 (rs8179172) [c]	A / T	low	-	-		-	
		high	-	-		-	
XPA (rs1800975)	C / T	low	36/187	1.00 (ref)	45/243	1.00 (ref)	
		high	33/179	0.65 (0.32-1.32)	39/254	0.73 (0.39-1.35)	0.63
XPA (rs3176644) [c]	G / T	low	-	-		-	
		high	-	-		-	
XPC (rs2227998)	C / T	low	41/247	1.00 (ref)	40/181	1.00 (ref)	
		high	37/233	0.78 (0.42-1.47)	35/197	0.58 (0.29-1.16)	0.57
XPC (rs2733537)	A / G	low	50/194	1.00 (ref)	31/236	1.00 (ref)	
		high	36/207	**0.46 (0.24-0.88)**	36/226	1.19 (0.61-2.33)	0.12
XRCC1 (rs1799778)	G / T	low	31/178	1.00 (ref)	50/251	1.00 (ref)	
		high	35/175	1.04 (0.50-2.15)	37/258	0.55 (0.30-1.03)	0.19
XRCC1 (rs1799782)	G / A	low	69/357	1.00 (ref)	12/73	1.00 (ref)	
		high	62/378	**0.60 (0.37-0.99)**	10/55	3.90 (0.68-22.32)	0.71
XRCC1 (rs2854508)	T / A	low	58/248	1.00 (ref)	23/182	1.00 (ref)	
		high	40/260	**0.46 (0.22-0.85)**	32/173	**1.11** (0.51-2.41)	**0.04**
XRCC1 (rs3213247) [c]	C / A	low	72/386	1.00 (ref)	9/44	1.00 (ref)	
		high	64/386	0.70 (0.43-1.14)	8/47	0.80 (0.14-4.41)	0.61
XRCC2 (rs3218522)	C / T	low	16/130	1.00 (ref)	65/300	1.00 (ref)	
		high	22/118	0.87 (0.32-2.42)	50/315	0.63 (0.38-1.07)	0.11

XRCC4 (rs10474079) [c] G / A	low	60/330	1.00 (ref)	21/99	1.00 (ref)	
	high	55/335	0.68 (0.40-1.15)	17/98	0.80 (0.28-2.26)	0.95
XRCC4 (rs28360135) [c] T / C	low	77/399	1.00 (ref)	4/31	1.00 (ref)	
	high	67/393	0.67 (0.42-1.09)	5/40	2.57 (0.20-32.89)	0.81
XRCC4 (rs35268) [c] T / C	low	61/331	1.00 (ref)	20/99	1.00 (ref)	
	high	55/339	0.80 (0.47-1.36)	17/94	0.40 (0.14-1.14)	0.99
XRCC4 (rs3777018) [c] A / G	low	67/382	1.00 (ref)	14/48	1.00 (ref)	
	high	61/387	0.76 (0.46-1.24)	11/46	0.89 (0.20-4.02)	0.98

[a] Categorized based on median split (< vs. ≥ median) among controls for African Americans as low: 1.54 – 9.30 mg/day and high: 9.31 – 44.62 mg/day; and for European Americans: 1.67 – 9.50 and 9.51 – 53.18 mg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[c] Results not presented for SNPs with deviation form Hardy-Weinberg equilibrium (p-value <0.05) and for SNPs with less than 5% minor allele frequency among controls in the respective race group.

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and dietary α-tocopherol level (< vs. ≥ median) in multivariable adjusted models.

Table 7.4 Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between dietary γ-tocopherol intake and prostate cancer aggressiveness, and stratified by genotype among African Americans

			African Americans				
			Cases/ Controls	OR (95% CI) [b]			
Dietary γ-tocopherol mg/day [a]		low	85/379	1.00 (ref)			
		high	103/381	0.89 (0.56-1.44)			
Stratified analysis by SNP			Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele			Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409)	T / G	low	39/152	1.00 (ref)	46/227	1.00 (ref)	
		high	42/153	0.78 (0.37-1.63)	61/228	1.01 (0.54-1.92)	0.62
ERCC8 (rs4647100)	A / G	low	75/310	1.00 (ref)	10/69	1.00 (ref)	
		high	81/315	0.82 (0.48-1.39)	22/66	1.51 (0.46-4.94)	0.26
ERCC8 (rs4647102)	T / C	low	34/153	1.00 (ref)	51/226	1.00 (ref)	
		high	41/162	0.76 (0.34-1.70	62/217	1.05 (0.57-1.94)	0.73
ERCC8 (rs976631)	T / C	low	45/214	1.00 (ref)	38/165	1.00 (ref)	
		high	56/220	0.93 (0.48-1..82)	47/160	0.98 (0.48-1.97)	0.92
GPX2 (rs4902346)	A / G	low	32/128	1.00 (ref)	53/251	1.00 (ref)	
		high	38/138	0.71 (0.31-1.64)	65/243	0.99 (0.54-1.80)	0.72
MUTYH (rs3219489)	C / G	low	46/219	1.00 (ref)	39/160	1.00 (ref)	

		high	61/215	0.91 (0.49-1.71)	42/166	0.87 (0.42-1.80)	0.68
NOS3 (rs1799983)	G / T	low	69/303	1.00 (ref)	16/76	1.00 (ref)	
		high	83/295	1.05 (0.62-1.77)	20/84	0.42 (0.11-1.56)	0.55
NOS3 (rs3918226) [c]	C / T	low	-	-		-	
		high	-	-		-	
NOX3||ARID1B (rs9372014) [c]	G / T	low	-	-		-	
		high	-	-		-	
NQO1 (rs689453)	C / T	low	78/341	1.00 (ref)	7/38	1.00 (ref)	
		high	93/332	0.85 (0.52-1.40)	10/49	1.69 (0.17-16.36)	0.69
OGG1 (rs1805373) [c]	G / A	low	65/328	1.00 (ref)	20/51	1.00 (ref)	
		high	86/320	1.05 (0.63-1.79)	17/61	0.30 (0.08-1.17)	**0.08**
PPARG (rs1801282) [c]	C / G	low	-	-		-	
		high	-	-		-	
RAD51C (rs304269)	G / A	low	49/201	1.00 (ref)	36/178	1.00 (ref)	
		high	55/199	1.26 (0.66-2.43)	48/182	0.54 (0.25-1.14)	0.70
RAD51C (rs6503874)	C / G	low	44/204	1.00 (ref)	41/175	1.00 (ref)	
		high	45/184	0.63 (0.30-1.34)	58/196	1.13 (0.60-2.13)	0.61
SOD2 (rs10370)	T / G	low	66/268	1.00 (ref)	19/111	1.00 (ref)	
		high	79/281	0.77 (0.44-1.33)	24/98	1.43 (0.51-3.97)	0.59
SOD2 (rs4880)	A / G	low	29/133	1.00 (ref)	55/245	1.00 (ref)	
		high	40/121	1.39 (0.62-3.14)	63/260	0.72 (0.39-1.31)	0.25

USP4||GPX1 (rs8179172) [c] A / T		low	69/321	1.00 (ref)	16/58	1.00 (ref)	
		high	84/319	0.83 (0.49-1.41)	19/62	1.24 (0.32-4.85)	0.65
XPA (rs1800975) C /T		low	53/237	1.00 (ref)	32/141	1.00 (ref)	
		high	68/233	0.97 (0.53-1.76)	35/148	0.76 (0.34-1.71)	0.64
XPA (rs3176644) [c] G / T		low	29/157	1.00 (ref)	55/222	1.00 (ref)	
		high	40/165	1.24 (0.57-2.70)	63/215	0.76 (0.40-1.42)	0.57
XPC (rs2227998) C / T		low	40/186	1.00 (ref)	45/193	1.00 (ref)	
		high	54/180	0.98 (0.48-1.98)	49/201	0.82 (0.42-1.59)	0.21
XPC (rs2733537) A / G		low	60/267	1.00 (ref)	25/112	1.00 (ref)	
		high	71/260	0.82 (0.46-1.46)	32/121	1.10 (0.42-2.86)	0.72
XRCC1 (rs1799778) G / T		low	47/226	1.00 (ref)	38/153	1.00 (ref)	
		high	55/236	0.98 (0.53-1.82)	47/141	0.77 (0.35-1.71)	0.69
XRCC1 (rs1799782) G / A		low	73/331	1.00 (ref)	12/48	1.00 (ref)	
		high	92/329	0.92 (0.56-1.53)	11/52	0.86 (0.15-4.93)	0.49
XRCC1 (rs2854508) T / A		low	64/237	1.00 (ref)	21/142	1.00 (ref)	
		high	59/241	**0.71** (0.39-1.29)	44/139	**1.60** (0.71-3.63)	**0.03**
XRCC1 (rs3213247) [c] C / A		low	-	-	-	-	
		high	-	-	-	-	
XRCC2 (rs3218522) C / T		low	53/266	1.00 (ref)	32/113	1.00 (ref)	
		high	69/263	0.97 (0.53-1.75)	34/118	0.78 (0.34-1.81)	0.30
XRCC4 (rs10474079) [c] G / A		low	-	-	-	-	

		high	-	-	-	-	
XRCC4 (rs28360135) [c]	T / C	low	-	-	-	-	
		high	-	-	-	-	
XRCC4 (rs35268) [c]	T / C	low	46/206	1.00 (ref)	39/173	1.00 (ref)	
		high	54/211	0.87 (0.46-1.65)	48/170	0.84 (0.40-1.75)	0.51
XRCC4 (rs3777018) [c]	A / G	low	-	-	-	-	
		high	-	-	-	-	

[a] Categorized based on median split (< vs. ≥ median) among controls for African Americans as low: 0.78 – 18.54 mg/day and high: 18.55 – 67.95 mg/day; and for European Americans: 2.68 – 17.22 and 17.23 – 55.13 mg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[c] Results not presented for SNPs with deviation form Hardy-Weinberg equilibrium (p-value <0.05) and for SNPs with less than 5% minor allele frequency among controls in the respective race group.

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and dietary γ-tocopherol level (< vs. ≥ median) in multivariable adjusted models.

Table 7.4 (continued) Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between dietary γ-tocopherol intake and prostate cancer aggressiveness, and stratified by genotype among European Americans

		European Americans				
		Cases/ Controls	OR (95% CI) [b]			
Dietary γ-tocopherol mg/day [a]	low	75/431	1.00 (ref)			
	high	78/432	0.77 (0.48-1.24)			
Stratified analysis by SNP		Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele		Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409) T / G	low	17/108	1.00 (ref)	58/323	1.00 (ref)	
	high	21/122	0.69 (0.25-1.92)	57/310	0.77 (0.44-1.33)	0.77
ERCC8 (rs4647100) A / G	low	39/243	1.00 (ref)	36/188	1.00 (ref)	
	high	48/238	1.06 (0.56-2.01)	30/194	0.48 (0.23-1.03)	0.20
ERCC8 (rs4647102) T / C	low	24/152	1.00 (ref)	51/279	1.00 (ref)	
	high	37/172	1.54 (0.71-3.32)	41/260	**0.48 (0.25-0.91)**	0.16
ERCC8 (rs976631) T / C	low	23/133	1.00 (ref)	52/296	1.00 (ref)	
	high	23/142	0.60 (0.25-1.42)	55/289	0.85 (0.48-1.52)	0.95
GPX2 (rs4902346) A / G	low	42/273	1.00 (ref)	33/158	1.00 (ref)	
	high	44/280	0.73 (0.39-1.37)	34/152	0.85 (0.39-1.82)	0.54
MUTYH (rs3219489) C / G	low	47/232	1.00 (ref)	28/199	1.00 (ref)	

		high	45/227	0.58 (0.31-1.11)	33/205	1.15 (0.55-2.40)	0.60
NOS3 (rs1799983)	G / T	low	26/184	1.00 (ref)	49/244	1.00 (ref)	
		high	33/198	0.78 (0.36-1.69)	45/231	0.72 (0.39-1.34)	0.75
NOS3 (rs3918226) [c]	C / T	low	57/361	1.00 (ref)	18/70	1.00 (ref)	
		high	69/364	0.88 (0.52-1.50)	9/68	0.37(0.11-1.22)	0.12
NOX3||ARID1B (rs9372014) [c]	G / T	low	41/201	1.00 (ref)	34/229	1.00 (ref)	
		high	36/202	0.58 (0.28-1.18)	42/230	0.97 (0.50-1.88)	0.17
NQO1 (rs689453)	C / T	low	69/363	1.00 (ref)	6/68	1.00 (ref)	
		high	69/368	0.69 (0.41-1.14)	9/64	3.34 (0.70-15.84)	0.32
OGG1 (rs1805373) [c]	G / A	low	-	-		-	
		high	-	-		-	
PPARG (rs1801282) [c]	C / G	low	63/330	1.00 (ref)	12/101	1.00 (ref)	
		high	59/340	0.58 (0.34-1.00)	18/92	2.02 (0.63-6.53)	0.10
RAD51C (rs304269)	G / A	low	24/179	1.00 (ref)	51/252	1.00 (ref)	
		high	33/181	0.98 (0.47-2.08)	45/251	0.67 (0.35-1.26)	0.27
RAD51C (rs6503874)	C / G	low	65/388	1.00 (ref)	10/43	1.00 (ref)	
		high	71/379	0.90 (0.55-1.48)	7/53	**0.15 (0.03-0.90)**	0.30
SOD2 (rs10370)	T / G	low	48/249	1.00 (ref)	27/182	1.00 (ref)	
		high	46/266	0.62 (0.33-1.15)	32/166	1.04 (0.47-2.27)	0.41
SOD2 (rs4880)	A / G	low	21/111	1.00 (ref)	54/320	1.00 (ref)	
		high	21/86	1.66 (0.62-4.43)	57/345	0.65 (0.37-1.13)	0.59

USP4||GPX1 (rs8179172) [c] A / T	low	-	-		-	
	high	-	-		-	
XPA (rs1800975) C / T	low	33/184	1.00 (ref)	42/247	1.00 (ref)	
	high	36/182	0.51 (0.23-1.11)	42/250	0.96 (0.51-1.79)	0.80
XPA (rs3176644) [c] G / T	low	-	-		-	
	high	-	-		-	
XPC (rs2227998) C / T	low	39/236	1.00 (ref)	36/193	1.00 (ref)	
	high	39/244	0.66 (0.34-1.29)	39/185	0.92 (0.45-1.86)	0.53
XPC (rs2733537) A / G	low	42/186	1.00 (ref)	33/245	1.00 (ref)	
	high	44/215	0.64 (0.33-1.26)	34/217	0.90 (0.45-1.80)	0.32
XRCC1 (rs1799778) G / T	low	28/178	1.00 (ref)	47/253	1.00 (ref)	
	high	38/175	0.91 (0.42-1.95)	40/256	0.67 (0.36-1.26)	0.26
XRCC1 (rs1799782) G / A	low	59/362	1.00 (ref)	16/69	1.00 (ref)	
	high	72/373	**1.06** (0.52-1.43)	6/59	**0.23** (0.04-1.38)	**0.04**
XRCC1 (rs2854508) T / A	low	51/250	1.00 (ref)	24/181	1.00 (ref)	
	high	47/258	0.76 (0.42-1.36)	31/174	0.73 (0.32-1.70)	0.35
XRCC1 (rs3213247) [c] C / A	low	66/387	1.00 (ref)	9/44	1.00 (ref)	
	high	70/385	0.76 (0.46-1.25)	8/47	1.39 (0.23-8.30)	0.81
XRCC2 (rs3218522) C / T	low	13/139	1.00 (ref)	62/292	1.00 (ref)	
	high	25/109	**1.20** (0.42-3.42)	53/323	**0.66** (0.39-1.14)	**0.02**
XRCC4 (rs10474079) [c] G / A	low	55/326	1.00 (ref)	20/105	1.00 (ref)	

		high	60/339	0.71 (0.41-1.24)	187/92	1.11 (0.40-3.06)	0.72
XRCC4 (rs28360135) [c]	T / C	low	72/402	1.00 (ref)	3/29	1.00 (ref)	
		high	72/390	0.72 (0.44-1.18)	6/42	2.48 (0.21-28.62)	0.66
XRCC4 (rs35268) [c]	T / C	low	54/335	1.00 (ref)	21/97	1.00 (ref)	
		high	62/335	1.04 (0.60-1.79)	16/96	**0.27 (0.08-0.83)**	0.19
XRCC4 (rs3777018) [c]	A / G	low	62/375	1.00 (ref)	13/56	1.00 (ref)	
		high	66/394	0.75 (0.45-1.24)	12/38	1.96 (0.37-10.28)	0.72

[a] Categorized based on median split (< vs. ≥ median) among controls for African Americans as low: 0.78 – 18.54 mg/day and high: 18.55 – 67.95 mg/day; and for European Americans: 2.68 – 17.22 and 17.23 – 55.13 mg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total dietary fat intake, and study site

[c] Results not presented for SNPs with deviation form Hardy-Weinberg equilibrium (p-value <0.05) and for SNPs with less than 5% minor allele frequency among controls in the respective race group.

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and dietary γ-tocopherol level (< vs. ≥ median) in multivariable adjusted models.

CHAPTER 8

LYCOPENE INTAKE AND PROSTATE CANCER AGGRESSIVENESS: EFFECT MODIFICATION BY POLYMORPHISMS IN DNA REPAIR AND OXIDATIVE STRESS RELATED GENES

8.1 INTRODUCTION

Prostate cancer (PCa) is the most common invasive cancer and a leading cause of cancer death among men in North America and Western Europe [514]. The aggressive forms of PCa occur most frequently in men of African ancestry, and these are often diagnosed at an early age [515, 516]. Epidemiologic studies suggest that the etiology of aggressive PCa may be different from that of non-aggressive PCa, including differences in genetic susceptibility [517, 518] and potential differences in dietary risk factors [17, 437]. Lycopene, an antioxidant carotenoid, is among the potentially beneficial dietary factors associated with a reduced risk of PCa [179, 440, 519]. Some studies show that lycopene may have a stronger inverse association with the aggressive forms of PCa compared with more indolent PCa [520, 521]. Genetic variants (i.e., single nucleotide polymorphisms, SNPs) in oxidative stress and DNA repair genes have also been associated with PCa risk and aggressiveness [332, 491, 492, 522]. However, lycopene intake and genetic risk variants, when examined in isolation, may explain only a small portion of the factors contributing to PCa aggressiveness. There is some evidence that the association between lycopene and PCa are modulated by polymorphic variants of genes involved in the biological processes of PCa, such as those in the oxidative stress and DNA repair pathways [2, 128, 466, 470].

Several reports indicate that oxidative stress-related genes may confer greater risk of PCa [478, 522]. Indeed, oxidative stress has been implicated in prostate tumorigenesis [470, 478] and is thought to promote PCa initiation and aggressiveness by causing damage to DNA [479]. Cellular response to oxidative DNA damage involves the activation of oxidative stress-mediating genes, including those involved in base excision repair, nucleotide excision repair, homologous re-combination repair, and non-homologous end-joining DNA repair genes to correct the damaged parts and restore genomic stability [125, 523]. The ability to recognize and repair oxidatively modified DNA is an important determinant of an individual's susceptibility to PCa [523]. Thus, individual differences in polymorphic variants of genes encoding oxidative stress and DNA repair functions may influence the incidence and aggressiveness of PCa [125, 478].

Lycopene, which is obtained mainly from tomatoes and tomato-based products, has been shown in laboratory studies to have many anticancer properties, including acting as an antioxidant and inhibitor of oxidative DNA damage [371, 524]. However, associations between lycopene and PCa overall are inconsistent (reviewed in [179, 393, 440, 519]). Of five prospective dietary studies [180, 437, 449, 525, 526], three [180, 437, 449] reported an inverse association between lycopene intake and PCa incidence, while two [525, 526] were not supportive of a beneficial role of lycopene in PCa. Among case-control studies, three [450, 527, 528] reported inverse associations with higher lycopene intake, while several others have reported null association ([461, 462, 529], also reviewed in [179, 393, 440, 519]). Similarly, some [520, 521], but not all [442, 530, 531], plasma- and serum-based studies have reported inverse associations between lycopene levels and PCa incidence. These somewhat inconsistent findings might be because these studies

consisted of a heterogeneous group of PCa cases, which can confound associations. It might also reflect the genetic risk variability of the studied populations. Examining gene-nutrient interaction in relation to PCa phenotype may therefore help clarify the association between lycopene and PCa.

The purpose of this study was to investigate whether polymorphisms in oxidative stress and DNA repair genes interact with lycopene to modulate PCa aggressiveness among African-American (AA) and European-American (EA) men in North Carolina and Louisiana. The hypothesis was that associations between lycopene intake and PCa aggressiveness are modified by SNPs in oxidative stress and DNA repair genes.

8.2 METHODS

Study Population

This study was conducted using data from the North Carolina-Louisiana Prostate Cancer Project (PCaP) [399]. PCaP is a large, population-based, cross-sectional, case-only study designed to examine biological, lifestyle, and socio-demographic factors associated with PCa aggressiveness among AAs and EAs. Between July 2004 and August 2009, PCaP enrolled 2,258incident PCa cases (AAs, n = 1,130; EAs, n = 1,128) from North Carolina (NC) and Louisiana (LA), with a median time between diagnosis and recruitment of 3.9 months. The inclusion criteria for PCaP were having a first diagnosis of histologically confirmed adenocarcinoma of the prostate, being of age 40–79 years at diagnosis, and self-identification of race as Black/AA or Caucasian American/White/EA. The eligible participants were also required to be able to complete the study interview in English; have sufficient physical and cognitive ability to participate; and not reside in an institution (e.g., nursing home). Institutional Review Board (IRB) approval was obtained

from all collaborating institutions, and all of the research subjects provided consent before enrollment [399]. The current analysis also was approved by the IRB of the University of South Carolina. The PCa cases included in this analysis were drawn from PCaP research subjects with available data on the SNPs of interest (n = 1,964; AAs n = 948; EAs n = 1,016).

Data Collection

Data on demographic, lifestyle, and health-related factors were obtained by trained research nurses using structured questionnaires during in-home visits [399]. The research nurses also obtained anthropometric measures and peripheral blood samples during each interview following a standardized protocol. Research subjects who could not provide blood samples were given the option to complete a buccal rinse for DNA analysis (approximately 5%). Information on the cancer stage at diagnosis, Gleason sum, and prostate-specific antigen (PSA) level at diagnosis were abstracted from the medical records obtained from diagnosing physicians. The medical records abstraction was standardized and performed by trained personnel, and included a duplicate abstraction of a random sample (about 10%) to ensure consistency between abstractors. PCa aggressiveness was classified as previously described [399] to be high aggressive (Gleason sum ≥8; PSA >20 ng/ml; or Gleason sum = 7 and stage T3–T4), low aggressive (Gleason sum <7 and stage T1–T2 and PSA<10 ng/ml), or intermediate aggressive (all other cases). These categories were used in a case-control study design to compare high aggressive PCa “cases” with low or intermediate aggressive PCa “controls,” as has been done in previous studies [532, 533].

Dietary Assessment

Dietary data were obtained using the National Cancer Institute Dietary History, Food-frequency Questionnaire (NCI-DHQ) [453], which was modified to include Southern foods. The modified 144-item questionnaire queried on frequency of food intake, usual portion size, and food preparation methods in the 12 months prior to diagnosis with PCa. Usual daily nutrient intake was estimated using the Diet*Calc software, which utilizes the NCI's nutrient database [399]. In addition, a validated questionnaire [454] was used to solicit information on multivitamins and single-nutrient supplement use in the 12 months prior to diagnosis. This information was used to calculate total lycopene intake by combining lycopene intake from food and supplements. None of the subjects included in this analysis had implausible values for calorie intake (i.e., < 500 or > 6000 kcal/day).

SNP Selection

Details of the methods used for SNP selection in PCaP has been reported [332]. For the current analysis, 34 candidate SNPs across 18 genes were selected from the PCaP genotype data repository for analyses. These include *NOS3* (rs1799983, rs3918201, rs3918226) and *NOX3||ARID1B* (rs9372014), which are implicated in oxidative stress, and *GPX2* (rs4902346), *NQO1* (rs689453), *PPARG* (rs1801282), *SOD2* (rs10370, rs4880), and *USP4||GPX1* (rs8179172), which are involved in antioxidant defense. Also included were *APEX1* (rs1048945, rs1130409), *APEX2* (rs28382675), *MUTYH* (rs3219489), *OGG1* (rs1805373), and *XRCC1* (rs1799778, rs1799782, rs2854508, rs3213247), which are located in the base excision repair pathway, and *ERCC8* (rs4647100, rs4647102, rs976631), *XPA* (rs1800975, rs3176644), and *XPC* (rs2227998,

rs2733537), which are located in the nucleotide excision DNA repair pathway. *RAD51C* (rs304269, rs6503874) and *XRCC2* (rs3218522), which are involved in homologous recombination, and *XRCC4* (rs10474079, rs28360135, rs28360248, rs35268, rs3777018), which is involved in non-homologous end-joining DNA repair, were also analyzed [489, 534]. These SNPs were selected because of known or suspected functional significance in oxidative stress or DNA repair in relation to cancer based on published literature [491, 492, 522, 535]. An *a priori* decision was made to exclude SNPs with low minor allele frequency (MAF, < 0.05). Based on this criterion, four out of the 34 SNPs were removed from the analyses for both AAs and EAs: *APEX1* (rs1048945), *APEX2* (rs28382675), *NOS3* (rs3918201), and *XRCC4* (rs28360248). Of the remaining SNPs, *OGG1* (rs1805373), USP4||GPX1 (rs8179172), and *XPA* (rs3176644) were excluded from analyses among EAs only, while *NOS3* (rs3918226), *NOX3*||*ARID1B* (rs9372014), *PPARG* (rs1801282), *XRCC1* (rs3213247), and *XRCC4* (rs10474079, rs28360135, rs3777018) were excluded among AAs only.

Genotyping

DNA was extracted from blood samples (n = 1,630) or buccal cells (n = 118) by the University of North Carolina (UNC, Chapel Hill) Biospecimen Processing Facility or from peripheral blood mononuclear cells immortalized by the UNC Tissue Culture Facility (n = 216). Genotyping was done at the National Institutes of Health (NIH) Center for Inherited Disease Research (CIDR) using a custom designed Illumina GoldenGate array. There was an excellent genotyping call rate (99.93%) and inter-assay agreement with blinded duplicates (99.99%). Further details of the genotyping process and quality control measures have been published [332].

Statistical Methods

All analyses were conducted separately for AAs and EAs because of significant interaction that was observed between race and lycopene intake. Distributions of research subjects' characteristics by the levels of PCa aggressiveness were examined using Students' *t*-test and chi-square tests for continuous and categorical variables, respectively. Unconditional logistic regression was used to calculate odds ratios (ORs) and corresponding 95% confidence intervals (CIs). In order to maximize sample size, a dominant model of inheritance was adopted to evaluate the effect of genotype on PCa aggressiveness by collapsing the three-level genotype variables into two groups. This was done by taking the most frequent genotype (i.e., homozygous common allele) as the reference category to estimate ORs for the homozygous and heterozygous variant genotypes (combined in one group), adjusting for age at diagnosis. The lycopene variables also were categorized into two groups (< *versus* ≥ median) to conserve sample size. Cutpoints for categorizing the dietary and total (diet + supplement) lycopene variables were based on race-specific distribution among low/intermediate aggressive cases.

All multivariable logistic regression models simultaneously adjusted for the following variables: age (continuous), study site (NC, LA), BMI (in kg/m^2), pre-diagnostic PSA screening history (0, 1–7, >7 screenings), smoking status (never, former, current), education (less than high school education, high school graduate/some college, college graduate), annual household income (< $20,000, $20,001–$40,000, $40,001–$70,000, >$70,000), regular NSAIDs use in the five years prior to diagnosis (yes, no), and total fat intake (grams/day). Other factors that were evaluated as potential

confounders but not included in the final adjusted models are: comorbidities (0, 1, 2, ≥3), a first degree family history of PCa (none, at least one), PCa treatment status (started treatment, not started), and physical activity (i.e., metabolic equivalents (METs) of light, moderate, and vigorous exercise in the year prior to diagnosis, ≤ 10.2, 10.3–29.0, >29.0 MET-hours/week).

To examine potential modification of associations between lycopene intake and PCa aggressiveness, stratified analyses were performed comparing greater than or equal to median (i.e. high) with less than median (i.e., low) intake within each stratum of the genotype groups (homozygous common allele *versus* heterozygous or homozygous minor allele). Interaction effects were assessed on the multiplicative scale using likelihood ratio tests to compare models with and without interaction terms of the dichotomous genotype and lycopene variables following the hierarchical principle. The threshold for a statistically significant interaction was set at P value < 0.10 to compensate for the small sample size in the stratified groups [336]. All other tests were considered statistically significant at the 0.05 level. Results were corrected for multiple testing using the false discovery rate (FDR) method [338]. All statistical tests were two-sided and performed using SAS® version 9.3 (SAS, Inc.).

8.3 RESULTS

In both race groups, research subjects with high aggressive PCa were older and less educated as compared to those with low or intermediate aggressive PCa (Table 8.1). EA research subjects with high aggressive PCa had a marginally higher BMI than EAs with low or intermediate aggressive PCa. AA research subjects with high aggressive PCa tended to have higher dietary fat intake and included greater proportions of current

smokers, low incomes, and those who have never had a PSA screening test prior to diagnosis with PCa as compared with AAs with low or intermediate aggressive PCa.

As shown in Table 8.2, evaluation of the main effect of the SNP variants in relation to PCa aggressiveness did not show a statistically significant beneficial or harmful effect for any of the SNPs, except for a marginally significant association for the *XRCC4* (rs3777018) SNP among EAs. The EA carriers of the minor allele of *XRCC4* (rs3777018, A > G) appeared to have greater odds of high aggressive PCa than those homozygous for the common allele (OR = 1.63, 95% CI = 1.00-2.64).

Dietary lycopene intake expressed as the median split variable was not significantly associated with PCa aggressiveness among EAs (OR = 0.77, 95% CI = 0.53–1.16, high *versus* low intake) or AAs (OR = 1.41, 95% CI = 0.96-2.07, high *versus* low intake) (Table 8.3). However, evidence existed of gene-nutrient interaction, which was particularly noticeable for the *XRCC1* (rs2854508, T > A) (*P* values for interaction = 0.01 and 0.06 for AAs and EAs, respectively). The pattern of the interaction was such that, in both AAs and EAs, high (compared to low) dietary lycopene intake tended to be associated with increased odds of high aggressive PCa among those heterozygous or homozygous for the minor allele, while higher lycopene intake was inversely related to high aggressive PCa among those homozygous for the common allele. *XPA* (rs1800975, C > T) also appeared to have a modifying effect on associations between dietary lycopene and PCa aggressiveness in that lycopene intake was associated with reduced odds of high aggressive PCa among EAs with one or two copies of the minor allele (OR = 0.56, 95% CI = 0.32–0.98, high *versus* low intake), but not those homozygous for the common allele (OR = 1.02, 95% CI = 0.56–1.87, high *versus* low intake; *P* value for interaction =

0.07). Analogous findings were observed among AAs, although statistical interaction was non-significant in this group. A similar pattern of lower odds of high aggressive PCa was observed among EAs who harbor the minor allele of *NQO1* (rs689453, C > T) and *XRCC2* (rs3218522, C > T) and had higher lycopene intake, which was not observed among EAs who were homozygous for the common allele (*P* values for interaction = 0.05 and 0.08, respectively). Among AAs only, higher dietary lycopene intake was associated with increased odds of high aggressive PCa among those homozygous for the common alleles of *ERCC8* (rs4647102, T > C), *RAD51C* (rs6503874, C > G), and *XRCC1* (rs1799778, G >T), but not those who were heterozygous or homozygous for the minor alleles of these SNPs. Among AAs heterozygous or homozygous for the minor alleles of *XPA* (rs3176644, G >T), *XPC* (rs2227998, C >T), and *XPC* (rs2733537, A > G) higher lycopene intake was associated with increased odds of high aggressive PCa.

Table 8.4 presents associations of total lycopene intake from diet, supplements, and PCa aggressiveness by genotype. Similar to dietary lycopene, there were no significant associations between total lycopene intake and PCa aggressiveness among EAs or AAs. Interactions observed with total lycopene were fewer, but largely mirror those observed with dietary lycopene. In particular, the genetic variants in *XRCC1* (rs2854508), *XPA* (rs1800975), and *NQO1* (rs689453) demonstrated identical interaction with total lycopene intake as observed with dietary lycopene. Similarly, the associations observed for *RAD51C* (rs6503874), *XPA* (rs3176644), and *XPC* (rs2733537) with dietary lycopene among AAs were consistent with the findings for total lycopene intake among AAs.

8.4 DISCUSSION

In this population-based, case-only study, there was evidence that an association between lycopene and PCa aggressiveness can be modified by genotype of SNPs in oxidative stress and DNA repair genes. The evidence was strongest for the *XRCC1* (rs2854508) SNP, whereby among research subjects with the variant genotypes (AT or AA), higher lycopene intake was associated with an increased odds of high aggressive PCa, while high lycopene intake was associated with lower odds of high aggressive PCa among those with the homozygous common genotype (TT). In addition to *XRCC1* (rs2854508), other SNPs that did not have independent association with high aggressive PCa appeared to interact with lycopene to influence PCa aggressiveness. Notably, *XPA* (rs1800975) and *NQO1* (rs689453), demonstrated statistical interaction with dietary and total lycopene intake, with similar patterns of association in both AAs and EAs. It is, however, worth noting that no study has yet examined these SNPs in gene-diet interaction studies. Hence, confirmatory studies are warranted.

Few data exist for interaction between lycopene and other polymorphisms in DNA repair and oxidative stress-related genes [2, 128, 335, 466, 497]. Goodman and colleagues [466] demonstrated that the *XRCC1* A399G polymorphism modifies association between lycopene and PCa risk such that the beneficial effect of lycopene was only evident in men with the AA genotype, not those with the AG or GG genotype. Li et al. [335] examined interaction between pre-diagnostic plasma lycopene level and genotype of the manganese superoxide dismutase (*MnSOD*, also referred to as *SOD2*) gene, but did not find independent association of *MnSOD* genotype or interaction with plasma lycopene level in relation to overall risk of PCa and risk for aggressive PCa. This

finding concurs with the current results for the *SOD2* SNPs (rs10370 and rs4880). However, in an analysis of combined plasma antioxidant status (sum of lycopene, selenium and α-tocopherol), the highest quartile of plasma antioxidant status (compared to lowest) was associated with a five-fold lower risk of PCa and ten-fold lower risk of aggressive PCa among men with the AA genotype, but no significant association was observed among those with AV or VV genotype [335]. Similar findings have been reported by Mikhak et al. [497]. Although different candidate SNPs have been examined in various studies, together, these data provide support for the gene-nutrient interactions observed in the current analyses, indicating that the association between lycopene and PCa can be modified by genotype of polymorphisms in relevant genes.

A nearly significant positive association with PCa aggressiveness was observed for the *XRCC4* (rs3777018) G allele though it did not modify the association of lycopene intake with PCa aggressiveness. While some of the SNPs did not show evidence of statistical interaction with lycopene, certain variants of the SNPs appear to work in tandem with lycopene to influence PCa aggressiveness. As suggested by Savas et al. [502] and Rebbeck [536], it is possible that some of these variants confer low-to-moderate risk or protection against cancer that becomes evident only under certain physiological conditions or environmental exposures.

Limitations of the study include the use of a food frequency questionnaire to measure lycopene intake, which in addition to identified response set biases [510-512], does not account for the bioavailability or bioefficiency of carotenoids because of inter-individual variability in absorption and metabolism [278]. The use of a single dietary assessment also might have introduced some misclassification of lycopene exposure.

However, because this is a case-only study, such misclassification would likely be non-differential, resulting in conservative OR estimates [384]. Another limitation includes the use of median cutpoints to categorized lycopene in an effort to conserve sample size; however, this may have resulted in too small contrast between high and low lycopene intake categories to observe a substantial effect of lycopene. Other limitations include the observational nature of the study, which precludes causal inferences. Because of the small sample size and multiple comparisons, chance findings cannot be excluded, considering that none of the *P* values retained statistical significance after FDR correction for multiple testing (data not shown). Therefore, replication of the findings in larger studies would be useful. Even though analyses were stratified by self-reported race, residual confounding by ethnicity due to genetic admixture cannot be completely ruled out [537, 538]. Additionally, *post hoc* analysis involving adjustment for African ancestry proportions did not materially change the study results (Supplementary Tables 3 and 4).

8.5 CONCLUSIONS

In summary, the results of the analysis suggest that EA men with AG or GG genotype of the *XRCC4* (rs3777018) SNP may be at a higher risk of developing high aggressive PCa compared to those with the AA genotype. The results also show that an association between lycopene and PCa aggressiveness can be modified by genotype of SNPs in oxidative stress and DNA repair genes, including *XRCC1* (rs2854508), *XPA* (rs1800975) and *NQO1* (rs689453). Additional work is needed to verify these findings and help determine their potential for targeted PCa interventions.

Table 8.1 Demographic and health-related characteristics of the research subjects by level of prostate cancer aggressiveness

Characteristics	**European Americans** N = 1016					**African Americans** N = 948				
	High aggressive (n=153)		Low/intermediate aggressive (n= 863)		P ‡	High aggressive (n=188)		Low/intermediate aggressive (n= 760)		P ‡
	Mean (SD)		Mean (SD)			Mean (SD)		Mean (SD)		
Age, years	67 (7.5)		64 (7.7)		<0.0001	63 (7.6)		62 (7.6)		0.007
Energy Intake, kcals/day	2360.1 (963.4)		2322.1 (878.2)		0.63	2792.8 (1200.8)		2614.7 (1153.1)		0.06
Dietary fat intake, grams/day	95.7 (43.2)		91.2 (39.7)		0.20	103.8 (51.6)		95.5 (48.2)		0.04
Body mass index (BMI), kg/m^2	30.3 (5.1)		29.0 (4.8)		0.002	30.0 (6.7)		29.2 (5.4)		0.10
	N	**%**	**N**	**%**		**N**	**%**	**N**	**%**	
Study Site										
NC	64	42	413	48	0.17	80	43	347	46	0.44
LA	89	58	450	52		108	57	413	54	
PSA Screening History										
0 screenings	35	23	140	16	0.13	111	59	278	37	<0.0001
1-7 screenings	63	41	385	45		46	24	321	42	
> 7 screenings	55	36	338	39		31	17	161	21	
Education										
Less than high school education	27	18	74	8	0.002	78	42	220	29	0.001
High school graduate/ some college	70	46	412	48		93	49	413	54	
College graduate	56	37	377	44		17	9	126	17	
Income Level										
≤ $20, 000	22	14	71	8	0.15	75	40	217	29	0.002
$20,001- $40,000	30	20	171	20		47	25	199	26	

$40,001- $70,000	37	24	211	24		22	12	158	21	
>$70,000	50	33	336	40		21	11	121	16	
Unknown	14	9	74	8		23	12	65	8	
Smoking Status										
Never	54	35	314	36	0.69	37	20	258	34	0.0003
Former smokers	82	54	472	55		99	52	358	47	
Current smokers	17	11	77	9		52	28	144	19	
NSAID Use										
No	52	34	289	34	0.92	76	41	336	44	0.38
Yes	101	66	572	66		110	59	420	56	

Prostate cancer aggressiveness defined by a combination of Gleason sum, clinical stage, and PSA level at diagnosis and classified as follows: High aggressive (Gleason sum ≥8 or PSA >20ng/ml or Gleason sum ≥7 AND clinical stage T3 -T4); Low /Intermediate aggressive: all other cases.

Abbreviations: PCa – prostate cancer; LA – Louisiana; NC –North Carolina; NSAIDs – nonsteroidal anti-inflammatory drugs; PSA – prostate-specific antigen; SD – standard deviation

[‡]Test for differences between high aggressive and low/intermediate aggressive prostate cancers performed using Student's t-test for continuous variables and chi-square tests for categorical variables.

Table 8.2 Associations of polymorphisms in DNA repair and oxidative stress-related genes in relation to prostate cancer aggressiveness among European Americans and African Americans

		African Americans			European Americans		
Gene (SNP ID)	Genotype	Case/ Controls [b] 188/760	OR (95% CI) [c]	*P*	Case/ Controls [b] 153/863	OR (95% CI) [c]	*P*
APEX1 (rs1130409)	TT	81/305	1.00 (ref)		38/230	1.00 (ref)	
	GT+GG	107/455	0.88 (0.64-1.22)	0.44	115/633	1.10 (0.74-1.64)	0.63
ERCC8 (rs4647100)	AA	156/625	1.00 (ref)		87/481	1.00 (ref)	
	AG+GG	32/135	0.97 (0.64-1.49)	0.91	66/382	0.96 (0.67-1.36)	0.81
ERCC8 (rs4647102)	TT	75/315	1.00 (ref)		61/324	1.00 (ref)	
	CT+CC	113/443	1.10 (0.79-1.53)	0.57	92/539	0.90 (0.63-1.29)	0.57
ERCC8 (rs976631)	TT	101/434	1.00 (ref)		46/275	1.00 (ref)	
	CT+CC	85/325	1.09 (0.79-1.51)	0.58	107/585	1.12 (0.77-1.63)	0.56
GPX2 (rs4902346)	AA	70/266	1.00 (ref)		86/553	1.00 (ref)	
	AG+GG	118/494	0.90 (0.64-1.25)	0.53	67/310	1.39 (0.98-1.97)	0.07
MUTYH (rs3219489)	CC	107/434	1.00 (ref)		92/459	1.00 (ref)	
	CC+GG	81/326	1.02 (0.74-1.41)	0.89	61/404	0.76 (0.53-1.08)	0.12
NOS3 (rs1799983)	GG	152/598	1.00 (ref)		59/382	1.00 (ref)	
	GT+TT	36/160	0.89 (0.59-1.33)	0.56	94/475	1.31 (0.92-1.87)	0.13
NOS3 (rs3918226) [d]	CC	-	-	-	126/725	1.00 (ref)	
	CT+TT	-	-	-	27/138	1.09 (0.69-1.73)	0.70

NOX3||ARID1B (rs9372014) [d]	GG	-	-	-	77/403	1.00 (ref)	
	GT+TT	-	-	-	76/459	0.86 (0.60-1.21)	0.38
NQO1 (rs689453)	CC	171/673	1.00 (ref)		138/731	1.00 (ref)	
	CT+TT	17/87	0.75 (0.44-1.31)	0.31	15/132	0.62 (0.35-1.10)	0.10
OGG1 (rs1805373) [d]	GG	151/648	1.00 (ref)		-	-	-
	AG+AA	37/112	1.45 (0.96-2.20)	0.08	-	-	-
PPARG (rs1801282) [d]	CC	-	-	-	122/670	1.00 (ref)	
	CG+GG	-	-	-	30/193	0.84 (0.54-1.29)	0.42
RAD51C (rs304269)	GG	104/400	1.00 (ref)		57/360	1.00 (ref)	
	AG+AA	84/360	0.92 (0.67-1.27)	0.61	96/503	1.21 (0.85-1.73)	0.30
RAD51C (rs6503874)	CC	89/388	1.00 (ref)		136/767	1.00 (ref)	
	CG+GG	99/371	1.20 (0.87-1.65)	0.27	17/96	1.00 (0.57-1.73)	0.99
SOD2 (rs10370)	TT	145/549	1.00 (ref)		94/515	1.00 (ref)	
	GT+GG	43/209	0.79 (0.54-1.15)	0.21	59/348	0.96 (0.67-1.37)	0.83
SOD2 (rs4880)	AA	69/254	1.00 (ref)		42/197	1.00 (ref)	
	AG+GG	118/505	0.88 (0.63-1.23)	0.45	111/665	0.77 (0.52-1.14)	0.19
USP4||GPX1 (rs8179172) [d]	AA	153/640	1.00 (ref)		-	-	-
	AT+TT	35/120	1.22 (0.81-1.86)	0.34	-	-	-
XPA (rs1800975)	CC	121/470	1.00 (ref)		69/366	1.00 (ref)	
	CT+TT	67/289	0.90 (0.64-1.25)	0.52	84/497	0.90 (0.64-1.28)	0.56
XPA (rs3176644) [d]	GG	69/322	1.00 (ref)		-	-	-
	GT+TT	118/437	1.28 (0.92-1.79)	0.14	-	-	-

XPC (rs2227998)	CC	94/366	1.00 (ref)		78/480	1.00 (ref)	
	CT+TT	94/394	0.92 (0.67-1.27)	0.62	75/378	1.17 (0.83-1.66)	0.36
XPC (rs2733537)	AA	131/527	1.00 (ref)		86/401	1.00 (ref)	
	AG+GG	57/233	0.96 (0.68-1.37)	0.84	67/462	0.71 (0.50-1.01)	0.05
XRCC1 (rs1799778)	GG	102/462	1.00 (ref)		66/353	1.00 (ref)	
	GT+TT	85/294	1.31 (0.95-1.81)	0.10	87/509	0.91 (0.64-1.29)	0.58
XRCC1 (rs1799782)	GG	165/660	1.00 (ref)		131/735	1.00 (ref)	
	AG+AA	23/100	0.93 (0.57-1.52)	0.79	22/128	0.98 (0.60-1.60)	0.93
XRCC1 (rs2854508)	TT	123/478	1.00 (ref)		98/508	1.00 (ref)	
	AT+AA	65/281	0.90 (0.64-1.26)	0.54	55/355	0.80 (0.56-1.14)	0.22
XRCC1 (rs3213247) [d]	CC	-	-	-	136/772	1.00 (ref)	
	AC+AA	-	-	-	17/91	1.06 (0.61-1.84)	0.84
XRCC2 (rs3218522)	CC	122/529	1.00 (ref)		38/248	1.00 (ref)	
	CT+TT	66231	1.25 (0.89-1.75)	0.20	115/615	1.20 (0.80-1.78)	0.38
XRCC4 (rs10474079) [d]	GG	-	-	-	115/665	1.00 (ref)	
	AG+AA	-	-	-	38/197	1.07 (0.71-1.60)	0.74
XRCC4 (rs28360135) [d]	TT	-	-	-	144/792	1.00 (ref)	
	CT+CC	-	-	-	9/71	0.77 (0.37-1.59)	0.48
XRCC4 (rs35268)	TT	100/417	1.00 (ref)		116/670	1.00 (ref)	
	CT+CC	87/343	1.08 (0.78-1.49)	0.63	37/193	1.05 (0.70-1.59)	0.80
XRCC4 (rs3777018) [d]	AA	-	-	-	128/769	1.00 (ref)	
	AG+GG	-	-	-	25/94	**1.63 (1.00-2.64)**	0.05

[a] The most common genotype used as the reference category
[b] Some categories may not sum to total sample because of missing data
[c] Adjusted for age
[d] Results are not presented for SNPs with less than 5% minor allele frequency in the respective race group

Table 8.3 Associations between dietary lycopene and prostate cancer aggressiveness stratified by genotype of SNPs in DNA repair and oxidative stress-related genes

			European Americans (n = 1,016)				
			Cases/ Controls	OR (95% CI) [b]			
Dietary lycopene mg/day [a]		low	85/431	1.00 (ref)			
		high	68/432	0.77 (0.53-1.16)			
Stratified analysis by SNP			Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele			Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409)	T / G	low	20/110	1.00 (ref)	65/321	1.00 (ref)	
		high	18/120	0.51 (0.22-1.18)	50/312	0.85 (0.54-1.35)	0.71
ERCC8 (rs4647100)	A / G	low	51/240	1.00 (ref)	34/191	1.00 (ref)	
		high	36/241	0.62 (0.37-1.07)	32/191	0.94 (0.51-1.73)	0.43
ERCC8 (rs4647102)	T / C	low	34/155	1.00 (ref)	51/276	1.00 (ref)	
		high	27/169	0.68 (0.36-1.29)	41/263	0.85 (0.51-1.42)	0.56
ERCC8 (rs976631)	T / C	low	26/144	1.00 (ref)	59/286	1.00 (ref)	
		high	20/131	0.81 (0.38-1.72)	48/299	0.76 (0.47-1.21)	0.57
GPX2 (rs4902346)	A / G	low	47/278	1.00 (ref)	38/153	1.00 (ref)	
		high	39/275	0.83 (0.49-1.42)	29/157	0.65 (0.36-1.19)	0.62
MUTYH (rs3219489)	C / G	low	55/229	1.00 (ref)	30/202	1.00 (ref)	
		high	37/230	0.64 (0.38-1.08)	31/202	1.04 (0.56-1.92)	0.39

NOS3 (rs1799983) G / T	low	31/188	1.00 (ref)	54/240	1.00 (ref)	
	high	28/194	0.99 (0.52-1.88)	40/235	0.65 (0.39-1.08)	0.45
NOS3 (rs3918226) [c] C / T	low	72/371	1.00 (ref)	13/60	1.00 (ref)	
	high	54/354	0.76 (0.49-1.18)	14/78	0.89 (0.33-2.41)	0.96
NOX3||ARID1B (rs9372014) [c] G / T	low	41/191	1.00 (ref)	44/240	1.00 (ref)	
	high	36/212	0.81 (0.47-1.41)	32/219	0.71 (0.40-1.27)	0.84
NQO1 (rs689453) C / T	low	75/378	1.00 (ref)	10/53	1.00 (ref)	
	high	63/353	0.89 (0.59-1.35)	5/79	0.19 (0.03-1.14)	**0.05**
OGG1 (rs1805373) [c] G / A	low	-	-	-	-	-
	high	-	-	-	-	-
PPARG (rs1801282) [c] C / G	low	67/336	1.00 (ref)	18/95	1.00 (ref)	
	high	55/334	0.79 (0.51-1.23)	12/98	0.69 (0.26-1.86)	0.83
RAD51C (rs304269) G / A	low	31/180	1.00 (ref)	54/251	1.00 (ref)	
	high	26/180	0.90 (0.46-1.76)	42/252	0.71 (0.43-1.17)	0.57
RAD51C (rs6503874) C / G	low	76/382	1.00 (ref)	9/49	1.00 (ref)	
	high	60/385	0.76 (0.50-1.15)	8/47	1.33 (0.26-6.79)	0.91
SOD2 (rs10370) T / G	low	53/258	1.00 (ref)	32/173	1.00 (ref)	
	high	41/257	0.77 (0.46-1.26)	27/175	0.77 (0.40-1.48)	0.97
SOD2 (rs4880) A / G	low	25/91	1.00 (ref)	60/339	1.00 (ref)	
	high	17/106	0.56 (0.25-1.26)	51/326	0.84 (0.53-1.33)	0.27
USP4||GPX1 (rs8179172) [c] A / T	low	-	-	-	-	-
	high	-	-	-	-	-
XPA (rs1800975) C / T	low	33/185	1.00 (ref)	52/246	1.00 (ref)	
	high	36/181	1.02 (0.56-1.87)	32/251	**0.56 (0.32-0.98)**	**0.07**

XPA (rs3176644) [c]	G / T	low	-	-	-	-	-
		high	-	-	-	-	-
XPC (rs2227998)	C / T	low	48/239	1.00 (ref)	37/190	1.00 (ref)	
		high	30/241	0.65 (0.38-1.12)	38/188	0.90 (0.49-1.65)	0.37
XPC (rs2733537)	A / G	low	51/202	1.00 (ref)	34/229	1.00 (ref)	
		high	35/199	0.65 (0.37-1.14)	33/233	0.96 (0.55-1.69)	0.47
XRCC1 (rs1799778)	G / T	low	37/181	1.00 (ref)	48/249	1.00 (ref)	
		high	29/172	0.74 (0.39-1.39)	39/260	0.75 (0.45-1.27)	0.82
XRCC1 (rs1799782)	G / A	low	74/365	1.00 (ref)	11/66	1.00 (ref)	
		high	57/370	0.71 (0.46-1.09)	11/62	1.15 (0.34-3.66)	0.47
XRCC1 (rs2854508)	T / A	low	63/266	1.00 (ref)	22/165	1.00 (ref)	
		high	35/242	**0.57 (0.34-0.95)**	33/190	1.22 (0.63-2.39)	**0.06**
XRCC1 (rs3213247) [c]	C / A	low	77/390	1.00 (ref)	8/41	1.00 (ref)	
		high	59/382	0.73 (0.48-1.12)	9/50	1.02 (0.30-3.40)	0.69
XRCC2 (rs3218522)	C / T	low	16/126	1.00 (ref)	69/305	1.00 (ref)	
		high	22/122	1.22 (0.53-2.80)	46/310	0.66 (0.42-1.05)	**0.08**
XRCC4 (rs10474079) [c]	G / A	low	65/333	1.00 (ref)	20/97	1.00 (ref)	
		high	50/332	0.74 (0.47-1.16)	18/100	0.80 (0.34-1.87)	0.71
XRCC4 (rs28360135) [c]	T / C	low	79/404	1.00 (ref)	6/27	1.00 (ref)	
		high	65/388	0.81 (0.54-1.21)	3/44	0.44 (0.04-4.56)	0.34
XRCC4 (rs35268) [c]	T / C	low	68/327	1.00 (ref)	17/104	1.00 (ref)	
		high	48/343	0.95 (0.41-1.22)	20/89	1.31 (0.71-4.10)	0.27
XRCC4 (rs3777018) [c]	A / G	low	74/382	1.00 (ref)	11/49	1.00 (ref)	
		high	54/387	0.68 (0.44-1.04)	14/45	1.31 (0.39-4.39)	0.23

[a] Categorized based on median split (< versus ≥ median) among controls for European Americans as low: 344.77 – 4626.17 mcg/day and high: 4626.18 – 100250.76 mcg/day; and for African Americans: 4.90 – 3319.69 and 3319.70 – 106071.98 mcg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total fat intake, and study site

[c] Results are not presented for SNPs with less than 5% minor allele frequency in the respective race group

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and dietary lycopene intake (<versus ≥ median) in multivariable adjusted models.

Table 8.3 (continued) Associations between dietary lycopene and prostate cancer aggressiveness stratified by genotype of SNPs in DNA repair and oxidative stress-related genes

		African Americans (n = 948)				
		Cases/ Controls	OR (95% CI) [b]			
Dietary lycopene mg/day [a]	low	80/380	1.00 (ref)			
	high	108/380	1.41 (0.96-2.07)			
Stratified analysis by SNP		Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele		Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409) T / G	low	32/153	1.00 (ref)	48/227	1.00 (ref)	
	high	49/152	1.71 (0.95-3.07)	59/228	1.25 (0.74-2.10)	0.50
ERCC8 (rs4647100) A / G	low	66/312	1.00 (ref)	14/68	1.00 (ref)	
	high	90/313	1.47 (0.97-2.25)	18/67	0.91 (0.33-2.49)	0.79
ERCC8 (rs4647102) T / C	low	28/161	1.00 (ref)	52/219	1.00 (ref)	
	high	47/154	**1.94 (1.01-3.73)**	61/224	1.13 (0.70-1.82)	**0.08**
ERCC8 (rs976631) T / C	low	45/211	1.00 (ref)	35/169	1.00 (ref)	
	high	56/223	1.28 (0.76-2.17)	50/156	1.45 (0.80-2.60)	0.64
GPX2 (rs4902346) A / G	low	29/127	1.00 (ref)	51/253	1.00 (ref)	
	high	41/139	1.49 (0.76-2.94)	67/241	1.39 (0.86-2.24)	0.95
MUTYH (rs3219489) C / G	low	40/212	1.00 (ref)	40/168	1.00 (ref)	
	high	67/222	1.65 (0.98-2.78)	41/158	1.19 (0.67-2.13)	0.31
NOS3 (rs1799983) G / T	low	68/297	1.00 (ref)	12/82	1.00 (ref)	

		high	84/301	1.25 (0.82-1.90)	24/78	1.90 (0.71-5.12)	0.20
NOS3 (rs3918226) [c]	C / T	low	-	-	-	-	-
		high	-	-	-	-	-
NOX3||ARID1B (rs9372014) [c]	G / T	low	-	-	-	-	-
		high	-	-		-	-
NQO1 (rs689453)	C / T	low	71/336	1.00 (ref)	9/44	1.00 (ref)	
		high	100/337	1.42 (0.95-2.13)	8/43	0.16 (0.25-5.37)	0.35
OGG1 (rs1805373) [c]	G / A	low	62/33	1.00 (ref)	18/47	1.00 (ref)	
		high	89/315	1.56 (1.00-2.39)	19/65	0.83 (0.31-2.26)	0.11
PPARG (rs1801282) [c]	C / G	low	-	-	-	-	-
		high	-	-	-	-	-
RAD51C (rs304269)	G / A	low	49/207	1.00 (ref)	31/173	1.00 (ref)	
		high	55/193	1.27 (0.75-2.14)	53/187	1.50 (0.82-2.74)	0.50
RAD51C (rs6503874)	C / G	low	38/203	1.00 (ref)	42/177	1.00 (ref)	
		high	51/185	**1.86 (1.03-3.35)**	57/194	1.11 (0.65-1.89)	0.37
SOD2 (rs10370)	T / G	low	61/263	1.00 (ref)	19/116	1.00 (ref)	
		high	84/286	1.32 (0.85-2.06)	24/93	1.47 (0.63-3.43)	0.81
SOD2 (rs4880)	A / G	low	32/137	1.00 (ref)	47/242	1.00 (ref)	
		high	37/117	1.28 (0.68-2.41)	71/263	1.57 (0.96-2.59)	0.83
USP4||GPX1 (rs8179172) [c]	A / T	low	66/321	1.00 (ref)	14/59	1.00 (ref)	
		high	87/319	1.30 (0.86-1.99)	21/61	1.28 (0.43-3.79)	0.89
XPA (rs1800975)	C / T	low	49/247	1.00 (ref)	31/133	1.00 (ref)	
		high	72/223	1.28 (0.88-2.06)	36/156	0.73 (0.53-1.84)	0.14
XPA (rs3176644) [c]	G / T	low	36/161	1.00 (ref)	44/218	1.00 (ref)	

		high	33/161	0.91 (0.49-1.69)	74/219	**1.92 (1.16-3.20)**	0.10
XPC (rs2227998)	C / T	low	40/179	1.00 (ref)	40/201	1.00 (ref)	
		high	54/187	1.12 (0.64-1.96)	54/193	**1.74 (1.01-3.01)**	0.84
XPC (rs2733537)	A / G	low	59/276	1.00 (ref)	21/104	1.00 (ref)	
		high	72/251	1.22 (0.77-1.94)	36/129	**2.26 (1.03-4.92)**	0.56
XRCC1 (rs1799778)	G / T	low	40/226	1.00 (ref)	40/152	1.00 (ref)	
		high	62/236	**1.77 (1.05-2.98)**	45/142	1.00 (0.55-1.84)	0.42
XRCC1 (rs1799782)	G / A	low	72/332	1.00 (ref)	8/48	1.00 (ref)	
		high	93/328	1.37 (0.91-2.05)	15/52	1.88 (0.42-8.37)	0.93
XRCC1 (rs2854508)	T / A	low	60/232	1.00 (ref)	20/148	1.00 (ref)	
		high	63/246	0.79 (0.63-1.66)	45/133	**2.50 (1.27-4.89)**	**0.010**
XRCC1 (rs3213247) [c]	C / A	low	-	-	-	-	-
		high	-	-	-	-	-
XRCC2 (rs3218522)	C / T	low	50/265	1.00 (ref)	30/115	1.00 (ref)	
		high	72/264	1.38 (0.93-2.49)	36/116	0.89 (0.53-1.48)	0.39
XRCC4 (rs10474079) [c]	G / A	low	-	-	-	-	-
		high	-	-	-	-	-
XRCC4 (rs28360135) [c]	T / C	low	-	-	-	-	-
		high	-	-	-	-	-
XRCC4 (rs35268) [c]	T / C	low	41/211	1.00 (ref)	39/169	1.00 (ref)	
		high	59/206	0.99 (0.55-1.78)	48/174	1.33 (0.97-2.97)	0.38
XRCC4 (rs3777018) [c]	A / G	low	-	-	-	-	-
		high	-	-	-	-	-

[a] Categorized based on median split (< versus ≥ median) among controls for European Americans as low: 344.77 – 4626.17 mcg/day and high: 4626.18 – 100250.76 mcg/day; and for African Americans: 4.90 – 3319.69 and 3319.70 –

106071.98 mcg/day, respectively.
[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total fat intake, and study site
[c] Results are not presented for SNPs with less than 5% minor allele frequency in the respective race group
‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and dietary lycopene intake (<versus ≥ median) in multivariable adjusted models.

Table 8.4 Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between total lycopene intake (diet + supplements) and prostate cancer aggressiveness, stratified by polymorphisms in DNA repair and oxidative stress-related genes

			European Americans (n = 1,016)				
			Cases/ Controls	OR (95% CI) [b]			
Total lycopene mg/day [a]		low	84/431	1.00 (ref)			
		high	69/432	0.81 (0.55-1.20)			
Stratified analysis by SNP			Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele			Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409)	T / G	low	20/109	1.00 (ref)	64/322	1.00 (ref)	
		high	18/121	0.46 (0.20-1.06)	51/311	0.90 (0.57-1.42)	0.59
ERCC8 (rs4647100)	A / G	low	51/239	1.00 (ref)	33/192	1.00 (ref)	
		high	36/242	0.61 (0.36-1.05)	33/190	1.05 (0.57-1.91)	0.29
ERCC8 (rs4647102)	T / C	low	34/156	1.00 (ref)	50/275	1.00 (ref)	
		high	27/168	0.68 (0.36-1.28)	42/264	0.91 (0.55-1.51)	0.47
ERCC8 (rs976631)	T / C	low	25/143	1.00 (ref)	59/287	1.00 (ref)	
		high	21/132	0.87 (0.41-1.83)	48/298	0.78 (0.49-1.25)	0.52
GPX2 (rs4902346)	A / G	low	47/278	1.00 (ref)	37/153	1.00 (ref)	
		high	39/275	0.82 (0.48-1.41)	30/157	0.70 (0.38-1.27)	0.76
MUTYH (rs3219489)	C / G	low	55/229	1.00 (ref)	29/202	1.00 (ref)	

		high	37/230	0.65 (0.39-1.10)	32/202	1.11 (0.60-2.07)	0.32
NOS3 (rs1799983)	G / T	low	30/187	1.00 (ref)	54/241	1.00 (ref)	
		high	29/195	1.12 (0.59-2.12)	40/234	0.64 (0.38-1.06)	0.30
NOS3 (rs3918226) [c]	C / T	low	71/371	1.00 (ref)	13/60	1.00 (ref)	
		high	55/354	0.80 (0.51-1.24)	14/78	0.90 (0.33-2.47)	0.81
NOX3||ARID1B (rs9372014) [c]	G / T	low	41/193	1.00 (ref)	43/238	1.00 (ref)	
		high	36/210	0.83 (0.48-1.44)	33/221	0.74 (0.41-1.31)	0.82
NQO1 (rs689453)	C / T	low	74/378	1.00 (ref)	10/53	1.00 (ref)	
		high	64/353	0.92 (0.61-1.39)	5/79	0.20 (0.03-1.22)	**0.06**
OGG1 (rs1805373) [c]	G / A	low	-	-	-	-	-
		high	-	-	-	-	-
PPARG (rs1801282) [c]	C / G	low	66/335	1.00 (ref)	18/96	1.00 (ref)	
		high	56/335	0.82 (0.53-1.27)	12/97	0.73 (0.27-1.97)	0.82
RAD51C (rs304269)	G / A	low	30/183	1.00 (ref)	54/248	1.00 (ref)	
		high	27/177	1.04 (0.53-2.03)	42/255	0.69 (0.41-1.13)	0.34
RAD51C (rs6503874)	C / G	low	78/382	1.00 (ref)	8/49	1.00 (ref)	
		high	58/385	0.76 (0.50-1.16)	9/47	1.81 (0.38-8.72)	0.59
SOD2 (rs10370)	T / G	low	52/260	1.00 (ref)	32/171	1.00 (ref)	
		high	42/255	0.82 (0.50-1.35)	27/177	0.75 (0.39-1.43)	0.84
SOD2 (rs4880)	A / G	low	25/91	1.00 (ref)	59/339	1.00 (ref)	
		high	17/106	0.58 (0.26-1.29)	52/326	0.87 (0.55-1.38)	0.26
USP4||GPX1 (rs8179172) [c]	A / T	low	-	-	-	-	-
		high	-	-	-	-	-

XPA (rs1800975) C / T	low	32/187	1.00 (ref)	52/244	1.00 (ref)	
	high	37/179	1.15 (0.63-2.09)	32/253	**0.56 (0.32-0.97)**	**0.04**
XPA (rs3176644) [c] G / T	low	-	-	-	-	-
	high	-	-	-	-	-
XPC (rs2227998) C / T	low	48/238	1.00 (ref)	36/190	1.00 (ref)	
	high	30/242	0.64 (0.37-1.11)	39/188	0.98 (0.54-1.79)	0.26
XPC (rs2733537) A / G	low	50/205	1.00 (ref)	34/226	1.00 (ref)	
	high	36/196	0.75 (0.43-1.31)	33/236	0.93 (0.52-1.63)	0.73
XRCC1 (rs1799778) G / T	low	37/183	1.00 (ref)	47/247	1.00 (ref)	
	high	29/170	0.76 (0.40-1.43)	40/262	0.79 (0.47-1.32)	0.89
XRCC1 (rs1799782) G / A	low	73/364	1.00 (ref)	11/67	1.00 (ref)	
	high	58/371	0.73 (0.48-1.12)	11/61	1.30 (0.39-4.33)	0.42
XRCC1 (rs2854508) T / A	low	62/264	1.00 (ref)	62/264	1.00 (ref)	
	high	36/244	**0.59 (0.36-0.99)**	36/244	1.24 (0.64-2.41)	**0.07**
XRCC1 (rs3213247) [c] C / A	low	77/390	1.00 (ref)	7/41	1.00 (ref)	
	high	59/382	0.73 (0.48-1.120	10/50	1.21 (0.36-4.07)	0.41
XRCC2 (rs3218522) C / T	low	16/125	1.00 (ref)	68/306	1.00 (ref)	
	high	22/123	1.20 (0.52-2.76)	47/309	0.70 (0.45-1.11)	0.11
XRCC4 (rs10474079) [c] G / A	low	64/333	1.00 (ref)	20/97	1.00 (ref)	
	high	51/332	0.77 (0.49-1.21)	18/100	0.82 (0.35-1.90)	0.76
XRCC4 (rs28360135) [c] T / C	low	78/404	1.00 (ref)	6/27	1.00 (ref)	
	high	66/388	0.84 (0.56-1.26)	3/44	0.44 (0.04-4.56)	0.32
XRCC4 (rs35268) [c] T / C	low	67/327	1.00 (ref)	17/104	1.00 (ref)	

	high	49/343	0.67 (0.42-1.05)	20/89	1.46 (0.74-3.21)	0.21
XRCC4 (rs3777018) [c] A / G	low	73/382	1.00 (ref)	11/49	1.00 (ref)	
	high	55/387	0.70 (0.46-1.08)	14/45	1.31 (0.39-4.39)	0.36

[a] Categorized based on median split (< vs. ≥ median) among controls for European Americans as low: 344.77 – 4698.17 mcg/day and high: 4698.18 – 100250.76 mcg/day; and for African Americans: 4.90 – 3358.85 and 3358.86 – 106071.98 mcg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total fat intake, and study site

[c] Results are not presented for SNPs with less than 5% minor allele frequency in the respective race group

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and total lycopene intake (<versus ≥ median) in multivariable adjusted models.

Table 8.4 (continued) Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between total lycopene intake (diet + supplements) and prostate cancer aggressiveness, stratified by polymorphisms in DNA repair and oxidative stress-related genes

		African Americans (n = 948)				
		Cases/ Controls	OR (95% CI) [b]			
Total lycopene mg/day [a]	low	81/380	1.00 (ref)			
	high	107/380	1.36 (0.92-1.99)			
Stratified analysis by SNP		Homozygous Common Allele		Heterozygous or Homozygous Minor Allele		
Gene (SNP ID), major/minor allele		Cases/ Controls	OR (95% CI) [b]	Cases/ Controls	OR (95% CI) [b]	P ‡
APEX1(rs1130409) T / G	low	32/153	1.00 (ref)	49/227	1.00 (ref)	
	high	49/152	1.69 (0.94-3.04)	58/228	1.15 (0.69-1.94)	0.42
ERCC8 (rs4647100) A / G	low	67/312	1.00 (ref)	14/68	1.00 (ref)	
	high	89/313	1.41 (0.92-2.14)	18/67	0.91 (0.33-2.49)	0.86
ERCC8 (rs4647102) T / C	low	29/161	1.00 (ref)	52/219	1.00 (ref)	
	high	46/154	1.77 (0.93-3.40)	61/224	1.11 (0.69-1.80)	0.11
ERCC8 (rs976631) T / C	low	45/211	1.00 (ref)	36/169	1.00 (ref)	
	high	56/223	1.25 (0.74-2.12)	49/156	1.34 (0.74-2.40)	0.74
GPX2 (rs4902346) A / G	low	29/126	1.00 (ref)	52/254	1.00 (ref)	
	high	41/140	1.48 (0.75-2.92)	66/240	1.31 (0.82-2.11)	0.87
MUTYH (rs3219489) C / G	low	41/211	1.00 (ref)	40/169	1.00 (ref)	
	high	66/223	1.53 (0.91-2.55)	41/157	1.20 (0.67-2.14)	0.42

NOS3 (rs1799983) G / T	low	69/296	1.00 (ref)	12/83	1.00 (ref)	
	high	83/302	1.18 (0.78-1.80)	24/77	1.94 (0.72-5.23)	0.16
NOS3 (rs3918226) [c] C / T	low	-	-	-	-	-
	high	-	-		-	-
NOX3||ARID1B (rs9372014) [c] G / T	low	-	-	-	-	-
	high	-	-	-	-	-
NQO1 (rs689453) C / T	low	72/337	1.00 (ref)	9/43	1.00 (ref)	
	high	99/336	1.38 (0.92-2.07)	8/44	0.79 (0.16-3.75)	0.29
OGG1 (rs1805373) [c] G / A	low	62/332	1.00 (ref)	19/48	1.00 (ref)	
	high	89/316	1.53 (0.99-2.34)	18/64	0.76 (0.28-2.09)	**0.07**
PPARG (rs1801282) [c] C / G	low	-	-	-	-	-
	high	-	-	-	-	-
RAD51C (rs304269) G / A	low	49/205	1.00 (ref)	32/175	1.00 (ref)	
	high	55/195	1.23 (0.73-2.07)	52/185	1.42 (0.78-2.58)	0.53
RAD51C (rs6503874) C / G	low	38/204	1.00 (ref)	43/176	1.00 (ref)	
	high	51/184	**1.81 (1.02-3.34)**	56/195	1.03 (0.61-1.75)	0.27
SOD2 (rs10370) T / G	low	62/263	1.00 (ref)	19/116	1.00 (ref)	
	high	83/286	1.25 (0.81-1.94)	24/93	1.47 (0.63-3.44)	0.73
SOD2 (rs4880) A / G	low	32/137	1.00 (ref)	48/242	1.00 (ref)	
	high	37/117	1.27 (0.67-2.39)	70/263	1.46 (0.89-2.39)	0.95
USP4||GPX1 (rs8179172) [c] A / T	low	67/319	1.00 (ref)	14/61	1.00 (ref)	
	high	86/321	1.23 (0.81-1.87)	21/59	1.43 (0.48-4.29)	0.74
XPA (rs1800975) C / T	low	50/249	1.00 (ref)	31/131	1.00 (ref)	

		high	71/221	**1.79 (1.09-2.95)**	36/158	0.85 (0.51-1.76)	0.13
XPA (rs3176644) [c]	G / T	low	36/161	1.00 (ref)	45/218	1.00 (ref)	
		high	33/161	0.91 (0.49-1.70)	73/219	**1.79 (1.08-2.96)**	0.13
XPC (rs2227998)	C / T	low	40/180	1.00 (ref)	41/200	1.00 (ref)	
		high	54/186	1.11 (0.64-1.94)	53/194	1.63 (0.95-2.81)	0.94
XPC (rs2733537)	A / G	low	60/274	1.00 (ref)	21/106	1.00 (ref)	
		high	71/253	1.14 (0.72-1.80)	36/127	**2.33 (1.06-5.10)**	0.43
XRCC1 (rs1799778)	G / T	low	41/225	1.00 (ref)	40/153	1.00 (ref)	
		high	61/237	1.63 (0.97-2.73)	45/141	1.01 (0.55-1.85)	0.56
XRCC1 (rs1799782)	G / A	low	73/331	1.00 (ref)	8/49	1.00 (ref)	
		high	92/329	1.29 (0.86-1.94)	15/51	2.24 (0.48-10.48)	0.83
XRCC1 (rs2854508)	T / A	low	61/230	1.00 (ref)	20/150	1.00 (ref)	
		high	62/248	0.95 (0.59-1.53)	45/131	**2.56 (1.30-5.02)**	**0.005**
XRCC1 (rs3213247) [c]	C / A	low	-	-	-	-	-
		high	-	-	-	-	-
XRCC2 (rs3218522)	C / T	low	50/264	1.00 (ref)	31/116	1.00 (ref)	
		high	72/265	1.48 (0.90-2.42)	35/115	1.06 (0.56-2.00)	0.34
XRCC4 (rs10474079) [c]	G / A	low	-	-	-	-	-
		high	-	-	-	-	-
XRCC4 (rs28360135) [c]	T / C	low	-	-	-	-	-
		high	-	-	-	-	-
XRCC4 (rs35268) [c]	T / C	low	42/210	1.00 (ref)	39/170	1.00 (ref)	
		high	58/207	1.00 (0.56-1.80)	48/173	1.61 (0.95-2.71)	0.62

XRCC4 (rs3777018) [c] A / G	low	-	-	-	-	-
	high	-	-	-	-	-

[a] Categorized based on median split (< vs. ≥ median) among controls for European Americans as low: 344.77 – 4698.17 mcg/day and high: 4698.18 – 100250.76 mcg/day; and for African Americans: 4.90 – 3358.85 and 3358.86 – 106071.98 mcg/day, respectively.

[b] Adjusted for age, PSA screening history, BMI, smoking status, education, income, NSAIDs use, total fat intake, and study site

[c] Results are not presented for SNPs with less than 5% minor allele frequency in the respective race group

‡ Interaction p-value based likelihood ratio tests with and without multiplicative interaction term between SNP genotype (homozygous common allele vs. homozygous variant + heterozygous) and total lycopene intake (<versus ≥ median) in multivariable adjusted models.

CHAPTER 9

SYNTHESIS

9.1 ANTIOXIDANT INTAKE AND PROSTATE SPECIFIC ANTIGEN IN MEN WITH BIOCHEMICAL RECURRENCE OF PROSTATE CANCER

It is estimated that about 94% of American men with prostate cancer present with clinically localized disease and often are treated with radical prostatectomy or radical radiation with curative intent [340, 341]. However, about 25–40% of these men develop biochemical recurrence of PCa within 5 years of treatment [342-345]. Biochemical recurrence of PCa, which is generally defined by continuous rise in serum PSA level on three or more successive tests, is often an early sign of metastasis and precedes metastasis by an average of eight years [348]. Unfortunately, there is no known cure for biochemical recurrence of PCa, but it is usually managed with surgical or medical androgen ablation with the hope of delaying the time to metastasis. Androgen ablation has been associated with severe and life-altering side effects [351, 539], which makes its use unappealing to both patients and clinics. Thus, the idea of using dietary agents as alternate therapy or a first line treatment to delay the use of androgen ablation is a prospect that would be attractive to most patients. Results from this study show that men with higher plasma levels of α-tocopherol, β-cryptoxanthin, *trans*-β-carotene, cis-lutein/zeaxanthin and *trans*-lycopene had lower PSA levels at follow-up timepoints compared to men with lower plasma levels of these nutrients. This suggests that greater intake of foods

containing these micronutrients might be beneficial to men with biochemical recurrence of PCa.

These micronutrients are abundantly available in many fruits and vegetables (food sources reviewed in [291, 540]), which raises the question of whether these micronutrients may have acted as surrogates for greater intake of fruits and vegetables, which contain other beneficial micronutrients and phytochemicals. Thus, replication of the findings in larger studies with longer follow-up would be useful. Other caveats that should be considered are noted below.

9.2 Associations of antioxidants and prostate cancer aggressiveness

Although the epidemiologic literature is replete with reports on associations between antioxidants intake and PCa incidence, much less is known about antioxidants effect on PCa aggressiveness. Antioxidants are thought to mitigate oxidative stress, thereby averting oxidative DNA damage and the potential for malignant transformation of normal prostate cells. Recent published data indicates that oxidative stress correlates with the extent of PCa aggressiveness, such that the highly aggressive forms of PCa tends to display markedly higher degree of oxidative stress than do the less aggressive forms PCa [123]. Thus, increasing antioxidant intake may boost the body's defenses against oxidative stress, and by extension, protect against PCa and its aggressiveness [394, 541].

Results from analysis of associations between antioxidants intake, supplemental antioxidant use, and adipose tissue antioxidants levels in relation to PCa aggressiveness revealed some important findings. While there were no significant findings for supplemental antioxidant use, higher dietary intake of α-, γ- and δ-tocopherol were

inversely associated with PCa aggressiveness among European American, but not African Americans. These racial differences in associations are likely due to variations in dietary patterns and the possibility of gene-diet interaction that may vary by race. For example, European Americans in this study were more likely to obtained α-tocopherols from healthy foods sources such as olive oils, nuts, seeds and vegetables, while African Americans often obtained α-tocopherol from less healthy foods such as potato and corn chips, and dark green vegetables prepared with fatback and lard. Thus, the source of tocopherol intake may explain some of these differences in associations by race. Higher adipose tissue concentration of α-tocopherol appeared to be associated with increased odds of high aggressive PCa among European Americans only, albeit statistically non-significant. It is worth noting that European Americans in this study had a 75% higher adipose α-tocopherol concentration than African Americans. It is, however, unclear what constitutes "normal" adipose α-tocopherol levels, although the mean α-tocopherol levels among European Americans in this study were slightly higher than those reported in breast tissue from Malaysian women [418] and lower than those reported in adipose tissue from European males in the EURAMIC study [419]. Nonetheless, it is reasonable to speculate that long-term use of dietary supplements (the most common source of α-tocopherol) may have been the major contributor of the adipose α-tocopherol levels among European Americans, especially since a much greater proportion of European Americans reported vitamin E supplement use compared to African Americans.

Carotenoids intake varied significantly between African Americans and European Americans, including higher intake of lycopene among European Americans and higher β-cryptoxanthin intake among African Americans. Dietary lycopene was associated with

lower odds of high aggressive PCa among European Americans, and β-cryptoxanthin was associated with lower odds of high aggressive PCa among African Americans. Adipose tissue α-carotene and lycopene (*cis* + *trans*) levels were higher among European Americans than African Americans, and marginally significant inverse linear trends were observed for adipose α-carotene and lycopene in relation to PCa aggressiveness among European Americans only. Given that inverse associations were only observed in the race group with higher dietary intake or adipose concentration of these nutrients, it stands to reason that the potential benefits of lycopene, β-cryptoxanthin and α-carotene may be acquired only at higher levels of intake. However, potential interactions with genetic variants in relevant genes cannot be ruled out and is described in section 5.3.

9.3 GENE-DIET INTERACTION

Associations between antioxidant intake and PCa incidence have varied across studies [17]. There is some evidence that polymorphic variants in oxidative stress and DNA repair genes modulate associations between antioxidant intake and PCa incidence [128, 466]. Therefore, the inconsistency in prior studies may be reflecting genetic risk variability of the studied populations. In this study, we investigated whether associations of α-tocopherol, γ-tocopherol and lycopene in relation to PCa aggressiveness are modulated by single nucleotide polymorphisms (SNPs) in oxidative stress and DNA repair genes, and whether effect modification varies by race.

There was evidence of effect modification by certain SNPs with some variations by race. *XRCC1* (rs2854508) genotype significantly modified association between α-tocopherol intake and PCa aggressiveness among African Americans and European

Americans, such that among those who possess the TT genotype, higher α-tocopherol intake was inversely related to PCa aggressiveness, while the opposite was observed among those with AA or AT genotypes. A similar pattern of effect modification by *XRCC1* (rs2854508) was observed for association between γ-tocopherol and PCa aggressiveness, but only among African Americans. *XRCC1* (rs2854508) genotype also demonstrated interaction with lycopene, in that higher lycopene intake was associated with increased odds of high aggressive PCa among African Americans who harbor the AT or AA genotype, but not African Americans with the TT genotype. By contrast, higher lycopene intake was inversely related to high aggressive PCa among European Americans with the TT genotype, and no association was observed among European Americans with the AT or AA genotype. Lower odds of high aggressive prostate cancer was observed among European Americans who had higher lycopene intake and possessed the CT or TT genotype of *NQO1* (rs689453), but not European Americans with the CC genotype, and there was no evidence of effect modification among African Americans.

Even though some of the SNPs did not show evidence of statistical interaction with antioxidants intake, certain variants of the SNPs appear to work in tandem with antioxidants to influence PCa aggressiveness. Notably, higher α-tocopherol intake was associated with significantly lower odds of high aggressive PCa among European Americans who were homozygous for the common allele of *APEX1* (rs1130409, T > G), *PPARG* (rs1801282, C > G), *XPC* (rs2733537, A > G) or *XRCC1* (rs1799782, G > A). This supports suggestions that the potential benefits of α-tocopherol as well as other antioxidants may be limited to men with certain genetic variants, and this may explain

some of the inconsistencies in the epidemiologic literature when diet is examined in isolation of genetic factors.

This study provides evidence of interactions between α-tocopherol, γ-tocopherol and lycopene intake and SNPs in oxidative stress and DNA repair genes in relation to PCa aggressiveness. The findings indicate that not all men may benefit equally from dietary interventions involving these nutrients. Thus, targeted interventions for a subgroup of men with certain genetic variants may be a future strategy for chemoprevention of aggressive PCa.

9.4 STRENGTHS AND LIMITATIONS

Biochemical Recurrence of Prostate Cancer

The examination of the potential benefits of exposure to carotenoids and tocopherols in relation PSA rise following radical prostatectomy is appealing because diet interventions provide a non-invasive, relatively inexpensive, and safe treatment alternative for management of the disease progression in a population with limited treatment options. Since the study participants had already undergone radical prostatectomy or radical radiation for the treatment of organ-confined disease, continuous rise in serum PSA level as defined in this study most likely reflects progressive disease, rather than residual normal tissue left from radiation or spared during prostatectomy. The use of biomarkers of nutrient intake provides more reliable measures of nutritional status relative to self-reported intake [384]. Additionally, several potential risk factors of PCa including BMI, smoking, physical activity, tumor grade and race also were controlled for in the analysis, which limits potential confounding of the observed associations.

Limitations of the study include the small study and the multiple comparisons made, thus some of the findings could be due to chance alone. There is also the possibility that the study results may be reflecting interactions between plasma nutrients, rather than the effect of a single nutrient *per se* [383]. Blood antioxidant levels reflect short-term intake rather than average intake over long periods, which may be more relevant to PCa progression. The short duration of the study and lack of carotenoid and tocopherol data at 6 months prohibited evaluation of temporal trends over long periods. Additionally, restricting the study to a subgroup of PCa patients with strictly defined disease attributes precludes generalizability of the findings to the larger population of men with PCa.

Antioxidants and Prostate Cancer Aggressiveness

Notable strengths the analysis of associations between antioxidants intake and PCa aggressiveness include the design to measure PCa aggressiveness, which minimizes potential confounding by disease heterogeneity (i.e., the mixing of different disease states). The evaluation of three complementary measures of antioxidant intake allowed for a more comprehensive assessment of antioxidant status in PCa aggressiveness. Additionally, the assessment of individual antioxidants helps delineate the role of different antioxidants in PCa aggressiveness. The use of an ethnically diverse population with approximately equal numbers of African Americans and European Americans also made it possible to explore whether associations between antioxidants and PCa aggressiveness differed by race. Moreover, the potential for selection bias and selective survival were minimized because participation rates were reasonably high at both study sites (62% for North Carolina, 72% for pre- and 63% for post-Hurricane Katrina

Louisiana) and research subjects were recruited shortly after diagnosis via rapid case-ascertainment; an average of five months from the time of diagnosis to time of interview.

However, imprecise measurements of dietary antioxidants could have influenced the study results to some extent. Because exposure assessment for antioxidants were done independent of the extent of PCa aggressiveness, differential misclassification bias is unlikely; however, non-differential exposure misclassification may have occurred, resulting in underestimation of ORs and failure to detect modest associations [422]. Diet was assessed using a food frequency questionnaire. It is known that these structured instruments may be biased according to response sets [423], which in turn, may be related to psychological traits that either may exert a direct effect on cancer outcomes or indirectly affect other factors that may influence carcinogenesis [424]. There is also the concern that adipose antioxidant levels may be altered by the presence of a tumor; however, a study examining the effect of breast tumor proximity on breast adipose antioxidant levels did not find significant differences in adipose antioxidant levels at different quadrants of breast tissue, including sites proximal and distal to the tumor [425]. Moreover, although adipose antioxidant levels are good markers for internal dose, they may not reflect prostatic antioxidant levels; thus, results should be interpreted with this in mind. Other limitations include the failure to control for cholesterol levels, in particular, low density lipoprotein which function as transport vehicles for antioxidants [388], and abdominal adiposity which may influence the adipose antioxidant levels. The influence of individual differences in metabolism and absorption, interactions between individual antioxidants compounds and other micronutrients, as well as potential modifying effects of genetic variants acting via similar mechanisms [383, 407, 426] were beyond the scope

of this study. In addition, the possibility exists that some of the findings may be spurious owing to the sample size and multiple testing.

Gene-Diet Interaction

Very few studies have examined gene-diet interaction in relation to PCa aggressiveness, thus this study contributes important information to the limited data. The aggressive forms of PCa tend to have a strong genetic influence, and thus, aggressive and non-aggressive forms of PCa may differ in their etiology [458]. Thus, examining PCa aggressiveness reduces confounding by disease heterogeneity. Considering the controversy over the use of antioxidant nutrients for the prevention and control of PCa, this study provides evidence that indicates cautious use of such interventions, with suggestions that it may be beneficial only to a subgroup of men dependent on genotype of certain polymorphisms. Although genotyping errors cannot be completely ruled out in any genetic study, the genotyped data had over 99% concordance with blinded duplicates [411], which adds to the strengths of the study.

Limitations include the use of median cutpoints to categorize antioxidant in an effort to conserve sample size; however, this may have resulted in too small contrast between high and low antioxidant intake categories to observe a substantial effect of antioxidant by genotype. The observational nature of the study also precludes causal inferences. Because of the small sample size and multiple comparisons, chance findings cannot be excluded, especially considering that none of the *P* values retained statistical significance after FDR correction for multiple testing. Single dietary assessment in the year prior to PCa diagnosis may have resulted in misclassification of dietary intake and

overall dietary pattern to some extent. However, such misclassification is not likely to differ by the extent of PCa aggressiveness, as it is improbable that the research subjects considered their disease severity in responding to questions about dietary patterns. Thus, nondifferential misclassification may have attenuated some of the effect estimates. Even though analyses were stratified by self-reported race, residual confounding by ethnicity due to genetic admixture cannot be completely ruled out [537, 538]. Additionally, post hoc analysis involving adjustment for African ancestry proportions did not materially change the study results.

9.5 Public Health Significance

The study findings have important public health implications. First, we observed that certain antioxidant micronutrients (i.e., α-tocopherol, β-cryptoxanthin, *trans*-β-carotene, cis-lutein/zeaxanthin, and *trans*-lycopene) might slow the progression of PCa in men with biochemical recurrence of the disease. This was evidence by lower serum PSA levels among men with higher plasma concentrations of these nutrients at various timepoints over a 6-month period. If confirmed by other studies, dietary interventions that emphasize greater intake of these nutrients could serve as an alternate therapy or neoadjuvant to delay the use of androgen ablation in these patients. This is particularly encouraging because of the severe side effects associated with the use of androgen ablation [14-16].

The epidemiologic data relating to the role of antioxidants in PCa aggressiveness is very limited. Although the majority of PCa patients are diagnosed with indolent disease owing to the widespread use of PSA blood test for early detection, an estimated 30% of

prostate tumors progress aggressively [6]. Men with highly aggressive PCa have about a 14-fold increased risk of dying from the disease as compared to those with less aggressive disease [3]. Therefore, distinguishing the modifiable risk factors of virulent PCa from that of indolent disease is of particular interest. This study showed that greater intake of β-cryptoxanthin was inversely associated with PCa aggressiveness among African Americans, while greater intake of α-, γ- and δ-tocopherol as well as lycopene were associated with lower odds of PCa among European Americans. Given that the biology of PCa may be different between African Americans and European Americans, and the fact that populations included in previous studies were predominantly of European decent and thus findings may not apply to African Americans, this study uniquely provides information on the potentially beneficial dietary factors for the prevention/control of aggressive PCa in specific race groups.

The combined work of Objective 1 & 2 also advances our understanding on how antioxidants may be influencing PCa aggressiveness in general, and in men with certain genetic profiles. The evaluation of gene-diet interaction clearly demonstrated that not all men would benefit from antioxidant intervention in relation to PCa aggressiveness. Indeed, there were suggestions of increased odds of aggressive PCa with higher intake of some antioxidants in men with certain genetic profiles. Although preliminary, these findings have much translational potential as it could help clinician in designing structured and monitored dietary intervention programs aimed at reducing the occurrence of aggressive PCa, particularly among African Americans, an underserved population who suffer a greater burden of virulent PCa.

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